



# Synthesis and characterization of hollow magnetic nanospheres modified with Au nanoparticles for bio-encapsulation



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## ABSTRACT

Hollow magnetic nanospheres modified with Au nanoparticles were successfully synthesized. Au/SiO<sub>2</sub> nanospheres fabricated by a radiochemical process were used as templates for ferrite templating. After the ferrite plating process, Au/SiO<sub>2</sub> templates were fully coated with magnetite nanoparticles. Dissolution of the SiO<sub>2</sub> core lead to the formation of hollow magnetic nanospheres with Au nanoparticles inside. The hollow magnetic nanospheres consisted of Fe<sub>3</sub>O<sub>4</sub> grains, with an average diameter of 60 nm, connected to form the sphere wall, inside which Au grains with an average diameter of 7.2 nm were encapsulated. The Au nanoparticles immobilized on the SiO<sub>2</sub> templates contributed to the adsorption of the Fe ion precursor and/or Fe<sub>3</sub>O<sub>4</sub> seeds. These hollow magnetic nanospheres are proposed as a new type of nanocarrier, as the Au grains could specifically immobilize biomolecules inside the hollow sphere.

## 1. Introduction

In recent years, porous hollow nanospheres fabricated from various inorganic materials have been widely studied for biomedical applications, especially as a new type of carrier for drug delivery systems [1–8]. These unique structures enable the encapsulation of biomolecules such as genes, amino acids, and proteins inside the hollow spheres. Among the many different kinds of hollow nanospheres, magnetic nanospheres are particularly interesting as they respond to an external applied magnetic field. Magnetic nanospheres consist of magnetic nanoparticles connected to form a porous sphere wall. Many researchers are working on the application of such structures for magnetic drug targeting, as they could be delivered and accumulated by applying a direct current magnetic field [6–8]. In addition, the application of an alternating current magnetic field to the magnetic particles can generate heat [9], which could control the functionality of the biomolecules inside the spheres.

Although encapsulation of biomolecules inside hollow nanospheres is expected to avoid activity loss, which is often caused by interactions with the immune system [2], elution of encapsulated biomolecules is sometimes a challenge. Therefore, carriers that could immobilize biomolecules only inside the sphere would be beneficial for both in-vivo and in-vitro applications of hollow spheres. In the case of using inorganic nanoparticles for carriers, biomolecules are usually immobilized onto surfaces via covalent bonding or noncovalent attachment [1].

However, this strategy cannot be used for hollow spheres, as a technique to functionalize the surface of nanoparticles only inside the sphere is not yet established.

The target of this study is to immobilize Au nanoparticles only inside the magnetic nanospheres. As is well known, Au nanoparticles show strong affinity for thiol groups, which enables simple and specific surface functionalization [1,10]. It has been reported that Au nanoparticles immobilized on iron oxide nanoparticles can be used as a specific tag for various molecules such as amino acids, nucleic acids, polymers, and enzymes without activity loss [10]. Therefore, Au nanoparticles inside the sphere are also applicable for the specific immobilization of biomolecules.

In order to fabricate magnetic nanospheres with Au nanoparticles inside, a ferrite plating method was employed using SiO<sub>2</sub> spheres modified with Au nanoparticles as templates (Au/SiO<sub>2</sub>). Immobilization of Au nanoparticles on the SiO<sub>2</sub> spheres was performed by a radiochemical process. The process of fabricating such nanospheres is discussed here in detail.

## 2. Experimental procedure

### 2.1. Materials

SiO<sub>2</sub> nanospheres modified with NH<sub>2</sub> groups (NH<sub>2</sub> SicaStar, 300 nm) were purchased from Corefront Corp. Hydrogen

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Tetrachloroaurate(III) Tetrahydrate ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ ), Polyvinyl alcohol (PVA,  $n = 500$ ), 2-propanol, sodium hydroxide (NaOH), sodium acetate ( $\text{CH}_3\text{COONa}$ ), and iron(II) sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) were purchased from Wako Pure Chemical Industries, Ltd. Sodium nitrate ( $\text{NaNO}_3$ ) was purchased from Kanto Chemical Co., Inc. Ultrapure water (Millipore Direct-Q) was used for all syntheses.

## 2.2. Preparation of Au/SiO<sub>2</sub> templates

Au/SiO<sub>2</sub> templates were prepared using the electron beam irradiation reduction method. The detailed procedure is reported elsewhere [11]. An aqueous solution of 26 mL containing 1.0 mM  $\text{HAuCl}_4$ , 26 mg SiO<sub>2</sub>, 1.0 wt% PVA, and 1.0 vol% 2-propanol was prepared in a 100 mL sealed glass bottle. The solution was irradiated with a high-energy electron beam (40 kGy, 4.8 MeV) to reduce the Au ions and form metallic Au nanoparticles, which were stabilized on the surface of SiO<sub>2</sub> supports. The irradiated samples were washed for several times with pure water using a centrifuging process. The Au/SiO<sub>2</sub> templates thus obtained were dispersed in 20 mL of ultrapure water.

## 2.3. Ferrite plating on Au/SiO<sub>2</sub> templates and dissolution of SiO<sub>2</sub> cores

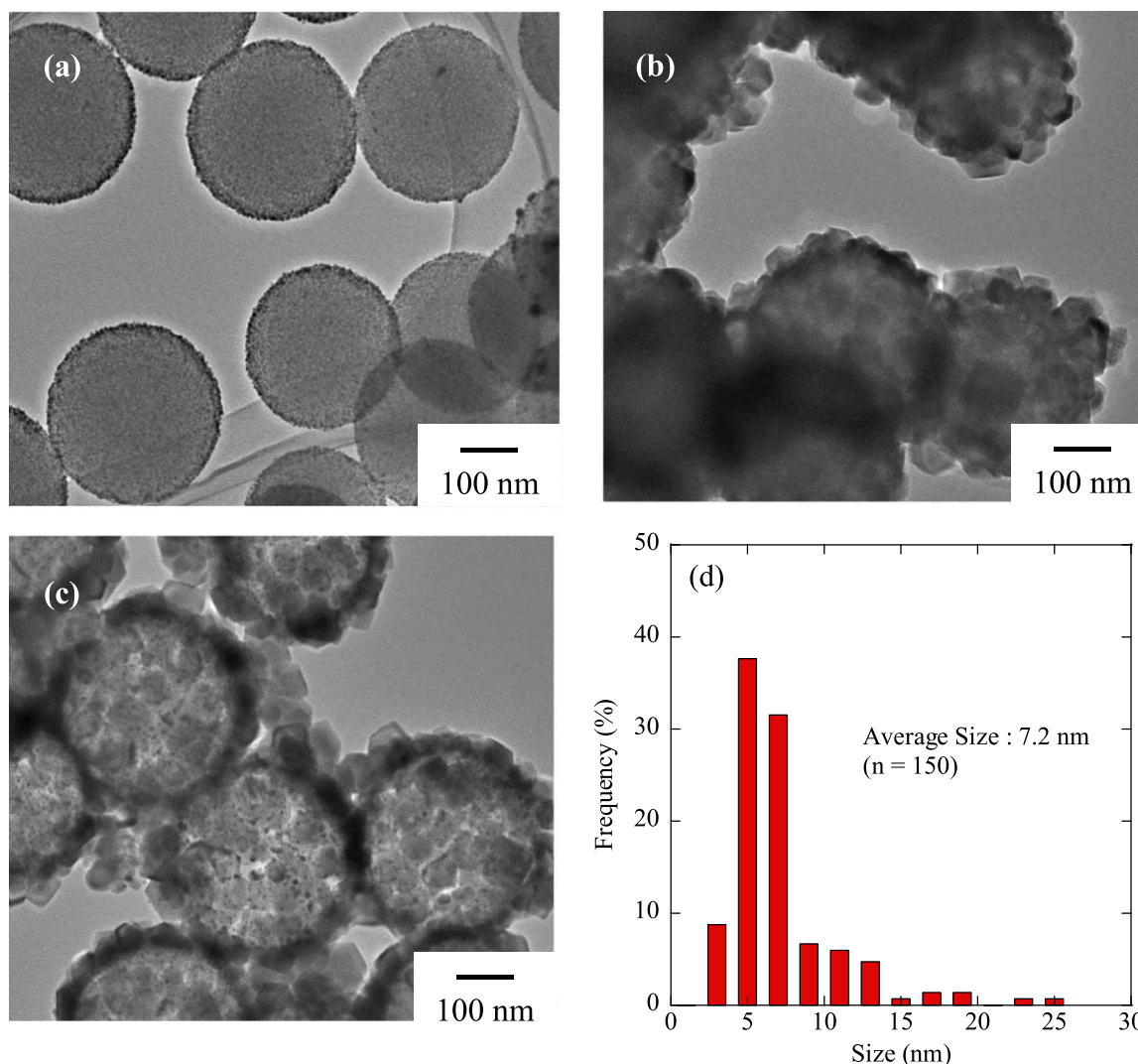
Ferrite plating on Au/SiO<sub>2</sub> templates were performed based on the

procedures give in a previous report [8]. All the pure water used in this process was purged with nitrogen gas. The aqueous dispersion of Au/SiO<sub>2</sub> templates were mixed with 180 mL of aqueous solution containing 16.4 g  $\text{CH}_3\text{COONa}$ , 111.2 mg  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.25 M  $\text{NaNO}_3$ . After the mixed solution was heated to 60 °C, an additional 111.2 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  was added to the solution and kept for 3 h. The product was collected by magnetic separation and washed with ultrapure water five times. The resulting powder was dispersed in 30 mL of 1.0 M NaOH solution at room temperature, left for 24 h, then washed in pure water with a magnetic decantation process. The resulting hollow magnetic nanospheres with Au nanoparticles inside were then used for further characterization process.

## 2.4. Material characterization

The morphologies of the nanospheres were analyzed by TEM (transmission electron microscopy, HITACHI H-8100T) and SEM (scanning electron microscopy, JEOL SM-7001). Elemental analysis was performed using an ICP-AES (Shimadzu, ICPS-7500). X-ray diffraction patterns were measured with a diffractometer (Rigaku RINT2100-Ultima+), using Cu-K $\alpha$  radiation. The magnetic properties were analyzed with a VSM (vibrating sample magnetometer, TOEI VSM-3S).

The surface activity of Au nanoparticles inside the hollow sphere



**Fig. 1.** TEM micrographs of (a) Au/SiO<sub>2</sub> template, (b) ferrite-plated Au/SiO<sub>2</sub> template, and (c) hollow magnetic nanospheres with Au nanoparticles inside. Size distribution of Au particles on SiO<sub>2</sub> is shown in (d).

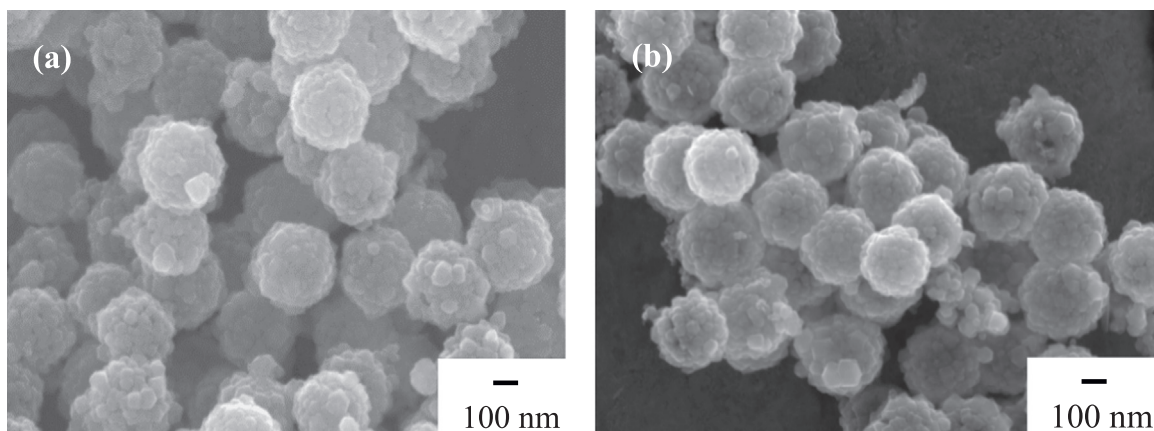


Fig. 2. SEM micrographs of (a) ferrite-plated Au/SiO<sub>2</sub> template and (b) hollow magnetic nanospheres with Au nanoparticles inside.

was confirmed by the adsorption of cysteine, which is a sulfur containing amino acid possessing thiol group. The dispersion of the magnetic hollow spheres were mixed with cysteine solution and then kept for 24 h. After the magnetic separation, spheres and adsorbed cysteine were dissolved in aqua regia for ICP-AES measurement. Amounts of adsorbed cysteine were determined by the sulfur content. For comparison, monolithic iron-oxide nanoparticles were also employed. The iron oxide nanoparticles were prepared by the ferrite plating onto the SiO<sub>2</sub> templates without Au grains.

### 3. Results and discussion

Figs. 1 and 2 show typical TEM and SEM images of the particles during various stages of the synthesis process. Fig. 1(a) shows a typical TEM image of prepared Au/SiO<sub>2</sub> templates. It was observed that Au particles were well dispersed on the surface of the SiO<sub>2</sub> particles. Size distribution of Au particles were shown in Fig. 1(d). Average size of Au particles was 7.2 nm. The aqueous dispersion of the Au/SiO<sub>2</sub> template particles was sonicated several times during the washing process and then dropped onto a Cu grid for TEM observation. The Au nanoparticles are firmly immobilized onto the SiO<sub>2</sub> support. After the ferrite plating process, the Au/SiO<sub>2</sub> templates were fully covered with magnetite nanoparticles as shown in the TEM and SEM images in Fig. 1(b) and Fig. 2(a). The spherical morphology of the SiO<sub>2</sub> templates was still observed. It should also be noted here that magnetite nanoparticles not attached to the Au/SiO<sub>2</sub> templates were rarely observed. After infiltrating the templates with a NaOH solution to dissolve the SiO<sub>2</sub> core, the resulting particles still keep the spherical shape, as shown in the SEM image in Fig. 2(c). The SEM-EDX analysis shown in Fig. 3 indicate that the cores of the SiO<sub>2</sub> spheres were successfully dissolved and removed. This means that small pores in the shell of the spheres acted as the flow channels for the NaOH solution. Also, the presence of Au was confirmed even after core dissolution. TEM observations shown in Fig. 1(c) revealed that a spherical hollow structure was successfully obtained, and Au nanoparticles (seen as darker grains) exist only inside the hollow structure. The average size of the ferrite particles determined by TEM observation was about 60 nm, which is almost the same as that before the core dissolution. Fig. 4 shows the XRD pattern of hollow ferrite particles modified with Au nanoparticles. Relatively sharp peaks corresponding to the Fe<sub>3</sub>O<sub>4</sub> phase were observed together with relatively weak and broad peaks of Au (111) around 38°. These material characterization results indicated that hollow magnetic spheres modified with Au nanoparticles were successfully synthesized using the described synthesis procedure.

Fig. 5 shows the magnetization curve of the samples. The magnetization values were normalized to the mass of Fe<sub>3</sub>O<sub>4</sub> in the samples. As shown in the figure, the magnetic properties did not change with the dissolution of the SiO<sub>2</sub> core. The magnetization value under an applied

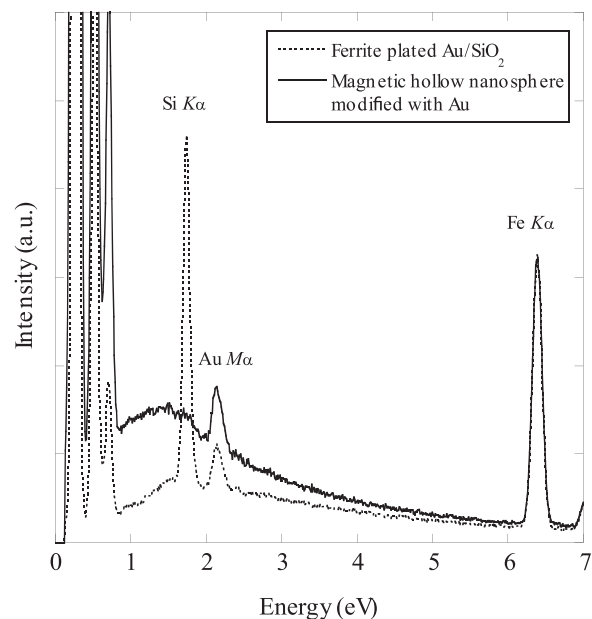


Fig. 3. SEM-EDX spectrum of the synthesized composite particles.

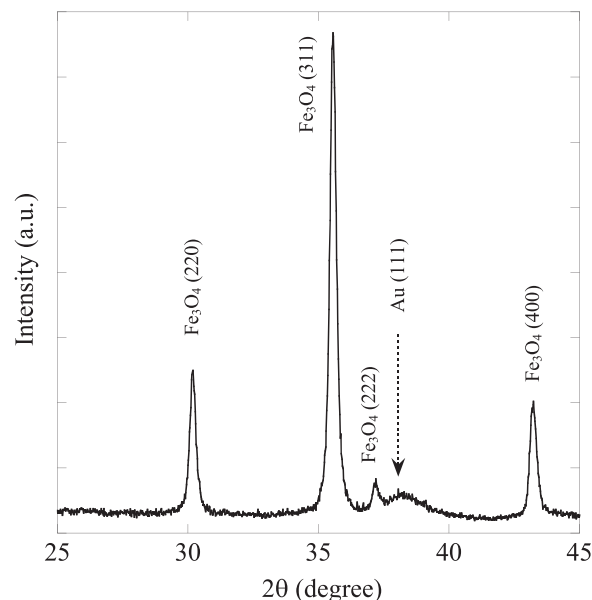


Fig. 4. XRD pattern of the hollow magnetic nanospheres with Au nanoparticles inside.

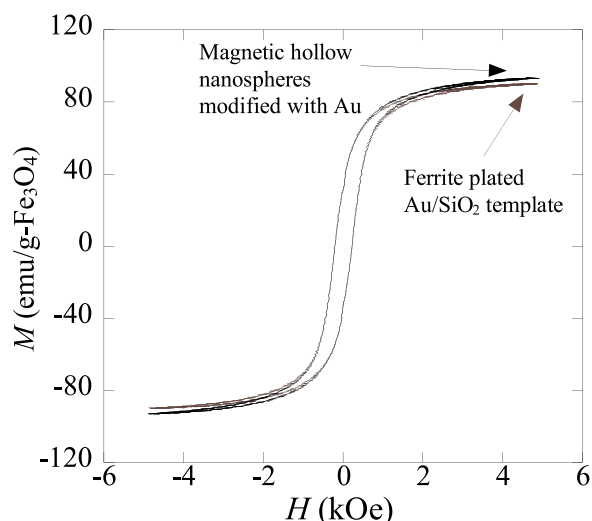


Fig. 5. Room-temperature magnetization curves of the synthesized composite particles.

Table 1

Adsorbed amount of cysteine onto magnetic hollow nanospheres.

	Fe <sub>3</sub> O <sub>4</sub> nanoparticle (control)	Au/hollow-Fe <sub>3</sub> O <sub>4</sub> (Au 8 wt %)	Au/hollow-Fe <sub>3</sub> O <sub>4</sub> (Au 14 wt %)
Adsorbed Cysteine (nmol/mg-Fe <sub>3</sub> O <sub>4</sub> )	14.1	38.2	56.4

magnetic field of 5 kOe was about 90 emu/g-Fe<sub>3</sub>O<sub>4</sub>, which is comparable to that of bulk magnetite [12]. The curves showed hysteresis loops and the remnant coercivity was about 200 Oe. These magnetic properties are consistent with the material characterization results showing that the Fe<sub>3</sub>O<sub>4</sub> nanoparticles are well crystallized and have grain sizes with multi-domains showing ferrimagnetism.

Adsorbed amount of cysteine onto hollow magnetic nanospheres were tabulated in Table 1. Two kinds of nanospheres with Au loading of 8 wt% and 14 wt% were used. Amount of Au nanoparticles in the hollow magnetic spheres were controlled by the washing time of Au/SiO<sub>2</sub> templates. Result obtained with monolithic iron-oxide nanoparticles was also shown for comparison. It was clearly shown that adsorbed amounts of cysteine significantly increased with Au NPs loading, indicating that the surface of Au grains were active even inside the hollow spheres. It was also indicated that the pores in the sphere wall acted as the flow channel for the cysteine, which is a relatively small molecule compared with various functional molecules such as enzymes antibodies, etc. Control of pore size would be necessary for the expression of molecular sieve effect.

The formation process of the composite nanoparticles will now be discussed. In conventional studies, SiO<sub>2</sub> particles modified with SH groups were used as templates for the ferrite plating method [8]. It was considered that the SH groups on the SiO<sub>2</sub> surface worked as bonding tags for the Fe ion precursor and/or Fe<sub>3</sub>O<sub>4</sub> seeds. In this study, SiO<sub>2</sub> particles modified with NH<sub>2</sub> groups were used. We have confirmed that ferrite plating onto this template was not successful, and monolithic ferrite particles free from the support were formed in significant

amounts. However, as shown in this study, surfaces of the Au/SiO<sub>2</sub> templates were successfully covered with Fe<sub>3</sub>O<sub>4</sub> nanoparticles. This result indicates that the Au nanoparticles immobilized on SiO<sub>2</sub> templates contributed to the adsorption of the Fe ion precursor and/or Fe<sub>3</sub>O<sub>4</sub> seeds. This finding could widen the application of ferrite plating to various kinds of support materials.

#### 4. Conclusion

In this paper, a novel procedure to synthesize hollow magnetic nanospheres with Au nanoparticles inside was reported. Au/SiO<sub>2</sub> nanospheres synthesized by a radiochemical process were used as templates for ferrite templating. TEM and SEM analyses indicated that the nanospherical structure and the Au nanoparticles remained after the removal of the SiO<sub>2</sub> cores. It is proposed that the Au grains worked as nucleation sites for Fe<sub>3</sub>O<sub>4</sub> grains in the ferrite plating method. The Au nanoparticles inside the hollow nanospheres showed high Au-S binding affinity. The obtained magnetic nanospheres are expected to be suitable as a new carrier for drug delivery systems, where the morphology could be optimized for specific applications.

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#### References

- [1] K.E. Sapsford, W.R. Algar, L. Berti, K.B. Gemmill, B.J. Casey, E. Oh, I.L. Medintz, Functionalizing nanoparticles with biological molecules: developing chemistries that facilitate nanotechnology, *Chem. Rev.* 113 (2013) 1904–2074.
- [2] I. Ortac, D. Simberg, Y.-S. Yeh, J. Yang, B. Messmer, W.C. Troglor, R.Y. Tsien, S. Esener, Dual-porosity hollow nanoparticles for the immunoprotection and delivery of nonhuman enzymes, *Nano Lett.* 14 (6) (2014) 3023–3032.
- [3] P.B. Landon, A.H. Mo, C. Zhang, C.D. Emerson, A.D. Printz, A.F. Gomez, C.J. DeLaTorre, D.A.M. Colburn, P. Anzenberg, M. Eliceiri, C. O'Connell, R. Lal, Designing hollow nano gold golf ball, *Appl. Mater. Interfaces* 6 (13) (2014) 9937–9941.
- [4] D. Wang, Z. Xu, Z. Chen, X. Liu, C. Hou, X. Zhang, H. Zhang, Fabrication of single-hole glutathione-responsive degradable hollow silica nanoparticles for drug delivery, *ACS Appl. Mater. Interfaces* 6 (2014) 12600–12608.
- [5] R. Kumar, A.N. Maitra, P.K. Patanjali, P. Sharma, Hollow gold nanoparticles encapsulating horseradish peroxidase, *Biomaterial* 26 (33) (2005) 6743–6753.
- [6] T. Fuchigami, R. Kawamura, Y. Kitamoto, M. Nakagawa, Y. Namiki, A magnetically guided anti-cancer drug delivery system using porous FePt capsules, *Biomaterial* 33 (5) (2012) 1682–1687.
- [7] K. Cheng, S. Peng, C. Xu, S. Sun, Porous hollow Fe<sub>3</sub>O<sub>4</sub> Nanoparticles for targeted delivery and controlled release of cisplatin, *J. Am. Chem. Soc.* 131 (30) (2009) 10637–10644.
- [8] M. Tada, T. Kanemaru, T. Nakagawa, H. Handa, M. Abe, Synthesis of hollow ferrite nanospheres for biomedical applications, *J. Magn. Magn. Mater.* 321 (2009) 1414–1416.
- [9] K. Hayashi, K. Ono, H. Suzuki, M. Sawada, M. Moriya, W. Sakamoto, T. Yogo, High-frequency, magnetic-field-responsive drug release from magnetic nanoparticle/organic hybrid based on hyperthermic effect, *ACS Appl. Mater. Interfaces* 2 (7) (2010) 1903–1911.
- [10] S. Seino, K. Watanabe, T. Shikakura, M. Abe, T. Nakagawa, Y. Koga, T.A. Yamamoto, Enzyme immobilization on gold/Fe-oxide composite nanoparticles using a methionine tag, *Colloids and Surf. A: Physicochem. Eng. Asp.* 459 (2014) 298–301.
- [11] S. Seino, T. Kinoshita, T. Nakagawa, T. Kohima, R. Taniguchi, S. Okuda, T.A. Yamamoto, Radiation induced synthesis of gold/iron-oxide composite nanoparticles using high-energy electron beam, *J. Nanopart. Res.* 10 (2008) 1071–1076.
- [12] M.R. Cornell, U. Schwertmann, *The Iron Oxides*, VCH, New York, 1996, p. 117.