

Fabrication and Surface-Modification of Silica/Gadolinium Compound/Silica Core-Shell Nanoparticles Discovered by Transmission Electron Microscopy

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Abstract - The present work describes methods for fabricating multilayered core-shell nanoparticles composed of silica (SiO_2) as a core, gadolinium compound (GdC) as an inner shell, and SiO_2 as an outer shell ($\text{SiO}_2/\text{GdC}/\text{SiO}_2$) toward colloidally-stable, inert magnetic resonance imaging contrast agent, and for surface-modifying the $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles toward immobilization of functional molecules on the $\text{SiO}_2/\text{GdC}/\text{SiO}_2$. SiO_2 nanoparticles (particle size by transmission electron microscopy (TEM): 28.6 ± 12.6 nm) were produced by a sol-gel method using tetraethylorthosilicate (TEOS) and methylamine in water/ethanol solution. GdC-coated SiO_2 nanoparticles (SiO_2/GdC) (GdC shell thickness by TEM: 35.9 ± 4.9 nm) were synthesized by a homogeneous precipitation method using water/propanol solution containing the SiO_2 nanoparticles, $\text{Gd}(\text{NO}_3)_3$, urea and polyvinylpyrrolidone. Succeeding SiO_2 -coating of the SiO_2/GdC nanoparticles was performed by using TEOS and NaOH in the presence of SiO_2/GdC nanoparticles. Consequently, $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles (SiO_2 shell thickness by TEM: 8.5 ± 2.7 nm) could be obtained. The $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles were successfully surface-modified by using 3-aminopropyltrimethoxysilane for amination, and by followingly using succinic anhydride for carboxylation.

Keywords: Multilayer, Core-shell, Nanoparticle, Silica, Gadolinium, Sol-gel, Homogeneous precipitation, Surface-modification

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1. Introduction

Because of magnetic resonance based on paramagnetism of gadolinium (Gd) ions, they function to increase contrast of images taken by a magnetic resonance imaging (MRI) technique [1]. Commercially-available Gd ion-related MRI contrast agents are solutions dissolving the Gd complexes homogeneously at molecular level. The contrast agents injected into living bodies flow in living bodies, and finally are discharged out of the living bodies. Since Gd complex molecules are smaller than the particles, their velocity may be large compared to nano- or submicron particles, which provides small drag force given from fluid. Thus, the discharge for the Gd complex molecules is quick, which makes it difficult to take high contrast images for a long period.

Particles containing Gd compounds (GdC) are promising as a new MRI contrast agent. The particles, which are larger than Gd ion-related molecules, are dragged from fluid to a direction opposite to the fluid due to their projected area larger than molecules, so that they can be expected to stay in living bodies for a long period due to controlled flow of the particles in liquid. Consequently, formation of the particles containing GdC will increase residence time of GdC in the living bodies, which makes it possible to take clear MRI images for a long term. The particles are required to unaggregate in living bodies, because aggregation of the particles increases their apparent size, which prevents them from flowing in body fluid. Since surface of silica (SiO_2)

particles prepared by a sol-gel method has a feature of colloidal stability, SiO₂ particles containing GdC may function as the MRI contrast agent that shows long residence time and non-aggregation in living bodies. Some researchers have studied on particles composed of GdC as core and silica as shell [2,3]. Our research group also has reported the fabrication of core-shell nanoparticles composed of core of GdC and shell of SiO₂ (GdC/SiO₂) [4].

Because strong interaction between water protons and GdC molecules promotes MRI properties of GdC [5], the GdC is desired to be present on or near surface of SiO₂ nanoparticles containing GdC. Accordingly, core-shell particles containing GdC as core are not necessarily suitable to MRI contrast agents. Then, several researchers have studied on core-shell particles composed of SiO₂ as core and GdC as shell [6,7], in which the colloidal stability of SiO₂ particles will stabilize the core-shell particles colloidally. The GdC on or near the particle surface may release free gadolinium ions through dissociation of the complexes. The release of gadolinium ions may provoke adverse reactions in some patients [8], though their toxicity is lower than that of iodine compounds. As one of methods for reducing the adverse reactions derived from GdC, coating of GdC surface with materials inert to living bodies as shell can be given, because contact of the GdC with living bodies may be controlled with a physical barrier of the shell materials. The SiO₂ is a candidate as the shell materials because of its inertness to living bodies. From this view point, our research group has reported the fabrications of core-shell nanoparticles composed of core of SiO₂ and shell of GdC (SiO₂/GdC), and of multilayered core-shell nanoparticles composed of core of SiO₂/GdC nanoparticles and shell of SiO₂ (SiO₂/GdC/SiO₂) [9]. The core-shell nanoparticles have another advantage. The GdC inside the particles can not function as the MRI contrast agent well, because it can not contact the protons in water. Thus, if the inside of the particles is replaced with core nanoparticles other than the GdC, the replacement will decrease the production cost of MRI contrast agent nanoparticles.

To use the MRI contrast agent nanoparticles practically, functional molecules such as polyethyleneglycol and antibody protein should be immobilized onto the particles for avoiding the particles from being recognized as foreign materials in living bodies and delivering the particles to antigen proteins due to a good affinity between the antibody proteins and the antigen proteins, respectively. Prior to the

immobilizations, the particles are desired to be surface-modified with functional groups such as amino group and carboxylic group, because some functional molecules are attracted to these functional groups electro-statically or many kinds of chemicals react with the functional groups. The present paper describes methods for fabricating SiO₂/GdC/SiO₂ nanoparticles, and for surface-modifying SiO₂/GdC/SiO₂ nanoparticles toward a precursor of MRI contrast agent nanoparticles on which various functional molecules are immobilized

2. Experimental

2. 1. Chemicals

Tetraethylorthosilicate (TEOS) (96%), aqueous methyl amine (MA) (40% solution) and ethanol (99.5%) were used for preparation of silica cores and silica-coating as monomer, catalyst and solvent, respectively. Gadolinium nitrate hexahydrate (Gd(NO₃)₃ · 6H₂O) (99.5%), urea (99.0%), polyvinylpyrrolidone (PVP) (average molecular weight 36,000), and 1-propanol were used as chemicals for Gd shell, a precipitation-inducer, a stabilizer of particles for fabrication of SiO₂/GdC nanoparticles, and solvent, respectively. A sodium hydroxide (NaOH) aqueous solution (1 M) was used as catalyst for formation of silica shells. (3-Aminopropyl)trimethoxysilane (APMS) (97%) and succinic anhydride (SA) (98.0%) were used for surface-modification of particles with amino groups and carboxyl groups, respectively. The solvent dissolving SA in the surface-modification with carboxyl groups was dimethylformamide (DMF) (99.5%). Salicylaldehyde (97.0%) was used for confirming the presence of amino groups in particle colloid solutions. The PVP and the APMS were purchased from Nacalai Tesque Ltd. (Kyoto, Japan) and Sigma-Aldrich Co. LLC (St. Louis, USA), respectively, all the other chemicals were purchased from Wako Pure Chemicals Ltd. (Osaka, Japan). All chemicals were used as received. Water that was ion-exchanged and distilled with Shimadzu SWAC-500 was used in all the preparations.

2. 2. Preparation of nanoparticles

A colloid solution of SiO₂ nanoparticles was prepared as follows. A solution of TEOS and ethanol was added to a solution of MA, water and ethanol under stirring in a hermetically sealed flask with a magnetic stirrer. The reaction temperature and time were 35°C and 24 h, respectively. Initial concentrations of TEOS, H₂O and MA were 0.2, 20, and 0.018M, respectively. The silica nanoparticles were washed by repeating

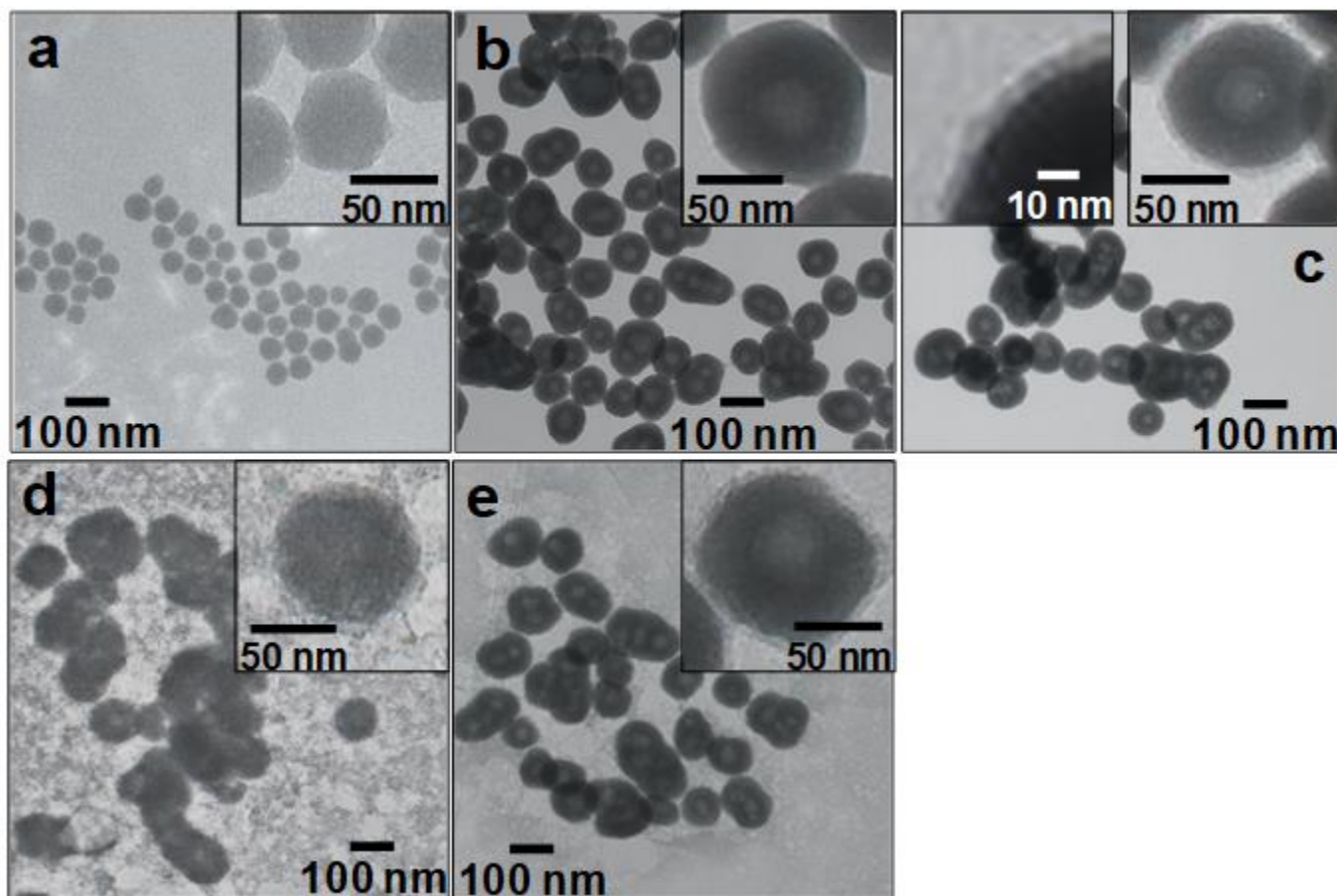


Figure 1. TEM images of (a) SiO_2 , (b) SiO_2/GdC , (c) $\text{SiO}_2/\text{GdC}/\text{SiO}_2$, (d) $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$, (e) $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-COOH}$.

centrifugation, removal of supernatant, addition of the water and sonication over three times.

A colloid solution of SiO_2/GdC nanoparticles was prepared as follows. The colloid solution of washed SiO_2 nanoparticles and PVP dissolved in 1-propanol were added to a water/1-propanol solution, and the mixture was aged for 24 h at room temperature under stirring in a hermetically sealed flask with a magnetic stirrer. A urea aqueous solution was added to the aged mixture, and then pH of the mixture was adjusted to 5 by adding nitric acid. A $\text{Gd}(\text{NO}_3)_3$ aqueous solution was added to the mixture. The mixture was aged for 3 h at 80°C under stirring. Initial concentrations were 0.001 vol% SiO_2 , 1 g/L PVP, 0.5 M urea and 0.001 M $\text{Gd}(\text{NO}_3)_3$, and a volume ratio of water : 1-propanol was 1 : 1.

A colloid solution of $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles was prepared as follows. The TEOS and the NaOH aqueous solution were added to the as-prepared colloid solution of SiO_2/GdC nanoparticles under stirring in a

hermetically sealed flask with a magnetic stirrer. The reaction temperature and time were 35°C and 24 h, respectively. Initial concentrations of TEOS and NaOH were 0.013 and 0.002 M, respectively.

2. 3. Surface-modification of nanoparticles

The $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles were surface-modified with amino groups ($\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$) as follows. The APMS was added to the as-prepared colloid solution of $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles. The reaction temperature and time were 35°C and 24 h, respectively. An initial APMS concentration was 0.03 M. The $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles were washed by repeating centrifugation, removal of supernatant, addition of solvent and sonication over two times. The solvents used in the second and the third washing processes were 1-propanol and DMF, respectively, which provided the replacement of solvent of colloid solution with DMF.

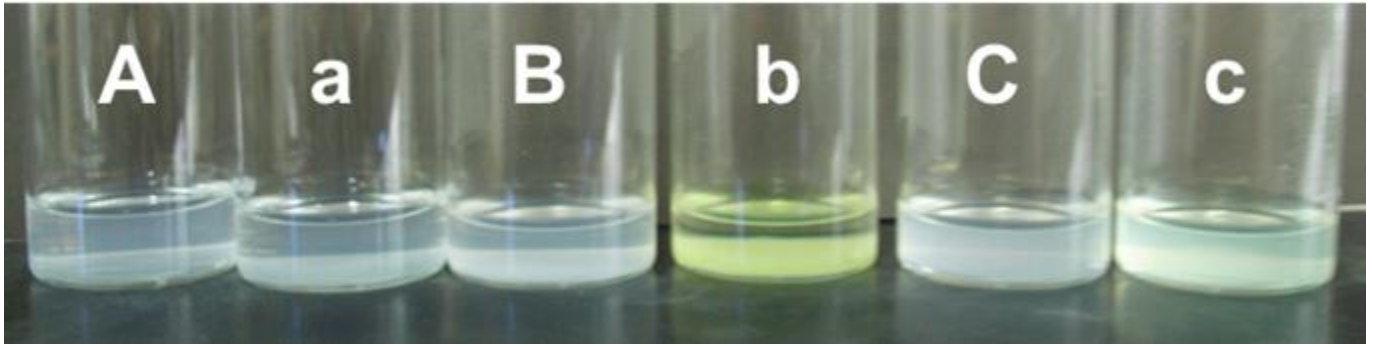


Figure 2. Photographs of various colloid solutions. (A) and (a): $\text{SiO}_2/\text{GdC}/\text{SiO}_2$. (B) and (b): $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$. (C) and (c): $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-COOH}$. The samples (A)-(C) and (a)-(c) were prior to and after the addition of salicylaldehyde, respectively.

According to the reference [10], the amino groups on $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles were transformed to carboxyl groups ($\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-COOH}$) as follows. The SA dissolved in DMF was added to the colloid solution of washed $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles at 35°C . An initial SA concentration was 0.05 M. After 24 h, the $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-COOH}$ particles were washed by repeating centrifugation, removal of supernatant, addition of water and sonication over three times, which provided the replacement of solvent of colloid solution with water.

2. 4. Characterization

Morphology of the particles was investigated by transmission electron microscopy (TEM). TEM was performed with a JEOL JEM-2000FX II microscope operating at 200 kV. Samples for TEM were prepared by dropping and evaporating the nanoparticle suspensions on a collodion-coated copper grid. Dozens of particle diameters in TEM images were measured to determine volume-averaged particle size, d_v , and standard deviation of particle size distribution, σ , defined by the following equations.

$$d_v = \left(\frac{\sum n_i d_i^3}{\sum n_i} \right)^{1/3} \quad (1)$$

$$\sigma = \left(\frac{\sum (d_i - d_v)^2}{\sum n_i} \right)^{1/2} \quad (2)$$

where n_i is the number of particles with a size of d_i . The measurement of particle diameter was performed by using a Mitsutoyo CD-15AX digimatic caliper. The presence of amino groups in particle colloid solutions was confirmed by yellowish coloration provided by a reaction between salicylaldehyde and amino group [11].

3. Results and Discussion

3. 1. Morphology of nanoparticles

The colloid solution of SiO_2 nanoparticles was prepared by a sol-gel method using TEOS as a SiO_2 source and MA as a catalyst. Fig. 1 (a) shows a TEM image of the SiO_2 nanoparticles. The silica nanoparticles were quasi spherical and were colloidally stable. They had an average size of 28.6 ± 12.6 nm by TEM. The SiO_2/GdC nanoparticles exhibited no aggregation during the GdC-coating of SiO_2 nanoparticles, which indicated that colloidal stability of SiO_2 nanoparticles prevented generation of aggregation.

The GdC-coating of SiO_2 nanoparticles, or fabrication of SiO_2/GdC nanoparticles was performed by a homogeneous precipitation method in the presence of the washed silica nanoparticles. Fig. 1 (b) shows a TEM image of SiO_2/GdC nanoparticles. The SiO_2 nanoparticles were coated with uniform GdC shell. An average particle size and an average shell thickness were 107.0 ± 23.0 and 35.9 ± 4.9 nm, respectively, by TEM. There was neither uncoated SiO_2 nanoparticles nor core-free particles.

The SiO_2 -coating of SiO_2/GdC nanoparticles, or fabrication of $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles was performed by a sol-gel method using TEOS as a SiO_2 source and NaOH as a catalyst. Fig. 1 (c) shows a TEM image of $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles. Prior to the TEM observation, the nanoparticles were washed by repeating centrifugation, removal of supernatant, addition of the water and sonication over three times. Only multilayered core-shell particles were observed, and there was neither SiO_2/GdC core-free silica nor SiO_2 shell-free GdC/ SiO_2 nanoparticles. An average particle size was 119.0 ± 39.0 nm by TEM. An average SiO_2 shell thickness was 8.5 ± 2.7 nm, according to a high magnification image (a left inset of Fig. 1 (c)). It was

confirmed by naked eye that no sedimentation took place for the $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticle colloid solution. This observation indicated that the SiO_2 shell contributed to their colloidal stability.

3. 2. Surface-modification

The $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles were surface-modified with amino groups by a reaction between an alkoxide group of APMS and hydroxide groups of the SiO_2 surface on $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles. Fig. 1 (d) shows a TEM image of $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles. Their multilayered core-shell structure of $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles was not damaged even with the surface-modification process using APMS. Many gel networks were also generated. Since the as-prepared $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles were used for the surface-modification, unreacted TEOS that did not form the SiO_2 shells had might been left in the colloid solution. Probably, SiO_2 nuclei were generated through hydrolysis and condensation of alkoxide groups of TEOS. Generation of silanol groups through the hydrolysis probably increased the ionic strength. Increased ionic strength has been reported to compress the double layer of colloidal particles [12]. The generation of silanol groups therefore decreased the double-layer repulsion between the nuclei. The increased ionic strength was thus primarily responsible for the aggregation and growth of nuclei in the present system, which formed the gel networks. A reaction between salicylaldehyde and amino group provides yellowish coloration of solution [11]. In the present work, the presence of amino groups in solutions was confirmed by the yellowish coloration. Figs. 2 (B) and (b) shows photographs of the $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticle colloid solutions prior to and after the addition of salicylaldehyde. The color of the colloid solution turned yellowish after the addition. In the case of the $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles, no color change took place, as shown in Figs. 2 (A) and (a). Probably, the color change took place by a reaction between salicylaldehyde and amino group. Accordingly, the color change confirmed that amino groups were introduced on the $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ particle surface, or the $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ particle surface was successfully surface-modified with amino groups with the amination process.

The amino groups on $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles were transformed to carboxyl groups by a reaction between a functional group of SA and the amino group on $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles, so that the particles were expected to be surface-modified with carboxyl groups. Fig. 1 (e) shows a TEM image of

$\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-COOH}$ nanoparticles. Their multilayered core-shell structure of $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles was not damaged even with the surface-modification process using SA. The amount of the gel network remarkably diminished compared to the $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$. Its weight was so light compared with the $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-COOH}$ nanoparticles that some of the gel networks did not sediment with the centrifugation process. Consequently, the repeated washing process gradually removed the non-sedimenting gel networks. Figs. 2 (C) and (c) shows photographs of the $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-COOH}$ nanoparticle colloid solutions prior to and after the addition of salicylaldehyde. The color of the colloid solution turned yellowish after the addition. The color change was slighter than that for $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles. The amino groups were transformed to carboxyl groups by a reaction between the functional groups of SA and the amino groups on $\text{SiO}_2/\text{GdC}/\text{SiO}_2\text{-NH}_2$ nanoparticles, which reduced the amino groups on particles. The reduction provided the slight color change, which indicated successful introduction of carboxyl groups onto particles, or surface-modification of particles with carboxyl groups.

4. Conclusion

Fabrication of multilayered $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ core-shell nanoparticles and surface-modification of the multilayered core-shell nanoparticles have been performed toward colloidally-stable, inert magnetic resonance imaging contrast agent. The SiO_2 nanoparticles with the size of 28.6 ± 12.6 nm (by TEM) fabricated through the sol-gel method were coated with GdC shells by means of the homogeneous precipitation method with the aid of stabilizer PVP, which produced the SiO_2/GdC nanoparticles that had the average GdC shell thickness of 35.9 ± 4.9 nm (by TEM). The SiO_2/GdC nanoparticles were coated with SiO_2 shells by the sol-gel method using the as-prepared SiO_2/GdC nanoparticle colloid solution, which formed the $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticles that had the average SiO_2 shell thickness of 8.5 ± 2.7 nm (by TEM). The $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ nanoparticle surface was aminated with APMS, and the amino groups of nanoparticle surfaces were transformed to carboxyl groups with SA, which aimed immobilization of functional molecules on the $\text{SiO}_2/\text{GdC}/\text{SiO}_2$ core-shell nanoparticles.

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