Molecular Wrapping of a Fluorescent Dye with TiO_2-Gel and Capping Reagents

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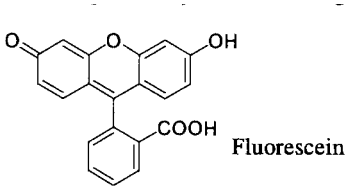
A fluorescent molecule, fluorescein, was covered with an ultrathin TiO_2-gel layer and the resulting nano-particles were stabilized by additional capping. Electron microscopy suggested aggregation and subsequent capping of wrapped fluorescein. The molecular wrapping was effective for suppressing fluorescence quenching by iodine.

Molecules and nano-particles that are spatially isolated from the surrounding medium have been attracting much attention because of their unique physical and chemical properties. For example, gold nano-particles coated with SnO_2 shell were demonstrated to realize long-term electron storing in the core.[1] Encapsulation of fluorescent dyes in hollow silica particles[2] and polymer beads[3] is employed to protect the dyes from outer environments, to probe biological cells, and to evaluate the intercellular event.[3] Coating of latex, metal oxide particles, and biocolloids by polyelectrolytes was similarly useful for controlling their solubility and reactivity, resulting in application in drug delivery, catalysis, and in designed particle assemblies.[4] We examined in this study encapsulation of fluorescent dyes into TiO_2 nano-particles that are formed in the conventional sol–gel process. Fluorescein molecule was employed for this purpose, since its carboxy group should possess high affinity towards TiO_2 gel.[5]



Fluorescein

Fluorescein has strong bright green fluorescence near 520 nm in 2-propanol (Figure 1a).[6] This peak shifted to 542 nm upon addition of 50 times excess titanium isopropoxide ($Ti(O^iPr)_4$), and the original yellow color turned yellowish red. This spectral change is apparently ascribed to complexation of titanium isopropoxide to the hydroxy and carboxy groups of fluorescein.[6,7] A small amount of water was then added at room temperature to produce TiO_2-gel by hydrolysis of the alkoxide group. The solution remained clear for a several hours without any color change and precipitation. It is clear that fluorescein molecules are bound to the particle of TiO_2-gel dispersed in 2-propanol.

Figure 1b compares decreases in fluorescence intensity due to addition of I_2. Iodine acts as a powerful quencher of fluorescein fluorescence, and about 70% of the fluorescence was quenched even at concentrations as low as 5×10^{-5} M for both species. This quenching effect was unchanged for a mixture of fluorescein and $Ti(O^iPr)_4$, and after further addition of water (entries **2** and **3**). From these results, we judged that TiO_2-gel

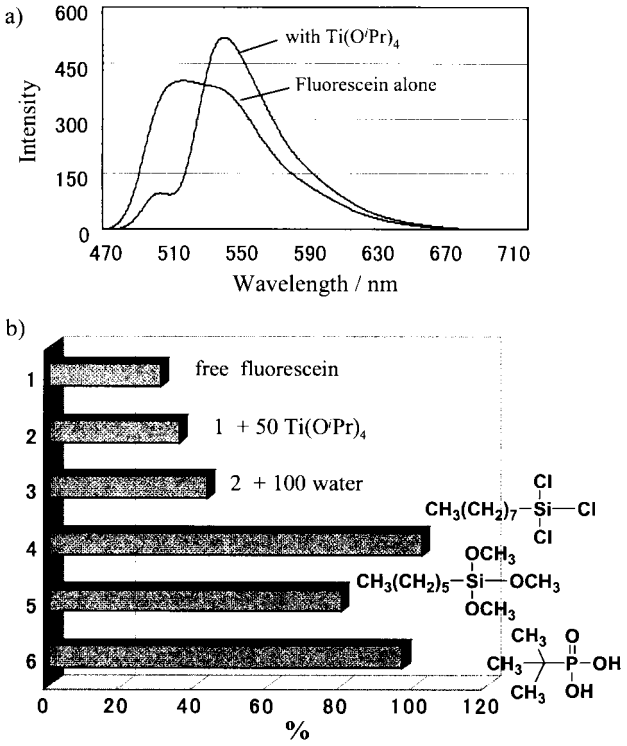


Figure 1. a) Fluorescence spectra of fluorescein and fluorescein / $Ti(O^iPr)_4$ mixture in 2-propanol. Fluorescein; 5×10^{-5} M, $Ti(O^iPr)_4$; 25 mM, excitation; 450 nm. b) Decreased fluorescence intensities at 560 nm after addition of I_2. Fluorescein and I_2 are 5×10^{-5} M for all cases. 1; free fluorescein, 2; fluorescein and 2.5×10^{-3} M $Ti(O^iPr)_4$, 3; sample 2 and 5.0×10^{-3} M water, 4; sample 3 and 2.5×10^{-3} M octyltrichlorosilane, 5; sample 3 and 2.5×10^{-3} M hexyltrimethoxysilane, 6; sample 3 and 2.5×10^{-3} M *tert*-butylphosphonic acid.

alone was insufficient to isolate fluorescein molecule from the outer environment. The TiO_2-gel particle forms aggregates very slowly under ambient humidity.

In order to improve the isolation and stability of the particles, we examined further capping by using reagents such as silicic acid and phosphonic acid derivatives that would effectively bind to the surface of nano-particles. The structure of capping molecules was crucial to stable dispersion of the fluorescein containing TiO_2-gel particles. For example, addition of dodecyltrimethoxysilane and octadecyltrimethoxysilane produced orange precipitates. Phenylphosphonic acid also gave orange precipitate, unlike *tert*-butylphosphonic acid that gave clear yellow solution. These results indicate that nano-particles coated with alkyl groups readily produce macroscopic aggregates in 2-propanol. However, short or branched alkyl chains, such as hexyl, octyl, and *tert*-butyl groups are relatively suitable for prevention of the agglomeration. The fluorescence quench-

ing with I_2 was remarkably suppressed, when these capping molecules were allowed to react (entries **4**–**6** in Figure 1b). The original fluorescence was recovered as much as 80% with hexyltrimethoxysilane and nearly 100% with octyltrichlorosilane and *tert*-butylphosphonic acid.[8] It is clear that the access of I_2 to the fluorescent dye is effectively suppressed only after reaction with the capping molecules.

We employed transmission electron microscopy (TEM) to elucidate the structure of the dispersed nano-particle. A TEM photograph shown in Figure 2a indicates formation of particles as capped with hexylsiloxane. Particles of 40–100 nm in diameter are partially aggregated. The corresponding high-resolution TEM image (Figure 2b) shows the presence of a core-shell structure in each particle, where an inner core of ca. 50 nm is coated with a pale-colored shell of 5–10 nm thick. The core contains dark spots of 2 nm size. These spots may be ascribed to relatively heavy titanium atoms. The more transparent outer shell must be mainly composed of multilayers of hexylsiloxane.

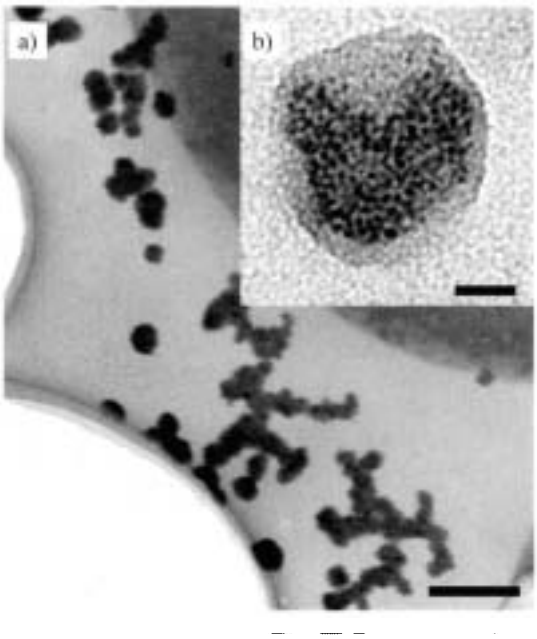


Figure 2. Transmission electron micrographs of fluorescein / TiO_2-gel particles capped with hexyltrimethoxysilane (entry 5 in Fig. 1b). a) Sample solution was dropped on a micro grid with a polymer support, and dried in vacuum. Scale bar; 300 nm. b) Sample solution was dropped on a silicon monoxide coated grid (Ted Pella Inc.), and dried. Scale bar; 20 nm. All the observations were made without staining.

It is noteworthy that most of the fluorescein is restrained from self-quenching even when the dye is concentrated in the core. For example, the nano-particle capped with hexylsiloxane shell gave a fluorescence intensity that was 40–45% of that of free fluorescein molecules in 2-propanol (entry **5**). This suggests that fluorescein molecules are dispersed in the TiO_2-gel matrix in a way to prevent self-quenching. Based on the fluorescence measurement and TEM observation, we propose the particle structure such as shown in Figure 3. Each fluorescein

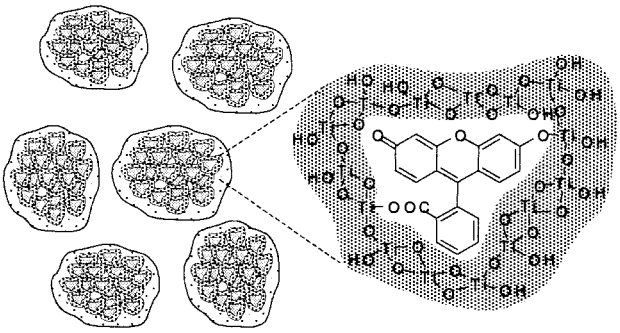


Figure 3. A schematic illustration of fluorescein / TiO_2-gel particles coated with capping reagents.

molecule is wrapped with thin titanium oxide layer, and the carboxy and hydroxy groups are bound to the titanium atom. The molecularly isolated dye molecule does not lose the individual molecular property, and emits the monomer fluorescence. Dark spots observed in high-resolution TEM correspond to the individual wrapped molecule. The initially formed TiO_2-gel must be small colloidal particles that allow penetration of solvent molecules. The fluorescent dye is incorporated in the coarse –Ti–O–Ti– network, which cannot prevent access of iodine. Physical isolation of dye molecules from the surrounding solvent and solute is achieved through surface coverage with capping molecules.

In conclusion, TiO_2-gel layer is shown to be a useful vehicle to encapsulate organic dye molecules. This approach will be, in principle, extended to a wide range of small molecules if they have affinities towards TiO_2-gel. Wrapped molecules are prevented from self-aggregation devoid of intermolecular interaction even in a condensed state. They would give unique molecular properties such as solubility, miscibility, and reactivity. Wrapping of other classes of molecules is in progress.

References and Notes

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6 A. Song, J. Zhang, M. Zhang, T. Shen, and J. Tang, *Colloid & Surface A*, **167**, 253 (2000).
7 Deprotonation of the hydroxy group of fluorescein causes a red shift in fluorescence spectra. Coordination of the hydroxy group to the titanium atom presumably induces a similar red shift.
8 Acidic octyltrichlorosilane and *tert*-butylphosphonic acid yield deep yellow and orange-colored solutions, respectively, due to neutralization of the hydroxy group of fluorescein.