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Synthesis of Amphiphilic Polyhedral Oligomeric Silsesquioxane Having a Hydrophobic Fluorescent Dye Group and Its Formation of Fluorescent Nanoparticles in Water

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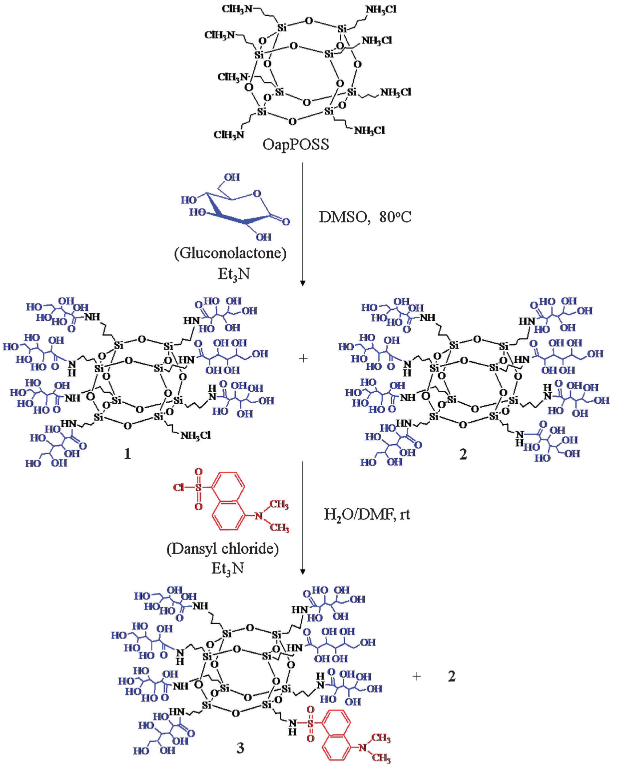
In this study, we synthesized an amphiphilic polyhedral oligomeric silsesquioxane (POSS) having controlled numbers of hydrophilic and fluorescent hydrophobic groups (seven and one, respectively) by the successive reactions of a starting compound, octa(3-aminopropyl)-POSS, with gluconolactone and with dansyl chloride under appropriate conditions. The product formed fluorescent nanoparticles with relatively well-defined morphology in water by self-aggregation, which was confirmed by SEM, DLS, and fluorescence measurements.

Amphiphilic molecules, of which soap is a typical example, possess antagonistic hydrophilic and hydrophobic moieties in the same molecule. In aqueous media, such molecules self-assemble into diverse aggregate morphologies, depending on the molecular shape and solution conditions.[1–3] Recently, we have reported synthesis of organic–inorganic amphiphilic hybrids based on the poly(2-aminopropylsiloxane) backbone.[4–6] The hybrids were synthesized by the successive reactions of amino groups in the polysiloxane with fatty acid chlorides and with sugar lactones. In the products, the former acted as hydrophobic groups, whereas the latter functioned as hydrophilic groups for the amphiphilic property. The further functionalization of the remaining free amino groups in the products with fluorescent dye moieties was conducted by reaction with fluorescein thioisocyanate to give the fluorescent-dye-labeled amphiphilic polysiloxanes.[6] We also confirmed the formation of nanoparticles from dispersion of the products in water, which exhibited fluorescence emission. Furthermore, cellular uptake of the amphiphilic polysiloxane-based nanoparticles in human aortic endothelial cells was observed by fluorescence microscopy. These results suggest that such organic–inorganic amphiphilic hybrids have a possible potential to use as biomaterials in biological and medical applications. Moreover, silicon-containing materials such as polydimethylsiloxane have various interesting properties conferring advantages as biomaterials, such as hemocompatibility, biocompatibility, and anti-inflammatory activity.[7] On the basis of the above viewpoints and backgrounds, in this study, we noted polyhedral oligomeric silsesquioxanes (POSSs)[8] in place of the polysiloxanes as the silicon-containing inorganic framework for the synthesis of new organic–inorganic amphiphilic hybrids. Because POSS derivatives do not exhibit polydispersity, which is different from polysiloxanes, the former would have an advantage over the latter in the formation of fluorescent nanoparticles with well-defined morphologies by self-aggregation. Indeed, we found that the amphiphilic POSS prepared in this study formed more morphologically controlled nanoparticles in water compared with the previously reported amphiphilic polysiloxanes.



Scheme 1. Synthesis of amphiphilic POSS **3**.

We employed an octa(3-aminopropyl)-POSS (OapPOSS)[9–11] as a starting POSS derivative and attempted reactions of eight amino groups in OapPOSS with appropriate hydrophilic and hydrophobic substrates to synthesize amphiphilic fluorescent POSS derivatives. It has been considered that the functionalization of the desired numbers of amino groups to respective hydrophilic and hydrophobic groups by controlling the reaction conditions possibly yields a POSS derivative with definite numbers of hydrophilic and hydrophobic groups. As sources for the hydrophilic and hydrophobic moieties, gluconolactone and dansyl chloride were employed due to the presence of the reactive sugar lactone and sulfonyl chloride groups, respectively, for the amino groups (Scheme 1). Because the dansyl group is a fluorescent dye, it can be expected to act not only as the hydrophobic group, but also as the fluorescent moiety for the formation of the fluorescent nanoparticles.

Accordingly, the reaction of OapPOSS with gluconolactone was first performed in varying feed molar ratios in DMSO solvent.[12] The MALDI-TOF MS measurements of the crude products were conducted to evaluate how many amino groups participated in the reaction with gluconolactone under each condition. Consequently, when the reaction was carried out in a 64:1 feed molar ratio of gluconolactone to OapPOSS in DMSO

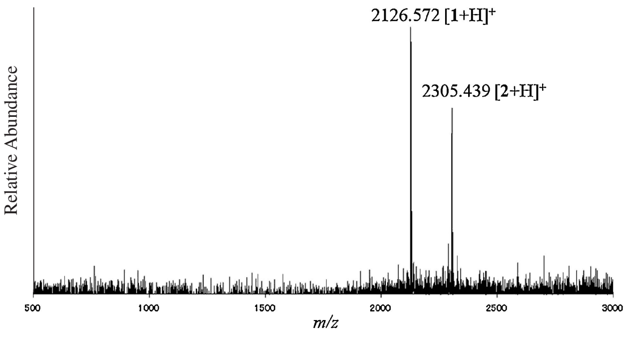


Figure 1. MALDI-TOF MS of the products obtained by the reaction of OapPOSS with gluconolactone in DMSO at 80 °C for 24 h (feed molar ratio of gluconolactone to OapPOSS = 64:1).

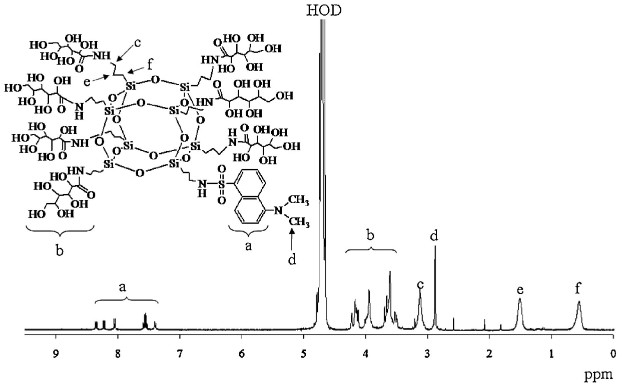


Figure 2. ^{1}H NMR spectrum of **3** in D_2O.

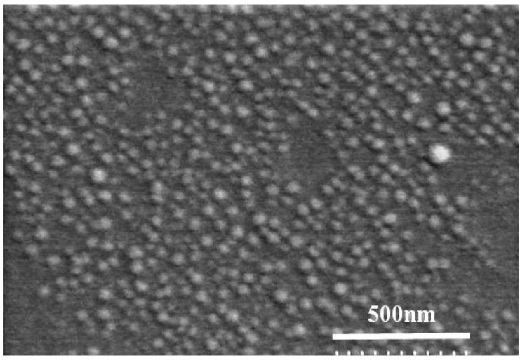


Figure 3. SEM image of the spin-coated sample prepared from an aqueous dispersion of **3**.

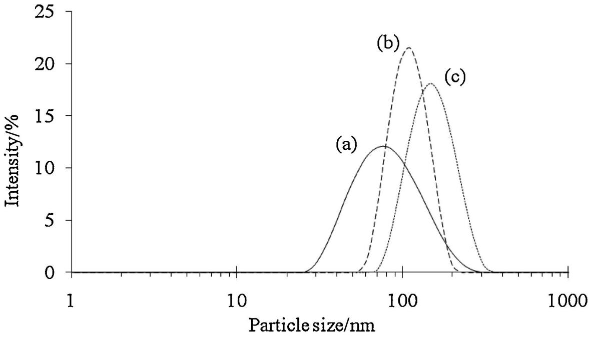


Figure 4. DLS profiles of aqueous dispersions of **3** in various concentrations; 1.0×10^{-4} (a), 1.0×10^{-3} (b), and 2.0×10^{-3} (c) mol L^{-1}.

at 80 °C for 24 h, the MALDI-TOF MS of the crude products, which were obtained as an acetone-insoluble fraction, showed only two peaks (*m/z* 2126.572 and 2305.439) corresponding to the masses of the POSS derivatives **1** and **2** (Figure 1), in which seven and eight amino groups were functionalized with hydrophilic groups, respectively. From the integrated ratio of the signal due to C*H*(OH)–C*H*(OH)–C=O derived from gluconolactone to the signal due to CH_2Si of OapPOSS in the ^{1}H NMR spectrum of the crude products (DMSO-d_6), the molar ratio of **1** to **2** was calculated as 0.215:0.785. These data indicated that one of the two products, i.e., the derivative **1**, still had an unreacted amino group, which was further used for the reaction with dansyl chloride. Because the two POSS products **1** and **2** could not be separated by the simple procedure, the following functionalization was conducted using the mixture of the two products.

The reaction of the amino group in **1** with dansyl chloride was performed by stirring a solution of the aforementioned products, dansyl chloride, and triethylamine in a mixed solvent of water and DMF at room temperature for 2 h. Thus, the resulting dansyl-functionalized POSS derivative was roughly separated from **2** by means of the difference in their solubility. In addition to the low content of **1** in the products obtained by the first reaction, complicated procedures including column chromatography on ion-exchange resin and dialysis were necessary to obtain **3** in high purity. The improvement of the isolation procedure of **3** for increasing the yield is now in progress. Figure 2 shows the ^{1}H NMR spectrum of the isolated POSS derivative in D_2O. In addition to signals due to $Si(CH_2)_3N$ at δ 0.53, 1.49, and 3.11, signals assignable to the hydrophilic groups derived from gluconolactone at δ 3.45–4.25 and signals ascribable to the dansylamide group at δ 2.86 (CH_3) and δ 7.37–8.33 (aromatics) were observed. The integrated ratio of the signals due to $SiCH_2$, the hydrophilic, and aromatic protons were 16:42:6. The ^{29}Si NMR spectrum of **3** in DMSO-d_6 showed four signals at δ −65.9, −66.0, −66.1, and −66.2, ascribable to four kinds of Si atoms in **3** (Figure S1[14]). Their chemical shifts were close to those of OapPOSS (δ −66.4), suggesting that the POSS framework was maintained in the product. The NMR data fully supported the structure of the POSS derivative **3** having seven hydrophilic groups and a hydrophobic dansyl group.

To confirm the self-aggregation of **3**, SEM and DLS measurements were conducted. Figure 3 shows the SEM image of a spin-coated sample from a dispersion of **3** in water. Nanoparticles with relatively narrow distribution of sizes were clearly seen. Average diameter and a standard deviation were calculated to be 37.9 and 3.29 nm, respectively. This standard deviation is much smaller than that calculated from the SEM image for the amphiphilic polysiloxane nanoparticles reported in our previous paper (Figure S2[14]).[5] These results indicated that **3** formed the more morphologically controlled nanoparticles in water compared with the amphiphilic polysiloxanes. The DLS measurement of a dispersion of **3** in water (1.0×10^{-4} mol L^{-1},

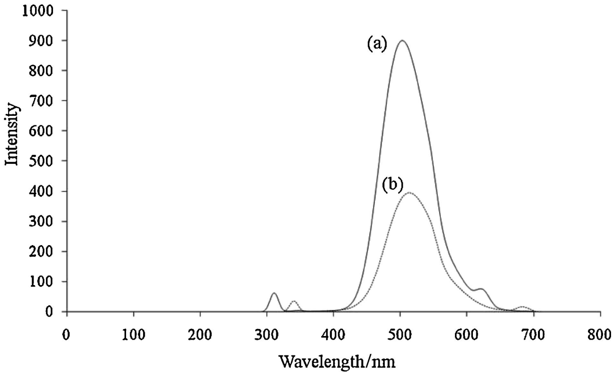


Figure 5. Fluorescence spectra of **3** excited at 310 nm in water (a) and *N*-butyl-5-(dimethylamino)naphthalene-1-sulfonamide excited at 340 nm in methanol (b): concentrations; 0.90×10^{-4} mol L^{-1}.

Figure 4a) also showed formation of nanoaggregates with mono modal profile and the average diameter was calculated to be 72.5 nm. The average diameters in the DLS profiles increased with increasing the concentration of the dispersion as shown in Figures 4b and 4c; 101.0 (1.0×10^{-3} mol L^{-1}) and 135.4 nm (2.0×10^{-3} mol L^{-1}), respectively. This is probably due to further aggregation under conditions of higher concentration of **3** in water. The average diameters calculated on the basis of the DLS measurement were always larger than those calculated by the SEM image. The difference was probably because of the different calculation methods for the average diameters in both the measurements, in which the average diameter in the DLS measurement was calculated from the intensity of scattering light, which was proportional to d^6 (d: diameter of a nanoparticle), whereas number distribution was used for calculation of the average diameter of nanoparticles in the SEM measurement.[13] Furthermore, the wet and dry sample conditions in these measurements probably affected the difference in the particle sizes.

The fluorescence spectrum of a dispersion of **3** in water (0.90×10^{-4} mol L^{-1}, a similar concentration as that in the DLS measurement (Figure 4a)) exhibited an emission maximum at 503 nm by excitation at 310 nm (Figure 5a), which was a typical fluorescence peak due to the dansyl moiety as appeared in the fluorescence spectrum of *N*-butyl-5-(dimethylamino)naphthalene-1-sulfonamide in methanol (Figure 5b, Ex. 340 nm, Em. 513 nm). The above SEM, DLS, and fluorescence analyses suggested the formation of fluorescent nanoparticles with relatively well-defined morphology by self-aggregation of the amphiphilic POSS derivative **3** in water.

In conclusion, we reported herein the synthesis of a fluorescent amphiphilic POSS derivative from OapPOSS as a starting compound. Under the appropriate conditions, the reaction of seven amino groups with gluconolactone and the subsequent reaction of an amino group with dansyl chloride successfully took place to give the amphiphilic OapPOSS **3** with controlled functionalities of the hydrophilic and hydrophobic groups. The product formed more morphologically controlled nanoparticles in water compared with the previously reported amphiphilic polysiloxanes, which was confirmed by the SEM and DLS measurements. Furthermore, the product exhibited fluorescence emission in water, because of the presence of the dansyl group. Further studies on the synthesis of different amphiphilic POSS having various hydrophilic and hydrophobic groups by the same procedure as that described here and the effect of their molecular structures on self-aggregation are now in progress.

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