Synthesis of iron oxide nanoparticles with different morphologies by precipitation method with and without chitosan addition

Oleg VASYLKIV,† Oleksii BEZDOROZHEV and Yoshio SAKKA
National Institute for Materials Science, 1–2–1 Sengen, Tsukuba, Ibaraki 305–0047, Japan

In this work, iron oxide nanoparticles with various morphologies were synthesized by the precipitation method from aqueous solutions of iron oxide chlorides and urea in the absence or presence of chitosan. Synthesis conditions were selected to yield magnetite nanoparticles with three different morphologies. By adding chitosan, we were able to change the morphologies of the Fe₃O₄ nanoparticles from cubo-octahedral, flower-like, and rod-like structures to cubic, quasi-spherical, and rice-seed-like structures, respectively. The size of these iron oxide structures were in the range of 28–125 nm, while the longitudinal sizes of the rod-like and rice-seed-like structures were 110–1000 and 75–290 nm, respectively. Transmission electron microscopy (TEM) results showed that the magnetite nanostructures synthesized in the presence of chitosan have a mesoporous structure and are composed of many nanocrystals. The mechanisms responsible for the formation of differently shaped iron oxides in the presence or absence of chitosan are analyzed and discussed. We observed that the growth mechanism changed from the classical route to the reverse growth mechanism, when chitosan was added to the synthesis solution.

1. Introduction

In recent years, magnetic nanoparticles have attracted considerable research interest owing to their unique magnetic properties and technological applications. Among the magnetic nanoparticles, iron oxide nanoparticles are extremely interesting for their applications in catalysts, pigments, water treatment, magnetic materials, sensors, and biomedical fields. The biomedical applications include targeted drug delivery, magnetic hyperthermia therapy, MRI contrast enhancement, bioseparation, and biosensors. The broad range of applications of iron oxide nanoparticles is attributed to their high saturation magnetization, magnetic susceptibility, non-toxicity, reactive surface, biodegradability, and biocompatibility. Owing to their excellent characteristics, considerable effort has been made to synthesize iron oxide nanoparticles with different sizes and shapes. For instance, nanoparticles with shapes such as cubes, spheres, rods, spindles, and nanobelts have been successfully synthesized. Numerous methods, such as sol–gel, precipitation, microemulsion, sonochemical, hydrothermal, electrochemical, nano-explosion, and aerosol/vapor methods have been developed to effectively control nanoparticle properties. Some of these chemical methods were optimized in our previous studies and successfully used for the synthesis of ceria- and zirconia-based nanoparticles with tunable properties. The precipitation method is usually used for the production of iron oxide nanoparticles due to its simplicity and cost-effectiveness. However, their synthesis has become a challenging area of research since the synthesis parameters play a key role in determining the particle size, shape, phase composition, and other properties of iron oxide nanoparticles. The addition of organic or inorganic compounds is also known to have a strong effect on the formation and growth processes of iron oxides in aqueous reaction mixtures. The biopolymer-assisted synthesis of nanomaterials is an attractive field of research based on green chemistry principles. As a natural biopolymer, chitosan has been applied in the synthesis of functional materials. Chitosan is a biocompatible, biodegradable, linear aminopolysaccharide, which is obtained through the alkaline N-deacetylation of natural chitin. Also, chitosan has high ability to chelate metal ions due to the presence of NH₂ and OH groups in its D-glucosamine units. For this reason, chitosan has been employed as a surface modifier of magnetic nanoparticles as well as a reducing and stabilizing agent in the synthesis of metallic nanoparticles.

It was found that the size, shape, porosity, and aggregation of nanoparticles can be controlled by varying the chitosan concentration owing to its interaction with the surface of particles. Moreover, Ritchie et al. showed that the presence of chitosan resulted in the reverse crystal growth of rhombohedral CaCO₃ through (I) the formation of large Ca⁺⁺-chitosan aggregates, (II) the nucleation of CaCO₃ on the surface of the aggregates, (III) their reaggregation into polycrystalline rhombohedral particles, and (IV) surface recrystallization to form a ‘core–shell’ structure, followed by (V) reverse crystal growth from the surface to the core via Ostwald ripening. However, despite the considerable number of publications on the synthesis of iron oxide/chitosan composites, there is still a lack of reports on the effect of chitosan on the synthesis and properties of iron oxides. Thus, in the present study, we report on the effect of chitosan addition on the morphology of iron oxide nanoparticles. We show the possibility of manipulating the morphology to form cubic, quasi-spherical, and rice-seed-like structures in the presence of chitosan.

2. Experimental procedure

2.1 Synthesis of iron oxide nanoparticles

Iron oxide nanoparticles were prepared by the chemical precipitation of Fe²⁺ and Fe³⁺ ions by urea in the presence or
absence of chitosan. For this purpose, aqueous solutions of FeCl₂·4H₂O and FeCl₃·6H₂O (99%, Wako Pure Chemical Industries Ltd., Japan) with a 1:2 molar ratio and concentrations of Fe²⁺ ions of 0.02 and 0.05 M were prepared. Urea (NH₂CONH₂, 99%, Kanto Chemical Co., Japan) was added as a precipitating agent to the previously prepared aqueous iron chloride solutions and the mixture was stirred at room temperature for 10 h. The weight ratio of metal chlorides to urea was 1:2. Chitosan acetic solution was obtained by dissolving 500 mg of chitosan with a molecular weight of 400 kDa and 87% degree of deacetylation (Fluka BioChemika, Japan) in 200 ml of 0.1 mol/l aqueous acetic acid solution under intensive agitation. To study the effect of chitosan addition on the morphology, size, and phase composition of iron oxide nanoparticles, an aqueous solution of iron chloride and chitosan acetic solution was also prepared by mixing for 10 h. The resulting solution was then used to precipitate iron oxide nanoparticles at 80–95°C for 2–50 h using a magnetic stirrer with a hot plate. To ensure that a pure Fe₃O₄ phase was obtained without any other iron oxide phases, the synthesis was carried out in a nitrogen atmosphere. The above synthesis parameters were intentionally chosen to yield iron oxide nanoparticles with different morphologies by precipitation method with and without chitosan addition on the morphology, size, and phase composition of iron oxide nanoparticles with various morphologies. For comparison, iron oxide nanoparticles were also precipitated in the same synthesis regimes as above but without the addition of chitosan acetic solution. After cooling the mixture, the precipitate was washed several times with distilled water and ethanol (99.5% reagent grade) to remove anion impurities and to suppress agglomeration. An ultrasonic horn and a centrifuge (10000 rpm for 5 min) were used to break up powder agglomerates and to separate the powder from the supernatant, respectively. After that, the dried products were annealed at 350°C in a N₂ atmosphere for 1 h to obtain well-crystallized powders.

2.2 Characterization

The crystal structure of the samples was determined by X-ray diffraction (XRD) using a Rigaku Ultima III diffractometer (Rigaku Corp., Japan) with CuKα radiation (λ = 1.5406 Å) in the 2θ range of 20–80° with 0.01 intervals at 40 kV and 40 mA. The size and morphology of the nanoparticles were characterized by transmission electron microscopy (TEM) using a JEM-2100-F microscope (JEOL Ltd., Japan) operated at 200 kV. The TEM samples were obtained by placing a drop of the solution with dispersed nanoparticles onto a copper mesh grid, which was followed by evaporation in air at room temperature.

3. Results and discussion

Figure 1(a) shows a TEM micrograph of the iron oxide nanoparticles synthesized from an aqueous solution of 0.02 M FeCl₂·4H₂O and 0.04 M FeCl₃·6H₂O at 80°C for 25 h. The nanoparticles have an octahedral shape with a shape factor (length/width ratio) between 1 and 1.6, indicating that the nanoparticles are not very elongated. A relatively small number of nanoparticles with a cuboidal morphology were also observed. The width of the nanoparticles varies from 35 to 96 nm and the length varies from 50 to 125 nm, while the mean width and length of the nanoparticles are 68 ± 8 and 95 ± 11 nm, respectively. The cube-octahedral shape of the nanoparticles is the most common for Fe₃O₄ and is a result of the minimization of the surface energy and also the synthesis conditions, which favor growth along the three crystallographic axes of Fe₃O₄. The surface energy of the nanoparticles is also minimized through their agglomeration due to van der Waals and dipole–dipole interactions. The observed size, shape, and phase composition of the iron oxide nanoparticles prepared with different synthesis parameters are compared in Table 1.

Figure 1(b) shows a TEM image of the iron nanoparticles synthesized at 80°C for 25 h with the same iron ion concentrations but with the addition of chitosan. In this case, the iron oxide nanoparticles self-assembled into polycrystalline mesoporous structures with a cubic shape. The size of these structures varies from 72 to 90 nm. Cube-octahedral clusters with sizes of 60–105 nm were also detected but in an insignificant quantity. In our experiments, we did not observe chitosan nanoparticles into TEM images.

![Fig. 1. TEM images of (a) cubo-octahedral nanoparticles and (b) cubic mesoporous structures precipitated in the absence and presence of chitosan, respectively.](image)

| Table 1. Properties of iron oxides precipitated at different synthesis parameters |
|-----------------|-----------------|-----------------|
| Synthesis parameters | Shape | Size (nm) | Phase composition |
|-----------------|-----------------|-----------------|
| 0.02 M Fe²⁺, 0.04 M Fe³⁺, 80°C, 25 h | cubo-octahedral cubic⁹⁺ | 50–125⁹⁺, 35–91¹¹ | α-Fe₂O₃, Fe₃O₄ |
| 0.02 M Fe²⁺, 0.04 M Fe³⁺, 95°C, 2 h | flower-like quasi-spherical⁹⁺ | 55–125²³ | Fe₃O₄ |
| 0.05 M Fe²⁺, 0.1 M Fe³⁺, 80°C, 50 h | rod-like rice-seed-like⁹⁺ | 110–1000⁹⁺, 22–44¹¹ | α-Fe₂O₃, Fe₃O₄ |

| a) Synthesis in the presence of chitosan; b) Length; c) Width. |
From the obtained results, it is clear that chitosan played a key role in the formation of the polycrystalline mesoporous structures, in contrast to the cubo-octahedral iron oxide nanoparticles prepared under the same synthesis conditions but without the addition of chitosan. Since chitosan has functional amino (NH₂) and hydroxyl groups, it has high affinity to bind with iron oxide nanoparticles. Thus, the presence of chitosan resulted in the capture of iron hydroxide groups by chitosan, inhibiting the growth of individual iron oxide nanocrystals. These individual nanocrystals are probably cubo-octahedral in shape since the synthesis conditions are favorable for this, as on Figs. 1(a) and 1(b).

As the reaction time was prolonged, the small nanoparticles aggregated to reduce the surface energy and form cubic mesoporous structures. Interestingly, even though the mesoporous structures were composed of many nanocrystals, a cubic shape was formed, implying that the iron oxide nanocrystals are well oriented.

The XRD pattern in Fig. 2(a) supports the conclusion that the synthesized iron oxide nanoparticles are Fe₃O₄ (DB card No. 9005836). The presence of sharp peaks indicates the high crystallinity of the Fe₃O₄ phase. However, some minor peaks corresponding to α-Fe₂O₃ (DB card No. 2101167) were also detected. The presence of the α-Fe₂O₃ phase is probably due to its amorphous nature and/or degradation during thermal treatment at 350°C in the N₂ atmosphere.

To further study the effect of chitosan on the morphology of iron oxide nanoparticles, the synthesis parameters were changed to obtain nanoparticles with other shapes. A TEM image of the nanoparticles prepared from an aqueous solution of 0.05 M FeCl₂·4H₂O and 0.1 M FeCl₃·6H₂O at 95°C for 2 h is shown in Fig. 3(a). The as-prepared sample had a self-oriented flower-like nanostructure, which formed from multiple randomly assembled nanosheets [Fig. 3(a)]. As evidenced from the TEM images, the diameter of these 3D flower-like nanostructures is 55–125 nm, while the thickness of the nanosheets is 2–4 nm. According to Zhu et al., the formation process for the flower-like structure proceeds through (I) nucleation and aggregation, (II) secondary oriented growth (self-assembly into nanosheets), (III) further growth (self-assembly into a 3D flower-like nanostructure), and (IV) Ostwald ripening (oriented growth of the nanosheets at the expense of adjacent smaller nanoparticles). The yield of flower-like nanostructures was nearly 100% for the selected synthesis parameters.

As can be seen from Fig. 3(a), the flower-like nanostructures tended to agglomerate owing to the van der Waals interaction between the particles. In this case, the complex morphology and comparatively large surface-to-volume ratio of the flower-like nanostructures promoted the agglomeration.

Figure 3(b) shows a TEM micrograph of the iron oxide nanoparticles precipitated at 95°C for 2 h in the presence of chitosan. As in the previous case, the addition of chitosan altered the morphology of the precipitated product. In contrast to the flower-like structures in Fig. 3(a), the precipitated nanostructures in Fig. 3(b) have a quasi-spherical shape, with a small number of particles with a nonspherical shape also present. The synthesis conditions were favorable for the direct formation of nanostructures with sizes ranging from 28 to 105 nm and an average size of 72 ± 10 nm.

The formation of quasi-spherical nanostructures in the presence of chitosan can be explained as follows. First, the iron hydroxide groups on the surface of iron oxide crystals are easily linked to chitosan molecules by the adsorption interactions, suppressing the nucleation and further growth of iron oxide crystals in the solution. Second, the formed nuclei aggregate to eliminate the interfaces and reduce the total energy of the system. Although the growth of individual iron oxide crystals inside the aggregates is inhibited, the surface of these aggregates provides the most active nucleation sites. Thus, the formed polycrystal-
line nanostructures are a result of slow crystal growth and the aggregation of iron oxide crystals, as can be seen from Fig. 3(b). Figure 3(b) also shows the mesoporous nature of the nanostructures, which supports the above conclusion. A possible reason for the formation of mesoporous quasi-spherical nanostructures instead of the flower-like ones is that the chitosan interacted with the surface of the iron oxide crystals, inhibiting their self-assembly into flower-like structures.

According to the XRD pattern in Fig. 4(a), the precipitated flower-like structures can be indexed to the pure phase of magnetite (DB card No. 9006242). No other peaks can be observed in the XRD pattern, confirming the high purity of the product. The intense peaks in the XRD pattern indicate that a well-crystallized structure was formed. An almost identical XRD pattern was obtained for the iron oxide structures precipitated in the presence of chitosan [Fig. 4(b)]. All peaks were assigned to Fe3O4 and were in good agreement with the standard data for Fe3O4 (DB card No. 9007644). No other crystalline phases were detected in the synthesized sample.

The precipitation of iron oxide particles from the aqueous solution of 0.05 M FeCl2·4H2O and 0.1 M FeCl3·6H2O at 80°C for 50 h in the absence of chitosan yielded rod-shaped particles [Fig. 5(a)]. The length of the rods was in the range of 110 nm to 1 μm with a width of 22–44 nm. No other particle morphology was observed under these experimental conditions. The primary nanorods showed a tendency to aggregate into larger rods. In some cases, the formation of dendrites was also observed.

The existence of rod-shaped structures implies the anisotropic growth of iron oxide nanoparticles. The rod-like shape of the iron oxide particles suggests their origin from goethite as the primary phase.32) For goethite, the preferential growth in the direction perpendicular to the (010) plane is responsible for the rod-like shape. The formation of the goethite phase is facilitated by theolation mechanism involving the condensation of the hydroxo and aquo-hydroxo complexes in solution.33) The as-prepared goethite precursor can be used to prepare porous iron oxide rods by heating. The goethite rods transform to Fe2O3 rods via a topotactic transformation without significant structural modification and the rod-like morphology is retained.34) During the thermal decomposition of goethite and the release of H2O, nanosize pores are generated, as can be seen from the insert in Fig. 5(a). The size of the pores is in the range of 4–30 nm. As evidenced from the XRD pattern [Fig. 6(a)], a mixture of α-Fe2O3 (DB card No. 2101167) and Fe3O4 (DB card No. 9000926) phases was formed after the thermal treatment of goethite at 350°C in N2 atmosphere. The presence of hematite was attributed to the partial oxidation of Fe3O4 to α-Fe2O3. All the strong and narrow diffraction peaks indicate the high crystallinity of the samples.

A TEM image of the iron oxide particles precipitated from the aqueous mixture of iron chlorides, urea, acetic acid, and chitosan at 80°C for 50 h is shown in Fig. 5(b). In contrast to the rod-like particles precipitated from the chitosan-free solution [Fig. 5(a)], the addition of chitosan leads to the formation of mostly rice-seed-like structures along with a very small fraction of quasi-spherical structures. The yield of rice-seed-like structures was nearly 80% for the selected synthesis parameters. The estimated average length and width of the structures are 206 ± 19 and
117 ± 13 nm, respectively. The shape factor varies between 1.1 and 2.5, indicating a wide length/width ratio distribution and the elongated nature of the structures. The quasi-spherical structures have a size ranging from 89 to 128 nm, as estimated from the TEM image.

It was concluded that each of the rice-seed-like structures has a rough surface composed of many closely packed nanoparticles, as evident from the inset in Fig. 5(b). In the previous synthesis of iron oxide nanoparticles in the presence of chitosan, the formation of rice-seed-like structures most likely proceeds via the adsorption of iron oxide crystals on the surface of chitosan molecules, their aggregation, slow crystal growth, and self-assembly into polycrystalline rice-seed-like structures. In contrast to the synthesis in a pure inorganic system, the crystal growth rate in the presence of chitosan is significantly reduced owing to the rapid adsorption of organic molecules onto the crystal surface. Under these experimental conditions, it is likely that the aggregation rate is faster and dominant over the crystal growth rate. Thus, the iron oxide crystals have relatively little time to form perfect rod-shaped crystals; instead they join together to form polycrystalline rice-seed-like and quasi-spherical structures.

In contrast to the two-phase rod-like particles [Fig. 6(a)], the rice-seed-like structures were found to consist of a pure Fe$_3$O$_4$ phase [Fig. 6(b)] since all the characteristic peaks were found to be consistent with the diffraction pattern of Fe$_3$O$_4$ (DB card No. 9006242). No diffraction peaks from other crystalline forms were detected, indicating that the formation of Fe$_3$O$_4$ is more probable than that of Fe$_2$O$_3$ under the selected synthesis conditions.

From the results, it is evident that chitosan markedly affected the growth mechanism as well as the morphology of the Fe$_3$O$_4$ nanoparticles. We consider that the growth mechanism changed from the classical route (single nucleation) to the reverse growth mechanism when chitosan was added to the synthesis solution. The formation of cubo-octahedral, flower-like, and rod-like iron oxide particles follows the classical theory of crystal growth, which is described by the Bravais-Friedel-Donnay-Harker law and the Hartman-Perdok approach. In this case, the crystal grows from a single nucleus through the layer-by-layer deposition of ions with different growth rates along different crystallographic orientations. However, the reverse growth mechanism is believed to be responsible for the formation of cubic, quasi-spherical, and rice-seed-like structures. Figures 7(a)–7(d) shows schematic illustrations of the formation process of iron oxide structures in the presence of chitosan. Figure 7(a) represents the adsorption and nucleation of iron oxide crystals on the surface of chitosan molecules. Figure 7(b) schematically shows the aggregation of crystallites. Figures 7(c) and 7(d) show slow crystal growth and self-assembly into polycrystalline structures, respectively. The adhesion and nucleation of iron oxide crystals [see Figs. 7(a)–7(d)] start on the surface of chitosan molecules, forming single crystals that aggregate during their subsequent growth and self-assembly into polycrystalline structures. It is expected that upon further prolonging the reaction time, the crystallization will extend from the surface to the core via Ostwald ripening, resulting in the formation of a single-crystalline particle.

4. Conclusions

This paper reports our investigation of the effect of chitosan addition on the morphology of iron oxide nanoparticles synthesized by the precipitation method. The precipitation was carried out at 80–95°C for 2–50 h from aqueous solutions of iron oxide chlorides and urea to prepare magnetite nanoparticles with various morphologies. It was shown that chitosan addition changes the cubo-octahedral morphology of Fe$_3$O$_4$ nanoparticles (50–125 nm) to cubic mesoporous structures with sizes of 60–105 nm. Moreover, the formation of polycrystalline quasi-spherical (28–105 nm) and rice-seed-like (75–290 nm) structures instead of flower-like (55–125 nm) and rod-like (0.11–1 μm) particles, respectively, was also observed when chitosan was added to the synthesis solution. The results of the present study clearly demonstrate that chitosan plays a key role in the formation of polycrystalline mesoporous structures. The addition of chitosan can alter the growth mechanism from the classical route (single nucleation) to the reverse growth mechanism. In this case, the free crystal growth in the early stages is inhibited by the capture of iron oxide nanoparticles by chitosan. To reduce the surface energy, the small crystals aggregate, and the surface of these aggregates provides the most active nucleation sites. This is followed by slow crystal growth on the surface of the aggregates and their self-assembly into polycrystalline mesoporous structures. The shape of these structures is determined by the morphology of the initial crystals. The obtained results may provide a deeper understanding of the growth mechanism of iron oxide particles in the presence of biopolymers for further tuning of their properties. Thus, high-quality iron oxide nanoparticles with various morphologies can be formed by a relatively simple, low-cost, and non-toxic method, enabling their use in various biomedical applications.

References