

## Research Article

# Quantitative Analysis and Efficient Surface Modification of Silica Nanoparticles

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Aminofunctional trialkoxysilanes such as aminopropyltrimethoxysilane (APTMS) and (3-trimethoxysilylpropyl)diethylenetriamine (DETAS) were employed as a surface modification molecule for generating monolayer modification on the surface of silica ( $\text{SiO}_2$ ) nanoparticles. We were able to quantitatively analyze the number of amine functional groups on the modified  $\text{SiO}_2$  nanoparticles by acid-base back titration method and determine the effective number of amine functional groups for the successive chemical reaction by absorption measurements after treating with fluorescent rhodamine B isothiocyanate (RITC) molecules. The numbers of amine sites measured by back titration were 2.7 and 7.7 ea/ $\text{nm}^2$  for  $\text{SiO}_2$ -APTMS and  $\text{SiO}_2$ -DETAS, respectively, while the numbers of effective amine sites measured by absorption calibration were about one fifth of the total amine sites, namely, 0.44 and 1.3 ea/ $\text{nm}^2$  for  $\text{SiO}_2$ -APTMS(RITC) and  $\text{SiO}_2$ -DETAS(RITC), respectively. Furthermore, it was confirmed that the reactivity of amino groups on the surface-modified silica nanoparticles could be maintained in ethanol for more than 1.5 months without showing any significant differences in the reactivity.

## 1. Introduction

Since Werner Stöber has developed the synthetic method for preparing silica particles, colloidal silica particles have been intensively investigated to understand the reaction mechanism and control the size and size uniformity [1], and they have been used in many areas such as silicon wafer polishing, beverage clarification, and composite materials [2]. Recently, silica nanoparticles were represented as one of the most widespread nanomaterials in use because they have several features: (i) ease of preparation through hydrolysis-condensation reaction from relatively low-priced precursor molecules such as tetraethyl orthosilicate (TEOS) in the presence of acid or base catalysts, (ii) possible surface modification with various organosilicon compounds, and (iii) its biocompatibility without showing acute toxicity [3–5].

The silanol groups, Si–OH, on the silica surface can be easily modified to various functional groups by treating with organotrialkoxysilane ( $\text{RSi}(\text{OR}')_3$ ) compounds or methylsilanes together with catalyst [6], and surface modification of the silica nanoparticles with biorecognition molecules can make a specific interaction with receptor sites of living

systems. Based on this surface modification technique, there has been a great amount of research effort to use the silica nanoparticles as carriers for drug or gene deliveries [7–10]. Due to its stability and good biocompatibility along with easy surface modification, silica was also used as a surface coating material for many nanomaterials [11]. Organotrialkoxysilanes produce silanol groups by hydrolysis, which can be condensed with surface Si–OH group to form stable siloxane bond, Si–O–Si, for surface modification. However, they can also engage in self-condensation to form gel or oily oligomers to be precipitated within few hours in the presence of base catalysts. Therefore, there is a dilemma whether to use the large excess amount of organotrialkoxysilane to maximize the coverage of  $\text{SiO}_2$  surface or to use minimum amount to prevent the formation of any unwanted self-condensed side products. These self-condensed side products, in practice, are usually removed by repeating centrifugation/redispersion processes because the actual size of self-condensation products are very small compared to the size of  $\text{SiO}_2$  nanoparticles. Therefore, there is always a possibility that these self-condensed products further condense with Si–OH groups of the silica surface, resulting in a thick coating layer instead of

generating a monolayer modification. Even though the thick coating layer of organotrialkoxysilane has the advantage, in some cases, of giving more reactive functional groups for reactions, precise control of the number of surface functional groups cannot be achieved in a reproducible and reliable manner.

Among the various organotrialkoxysilane molecules for the modification of silica surfaces, 3-aminopropyltrimethoxysilane (APTMS) and 3-aminopropyltriethoxysilane (APTES) were widely explored by many researchers. The aminofunctional groups are known to enhance the dispersibility and miscibility of silica fibers [12], and to be used as a linkage unit to attach other functional molecules [13]. The surface modification of silica has been investigated in bulk surfaces and micron size silica beads by many characterization techniques [14–19]. A reaction mechanism was suggested as through the interaction between the amino group of APTES and surface Si–OH group in anhydrous condition [14, 20–22] or through the self catalytic effect of the amino group of APTES in polar alcoholic condition [23]. Interestingly, it was known that aminofunctional trialkoxysilanes such as APTMS and APTES are readily soluble in water to give solutions of unlimited stability at their natural pH in which normal organotrialkoxysilanes cause rapid condensation of Si–OH groups to form insoluble gels, and the internal hydrogen bonding was suggested to explain their lack of reactivity [12].

In this study, we have investigated the characteristic feature of aminofunctional ligand molecules such as APTMS and DETAS in ethanol solution, forming monolayer on the surface of SiO<sub>2</sub> nanoparticles instead of generating a thick coating layer. The total number of amino groups on the surface of SiO<sub>2</sub> nanoparticle was quantitatively analyzed by a simple acid-base back titration method and the effective number of amino groups for the successive chemical reactions was also determined by spectroscopic measurements after treating with fluorescent Rhodamine B isothiocyanate (RITC) molecule. It was also confirmed that the number of amino groups on the surface-modified silica nanoparticles, and their reactivity could be maintained in ethanol at room temperature for more than 1.5 months without showing any significant differences.

## 2. Experimental

**2.1. Materials.** 3-Aminopropyltrimethoxysilane (APTMS), 3-aminopropyltriethoxysilane (APTES), and (3-trimethoxysilylpropyl)diethylenetriamine (DETAS) were purchased from Gelest. Tetraethylorthosilicate (TEOS) was purchased from TCI. Rhodamine B isothiocyanate (RITC), methyl acrylate (MA), and ethylenediamine (EDA) were purchased from Aldrich. Ammonia solution was purchased from Samchun. All organic solvents were used as received without any further purification.

The size and shape of silica nanoparticles were characterized by transmission electron microscope (TEM; Carl Zeiss, LIBRA 120) and field emission scanning electron microscope (FE-SEM; Hitachi, S-4300), dynamic light scattering spectrophotometer (DLS; Qudix, scatteroscope I).

Fourier transform infrared (FT-IR) spectra were recorded using a JASCO FT/IR-600 Plus spectrometer for wavelengths ranging from 400 to 4000 cm<sup>-1</sup> to study the surface of silica nanoparticles. The absorption spectra of surface modified silica nanoparticles decorated with RITC were measured by UV-visible spectrometer (Sinco, S-3100).

**2.1.1. Synthesis of Silica Nanoparticles.** To a TEOS (2.5 mL) solution in 115 mL of dried ethanol, 3.75 mL of aqueous ammonium hydroxide solution (14.6 M) and 3.75 mL of water were added while stirring. After 12 h of stirring, silica nanoparticles were isolated by centrifugation at a speed of 15,000 rpm and the supernatant was removed. The isolated products were redispersed in ethanol. The washing process with centrifugation/redispersion was repeated 3 times. Finally, the redispersed nanoparticle solution was centrifuged at a speed of 2,000 rpm to remove any aggregated particles. The purified SiO<sub>2</sub> nanoparticles were homogeneously dispersed in ethanol. The size and shape of the nanoparticles were characterized by TEM, FE-SEM, and DLS.

**2.1.2. Surface Modification of Silica Nanoparticles with Aminofunctional Trimethoxysilanes.** 10 mL of SiO<sub>2</sub> nanoparticle solution (5 mg/mL of ethanol) was added into ten different vials. Appropriate amounts of APTMS (or DETAS) were added into each vial to maintain the conditions of surface modification; weight ratios of SiO<sub>2</sub>:APTMS (or DETAS) were varied from 1:0.01 to 1:0.1. After 12 hr of stirring at room temperature, modified silica nanoparticles were isolated and purified by centrifugation/redispersion processes (for 10 min at 15,000 rpm, 3 times) to remove the excess APTMS (or DETAS). Finally, the purified SiO<sub>2</sub>-APTMS (or SiO<sub>2</sub>-DETAS) nanoparticles were kept dispersed in ethanol.

**2.1.3. Quantification of the Number of Amine Sites on the Modified SiO<sub>2</sub> Nanoparticles by Back Titration.** 10 mg of modified silica nanoparticles was dispersed in 20 mL of 1.0 mM HCl solution and stirred for 30 min. Nanoparticles was separated by centrifugation at 15,000 rpm for 10 min, and 10 mL of supernatant was collected to be titrated with the standardized 1.0 mM NaOH solution in the presence of phenolphthalein indicator. From the difference of HCl concentration after treating with modified SiO<sub>2</sub> nanoparticles, the molar concentration of amine sites on the modified SiO<sub>2</sub> nanoparticles (10 mg) was calculated. This value was converted into the number of amine sites per unit area (nm<sup>2</sup>) of the surface of SiO<sub>2</sub> nanoparticle based on the density of bulk silica (2.2 g/mL) and the surface area of 100 nm SiO<sub>2</sub> nanoparticle.

**2.1.4. Quantification of the Number of Amine Sites on the Modified SiO<sub>2</sub> Nanoparticles by Absorption Measurement after Coupling with RITC.** 10 mL of the dispersed SiO<sub>2</sub>-APTMS (or SiO<sub>2</sub>-DETAS) nanoparticles in ethanol (5 mg/mL solution), prepared from various ratios of SiO<sub>2</sub>:APTMS (or DETAS), was treated with RITC (34.4 mg, 6.42 × 10<sup>-2</sup> mmol), and the mixed solution was stirred for 12 hr at RT. SiO<sub>2</sub>-APTMS(RITC) {or SiO<sub>2</sub>-DETAS(RITC)} nanoparticles were

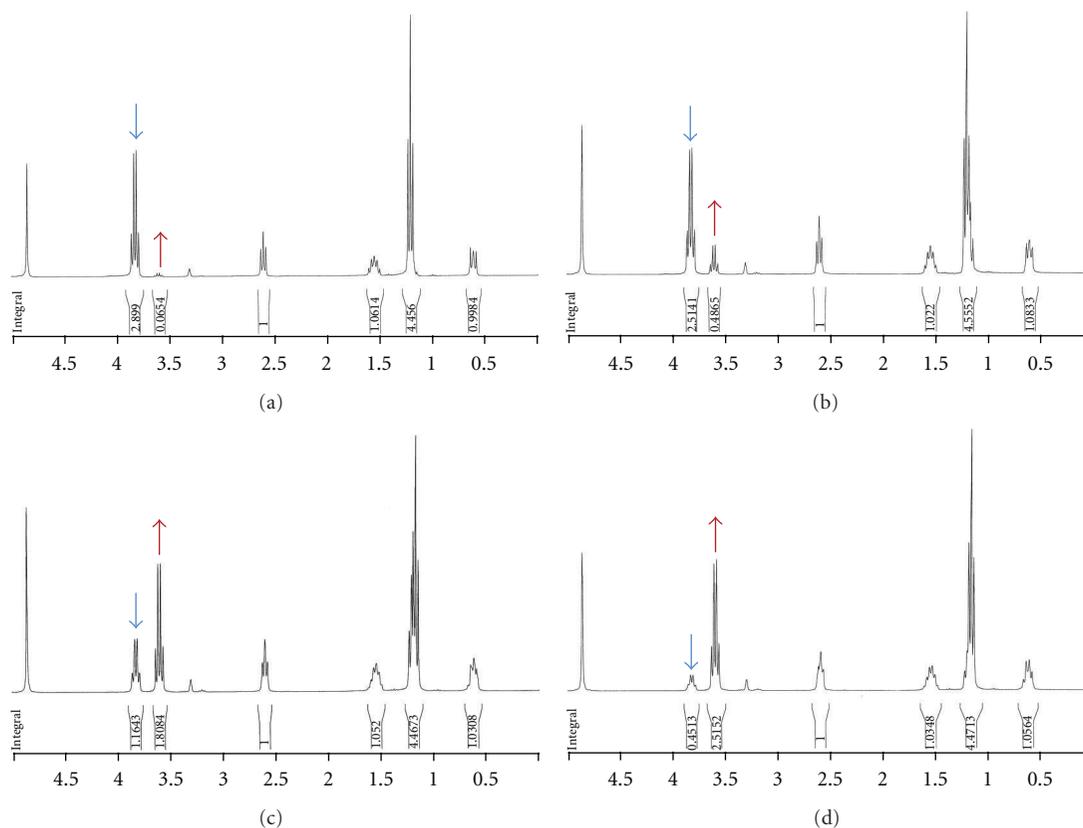


FIGURE 1: <sup>1</sup>H-NMR spectra of APTES in CD<sub>3</sub>OD after (a) just mixing, (b) 30 min, (c) 3 h, and (d) 6 h.

isolated by centrifugation at a speed of 15,000 rpm and repeatedly washed by centrifugation and redispersion until no RITC was detected from the supernatant. All the purified SiO<sub>2</sub>-APTMS(RITC) {or SiO<sub>2</sub>-DETAS(RITC)} nanoparticles, prepared from different ratios of SiO<sub>2</sub>: APTMS (or DETAS), were redispersed in 10 mL of ethanol. Small portions of each solution of modified nanoparticles (0.5 mL of SiO<sub>2</sub>-APTMS(RITC) or 0.3 mL of SiO<sub>2</sub>-DETAS(RITC), resp.) were diluted into 5 mL of ethanol for the absorption measurements. The absorptions of each sample were measured by UV-Vis spectrophotometry. A series of diluted solutions of RITC in ethanol was prepared to make the calibration curve and the absorption values were also measured by UV-Vis spectrophotometry.

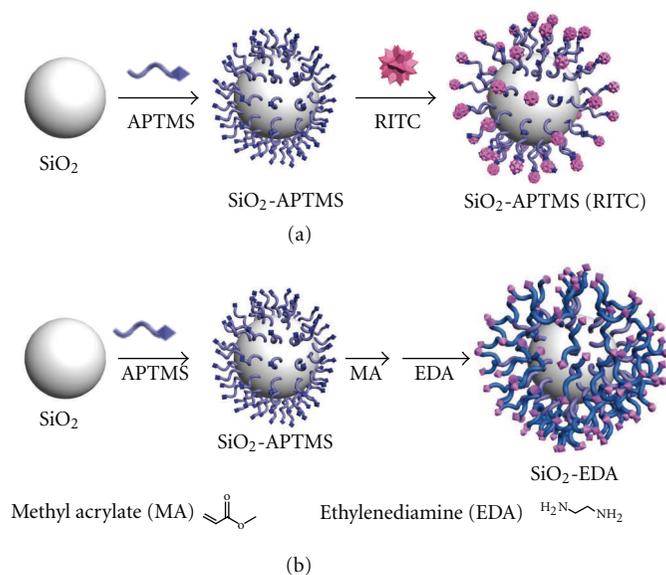
**2.1.5. Reaction of the Surface Amine Sites of SiO<sub>2</sub>-APTMS by a Process Analogous to Dendrimer Synthesis.** 50 mg of SiO<sub>2</sub>-APTMS nanoparticles was stirred with methyl acrylate (MA; 1.8 mL) in 10 mL of methanol in a vial at 25°C, with hydroquinone as an inhibitor. After 48 hr of stirring, the modified silica nanoparticles were purified by centrifugation/redispersion in methanol (for 10 min at 15,000 rpm, 3 times) to remove the excess MA. The purified SiO<sub>2</sub>-MA nanoparticles were dispersed in methanol and treated with ethylenediamine (EDA; 5.01 mL, concentration 7.5 M) at 25°C. After 48 hr of stirring, the SiO<sub>2</sub>-EDA nanoparticles were purified by centrifugation/redispersion in methanol

(for 10 min at 15,000 rpm, 3 times), and the purified SiO<sub>2</sub>-EDA nanoparticles were kept dispersed in methanol.

**2.1.6. Long Term Stability Test of Aminofunctional Groups on SiO<sub>2</sub>-DETAS by Coupling with RITC.** 10 mL of the dispersed SiO<sub>2</sub>-DETAS nanoparticles in ethanol (5 mg/mL solution), after storing for 1.5 months, was treated with RITC (34.4 mg,  $6.42 \times 10^{-2}$  mmol) and the mixed solution was stirred for 12 hr at RT. SiO<sub>2</sub>-DETAS(RITC) nanoparticles were isolated by centrifugation at a speed of 15,000 rpm and repeatedly washed by centrifugation and redispersion until no RITC was detected from the supernatant. The purified SiO<sub>2</sub>-DETAS(RITC) nanoparticles were redispersed in 10 mL of ethanol. 0.3 mL of SiO<sub>2</sub>-DETAS(RITC) nanoparticles were diluted into 5 mL of ethanol for the absorption measurements. The absorption of SiO<sub>2</sub>-DETAS(RITC) was measured by UV-Vis spectrophotometry.

### 3. Results and Discussion

To check the stability of aminofunctional trialkoxysilanes in aqueous and alcoholic solutions as reported in the literature [12], APTES was dissolved in deuterated methanol (CD<sub>3</sub>OD) and the changes were monitored by <sup>1</sup>H-NMR. As shown in Figure 1, new methylene peaks next to oxygen atom were increased at 3.6 ppm with time, corresponding to free ethanol molecule resulting from either alcohol exchange



SCHEME 1: Illustration of the surface modification of silica nanoparticle by APTMS followed by treatment with (a) RITC to measure the absorption and (b) methyl acrylate and ethylenediamine to increase the number of amine sites.

with methanol solvent or hydrolysis with small amount of water in the system. Even though almost all the ethoxy groups were replaced from APTES after 6 h, the clear solution remained without forming an insoluble gel by condensation reaction. A similar but much faster hydrolysis reaction was observed in D<sub>2</sub>O solution (see Figure S1 in supplementary materials available online at doi:10.1155/2012/593471), and again no gel was formed. When the concentration of APTES was increased as high as 0.1 M where silica nanoparticles were usually prepared in the presence of basic NH<sub>4</sub>OH catalyst, a clear solution was maintained for several weeks. The methanol solution of APTES was also checked by electrospray ionization mass spectrometry (ESI-MS). The very slow alcohol exchange and hydrolysis reaction was confirmed again and no higher molecular ion peaks (higher than 300) were detected after 6 h as shown in Figure S2.

Based on the results of stability tests of APTES in alcoholic solution confirming no self-condensation of APTES in a meaningful speed, the surface modification of silica nanoparticles having an average size of  $100 \pm 15$  nm was carried out with aminopropyltrimethoxysilane (APTMS) and (3-trimethoxysilylpropyl)diethylenetriamine (DETAS) in ethanol solution (Scheme 1(a)). Silica nanoparticles were prepared by the known method after slight modifications [1, 12], and dispersed in ethanol (5 mg/mL of EtOH). 10 mL of this solution was treated with different amounts of APTMS in the range of 1 to 10 wt%. After the mixed solution was stirred for 12 h, the modified silica nanoparticle, SiO<sub>2</sub>-APTMS, was isolated and washed by centrifugation 3 times and redispersion in ethanol. To easily check the number of effective amine groups on the surface of silica nanoparticle, these modified silica nanoparticles were reacted

with a large excess amount (more than 10<sup>3</sup> times excess compared to the amounts of used APTMS) of Rhodamine B isothiocyanate (RITC) to attach fluorescent rhodamine units by forming thiourea linkage, which has become one of the useful bioconjugation methods [24]. SiO<sub>2</sub>-APTMS(RITC) nanoparticles were again purified by centrifugation and redispersion in ethanol until no fluorescence from free RITC was detected in the supernatant. The absorption intensities from SiO<sub>2</sub>-APTMS(RITC) nanoparticles clearly showed that the absorption from chemically attached RITC units had increased as a function of the amount of APTMS used for the surface modification and seemed to reach the saturation point at a mixing ratio of around 1 : 0.05 (SiO<sub>2</sub> : APTMS, wt ratio) (Figure 2(a)). A very similar trend was observed from the modification experiments with DETAS, even though the absorption intensities were much higher due to the multiple amine sites (Figure 2(b) and Figure S3). These results showing saturation in absorption intensity seem to imply the formation of monolayer of APTMS and DETAS instead of the generation of a thick coating layer in our modification condition.

To confirm the formation of monolayer modification, silica nanoparticles before and after the surface modifications were precisely characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS) spectrophotometer. As shown in Figures 3(a)–3(c), TEM images of SiO<sub>2</sub>, SiO<sub>2</sub>-APTMS, and SiO<sub>2</sub>-DETAS did not show any significant size differences after the surface modifications. However, the size measured by DLS clearly showed the increment of the hydrodynamic sizes of SiO<sub>2</sub> nanoparticles in water from 137 to 141, and finally to 169 nm (Figures 3(d)–3(f)); the size from DLS is usually reported to be bigger

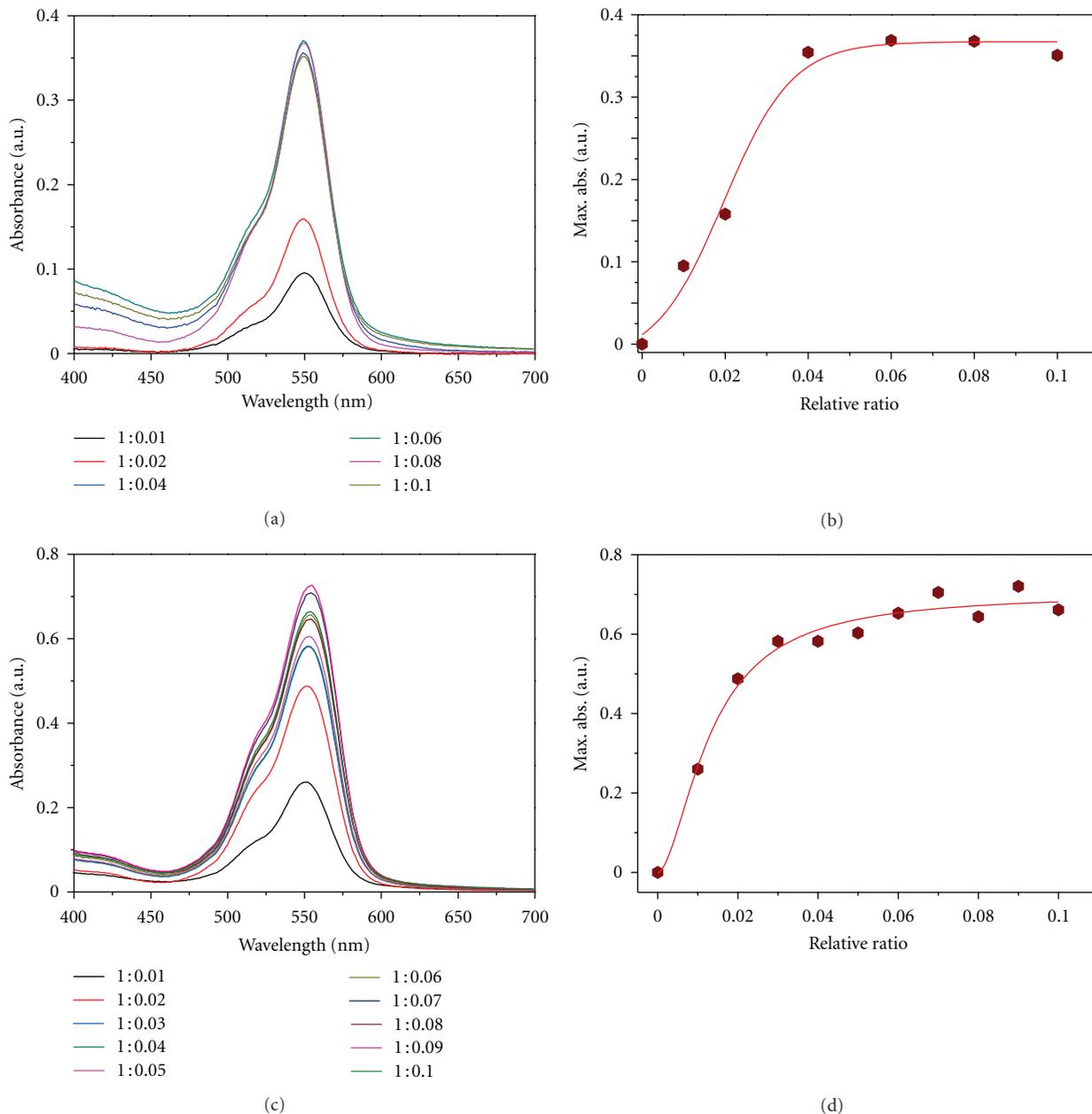


FIGURE 2: The absorption spectra of (a) SiO<sub>2</sub>-APTMS(RITC) and (c) SiO<sub>2</sub>-DETAS(RITC) nanoparticles prepared with various amounts of surface modification ligands, (b) and (d) relative intensity plots for each case clearly showing the absorption from chemically attached RITC units was increased as a function of the amount of APTMS used for the surface modification and seemed to reach the saturation point at around the mixing ratio of 1 : 0.05. 0.5 mL of SiO<sub>2</sub>-APTMS(RITC) or 0.3 mL of SiO<sub>2</sub>-DETAS(RITC) was diluted into 5 mL of ethanol for the absorption measurements.

than that from TEM due to the surrounded water molecules and swelling effect of surface molecules. A comparison of FT-IR spectra of SiO<sub>2</sub>-DETAS with SiO<sub>2</sub> clearly showed that the functional groups from DETAS molecule were observed after the surface modification (Figure 4).

Since the surface-to-volume ratio of nanoparticles is dramatically increased compared to bulk materials, the actual number of aminofunctional groups in the modified silica samples would be reasonably high enough to be directly

measured by simple acid-base titration. Due to the feature of amino group as a weak base and the possible aggregation and scattering problems during the titration, back titration method was employed for the direct measurement of amino groups on the surface of silica nanoparticles. Back titration is a technique designed to resolve problems when analytes are in nonsoluble solid phase having either too weak or too slow characteristics to give a valid reaction; the unknown concentration of a base sample is determined by reacting it with

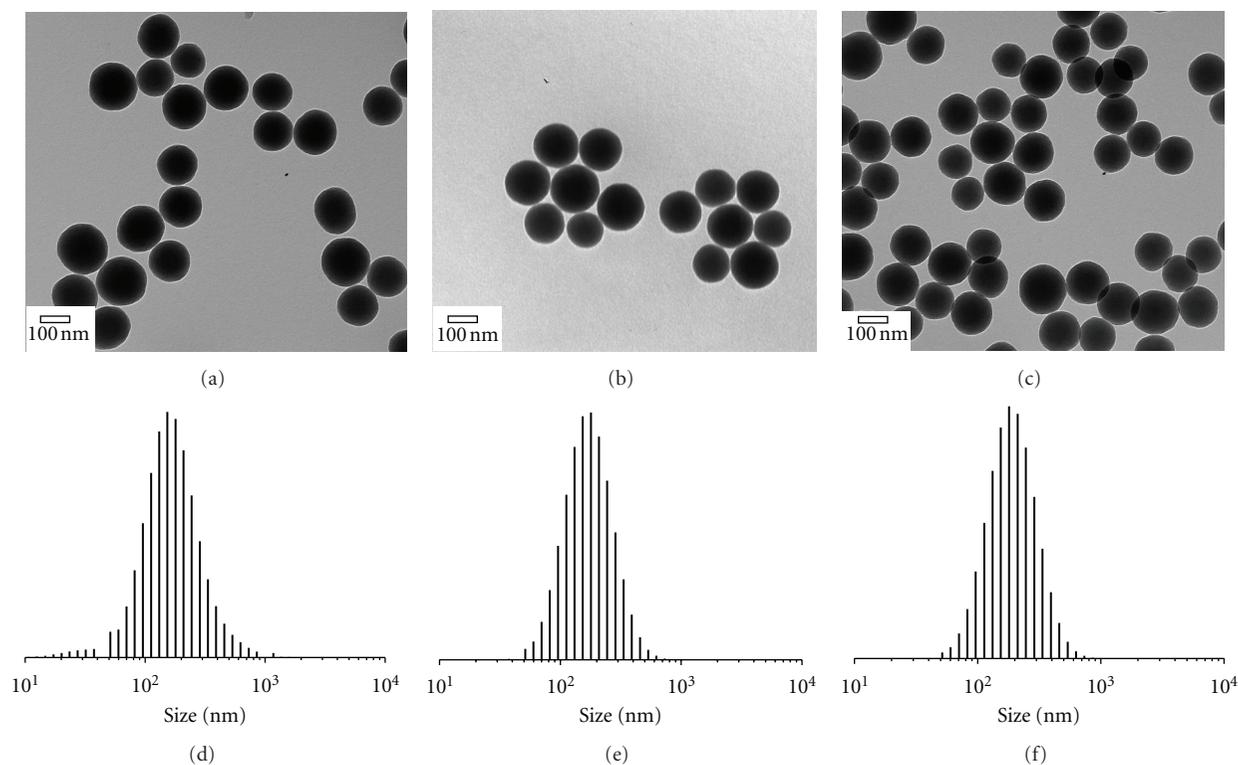


FIGURE 3: TEM images of (a) SiO<sub>2</sub>, (b) SiO<sub>2</sub>-APTMS, and (c) SiO<sub>2</sub>-DETAS nanoparticles, and size distributions measured by DLS for (d) SiO<sub>2</sub> (137 nm), (e) SiO<sub>2</sub>-APTMS (141 nm), and (f) SiO<sub>2</sub>-DETAS (169 nm) nanoparticles.

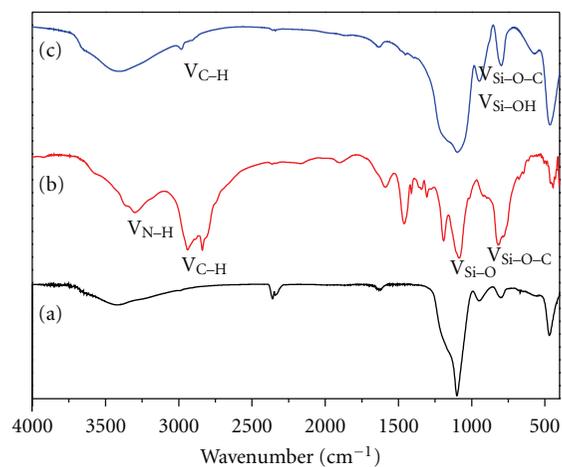


FIGURE 4: FT-IR spectra of (a) SiO<sub>2</sub> nanoparticle, (b) DETAS ligand molecule, and (c) SiO<sub>2</sub>-DETAS nanoparticle.

an excess volume of acid with a known concentration and then titrating the resulting mixture with the standardized base solution to give the differences of the acid concentration which corresponds to the concentration of unknown base sample [25]. 10 mg of modified silica nanoparticles was dispersed in 20 mL of 0.01 M HCl solution and stirred for 30 min. Nanoparticles were separated by centrifugation at 15,000 rpm for 10 min, and 10 mL of supernatant was

TABLE 1: Measured numbers of silanol and amine sites on the various silica surfaces from references as well as this work.

Type of silica	Number of silanol sites (ea/nm <sup>2</sup> )	Number of amine sites (ea/nm <sup>2</sup> )	Method (reference)
(1) Aerosol	4.8	1.6	IR [26]
(2) Fumed silica	7	2.3	Inverse reaction chromatography [27]
(3) Colloidal silica	3.3 ~ 6.6	1.2 ~ 2.2	IR [28]
(4) SiO <sub>2</sub> -APTMS		2.7 ± 0.35	Titration
(5) SiO <sub>2</sub> -DETAS		7.7 ± 0.19	"
(6) SiO <sub>2</sub> -EDA		6.8 ± 1.10	"
(7) SiO <sub>2</sub> -APTMS		0.44 ± 0.01	Absorption
(8) SiO <sub>2</sub> -DETAS		1.3 ± 0.08	"
(9) SiO <sub>2</sub> -EDA		0.99 ± 0.07	"

collected to be titrated with the standardized 0.01 M NaOH solution in the presence of phenolphthalein indicator (see Table S1 for the summary of titration results and calculations).

Table 1 summarizes the number of amine sites measured by the titration as well as the calculated values from the absorption measurement after treating with RITC. It has been reported that the number of surface silanol ( $\equiv\text{Si}-\text{OH}$ )

groups on the silica surface could be measured by various techniques such as IR spectroscopy and inverse reaction chromatography; surface Si–OH concentrations vary in the range 3.3~7 ea/nm<sup>2</sup> [26–28]. It can be assumed that one APTMS reacts with three Si–OH sites on the surface to give the minimum values for the number of amine sites in the range 1.2~2.3 ea/nm<sup>2</sup>, which are comparable to the values obtained from the back titration method in our measurements (2.7 ea/nm<sup>2</sup>). From the saturation value of SiO<sub>2</sub>-APTMS(RITC) nanoparticles in Figure 2(a), the number of effective amine sites reacting with RITC can be also calculated as 0.44 ea/nm<sup>2</sup> using the calibration curve of free RITC in ethanol solution (see Figure S4, based on the assumption that the absorption coefficient of RITC is not very different when it is attached on the surface). The differences from both analyses can be explained that only one out of six amino (–NH<sub>2</sub> and –NH–) groups can react with RITC probably due to the steric hindrance from the bulky and flat RITC molecule. Similar quantitative analyses were carried out for SiO<sub>2</sub>-DETAS and SiO<sub>2</sub>-DETAS(RITC) nanoparticles, which had three amine sites per DETAS ligand molecule. As expected, the number of amine sites measured by back titration was 7.7 ea/nm<sup>2</sup> and that of effective amine sites measured by absorption calibration was 1.3 ea/nm<sup>2</sup>, almost three times larger than SiO<sub>2</sub>-APTMS cases. To check the reproducibility and reliability of our quantitative analysis methods, a further chemical reaction was carried out from the amine sites of SiO<sub>2</sub>-APTMS, and the number of new functional sites was measured. For the quantitative conversion of each generation in the amine-terminal dendrimer synthesis, it is known that a terminal primary amine could be successively reacted with methyl acrylate (MA) and ethylenediamine (EDA) to form one tertiary amine and two terminal primary amines (Figure S5) [29]. The same reaction was carried out with SiO<sub>2</sub>-APTMS as illustrated in Scheme 1(b), and the number of amine sites in resulting SiO<sub>2</sub>-EDA was again measured by back titration. The number of amine sites per nm<sup>2</sup> was increased from 2.7 for SiO<sub>2</sub>-APTMS to 6.8 for SiO<sub>2</sub>-EDA, as shown in Table 1, corroborating that the conversion reactions proceeded almost quantitatively.

It is well known that the actual number of aminofunctional groups on the modified surfaces is changed with time due to the instability of surface layers. It has been also proposed that some amino group on the surface is bent toward the surface, and the bent amino group is interacted with circumjacent silanol group by hydrogen bonding or acid-base interaction to form ion pairs. Such interactions can reduce the actual reactivity of amine groups [30]. In our surface modification method forming monolayer with amino-functionalized trialkoxysilanes, however, these types of interaction of amine groups with surface silanol groups (so-called backbiting interactions) were reduced significantly and the reactivity of amino group could be maintained for a long period of time without showing any differences in the reactivity. After the surface modification, SiO<sub>2</sub>-DETAS nanoparticles were stored in ethanol for 1.5 month. Then, SiO<sub>2</sub>-DETAS was treated with RITC and SiO<sub>2</sub>-DETAS(RITC) nanoparticles were purified by centrifugation/redispersion

processes. The absorption intensities from Rhodamine B units from SiO<sub>2</sub>-DETAS(RITC) nanoparticles did not show any significant differences even after 1.5 month storage of SiO<sub>2</sub>-DETAS in ethanol solution, proving the excellent stability of surface aminofunctional groups and the reliable control of surface functionality in our modification method (Figure S6).

## 4. Conclusion

It has been demonstrated that a monolayer modification on the surface of SiO<sub>2</sub> nanoparticles can be obtained by using stable ethanol solutions of organofunctional trialkoxysilanes such as APTMS and DETAS. Precise characterizations with transmission electron microscopy (TEM) and dynamic light scattering (DLS) spectrophotometer have confirmed the formation of monolayer modification. The total number of amino groups on the surface of SiO<sub>2</sub> nanoparticle were quantitatively analyzed by a simple acid-base back titration method and the effective number of amino groups for the successive chemical reactions was also determined by spectroscopic measurements after treating with fluorescent Rhodamine B isothiocyanate (RITC) molecule, showing well-matched results with known values from literatures. Furthermore, the reproducibility and reliability of our quantitative analysis methods were confirmed by checking the change of amine sites of SiO<sub>2</sub>-APTMS after the quantitative conversion of the terminal primary amine to one tertiary amine and two terminal primary amines by successive reactions with methyl acrylate (MA) and ethylenediamine (EDA), a process well-developed in dendrimer synthetic methods. We believe that our method to generate monolayer modification and analyze the number of amine sites on nanoparticle surface will be useful in nano-bio research applications, such as sensors, diagnosis, and drug or gene deliveries, where reliable and reproducible modification and quantitative analysis are very critical.

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