

Novel Biocompatible Nanoreactor for Silica/Gold Hybrid Nanoparticles Preparation

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Abstract: A new approach to the preparation of PEGylated [PEG: poly(ethylene glycol)] SiO₂/Au hybrid nanoparticles was investigated. The synthesis of a PEGylated nanogel containing SiO₂/Au hybrid nanoparticles was performed using matrix-catalyzed hydrolysis of tetraethyl orthosilicate, followed by the reduction of HAuCl₄. UV-vis absorption of the prepared hybrid particles was obtained at 618 nm, which is a much longer wavelength than that of a nanogel containing only Au nanoparticles (523 nm). High-angle annular dark field images of the prepared particles observed using transmission electron microscopy and energy-dispersive X-ray spectroscopy confirmed the coexistence of Si and Au in the same particle. The presence of Si and Au in the prepared particles was also confirmed by inductively coupled plasma atomic emission spectroscopy. Dynamic light-scattering measurements of the particles in a highly ionic medium showed that they have high stability in both acidic and basic regions.

Keywords: SiO₂/Au hybrid nanoparticle, PEGylated nanogel, Nanoreactor, Bioimaging

1. Introduction

Nanoparticles have been extensively investigated during the last few decades because of their unique properties and potential applications. In recent years, metal nanoparticles such as Au [1, 2], Ag [3, 4], Pt [5, 6], and Fe oxide [7, 8] nanoparticles have attracted widespread interest in biomedical applications, including disease diagnosis and treatments. They have unique mechanical, electronic, magnetic, optical, and chemical properties. More recently, hybrid nanoparticles composed of metals and dielectric materials (usually SiO₂) have been shown to have potential applications in biomedical areas. The hybrid nanoparticles can exhibit a strong optical resonance at a much longer wavelength than the corresponding optical resonance of metal nanoparticles. In particular, new hybrid nanocomposites consisting of dielectric SiO₂ and Au show strong optical resonance in a wide region (from visible to near-infrared) of the electromagnetic spectrum [9–11]. This property makes them promising building blocks with many biomedical applications, such as in biological imaging [12, 13], thermal ablative cancer therapy [14, 15], immunoassays, and photothermally activated drug delivery [16]. These SiO₂/Au hybrid particles are biologically inert [17] and have low toxicity [18]. However, the SiO₂/Au particles are unstable in physiological environments, so their stabilities need to be increased for in vivo applications. As a result of the steric

stabilization and stealth properties of poly(ethylene glycol) (PEG), PEGylation of SiO₂/Au hybrid nanoparticles is widely used to make the particles suitable for in vivo applications [19–21]. However, PEGylation of nanoparticles by mono-end-functionalized PEG often causes de-PEGylation under physiological conditions [22], resulting in aggregation of the particles.

We previously reported that a PEGylated nanogel composed of a cross-linked poly[2-(*N,N*-diethylamino)ethyl methacrylate] (PEAMA) core and a tethered PEG shell [23] acts as a nanoreactor as well as a stabilizer for Au nanoparticles by autoreduction of chloroaurate ions within the cross-linked PEAMA core [24]. Coordination of the amino lone-pair of the PEAMA chain effectively stabilizes the Au nanoparticles in the nanogel. The PEGylated nanogel shows high stability against extremely dilute and high salt conditions, excellent biocompatibility, negligible toxicity, and prolonged blood circulation [25]. The nanogel also shows significant volume phase transitions (swelling/deswelling) in response to pH changes [23, 26]. Accordingly, if other metal nanoparticles are prepared by template preparation inside nanogels, the range of in vivo applications of PEGylated nanogels for the diagnostic and disease treatments will be further expanded. In this report, we describe a simple method for the synthesis of SiO₂/Au hybrid nanoparticles by matrix-catalyzed hydrolysis of tetraethyl orthosilicate (TEOS), followed by the reduction of HAuCl₄ in presence of PEGylated polyamine nanogel. SiO₂/Au hybrid nanoparticles were obtained in the nanogel core. The surface plasmon band of the obtained hybrid particles was observed at a longer wavelength (618 nm) than that of a PEGylated nanogel containing Au nanoparticles (523 nm) under specific conditions, and the prepared PEGylated nanogel containing SiO₂/Au nanoparticles showed excellent stability under physiological conditions.

2. Experimental

2.1. Materials

Ethylene glycol dimethacrylate (EGDMA; Wako, Tokyo, Japan) and 2-(*N,N*-diethylamino)ethyl methacrylate (EAMA, Wako) were distilled over CaH₂ under reduced pressure. The PEG macromonomer (acetal-PEG-PhCH=CH₂; $M_n = 6300$) was synthesized according to our previous report [23]. Potassium persulfate (KPS; Wako) was purified by recrystallization from water and then dried in vacuo. TEOS (Sigma-Aldrich, St. Louis, MO, U.S.A.) and tetrachloroauric acid (HAuCl₄; Sigma-Aldrich) were used without further purification. Water was purified using a Milli-Q system (Millipore,

Molsheim, France).

2.2 Synthesis of PEGylated nanogel containing SiO₂ nanoparticles (SiO₂NG)

The nanogel was prepared as previously described [23]; briefly, EAMA was emulsion polymerized with EGDMA (3 mol %) in the presence of acetal-PEG-PhCH=CH₂, which acts as both an emulsifier and a comonomer. KPS was used as an initiator. The copolymerization was carried out at room temperature for 24 h. In a typical procedure for the preparation of the pH-responsive PEGylated SiO₂NGs at N/Si ratios of 1, 0.5, 0.2, and 0.1, 20 mg of nanogel in 1 mL of H₂O ([N] = 38 μmol) were put in a glass vial and the vial was placed on a magnetic stirrer at 350 rpm. The desired amount of TEOS ([Si] = 38, 76, 190, and 380 μmol) was added and the reaction mixture was stirred for 24 h at room temperature. The prepared SiO₂NG was purified by dialysis against 2 L of water for 2 d (the water was changed after 1, 2, 4, 8, 12, and 24 h) using a membrane with a molecular weight cutoff size of 1 million. A blank experiment was performed in order to check the purification method; the highest amount of TEOS (380 μmol) used for SiO₂NG preparation was mixed with 1 mL of H₂O. After 24 h of stirring, the mixture was purified by dialysis as in the SiO₂NG purification.

2.3 Synthesis of PEGylated nanogel containing SiO₂/Au hybrid nanoparticles (SiO₂/AuNG)

For the synthesis of the PEGylated nanogel containing SiO₂/Au hybrid nanoparticles, 5 mg (1 mL) of the prepared SiO₂NG (Table1; Run-2) were placed in a glass vial and the pH was adjusted to 9.0 by adding 1 mL of 0.01 M tetraborate buffer. Then 4 mg (in 1 mL of H₂O) of HAuCl₄ were added and the resulting mixture was stirred for 24 h at room temperature. The prepared particles were purified by dialysis against 2 L of water for 2 d (the water was changed after 1, 2, 4, 8, 12, and 24 h) using a membrane with a molecular weight cutoff size of 1 million. A blank experiment was performed in order to check the purification method; 4 mg (in 1 mL of H₂O) of HAuCl₄ were mixed with 1 mL each of H₂O and tetraborate buffer (0.01 M; pH 9.0). After stirring for 24 h, the mixture was purified by dialysis using the same method as for the SiO₂/AuNG purification.

2.4 Characterization

The particle sizes of the SiO₂NG and PEGylated nanogel containing SiO₂/Au nanoparticles were evaluated by dynamic light-scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, Ltd., Malvern, U.K.) equipped with a 4-mW He–Ne ion laser ($\lambda = 633$ nm). The DLS measurements were carried out at 37.0 °C at a detection angle of 173°. To determine the amount of Si immobilized in the pH-responsive PEGylated nanogels, inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis of the SiO₂NG was carried out using a Shimadzu (Kyoto, Japan) ICPS-8100 plasma emission spectrometer. To characterize the optical properties of the SiO₂NG and the pH-responsive nanogel containing SiO₂/Au nanoparticles, UV-vis spectra were recorded using Thermo Scientific (Waltham, MA, U.S.A.) SkanIt[®] Software for a Varioskan[®] Flash version 2.4.3 spectrometer. Transmission electron microscope (TEM) samples were prepared by mounting a drop of the solution on a carbon-coated Cu grid and allowing it to dry in air. TEM analysis of the SiO₂NG was carried out using a JEOL (Tokyo, Japan) JEM-1400 instrument operated at 80 kV. For the SiO₂/AuNG sample, TEM analysis was carried out using a JEOL JEM-2010F instrument equipped with an energy-dispersive X-ray spectrometer (EDS; BRUKER XFlash 5030, Berlin, Germany) operating at 200 kV.

3. Results and discussion

A schematic diagram of the synthesis of the SiO₂/Au hybrid nanoparticles inside the pH-responsive PEGylated nanogel is shown in Figure 1. First, a PEGylated nanogel containing SiO₂ nanoparticles (SiO₂NG) was prepared by hydrolysis of TEOS by the polyamine core of the PEGylated nanogel. The prepared SiO₂NG was purified by dialysis against water using a membrane with a molecular weight cutoff size of 1 million. To check the purification efficiency, a blank experiment was performed, as described in the experimental section. Elemental analysis using ICP-AES of the blank sample after dialysis does not show any Si (Table S1 in the Supporting Information), confirming the successful purification of the prepared SiO₂NG by dialysis. After purification of the prepared SiO₂NG, the amount of Si was analyzed using ICP-AES. The quantitative data are summarized in Table 1. Note that the amount of immobilized Si increased with decreasing N/Si ratio (increasing amount of TEOS) and it becomes constant after the N/Si ratio reaches 0.2.

The SiO₂NG was also analyzed by TEM, as shown in Figure 2. Some smaller and high contrast particles can be seen in the nanogel, which were detected as higher and lower contrast particles in the image, suggesting the formation of SiO₂ particles in the nanogel core. The size of the SiO₂ nanoparticles is about 18 nm.

Secondly, SiO₂/Au hybrid particles were prepared in the nanogel core. We have previously reported that amino groups in a nanogel core reduce aurate cations effectively to form Au nanoparticles in the nanogel [24]. If the amine-assisted aurate reduction takes place in the SiO₂NG as it does in the NG, it is anticipated that a new SiO₂/Au hybrid will be formed in the nanogel. The reduction of aurate ions was examined by simple addition of HAuCl₄ to a previously prepared SiO₂NG at various pH values. After the reaction, the reaction mixture was dialyzed against water and the amount of Au in SiO₂NG was determined from ICP-AES analysis, as shown in Table 2. The obtained data confirm the presence of Au in the prepared particles. The purification efficiency was also checked by elemental analysis of a blank sample by ICP-AES. The preparation of the blank sample is described in the experimental section. The analysis results (See Table S2 in the Supporting Information) confirm that there is no Au present in the blank sample after dialysis.

Figure 3 shows the UV-vis spectra of the PEGylated nanogel containing SiO₂/Au hybrid particles prepared at various pH values. A typical absorption based on the plasmon band of the Au surface was observed at around 520 nm for most of the samples. It is interesting to note that the absorption was observed at a much longer wavelength (618 nm) for the sample prepared at pH 9.0, suggesting the formation of SiO₂/Au hybrid particles. The changes in the UV-vis spectra of the particles as a function of time are shown in Figure 4. The λ_{\max} of the particles was observed at around 540 nm initially, suggesting the formation of primary Au particles [27] from the reduction of Au (III) ions by the polyamine core of the nanogel. The λ_{\max} changed to 618 nm after 40 min in the case of the particles prepared at pH 9.0 [Figure 4(a)]. This observation suggests that after formation, the primary Au particles interacted with the SiO₂ particles and formed stable SiO₂/Au hybrid particles in the nanogel core. In contrast, the λ_{\max} values of the particles prepared at neutral or acidic pHs [Figure 4(b)] gradually shifted to 520 nm with time, suggesting the formation of stable Au nanoparticles through the ion-exchange reaction between protonated amino group of the nanogel core ($-N^+Me_2HCl^-$) and the $AuCl_4^-H^+$, followed by the reduction of aurate ions by the polyamine core [24].

A high-angle annular dark field TEM image of the PEGylated nanogel containing SiO₂/Au hybrid particles is shown in Figure 5(a). The SiO₂/Au hybrid particles (brighter particles) are clearly observed in the nanogel (low contrast

particles). The EDS spectra [Figure 5(b)] highlighted the brighter particles (indicated by “+”) in Figure 5(a), showing the presence of both Si and Au in the same particle; this observation confirmed the formation of SiO₂/Au hybrid particles in the nanogel core. On the basis of these results, viz., the UV-vis and the EDX data, it is concluded that Au/SiO₂ hybrid was truly formed in the core of nanogel.

The size distributions of the PEGylated nanogels containing SiO₂/Au hybrid particles at different pH values, measured using DLS, are shown in Figure 6. Measurements carried out in water containing 0.15 M NaCl at 37 °C showed a unimodal size distribution [polydispersity index (PDI) < 0.15] under both acidic and basic pH conditions; this indicates high stability of the particles in high ionic strength environments over a wide pH range. Because the nanogel has amino groups in its core, it shows pH-dependent volume phase transitions [23, 26]. This phenomenon is very useful in biomedical applications such as in vivo drug delivery systems [28]. Figure 7 shows the pH dependence of the diameter of the PEGylated nanogel containing SiO₂/Au hybrid particles, measured using DLS. The diameter of the PEGylated nanogel containing SiO₂/Au hybrid particles increased proportionally, with a unimodal size distribution, with decreasing pH, reaching a hydrodynamic volume at pH < 7.5 (diameter = 137.2 nm); this is 1.4 times that at pH > 7.5 (diameter = 122.4 nm). This confirmed that the SiO₂/AuNG also shows volume phase transitions similar to those of the original nanogel, although the degree of swelling was lower than that of the original nanogel as a result of coordination of some of the amino groups on the nanoparticle surfaces.

4. Conclusions

In conclusion, the synthesis of a pH-responsive PEGylated nanogel containing SiO₂/Au hybrid nanoparticles was successfully performed using matrix-catalyzed hydrolysis of TEOS, followed by addition of HAuCl₄ at pH 9.0. The surface plasmon band of the PEGylated nanogel containing SiO₂/Au hybrid nanoparticles was observed at a longer wavelength than that of a PEGylated nanogel containing Au nanoparticles. TEM-EDS data showed the formation of SiO₂/Au hybrid particles in the nanogel. The prepared PEGylated nanogel containing SiO₂/Au hybrid nanoparticles had high stability under physiological conditions, and showed reversible volume phase transitions (swelling) in response to pH. This pH-responsive PEGylated nanogel containing SiO₂/Au hybrid nanoparticles may have important applications in biomedical fields.

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Supporting information

Elemental analysis data of the blank samples (described in the experimental section) obtained from ICP-AES.

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Table 1:
Analysis of PEGylated nanogel containing SiO₂ nanoparticles by ICP-AES.

Run	Sample code	Added Si (μmol)	N/Si ratio	Immobilized Si (μmol)
Run-1	PEGylated Nanogel	0	-	0.00
Run-2	SiO ₂ NG-1	38	1	5.36
Run-3	SiO ₂ NG-0.5	76	0.5	10.36
Run-4	SiO ₂ NG-0.2	190	0.2	13.93
Run-5	SiO ₂ NG-0.1	380	0.1	13.57

Table 2:
Analysis of PEGylated nanogel containing SiO₂/Au nanoparticles by ICP-AES.

Run	Amount of SiO ₂ NG-1 (mg)	Added HAuCl ₄ (mg)	Added Au (μmol)	Immobilized Au (μmol)
Run-1		0	0	0.00
Run-2		1	2.54	2.34
Run-3	5	2	5.08	4.62
Run-4		3	7.61	6.60
Run-5		4	10.15	7.97

Figure 1: Schematic representation of the synthesis of PEGylated nanogel containing SiO₂/Au hybrid nanoparticles.

Figure 2: TEM photographs of SiO₂NG.

Figure 3: UV-vis absorption spectra of the pH-responsive PEGylated nanogel containing SiO₂/Au hybrid nanoparticles prepared at various pH conditions.

Figure 4: UV-vis absorption spectra of the pH-responsive PEGylated nanogel containing SiO₂/Au hybrid nanoparticles as a function of reaction time; particles prepared at (a) pH 9.0, (b) pH 6.0.

Figure 5: (a) High angle annular dark field (HAADF) image of PEGylated nanogel containing SiO₂/Au hybrid particles; (b) EDS spectra of the sample pointed at brighter particles (indicated by “+”) in Figure 5 (a).

Figure 6: Size distribution of the pH-responsive PEGylated nanogel containing SiO₂/Au hybrid nanoparticles at different pH conditions, measured by DLS in water containing 0.15 M NaCl at 37 °C; direction angle, 173°.

Figure 7: pH dependency of the diameters of the pH-responsive PEGylated nanogel containing SiO₂/Au hybrid nanoparticles measured by DLS in water containing 0.15 M NaCl at 37 °C; direction angle, 173°.

Figure 1:

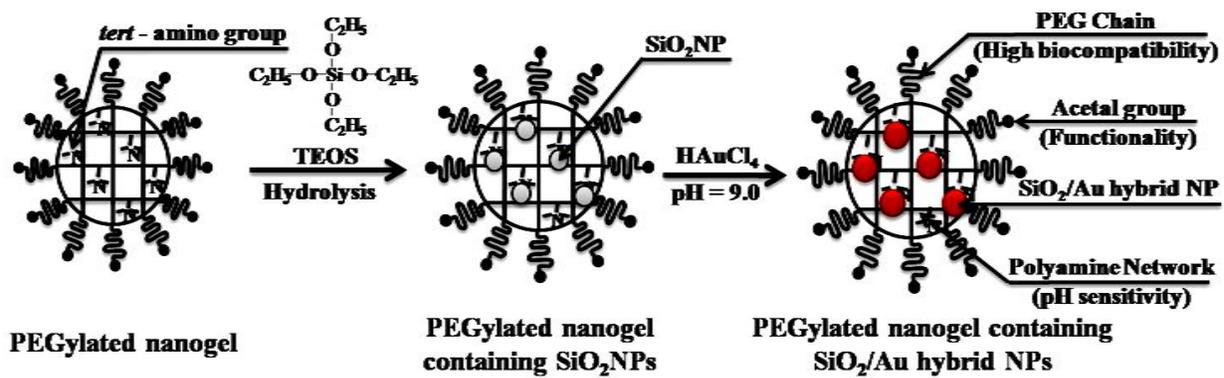


Figure 2:

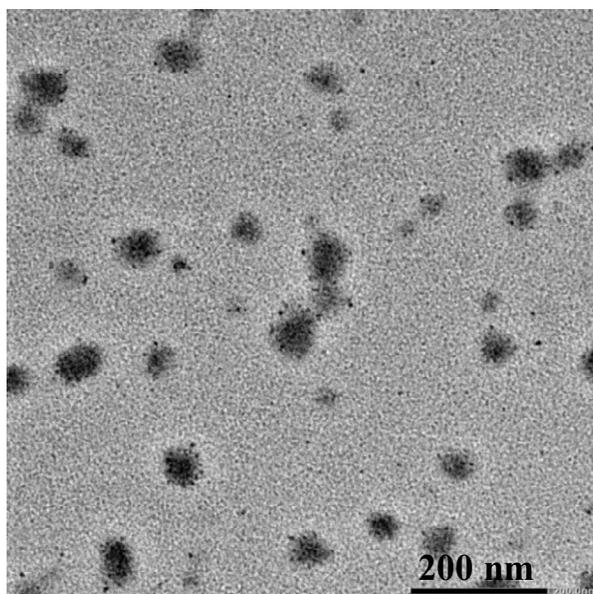


Figure 3:

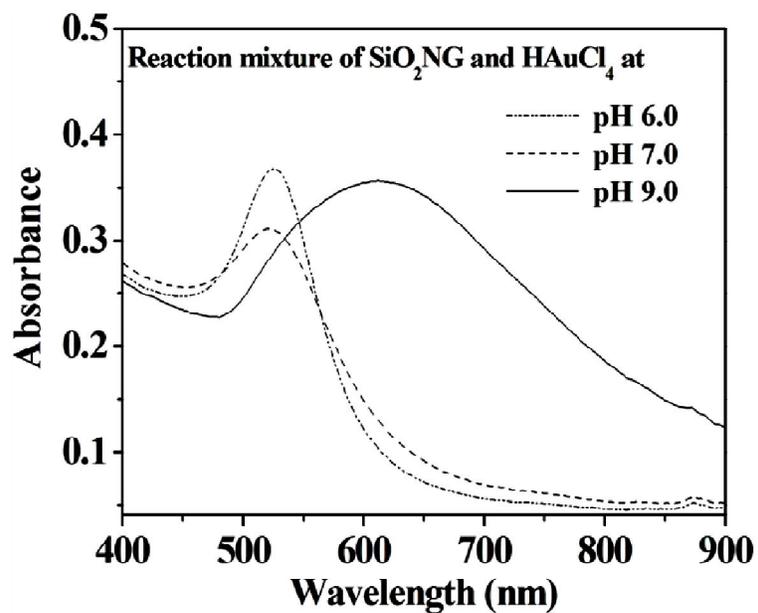


Figure 4:

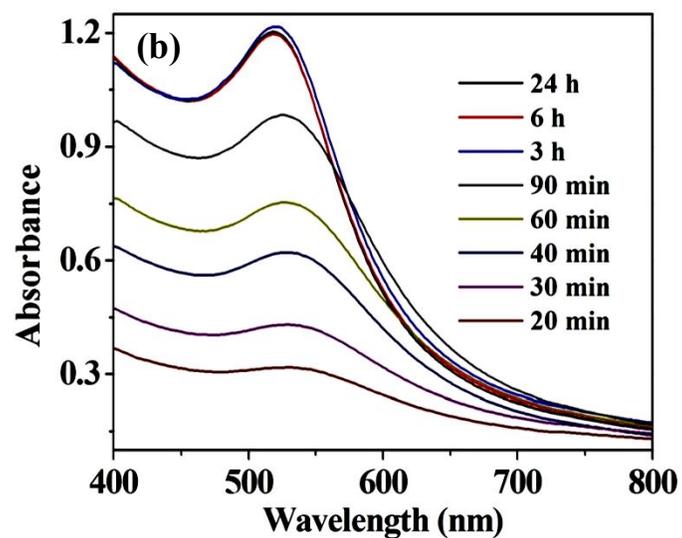
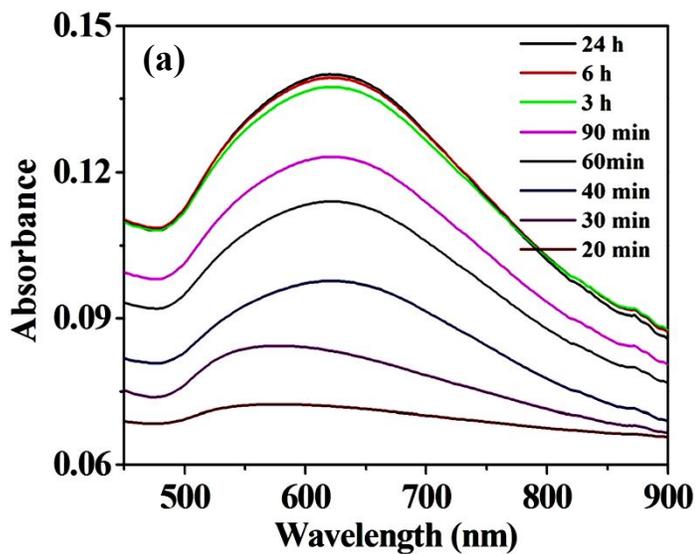


Figure 5:

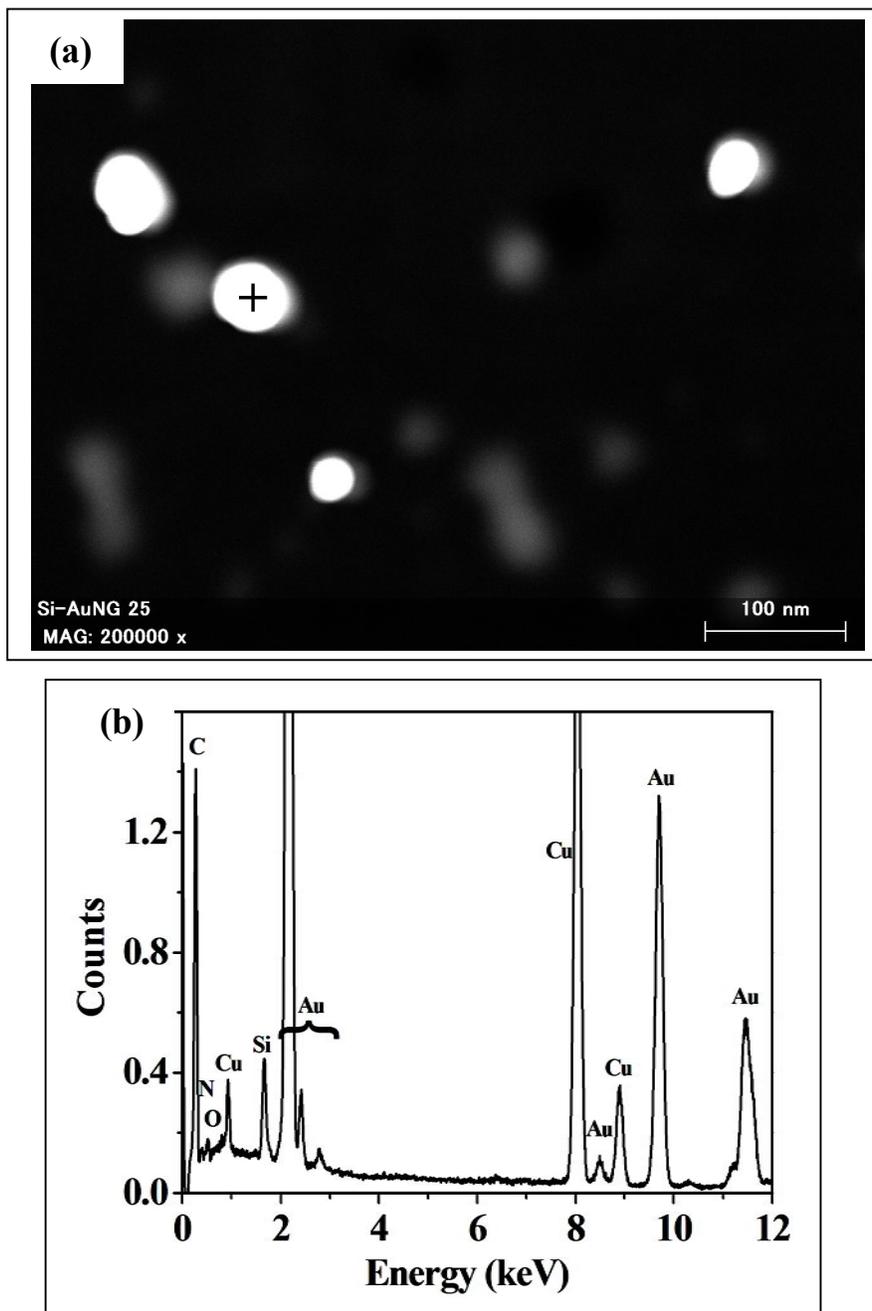


Figure 6:

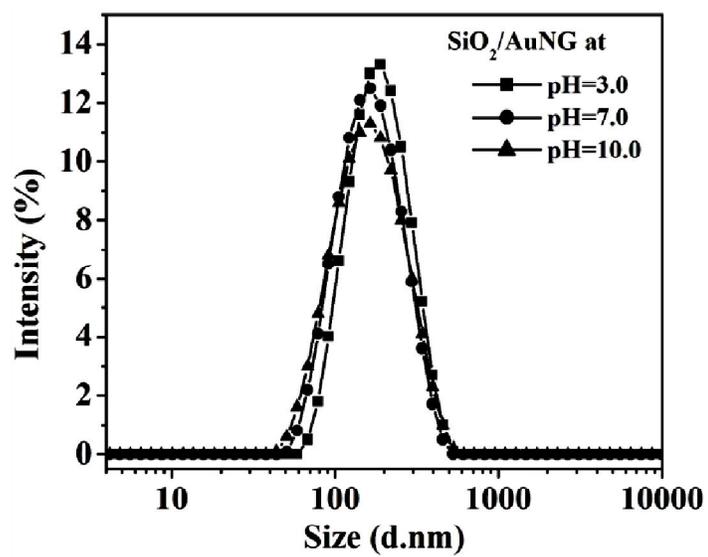


Figure 7:

