One-pot synthesis and surface modifications of organically modified silica (ORMOSIL) particles having multiple functional groups

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Here we report a facile one-pot synthetic method for organically modified silica (ORMOSIL) particles having multiple functional groups and demonstrate the homogeneous distribution of functional groups in ORMOSIL particles by chemical reactions of each surface functional group with fluorescent dyes such as fluorescein and rhodamine B isothiocyanate. Dye-tagged ORMOSIL particles having tri-functional groups are exhibited two fluorescent emission peaks at 475 (blue) and 570 nm (red), indicating the positions of functional groups. The surface reaction of these functionalized ORMOSIL particles with various organic or inorganic materials not only endowed additional functionalities and physical properties, but also produced metallic hybrid composite particles. Chemical and physical properties of functionalized ORMOSIL particles were characterized by FT-IR, solid state 13C and 29Si NMR, thermogravimetric analysis (TGA), electron microscopy (SEM and TEM), and X-ray diffraction (XRD) analysis.

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1. Introduction

Multifunctional colloidal particles with the submicron size have recently emerged with great promise not only for industrial materials such as catalyst, electronics, and pigment [1–3], but also in biological applications including bio-labeling, medical diagnostics, and drug delivery [4–7]. These particles are non-toxic and have size comparable to biomolecules, becoming very suitable for biomedical applications. The particle structures can be engineered to perform multicolor imaging, as well as multiplex diagnostics, and efficient preparation method for ORMOSIL particles having multiple functional groups and demonstrate the homogeneous distribution of functional groups. Moreover, the further surface reactions of functionalized ORMOSIL particles with various types of functionalities is of great importance.

Here we report a "one-pot" synthesis of ORMOSIL particles of monodispersed colloidal silica particles uniform in composition. These particles have been prepared by the hydrolysis and condensation of silicon alkoxides leading to the stable suspension. Since Stöber et al. developed a system of chemical reactions to produce uniform silica particles by hydrolysis and condensation and subsequent growth of particles in alcoholic solutions with a morphological catalyst such as ammonia [24], a large number of researches have been carried out to elucidate the hydrolysis and condensation mechanism and find the optimal conditions for the preparation of particles with uniform size and morphology [28–30]. Organically modified silica (ORMOSIL) particles are organic–inorganic hybrid materials in which the organic component may be chemically bonded to a silica matrix. The resulting mechanical, electrical, and optical properties of the ORMOSIL particles are then governed by the type and concentration of organics used. Furthermore the utilization of nanosized ORMOSIL powders for the production of advanced ceramic materials requires high quality in chemical purity, crystallinity, homogeneity, morphology, and controlled state of agglomeration as well as low production cost. ORMOSIL particles have been prepared by the surface modification of silica particles from tetraethylorthosilicate (TEOS) or using an organo-silane monomer as a starting material. However, surface modification of silica particles for the preparation of ORMOSIL particles needed time-consuming long and multiple sequential steps and resulting ORMOSIL particles usually contain only a single type of functional group. Therefore, multiple modification processes have to be employed in order to attach multitasking functional components such as imaging components, drugs and targeting moieties. For wider economical and biological applications, the development of a simple and efficient preparation method for ORMOSIL particles having various types of functionalities is of great importance.

Herein, we report a "one-pot" synthesis of ORMOSIL particles of uniform size and morphology with multiple, homogeneously distributed functional groups. Moreover, the further surface reactions
of ORMOSIL particles with various organic or inorganic materials can provide the introduction of additional functionalities on particles as well as the preparation of metallic hybrid composite particles. The existence of multiple functionalities in ORMOSIL particles are investigated by a selective tagging reaction of fluorescent dye molecules reactive to specific functional groups on the particle’s surface. These functionalized ORMOSIL particles tagged with various multitasking components can be applied in various academic and industrial applications including detection, selective binding, and separation of catalysts and biological materials, in an easier and more flexible way than those particles with a single functional group.

2. Experimental

2.1. Materials

Phenyltrimethoxysilane [PTMS] (94%, Aldrich), vinyl-trimethoxysilane [VTMS] (98%, Aldrich), 3-mercaptopropyltrimethoxysilane [MPTMS] (97%, Fluka), (3-trimethoxysilyl)propylmethacrylate [TMSPMA] (98%, Aldrich), 3-aminopropyltrimethoxysilane [APMTS] (99%, Aldrich), 3-glycidoxypropyltrimethoxysilane [GPTMS] (98%, Aldrich), glutaric anhydride (95%, Aldrich), rhodamine B isothiocyanate (Fluka), fluorescamine (Sigma), silver nitrate (AgNO₃, 99%, Junsei), and hydrazine monohydrate (H₂NNH₂·H₂O, Sam-Chun Chemicals) were used without further purification. Ammonium hydroxide solution (30 wt. % as NH₃), nitric acid (60%), and sulfuric acid (95%) were obtained from Sam-Chun Chemicals. Methyl alcohol (MeOH, 99.5%, Sam-Chun Chemicals) and ethyl alcohol (EtOH, 95%, Sam-Chun Chemicals) were used as a solvent, respectively.

2.2. Typical synthesis of ORMOSIL particles having multiple functional groups

A typical synthetic procedure for monodispersed ORMOSIL particles with di- and tri-functional groups was consisted of two consecutive steps; in the first step, an aqueous acidic solution (1.8 × 10⁻² M HNO₃) was placed in an isothermal water bath at 40 °C. Two or three silane monomers for di- or tri-functional ORMOSIL particles were added sequentially to the solution with stirring. This mixture was stirred at a rate of 300 rpm for 1 min. In the second step, an aqueous NH₄OH solution (1.55 M) was added to the resultant homogeneous solution. The transparent mixture became turbid, indicating the formation of colloidal particles. The suspension was stirred for 4 h and the product was obtained after filtration and washing.

2.2.1. Vinyl and methacrylate based di-functional ORMOSIL particles

One hundred and fifty milliliters of de-ionized water was placed in a 250 mL three-neck round bottom flask while maintaining a temperature of 40 °C, and 0.2 mL of nitric acid (60 wt. %, 2.67 mmol) was added with stirring at 300 rpm. Five milliliters of VTMS (32.06 mmol) was added to an acidic aqueous solution, followed by the addition of TMSPMA (0.5 mL, 2.06 mmol). After stirring for 1 min, 40 mL of ammonium hydroxide (30 wt. %, 306 mmol) was poured into the reaction mixture to induce condensation. The solution became turbid, indicating the formation of colloidal particles. After stirring for 4 h, the suspension was filtered through a membrane filter (Osmonics, pore size of 400 nm) and the filtered product was washed with 10 mL of de-ionized water and 10 mL of ethanol twice. The product was dried under vacuum and 2.7 g of vinyl and methacrylate based di-functional ORMOSIL particles was obtained.

2.2.2. Phenyl-amine-methacrylate based tri-functional ORMOSIL particles

A 250 mL Erlenmeyer flask maintained at 40 °C was charged with 150 mL of de-ionized water and 0.2 mL of nitric acid (2.67 mmol, 60 wt. %) was added while stirring at 300 rpm. Five milliliters of PTMS (24.89 mmol) was first injected to an acidic aqueous solution, followed by the addition of 0.5 mL APTMS (2.75 mmol) and 0.5 mL TMSPMA (2.06 mmol). The resulting solution was stirred for 1 min to allow hydrolysis followed by the addition of 40 mL of ammonium hydroxide (305 mmol, 30 wt. %) to induce condensation. The mixture became turbid and was stirred for an additional 4 h. The resulting multifunctional hybrid silica particles were filtered through a membrane filter and washed with 10 mL of de-ionized water and 10 mL of ethanol several times. The filtered particles were dried under vacuum and 2.8 g of phenylamine-methacrylate based tri-functional ORMOSIL particles was obtained.

2.3. Surface modification reactions of ORMOSIL particles having multiple functional groups

2.3.1. Organic modification with glutaric anhydride

Vinyl based ORMOSIL particles with mercapto and amine functional groups (0.2 g) were placed in a 250 mL three-neck round bottom flask and the reaction vessel was heated to 85 °C. Glutaric anhydride (0.5 g, 4.16 mmol) was added to ORMOSIL particles and the resulting mixture was stirred for 3 h. One hundred milliliters of de-ionized water and 100 mL of ethanol were poured into the reaction flask for dilution and dispersion of the resulting mixture. The suspension was stirred for 5 min and filtered through a membrane filter. The product was dried in air and 0.2 g of surface modified ORMOSIL particles was obtained.

2.3.2. Hydrophilic modification with nitric acid

Three grams of phenyl-based ORMOSIL particles with mercapto and amine functional groups were placed in a round bottom flask in an ice bath and 6 mL (60%, 80 mmol) of nitric acid was added to the flask. A solution containing 0.5 mL (98%, 10 mmol) of sulfuric acid was added drop-wise and the reaction mixture was stirred for 2 h. For dilution, 500 mL of cold water was poured into the reaction flask and after 10 min, the resulting particles were collected with a membrane filter. The particles were dried in a vacuum oven at 60 °C for 4 h and 2.8 g of surface modified ORMOSIL were obtained.

2.3.3. Inorganic modification with AgNO₃ and the reduction to Ag nanoparticles with hydrazine

Phenyl-based ORMOSIL particles with mercapto and amine functional groups (0.2 g) were dispersed in 100 mL of ethanol. AgNO₃ (0.2 g, 1.18 mmol) in 10 mL of de-ionized water was added and the resulting solution was stirred for 4 h. To remove unreacted AgNO₃, the reaction mixture was filtered and the obtained particles were washed with 10 mL of de-ionized water at least three times. The resulting particles were re-dispersed in 100 mL of ethanol and 0.2 mL of hydrazine monohydrate (4.19 mmol) was added to reduce the Ag⁺ ions attached to thiol functional groups. The Ag nanoparticle coated ORMOSIL particles were filtered through a membrane filter and washed with de-ionized water and ethanol several times. The particles were air-dried and 0.2 g of the metal hybrid composite particles was obtained.

2.3.4. Fluorescent dye-tagging reaction of ORMOSIL particles having multiple functional groups

Phenyl-based ORMOSIL particles with amine and mercapto functional groups (0.05 g) were dispersed in 50 mL of ethanol and 50 mL of de-ionized water was added. Fluorescamine (Sigma, 0.05 g, 0.02 mmol) in 20 mL of an ethanol/water/dichloromethane
ORMOSIL particles, (c) phenyl based tri-functional ORMOSIL particles, and (d) mercapto based tri-functional ORMOSIL particles.

Structures and SEM images of typical di- and tri-functional ORMOSIL particles: (a) phenyl based di-functional ORMOSIL particles, (b) vinyl based di-functional ORMOSIL particles, (c) phenyl based tri-functional ORMOSIL particles, and (d) mercapto based tri-functional ORMOSIL particles.

2.4. Characterizations

The thermal analyses of the products were performed with the thermo-gravimetric analyzer (TGA, Perkin–Elmer and TA Instrument), the Fourier transform infrared spectrometer (FT-IR, ABB FTIR2000) and nuclear magnetic resonance spectrometer (NMR, Varian UnityInova 300 and Bruker Avance 400) were used for the chemical characterization. X-ray powder diffractometer (XRD, Rigaku, D/RAD-C diffractometer with CuKα radiation) was used to investigate the structures of products. The sizes and morphologies of ORMOSIL particles and surface modified products were examined with field emission scanning electron microscope (FE-SEM, JEOL JEM-6340F) and transmission electron microscope (TEM, JEOL EM-2000EXII). The Confocal Laser Scanning Microscope (Bio-Red, MRC-1204 and Radiance2000/MP) was used to obtain fluorescence images of dye tagged ORMOSIL particles.

3. Results and discussion

3.1. Preparation of ORMOSIL particles having multiple functional groups

Monodispersed ORMOSIL particles with di- and tri-functional groups were prepared by two step acid–base sol gel process. In acidic conditions, the hydrolysis of a mixture of various silane monomers occurred simultaneously and emulsion droplets with homogeneous silane mixtures were formed. In basic conditions, co-condensation between the hydrolyzed silane mixtures in the emulsion droplets occurred. The mixture of silane monomers for ORMOSIL particles with multiple functional groups included phenyltrimethoxysilane, vinyltrimethoxysilane, and mercaptopropyltrimethoxysilane as a major functional component and (3-trimethoxysilyl)propyl methacrylate, 3-aminopropyltrimethoxysilane, 3-glycidoxypropyl trimethoxysilane, or other functional organo silanes as minor functional components. For most ORMOSIL particles having multiple functional groups, we found that the relative concentration of major silane monomers, when compared to minor functional silanes, should be greater than 70% of the total silane concentration to obtain monodispersed, spherical particles. When the relative concentration of any major component was less than 70%, spherical particles were not formed. Structural drawings and SEM images of typical di- and tri-functional ORMOSIL particles are shown in Fig. 1, indicating that all of the ORMOSIL particles exhibit mono-disperisty and spherical morphologies.

By changing reaction conditions, such as silane concentrations, temperature, and reaction time, the size of the ORMOSIL particles could be controlled from 200 nm to 1.4 μm. For example, the size of ORMOSIL particles was reduced when the total concentration of silane monomers was decreased as can be seen in Fig. 2. The size of the ORMOSIL particles also decreased when the reaction temperature increased. Increasing the stirring rate yielded smaller particles, although spherical particles were not obtained at a higher stirring rate. The reaction time also affected the size and morphology of the ORMOSIL particles. When the reaction time was short, polydispersed particles were obtained, while the size of the ORMOSIL particles increased with prolonged reaction time. Among the reaction variables, the concentration of monomer had the strongest effect on the size of resulting ORMOSIL particles.

The existence of multiple functional groups in ORMOSIL particles was confirmed by FT-IR and solid state NMR spectra (see Supplementary material). The IR spectra of ORMOSIL particles exhibited the characteristic stretching vibrational absorption peaks for the corresponding functional groups. For example, phenyl based glycidoxy di-functional ORMOSIL particles exhibited aromatic (3054 cm⁻¹) and aliphatic (2935 cm⁻¹) ν(C–H) stretching vibrational absorption peaks from phenyl and epoxy groups, respectively. Furthermore, ν(N–H), ν(S–H), and ν(C=O) stretching vibrational absorption peaks from each functional group were observed at 3370, 2554, and 1660 cm⁻¹, respectively, for the mercapto-based amine and isocyanate tri-functional ORMOSIL particles. Solid-state ¹³C NMR spectra also showed typical carbon resonance peaks for corresponding functional groups. For multifunctional ORMOSIL particles having phenyl or vinyl groups as a major component, strong absorption peaks near 135 ppm were observed from unsaturated carbons, while aliphatic carbon peaks between 0 and 50 ppm were observed for ORMOSIL particles having mercapto groups as a major component. In addition, relatively weak carbon absorption peaks from minor functional groups such as minor functional components. For most ORMOSIL particles having multiple functional groups, we found that the relative concentration of major silane monomers, when compared to minor functional silanes, should be greater than 70% of the total silane concentration to obtain monodispersed, spherical particles. When the relative concentration of any major component was less than 70%, spherical particles were not formed. Structural drawings and SEM images of typical di- and tri-functional ORMOSIL particles are shown in Fig. 1, indicating that all of the ORMOSIL particles exhibit mono-disperisty and spherical morphologies.

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![Fig. 1. Structures and SEM images of typical di- and tri-functional ORMOSIL particles: (a) phenyl based di-functional ORMOSIL particles, (b) vinyl based di-functional ORMOSIL particles, (c) phenyl based tri-functional ORMOSIL particles, and (d) mercapto based tri-functional ORMOSIL particles.](image-url)
Fig. 2. SEM images of tri-functional ORMOSIL particles prepared at different reaction conditions: top – silane concentrations of (a) 5.915 mmol, (b) 29.6 mmol; bottom – reaction temperature of (c) 50 °C, (d) 70 °C.

Fig. 3. SEM and TEM images of phenyl based amine and methacrylate tri-functional ORMOSIL particles (a) before and (b) after leaching with dimethyl sulfoxide.

Fig. 4. TGA curves and SEM images of typical di- and tri-functional ORMOSIL particles: top – TGA curve for (a) vinyl based and (b) phenyl based tri-functional ORMOSIL particles; bottom – phenyl based tri-functional ORMOSIL particles (c) before and (d) after heat treatment at 800 °C.
as amine, epoxy, and methacrylate were observed in corresponding multifunctional ORMOSIL particles. The solid-state $^{29}$Si NMR spectrum showed two peaks at 78.1 and 69.4 ppm, indicating that the main structure of multifunctional ORMOSIL particles consisted of $T_2$ and $T_3$ units from major functional component, similar to those in PTMS and PTMS/VTMS composite particles [31–33]. However, the relative amount of $T_2$ units is reduced for thiol and vinyl based ORMOSIL particles compared to those in phenyl-based ORMOSIL particles. The relatively large intensity of $T_2$ units in phenyl-based ORMOSIL particles might indicate that the degree of condensation was not high compared to that in vinyl or thiol based ORMOSIL particles. SEM and TEM images of phenyl based amine and methacrylate tri-functional ORMOSIL particles before and after leaching with dimethyl sulfoxide confirmed that porous ORMOSIL

![Scheme 1. Surface modification reactions of tri-functional ORMOSIL particles: (a) organic modification, (b) hydrophilic modification, (c) inorganic modification, and (d) selective dye tagging reaction.](image)

![Fig. 5. XRD pattern and SEM image of composite particles after reaction with AgNO₃ followed by the reduction with hydrazine using phenyl based ORMOSIL particles with mercapto and amine functional groups.](image)
particles were formed as shown in Fig. 3. $^1$H and $^{13}$C NMR spectra of solvent extract also exhibited absorption peaks from dissolved phenyl functional groups. In addition to two absorption peaks from a major silane component, additional peaks for $T^2$ and $T^3$ units from minor components were also observed for multi-functional ORMOSIL particles.

Thermal behaviors of the ORMOSIL particles with multiple functional groups were investigated using thermo-gravimetric analysis (TGA). While most of the ORMOSIL particles with a mono-functional group exhibited a single and relatively sharp weight loss at a decomposition temperature of the corresponding functional group, ORMOSIL particles with multi-functional groups showed broad weight loss patterns indicating multiple decompositions from major and minor silane components as shown in Fig. 4. The decomposition for multi-functional ORMOSIL particles occurred between 300 and 700 °C. The amount of residue after heat treatment for vinyl based ORMOSIL particles is higher than that for PTMS based particles, as expected. SEM images of ORMOSIL particles before and after the heat treatment at 800 °C showed that the spherical morphologies of the ORMOSIL particles were maintained. The N$_2$ physisorption experiment for ORMOSIL particles has been performed and the specific surface area of 3.945 m$^2$/g, pore size of 1.912 nm, and pore volume of 0.906 cm$^3$/g have been obtained.

3.2. Surface modification reactions of ORMOSIL particles having multiple functional groups

Chemical reactions of surface functional groups on ORMOSIL particles with various organic and inorganic materials were performed to endow additional functionalities and modified physical properties to the particle surfaces.

As shown in Scheme 1a, amine functional groups on the surface of tri-functional ORMOSIL particles could be converted to carboxyl groups by the reaction with glutaric anhydrides [34]. The IR spectra of surface reacted ORMOSIL particles showed two $\nu$(C=O) stretching vibrational absorption peaks from amide and carboxylic groups at 1685 and 1725 cm$^{-1}$, respectively. The CP-MAS $^{13}$C NMR spectrum also exhibited an additional resonance peak from a C=O functionality (see Supplementary material). The hydrophobic phenyl based ORMOSIL particles showed $\nu$(N–O) stretching vibrational peaks at 1350 and 1530 cm$^{-1}$. The hydrophilic substitution reaction of ORMOSIL particles was also confirmed by investigating the dispersion properties. Nitro-phenyl based ORMOSIL particles could be easily dispersed in water, while phenyl-based ORMOSIL particles floated on the top of the water. SEM image of nitro-phenyl based ORMOSIL particles indicated that the morphology of particles had not been changed after the substitution reaction (see Supplementary material). Surface modification of the ORMOSIL particles with inorganic materials, such as AgNO$_3$, produced metallic hybrid composite particles, as shown in Scheme 1c. Phenyl based ORMOSIL particles with mercapto and amine functional groups were treated with an aqueous AgNO$_3$ solution, converting the S–H functionality to S–Ag+. The resulting ORMOSIL particles were then reduced with hydrazine to produce Ag nanoparticles on the surface of the ORMOSIL particles after the removal of physically adsorbed Ag species through filtration and washing. The SEM image of ORMOSIL particles with S–Ag$^+$ functionalities did not reveal any noticeable change in the surface morphology compared to untreated particles, while nanosized Ag particles were observed on the surface of reduced ORMOSIL particles. The XRD spectrum of Ag nanoparticle-doped ORMOSIL particles confirmed the formation of metallic silver particles on the surface of ORMOSIL particles, while that of S–Ag$^+$ functional ORMOSIL particles only exhibited an amorphous phase (Fig. 5).

To investigate the homogeneous distribution of functional groups on ORMOSIL particles having multiple functional groups, selective tagging reactions of functionalities with fluorescent dye molecules were performed (Scheme 1d). Phenyl-based ORMOSIL particles containing amine and mercapto functional groups were first treated with an excess amount of fluorescein dye molecules to cover all the amine functionalities on the surface of the ORMOSIL particles, and then further reacted with rhodamine B isothiocyanate through surface mercapto groups [36,37]. Fig. 6 shows structural drawing, SEM image, excitation/emission spectrum, and confocal fluorescence spectra of dye-tagged phenyl-based tri-functional ORMOSIL particles with amine and mercapto functionalities. The ORMOSIL particles tagged with two different dyes exhibited two fluorescent emission peaks (blue and red$^1$) at 475 and 570 nm using excitation wavelengths of 390 and 540 nm,

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$^1$ For interpretation of color in Figs. 5 and 6, the reader is referred to the web version of this article.
respectively, while those tagged with fluoresceamine dye only exhibited a blue fluorescent emission peak at 475 nm by excitation at 390 nm. This indicated that the surfaces of the ORMOSIL particles were covered by multiple functional groups, demonstrating a better and more simplified control over the amount of multiple dyes tagging on the particle surface than ORMOSIL surfaces with only a single type of functional group. Therefore, these multi-dye tagged ORMOSIL particles have excellent potential for applications to bio- and nanotechnologies such as multiplex signaling and combinatorial chemistry.

4. Conclusions

In conclusion, monodispersed ORMOSIL particles with di- and tri-functional groups were prepared by facile one-pot synthesis. The types and numbers of functional groups on the surface and inside ORMOSIL particles can be controlled with great flexibility and the size of the particles can also be controlled between 200 nm and 1.4 μm with a narrow size distribution. The surface of ORMOSIL particles can be further modified in order to introduce additional organic functional groups or to carry various dyes or molecules such as fluorescent image contrast agents, targeting ligands or drugs, making them useful for various biomedical applications such as multicolor imaging and multitasking application. In addition, the metallic hybrid composite particles could also be prepared by the reaction of these ORMOSIL particles with various types of inorganic and metallic precursors.

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Appendix A. Supplementary material


References