



Synthesis and Surface Coating of Magnetic Iron Oxide Nanoparticles for Biomedical Applications

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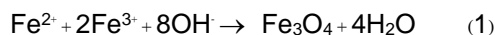
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Introduction

Magnetic iron oxide nanoparticles (MIONs) with appropriate surface chemistry have been widely used for numerous applications such as magnetic resonance imaging (MRI) contrast enhancement, immunoassay, hyperthermia, gene and drug delivery, cancer treatment, and cell targeting and separation. MIONs are required to have high magnetization, small size and good colloidal stability. Surface coating is an alternative way to increase colloidal stability, biocompatibility and targetability of MIONs. Magnetite (Fe₃O₄), a common magnetic iron oxide, can be synthesized through a co-precipitation of Fe²⁺ and Fe³⁺ at a 1:2 molar ratio and following by addition of a base. The overall reaction maybe written as described below:



In this study, we aimed to investigate factors affecting iron oxide nanoparticle synthesis, determine proper coating materials to provide good colloidal stability and reactive functional groups for further conjugation for targeted delivery application. The coating materials include oleic acid, citric acid, sodium carboxymethyl cellulose (SCMC), and polyethylene glycol (PEG). Iron oxide nanoparticles were characterized using Fourier Transform Infrared spectroscopy (FTIR), dynamic light scattering (DLS), optical microscope and colloidal stability was subsequently monitored.

Methods

Synthesis of iron oxide nanoparticles (IONs)

IONs were prepared using co-precipitation method. Ferric chloride (FeCl₃·6H₂O, 3.7 mmol) and ferrous sulfate (Fe₂SO₄·7H₂O, 1.53 mmol) were dissolved separately in 10 mL deionized water. The solutions were mixed with vigorously stirring for 15 min under nitrogen atmosphere. The mixture was allowed to react at room temperature, 60°C, or 90°C for 30 min. A base (ammonium hydroxide, NH₄OH, or sodium hydroxide solution, NaOH) was quickly dropped into the mixture and stirred for additional 30 min. Black precipitates were separated using centrifugation. The nanoparticles were subsequently washed for 3 times with water.

Surface coating of IONs

Coating with oleic acid¹

IONs were dispersed with 10 mL of 10% w/v oleic acid solution in hexane under vigorous stirring. The dispersion was concentrated by evaporating hexane. IONs were washed with 90% ethanol, centrifuged, and sonicated. Subsequently, 50 mL of 2% w/v aqueous solution of oleic acid and 0.1% NH₄OH were mixed and heated to 80°C for 15 min. One milliliter of the solution was added to IONs and stirred for 30 min at 80°C. IONs were washed with 90% ethanol, centrifuged, and sonicated.

Coating with citric acid²

IONs were dispersed in 100 mL of water and 10 mL of 1% or 2% or 3% w/v citric acid solution was added. The temperature was raised to 90°C under continuous stirring for 30 min.

Coating with SCMC³

IONs were dispersed in 100 mL of water and 0.05 or 0.10 or 0.15 g of SCMC was added. The solution was stirred for 30 min at 80°C.

Coating with PEG⁴

IONs were dispersed in 30 mL of water and 1.23 or 2.07 g of PEG400 or PEG6000 was added. The solution was stirred for 30 min at room temperature.

Characterization of iron oxide nanoparticles

Size and charge of IONs were determined using DLS (Marvern nanosizer, Nano-ZS90). The IONs were analyzed under a FTIR spectrometer (Nicolet iS5, Thermo Scientific) to monitor changes in chemical structure. Spectra were recorded in the range of 4,000-400 cm⁻¹. The IONs were also visualized under a light microscope.

Results**Preliminary study for IONs synthesis**

ION synthesis was carried out under various reaction conditions, for example, with different type and concentration of base (14.5% NH₄OH, 29% NH₄OH, 13M NaOH, 26M NaOH) and reaction temperature (room temperature, 60°C, 90°C). After adding different type and concentration of base, pH values of reaction mixtures were 12-14, which was a good pH range for IONs synthesis according to previous studies. However, reactions using NH₄OH resulted in greater yield (67-69%) compared to reactions using NaOH (50-59%). In addition, IONs from reaction using NH₄OH revealed higher magnetic property compared to IONs from reaction using NaOH. IONs from reaction using 14.5% NH₄OH were also slightly smaller in size compared to IONs from reaction using 29% NH₄OH. The synthesis, which was carried out at room temperature, resulted in smaller size (377-925 nm) compared to reactions that were carried out at 60°C (1,002-1,513 nm) and 90°C (1,157-1,505 nm). Therefore, 14.5% NH₄OH and room temperature were chosen to use for further synthesis.

Effect of coating

Coating is a good strategy to improve colloidal stability of IONs and also instantly result in desired functional groups on the surface depending on coating material used. Oleic acid, citric acid, SCMC, and PEG were chosen based on previous studies and availability in our lab. Prior to coating, IONs had an average size of 300 nm and negatively charged (-20 mV). IONs which were coated with oleic acid and SCMC were slightly larger in size after coating and their size remained unchanged after 1 month-storage at 4°C suggesting good colloidal stability (Fig. 1A and 1B). Whereas, IONs which were coated with citric acid showed similar size after coating, but their size became 3.5 to 5.5 times bigger after 1 month-storage at 4°C (Fig. 1A). In addition, IONs which were coated with PEG showed relatively large size after coating and 1 month-storage (Fig. 1C).

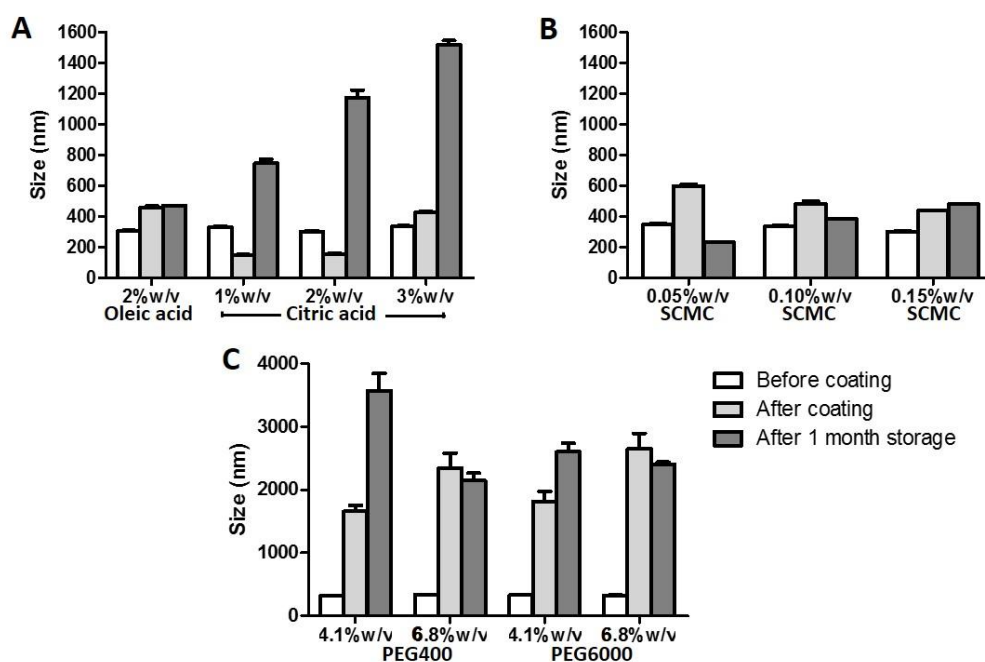


Figure 1 Size of IONs after coating with (A) oleic acid and citric acid (B) SCMC and (C) PEG400 and PEG6000. Data points represent mean±SD of three experiments.

After coating with oleic acid and SCMC, IONs had increased negative charge (-40 mV) suggesting the presence of coating materials on the surface (Fig. 2A and 2B). While, IONs coated with citric acid and PEG showed unchanged surface charge compared to IONs before coating (Fig. 2A and 2C).

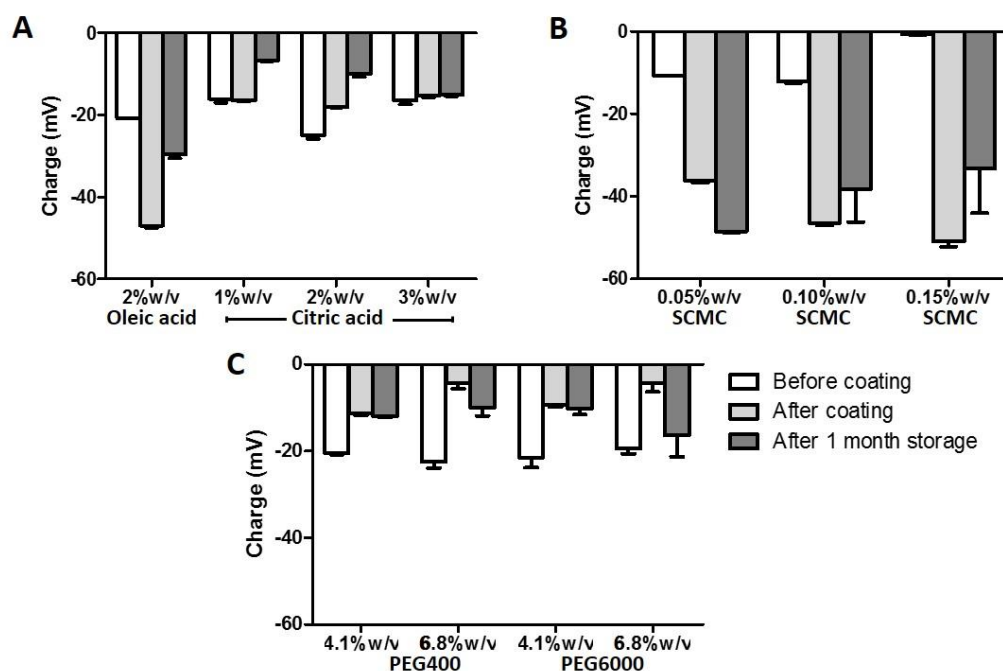


Figure 2 Charge of IONs after coating with (A) oleic acid and citric acid (B) SCMC and (C) PEG400 and PEG6000. Data points represent mean \pm SD of three experiments.

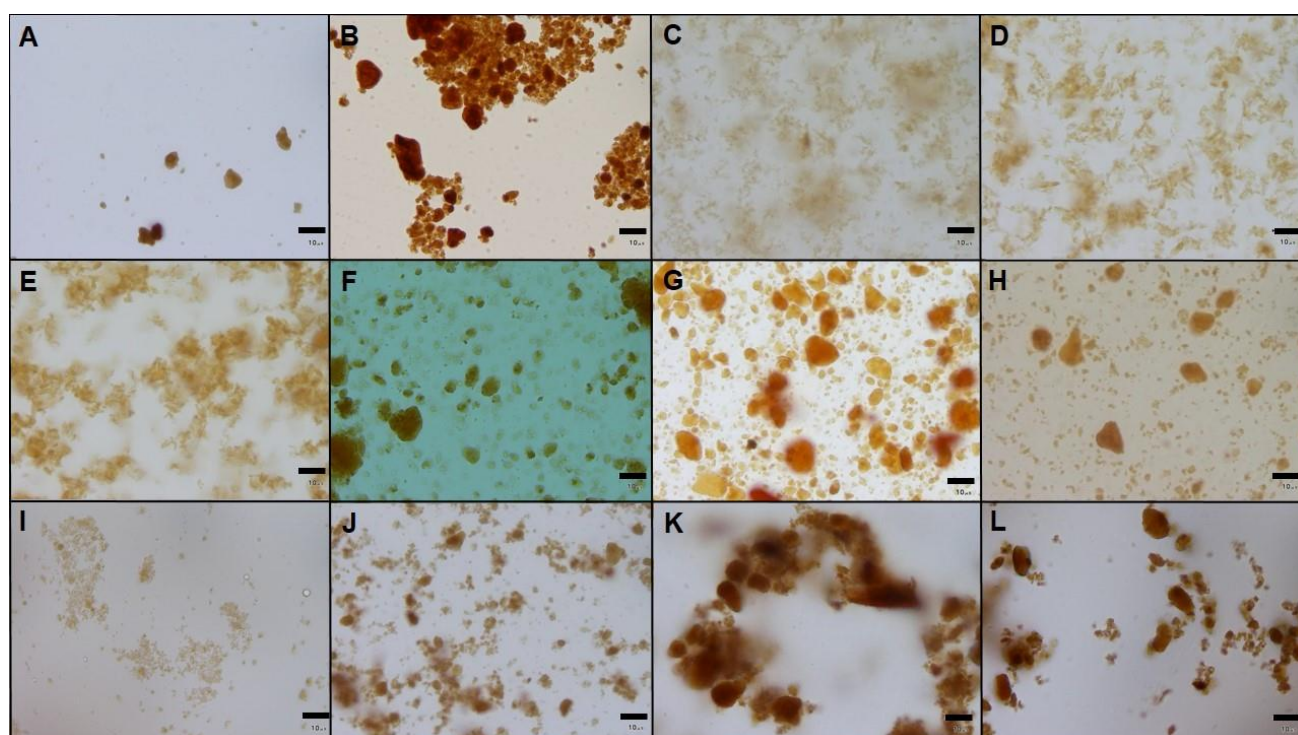


Figure 3 Iron oxide particles with different coating materials under microscope (A) non-coating (B) 2%w/v oleic acid (C) 1%w/v citric acid (D) 2%w/v citric acid (E) 3%w/v citric acid (F) 0.05%w/v SCMC (G) 0.10%w/v SCMC (H) 0.15%w/v SCMC (I) 4.1%w/v PEG400 (J) 6.8%w/v PEG400 (K) 4.1%w/v PEG6000 (L) 6.8%w/v PEG6000. Scale bars are 10 μ m.

Under a light microscope, IONs coated with oleic acid (Fig. 3B) and SCMC (Fig. 3F-3H) showed superior uniformity in size and less agglomeration which was in good agreement with DLS data. From Infrared spectra, all IONs showed both iron oxide and coating material characteristic (Fig. 4) indicating the presence of

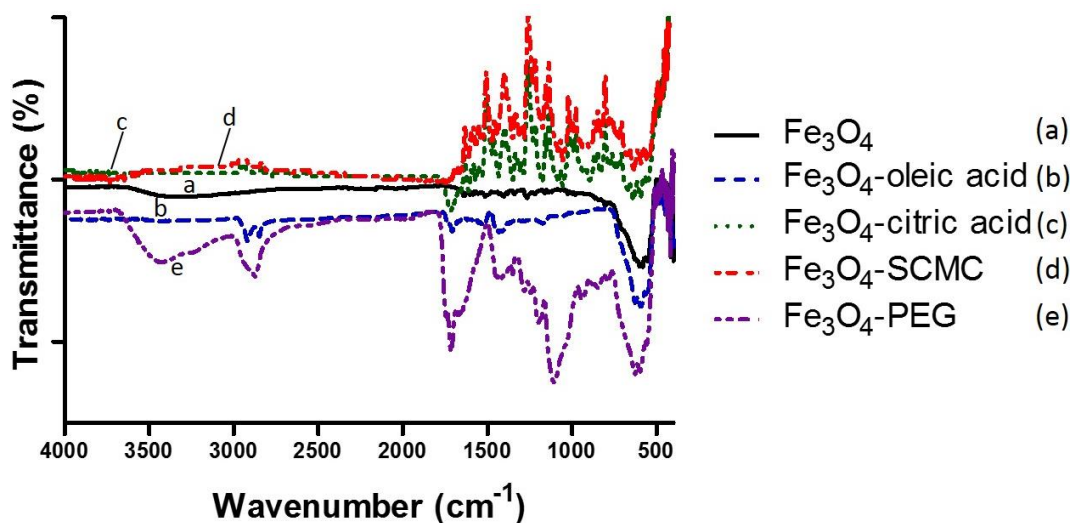


Figure 4 Infrared spectra of (a) Fe_3O_4 (b) Fe_3O_4 coated with oleic acid (c) Fe_3O_4 coated with citric acid (d) Fe_3O_4 coated with SCMC and (e) Fe_3O_4 coated with PEG.

oleic acid, citric acid, SCMC, and PEG. The characteristic absorption peak of Fe-O was observed at 580 cm^{-1} . The peak at 1700 cm^{-1} was assigned to C=O vibration of citric acid, oleic acid, and SCMC. The peak at $2800\text{--}2900\text{ cm}^{-1}$ was assigned to C-H vibration of CH_2 groups of oleic acid and PEG. The wide band at 3400 cm^{-1} was assigned to O-H vibration of PEG.

Discussion

In this study, IONs with different coating materials were successfully developed. Before coating, IONs had an average size of 300 nm and negatively charged (-20 mV). This size was similar to IONs reported by Oliveira et.al. (160-200 nm)¹. However, size of IONs from other studies were smaller and varied (10-60 nm)²⁻⁴. Our result suggested that there was some agglomeration that is consistent with morphology seen under a microscope. However, IONs which were coated with oleic acid and SCMC were slightly larger in size after coating and their size remained unchanged after 1 month-storage at 4°C suggesting good colloidal stability.

Conclusion

This study verified that oleic acid and SCMC are good coating materials resulting IONs with proper size and stable aqueous dispersion. However, further refinement, such as more precise size control, magnetization, in vitro MR imaging, drug encapsulation or targeting moiety conjugation, is essential to improve the effectiveness of these nanoparticles.

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