

Radiation-induced synthesis of Au/Fe oxide nanocomposite particles for magnetic separation of biomolecules

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Nanocomposite particles composed of gold and iron oxide were synthesized with various ratios of gold and iron oxide in an aqueous phase using γ -rays. Iron oxide nanoparticles of γ -Fe₂O₃ or Fe₃O₄ were used as a magnetic phase on which gold nanoparticles with average diameters of 5-10 nm were supported. The efficiency of magnetic separation of biomolecules by the present nanocomposite particles was investigated using glutathione as a model compound. The amount of glutathione magnetically separated was significantly enhanced by the supported gold nanoparticles.

Key words: γ -Fe₂O₃, Fe₃O₄, Au, nanocomposite particle, γ -ray, magnetic separation.

Introduction

In recent years, much attention has been paid to magnetic nanoparticle materials aiming at their biomedical applications such as DNA separation and drug delivery systems. In most cases, iron oxide particles such as γ -Fe₂O₃ and Fe₃O₄ were used as a magnetic phase because they are stable and harmless. In order to make them combine with various kinds of biomolecules, their surface must be coated with compounds such as dextran or starch [1-3]. Recently such micrometer sized particle materials can be commercially obtained [4, 5]. On the other hand, gold nanoparticles are also widely studied as a material for probing biomolecules because gold firmly combines with biomolecules possessing the mercapto group. It is also important that a colloidal solution of gold nanoparticles with diameters of 5-20 nm exhibits a red color due to surface plasmon resonance, and the color changes when gold nanoparticles aggregate [6, 7].

More recently, we have succeeded in synthesizing nanocomposite particles composed of γ -Fe₂O₃ and gold in an aqueous solution using γ -rays [8]. It is well known that noble metal nanoparticles can be synthesized by the radiation-induced reduction of noble metal ions [9, 10]. We have extended this procedure to the synthesis of nanocomposite particles. These nanocomposite particles are expected to be very useful for biomedical applications, because the gold part firmly combines with

biomolecules via mercapto groups and the magnetic part can be easily manipulated by an external magnetic field. In this paper, we report on the detailed synthesis procedure of the nanocomposite particles and their efficiency for the magnetic separation of biomolecules.

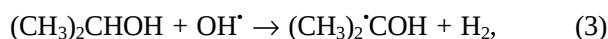
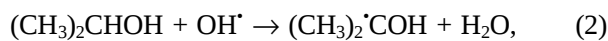
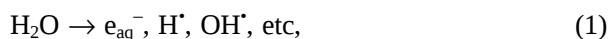
Experimental

The present Au/Fe oxide nanocomposite particles were synthesized as follows. The chemicals used were HAuCl₄, 2-propanol and polyvinyl alcohol (PVA) purchased from Wako Pure Chemical Industries Ltd. Iron oxide nanoparticles of γ -Fe₂O₃ and Fe₃O₄ obtained by a physical vapor synthesis process were used as the magnetic phase. The average diameters of γ -Fe₂O₃ and Fe₃O₄ nanoparticles were 21 nm and 100 nm, respectively. The nanoparticle materials were dispersed in aqueous solutions containing HAuCl₄, 2-propanol and PVA, and then closed up in glass vials. The aqueous solutions generally contained 0.5 mmol·l⁻¹ of HAuCl₄, 0.125 mol·l⁻¹ of 2-propanol and 10 g·l⁻¹ of PVA. The ratio of iron oxide and gold was controlled by the amount of iron oxide nanoparticles dispersed in the suspension. To avoid the photochemical reduction of gold ions, the suspensions were kept in a dark place before γ -ray irradiation. The suspensions were ultrasonically dispersed for 15 minutes by an ultrasonic bath just before γ -ray irradiation to disperse the iron oxide nanoparticles. The suspensions were irradiated at room temperature by a ⁶⁰Co γ -ray source with a dose rate of 3 kGy/h for 6 hours. During the irradiation, the suspensions were continuously stirred to maintain the dispersion.

The γ -ray irradiation causes radiolysis of water and

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2-propanol in the aqueous phase. The processes are expressed as follows [10]:



where equation (1) represents the radiolysis of water; and equations (2) and (3) the radiolysis of 2-propanol. Gold ions were reduced by H radicals, hydrated electrons and organic radicals generated by the irradiation to form metallic gold nanoparticles.

After the irradiation the color of the suspension changed to reddish, reflecting the formation of nano-sized gold particles. The suspension was magnetically separated into a magnetic component and a nonmagnetic component by a permanent magnet to confirm the formation of nanocomposite particles. The absorption spectra of the components were measured with a UV-vis spectrometer, Varian Cary 50. The X-ray diffraction (XRD) pattern of the nanocomposite powder obtained by drying the magnetic component was measured with a diffractometer, Rigaku RINT2100-Ultima⁺, by the use of Cu-K_α radiation. The size and shape of the nanocomposite particles were investigated by a transmission electron microscope (TEM), HITACHI H-8100T.

To investigate the magnetic separation efficiency of biomolecules by the present nanocomposite particles, glutathione (GSH, purchased from Wako) was used as a model compound of a biomolecule possessing a mercapto group. GSH is a kind of tripeptide, which consists of glutamic acid, cysteine and glycine, and a large amount of it exists in most cells. GSH was added to the suspensions of the nanocomposite particles, so that the concentration of GSH was 100 μmol·l⁻¹. After stirring for two hours, the nanocomposite particles and adsorbed GSH were separated by a magnetic separation column, MIDI MACS® [11]. To determine the separated amount of GSH, the concentration of GSH remaining in the nonmagnetic solution was measured by an enzymatic recycling technique [12, 13].

Results and Discussion

Absorption spectra of the suspensions of the Au/Fe oxide nanocomposite particles

Figures 1 and 2 shows the UV-vis absorption spectra of the magnetic and nonmagnetic component of the aqueous suspension of the Au/γ-Fe₂O₃ nanocomposite particles, respectively. Before irradiation, the mass ratios of Au/γ-Fe₂O₃ in the suspension were controlled within a range of 1/1 to 1/10. As shown in the Fig. 1, absorption bands were observed around 540 nm in all spectra, which are ascribed to the surface plasmon absorption peak of nano-sized metallic gold particles. It should be noted here that nano-sized gold particles exhibiting the

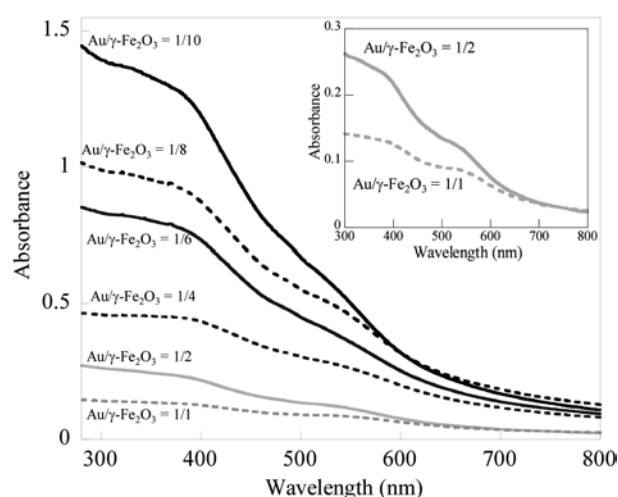


Fig. 1. UV-vis absorption spectra of the magnetic component of the aqueous suspension of the Au/γ-Fe₂O₃ nanocomposite particles.

plasmon peak are attracted by a magnet. This is a clear evidence for the formation of the nanocomposite particles.

As shown in Fig. 2, the nonmagnetic component of the suspension was substantially transparent when the γ-Fe₂O₃ nanoparticles were sufficiently dispersed in the suspension. The absence of a surface plasmon peak in the nonmagnetic components indicates that all the gold nanoparticles generated were supported on the γ-Fe₂O₃ nanoparticles. By contrast, when the amounts of γ-Fe₂O₃ nanoparticles were relatively small, clear absorption peaks around 525 nm were observed. This result indicates the formation of monolithic gold nanoparticles that are not supported on the γ-Fe₂O₃ nanoparticles. It is assumed that the formation of the nanocomposite particles occurs just after the reduction of gold ions, and iron oxide nanoparticles must be present near the

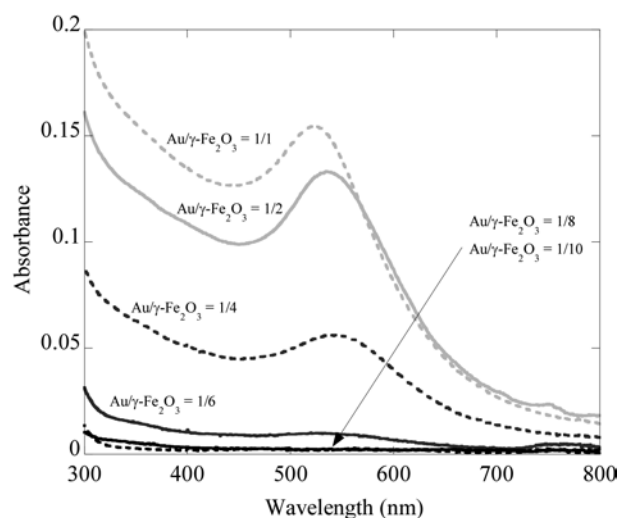


Fig. 2. UV-vis absorption spectra of the nonmagnetic component of the aqueous suspension of the Au/γ-Fe₂O₃ nanocomposite particles.

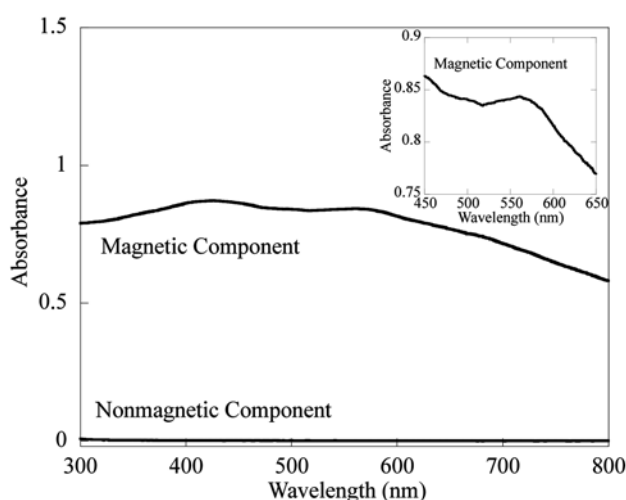


Fig. 3. UV-vis absorption spectra of the magnetic and nonmagnetic component of the aqueous suspension of the Au/Fe₃O₄ nanocomposite particles.

gold nanoparticles formed.

Figure 3 shows the UV-vis absorption spectra both of the magnetic and nonmagnetic components of the aqueous suspension of the Au/Fe₃O₄ nanocomposite particles. Before irradiation, the mass ratio of Au/Fe₃O₄ in the suspension was prepared to be 1/10. Similar to the Au/ γ -Fe₂O₃, the absorption band due to metallic gold nanoparticles observed around 555 nm in the magnetic component and the nonmagnetic component was transparent. These results clearly indicate the formation of nanocomposite particles. However, due to the low dispersibility of the Fe₃O₄ nanoparticles, the absorption spectrum of the magnetic component was relatively unclear.

XRD patterns of the Au/Fe oxide nanocomposite particles

Figure 4 shows the XRD patterns of powders obtained by drying the magnetic components of Au/ γ -Fe₂O₃ and Au/Fe₃O₄. In both cases, a broad peak around 38 degrees due to metallic gold is observed and all the

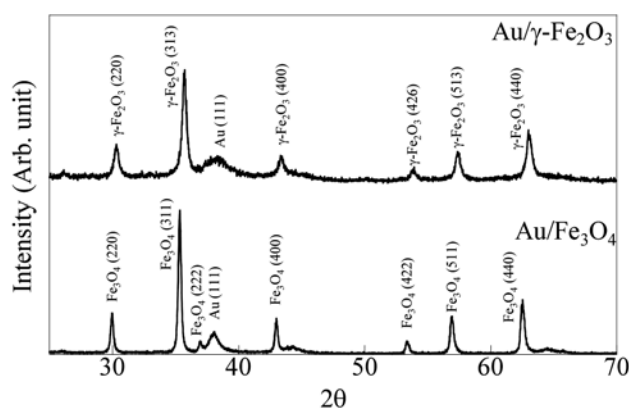


Fig. 4. XRD patterns of the Au/ γ -Fe₂O₃ and Au/Fe₃O₄ composite nanoparticles.

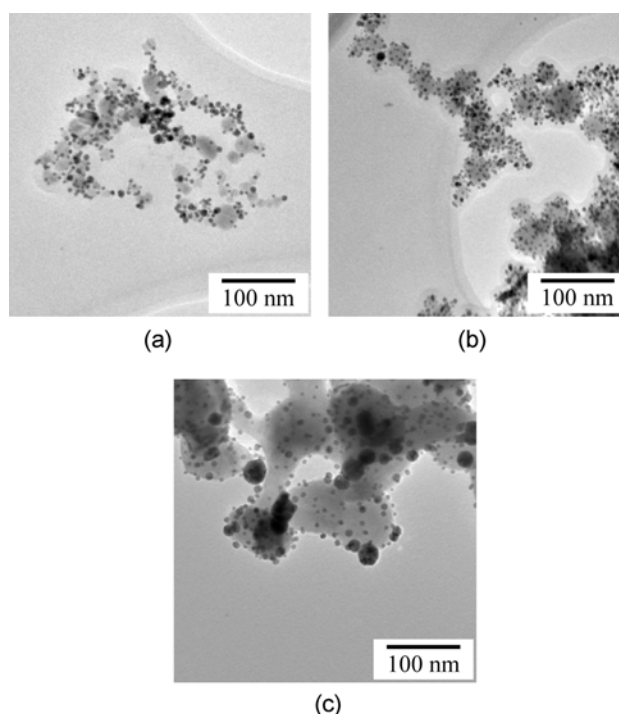


Fig. 5. (a)-(c) TEM micrographs of the Au/Fe oxide nanoparticles. (a); Au/ γ -Fe₂O₃ with a initial mass ratio of 1/10. (b); Au/ γ -Fe₂O₃ with a initial mass ratio of 1/1. (c); Au/Fe₃O₄ with a initial mass ratio of 1/10.

other peaks are ascribed to iron oxide phase. This is also a clear evidence for the formation of the nanocomposite particles.

TEM micrographs of the Au/Fe oxide nanocomposite particles

Figure 5 shows typical TEM micrographs of the Au/Fe oxide nanocomposite particles. The magnetic components of the suspensions of nanocomposite particles were deposited and dried on carbon-coated copper grids. Figure 5(a) and (b) show the Au/ γ -Fe₂O₃ nanocomposite particles with the initial mass ratios of 1/10 and 1/1, respectively. These micrographs clearly show that individual nano-sized gold particles, which are seen as smaller and darker grains, are well dispersed on the surface of the γ -Fe₂O₃ particles. In these micrographs, monolithic gold nanoparticles were scarcely observed. The number of gold nanoparticles supported on the γ -Fe₂O₃ particles are drastically different between (a) and (b), reflecting the initial mass ratio of Au/ γ -Fe₂O₃. The average diameters of gold nanoparticles were almost the same, about 5 nm. Figure 5(c) shows the Au/Fe₃O₄ nanocomposite particles with an initial ratio of 1/10. Small gold nanoparticles with average diameters of about 10 nm were supported on the Fe₃O₄ particles as well. The lower dispersibility of Fe₃O₄ nanoparticles might lead to the formation of relatively larger gold nanoparticles.

Magnetic Separation of GSH

Table 1. Amount of magnetically separated GSH by the magnetic nanoparticles

Nanoparticles	Amount (g·l ⁻¹)	PVA (10 g·l ⁻¹)	Separated GSH (%)
*Au/γ-Fe ₂ O ₃	1.1	Contained	72%
γ-Fe ₂ O ₃	1.0	Contained,	23%
γ-Fe ₂ O ₃	1.0	None	42%

Initial concentration of the GSH; 100 μmol l⁻¹*Mass ratio of Au/γ-Fe₂O₃; 1/10

Table 1 shows the amount of GSH separated by the present nanocomposite particles. The nanocomposite particles examined here were Au/γ-Fe₂O₃ with an initial mass ratio of 1/10. For comparison, the amounts of GSH separated by the monolithic γ-Fe₂O₃ nanoparticles dispersed in pure water or PVA solution are also given. Here, the amount is related to the percentage of the initial concentration of the GSH. The Au/γ-Fe₂O₃ separated GSH best, about 72%, reflecting the strong affinity between gold and the mercapto group. The monolithic γ-Fe₂O₃ nanoparticles also adsorbed GSH, but coexisting PVA seemed to prevent the adsorption. It should be noticed that the amount of the glutathione separated by the γ-Fe₂O₃ nanoparticles was significantly enhanced, about three times, by only 10 wt.% of supported gold nanoparticles. This is a promising feature of these nanocomposite particles for future biomedical applications.

Conclusions

Au/Fe oxide nanocomposite particles were successfully synthesized regardless of the phase of the magnetic iron oxide nanoparticles, and the ratio of gold and iron oxide nanoparticles can be controlled by the amount of iron oxide nanoparticles dispersed in the suspension.

Gold nanoparticles of 5-10 nm size were well dispersed on the surface of the iron oxide nanoparticles. A model compound of biomolecules, GSH, was well adsorbed on the nanocomposite particles and easily separated by a magnetic separation technique. All of these results indicate that the radiation-induced synthesis of Au/Fe oxide nanoparticles can provide very useful materials for various kinds of biomedical applications.

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