Silica-coating of fluorescent polystyrene microspheres by a seeded polymerization technique and their photo-bleaching property

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Abstract

This paper describes silica-coating of polystyrene microspheres incorporated with fluorescence dyes (fluorescent microspheres) by means of a seeded polymerization technique based on Stöber method. The silica-coating of the fluorescent microspheres was performed in the presence of 0–10 g/l polyvinylpyrrolidone (PVP), 1.13–17 M water, 0–1.2 M aqueous ammonia and 0.00038–0.2 M tetraethoxyorthosilicate (TEOS). The addition of PVP was found to suppress the generation of free silica particles and improve the uniformity of shell thickness. The silica shell thickness increased from 13 to 138 nm with an increase in TEOS concentration at 10 g/l PVP, 0.4 M aqueous ammonia and 10.9 M water. The thickness also increased with the ammonia concentration and the water concentration. However, excess ammonia and water caused aggregation of free silica particles and the polystyrene microspheres. The silica-coated fluorescence microspheres showed more stable fluorescence to laser-irradiation than uncoated microspheres.

Keywords: Fluorescent microsphere; Core-shell; Silica-coating; Sol–gel; Seeded polymerization

1. Introduction

Microspheres incorporated with fluorescence dyes (fluorescent microspheres) have been used widely as cell-surface antigen detection, neutral retrograde tracers, phagocytosis tracers, sensitive diagnostic reagents and blood flow measurements [1–4]. It is desirable that fluorescences of dyes in the microspheres are strong and persistent for long periods. The photostability of the dyes is environmentally sensitive, and single state oxygen molecules play the main role of photo-bleaching of the fluorescence dye molecules in the excited state [5–7]. Core-shell type particles are good candidates for preventing decomposition because the shell materials can keep dyes from contact with oxygen molecules.

The core-shell types particles show various unique properties owing to their composite structures. They are applicable to a wide variety of materials such as magnetism [8–12], electronics [13–16] and optics [17–19]. Liz-Marzán and co-workers demonstrated silica-coating on CdS nanoparticles inhibited light-induced surface reactions, so that photostability of CdS was improved [20]. Our group also reported a protection effect of silica shell using silica-coated Co nanoparticles, in which the silica-coating prevented Co nanoparticles from oxidization and provided crystallization to cubic metal Co phase that showed magnetic properties [21]. In addition, we employed direct silica-coating on gold nanoparticles by a seeded polymerization techniques [22].

In this article, the silica-coating technique is extended to the fluorescence microspheres. The fluorescence microspheres have been coated with silica shell at different concentrations of polyvinylpyrrolidone (PVP), water, ammonia and tetraethoxysilane (TEOS). The photo-bleaching of the
fluorescence dyes within the microspheres has been monitored under irradiation of laser in the presence of air.

2. Experimental

2.1. Chemicals

FluoSpheres® beads (F-8803) commercially available from Molecular Probes Inc. were used as fluorescent microspheres. The fluorescent microspheres were composed of a host matrix of polystyrene and a dopant of fluorescent dyes. Fig. 1 shows their fluorescence spectrum and photograph taken by a transmission electron microscope (TEM). The fluorescent microspheres have a fluorescence peak at 512 nm and an average size of 193 nm. The chemicals of ethanol (99.5%), NH₄OH (25% aqueous solution) and tetraethylorthosilicate (TEOS, 95%) obtained from Wako Pure Chemicals Ltd., and polyvinylpyrrolidone (PVP, average molecular weight: 36000) from Nacalai Tesque Ltd. were used as received. Ultrapure deionized water (resistivity higher than 18 MΩ cm) was used in the preparations.

2.2. Preparation of materials

Silica-coating of the fluorescent microspheres was carried out with ammonia-catalyzed reaction of TEOS in ethanol-water solution in a hermetically sealed reactor equipped with a magnetic stirrer at room temperature. Ethanol solution of TEOS was added to aqueous PVP solution under vigorous stirring after addition of the suspension of the fluorescent microspheres. Hydrolysis reaction of TEOS was initiated by the addition of the aqueous ammonia solution to form silica shell on the microspheres, which was reacted for 24 h under stirring. Concentrations of PVP, water, ammonia and TEOS were in ranges of 0–10 g/l, 1.13–17 M, 0–1.2 M and 0.00038–0.2 M, respectively.

2.3. Characterization

The silica-coated fluorescent microspheres were observed with a transmission electron microscope (TEM) (Zeiss LEO 912 OMEGA) operated at 100 kV accelerating voltage. Samples for TEM were prepared by dropping the suspension of the fluorescent microspheres onto the top of a collodion-coated copper grid and drying. Fluorescence spectra were measured with a Hitachi F-4500 fluorophotometer. Fig. 2 shows a set-up for analysis of photo-bleaching. The silica-coated microspheres in a quartz cuvette were irradiated by an argon ion laser (Coherent, INOVA90) with an emission wavelength of 488 nm and a power of 300 mW. Fluorescence at 541 nm was selected using a band pass filter with a bandwidth of 10 nm and detected with an Si photodiode (Anritsu, MA9411A) connected with an Anritsu ML9001 optical power meter.

3. Results and discussion

3.1. Effect of PVP concentration

Fig. 3 shows TEM micrographs of silica-coated fluorescent microspheres prepared at various PVP concentrations. In whole images, many core-free silica particles with sizes of 50–80 nm were observed. According to Kawahashi and Shiho [23–25], PVP is required for preventing aggregation of particles. However, no aggregations of the fluorescent microspheres were observed even without the addition of PVP (Fig. 3(a)). The fluorescent microspheres used have carboxyl groups on their surfaces according to a commercial catalog of FluoSpheres® beads. These carboxyl groups probably prevent such aggregation. In Fig. 3(c) and (b), silica particles with sizes of 45–90 nm deposited on the surfaces of fluorescent microspheres, which indicated that silica did not have a strong affinity for the fluorescent microsphere surfaces during growth from silica nuclei to silica nanoparticle. Such deposition decreased with the increase in PVP concentration. In Fig. 3(c)–(e), the silica shell with a size of 40–45 nm was formed on the surfaces, though the deposited...
Fig. 3. TEM images of fluorescence microspheres coated with silica for 10.9 M water, 0.4 M ammonia and 0.02 M TEOS at PVP concentrations of (a) 0 g/l, (b) 0.01 g/l, (c) 0.1 g/l, (d) 1.0 g/l and (e) 10 g/l.

Silica particles were still observed in Fig. 3(c). The surfaces of silica shell were smoother with the increase in PVP concentration. In our research, it can be considered that PVP improved an affinity between the silica nuclei and the fluorescent microsphere surfaces.

3.2. Effect of water concentration

Fig. 4 shows TEM micrographs of silica-coated fluorescent microspheres prepared at various water concentrations. In Fig. 4(a)–(c), homogeneous silica shells were observed on the surfaces of the fluorescent microspheres and their thickness increased from 13 to 60 nm with the water concentration. As Bogush and Zukoski reported [26], an increase in water concentration in TEOS/NH₃/water/ethanol solution dissociates ammonium hydroxide and brings about an increase in electric conductivity that corresponds to ionic strength. Since the increase in ionic strength reduces electrostatic repulsion between particles, the growth of silica shells was probably promoted.
Many core-free silica particles were also observed and their average sizes increased from 41 to 92 nm with the water concentration. In Fig. 4(d) and (e), such silica particles adhered to the fluorescent microsphere surfaces and no more homogeneous shell was observed, which can not be explained by the change in ionic strength that was the factor causing the silica shell growth as shown in Fig. 4(a)–(c). Because the dielectric constant of water/ethanol mixture increases with water concentration, silanol groups on the silica particle surface probably tend to ionize with increasing water concentration. This might increase affinity of the silica particles for the dispersant. Therefore, silica nuclei generated during the early stages of the sol–gel reaction probably grew as stable core-free silica particles.

3.3. Effect of ammonia concentration

Fig. 5 shows TEM micrographs of silica-coated fluorescent microspheres prepared at different ammonia concentrations. At an ammonia concentration of 0 M (Fig. 5(a)), no silica shell and no silica particle was observed because of a shortage of catalyst. At ammonia concentrations of 0.2–0.8 M (Fig. 5(b)–(d)), the thickness of silica shell increased from 38 to 43 nm with the increase in ammonia concentration. Addition of ammonia increases the ionic strength of the solution and catalyzes the hydrolysis and condensation of the alkoxysilanes [27]. Thus, the high ammonia concentration should reduce the double layer repulsion between the fluorescent microspheres and the silica nuclei. As a result, the silica shells grew on the microsphere surfaces. At an ammonia concentration as high as 1.2 M, the fluorescent microspheres aggregated with the secondary generated silica particles (Fig. 5(e)). The high ammonia concentration extensively accelerated the sol–gel reaction of TEOS and then the core-free silica particles were generated from the silica nuclei and grew much before the silica nuclei was used for the silica shell formation.

3.4. Effect of TEOS concentration

Fig. 6 shows TEM micrographs of silica-coated fluorescent microspheres formed at various TEOS concentrations. Some silica particles were observed and their size tended to increase with the TEOS concentration. The ionic strength decreases as a sol–gel reaction of TEOS proceeds and then secondary silica particles are generated [28, 29]. Since the high TEOS concentration should increase a source of silica, the silica shell grew. The silica shell thickness was varied from 13 to 138 nm as initial TEOS concentration increased from 0.00038 to 0.2 M. This means the shell thickness can be controlled within a certain threshold. The thickness of silica was smaller than those estimated from initial TEOS concentrations, because of the generation of the core-free silica particles.

3.5. Photo-bleaching

Fig. 7 shows the time-dependence of the fluorescence intensity. The fluorescence intensity of the silica-coated fluorescent microspheres was lower than that of the uncoated ones up to 15 min. However, the laser-irradiation over 15 min reversed the order of the fluorescence intensities. For making clear a difference between the silica-coated microspheres and the uncoated ones, the fluorescence intensities were normalized by the value of fluorescence intensity measured before the laser-irradiation, as shown in
Fig. 6. TEM images of fluorescence microspheres coated with silica for 10 g/l PVP, 10.9 M water and 0.4 M ammonia at initial TEOS concentrations of (a) 0.00038 M, (b) 0.0015 M, (c) 0.009 M, (d) 0.02 M and (e) 0.2 M. The inset shows fluorescence intensity normalized by the value of fluorescence intensity measured before the laser-irradiation.

Fig. 7. Fluorescence intensities of silica-coated fluorescent microspheres and uncoated ones as a function of laser-irradiation time. The silica-coating was employed at 10 g/l PVP, 10.9 M water, 0.4 M ammonia and 0.02 M TEOS. The inset shows fluorescence intensity normalized by the value of fluorescence intensity measured before the laser-irradiation.

4. Conclusion

A synthetic method was developed for the stabilization of fluorescent microspheres. The method was based on the deposition of a silica shell on the fluorescent microsphere cores. The silica-coating was performed with a sol–gel reaction of TEOS in the presence of PVP and the fluorescent microspheres. Homogeneous silica shells were formed on the fluorescent microspheres in the presence of PVP. At high water and ammonia concentrations, no formation of homogeneous silica shells could be performed. With increasing TEOS concentration, the silica shell thickness increased.

Concentration effects can probably be explained by differences in ionic strength of the solution. It was observed that the silica-coated fluorescent microspheres provided high luminescence stability, compared with uncoated ones. This property is significant for biomedical application.

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