厚生労働科学研究費補助金（萌芽的先端医療技術推進研究事業）
（分担）研究報告書

# ナノサイズ・センシングカプセルの新規開発と医療応用
# 新規シリカコーティング蛍光ビーズおよびX線造影剤開発に関する研究

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研究要旨
センシングナノカプセルの創製による新たな診断治療薬の開発を目指して、AgI ナノ粒子のシリカカプセル化法の開発を行った。

## A 研究目的

本研究はアレルギー等の副作用が問題となっている検査薬品の副作用を完全に取り除く技術の確立を目的とする。すなわち、本研究は、センシングマテリアル、例えばヨード含有粒子のシリカカプセル化法の開発とその医療分野への応用を目的とする。

## B 研究方法

1．シリカカプセル化AgIナノ粒子の作成：Ag塩水溶液とKI水溶液の混合により合成したAgIナノ粒子分散液にシリコンアルコキシドとアミン系触媒を作用させてシリカカプセル化した。このとき種々の操作条件の最適化を行った。

2．キャラクタリゼーション

武田らと共同で、CTによるX線撮影や動物実験を行い、X線造影能や粒子サイズ等の条件を検討した。

## 倫理面への配慮

本研究は現在までのところ人体を対象とした実験を行っていないため倫理的問題は生じない。また、動物実験は全て麻酔下で行い，動物愛護に十分配慮してある。

## C 研究結果

種々の試薬濃度条件を変化させてシリカカプセル化を行ったところ、30-90nm程度のカプセル化粒子の合成に成功した。これに関しては、Colloids and Surfaces A: Physiochemical and Engineering Aspects誌と第57回コロイドおよび界面化学討論会および第13回高分子ミクロスフェア討論会で報告した。また、武田らと共同で動物モデルおよびX線撮影装置による有用性評価を行い、良好なX線造影能を有することがわかった。この内容については平成16年度第104回日本外科学会総会で報告した。また、特許出願を行った。

## D 考察

カプセル化粒子は良好なX線造影能を有することから、シリカカプセル化蛍光ビーズと同様に、各種センシングへの応用が期待できる。実用的にはさらに濃縮された粒子分散液を用いる必要があるため、

今後は濃縮技術の開発が必要となる。

E 結論

以上の実験によりシリカカプセル化ナノサイズセンシング粒子は新たな医療診断材料として利用可能であることがわかった。今後、さらに新しい応用や新しい診断技術の開発が期待される。

F 健康危惧情報

現在までのところ、本研究は人間を対象としたものではないため、健康に対する害は生じない。

G 研究発表

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Ⅱ．知的財産権の出願登録状況

発明の名称　センチネルリンパ節検出剤及びその検出方法

登録日　平成16年03月08日

登録番号　2004 - 269439

発明の名称　X線造影剤及び造影方法
出願日　平成17年02月18日
出願番号　特願2005-42634号

# Ⅲ. 研究成果の刊行に関する一覧表

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特許財産権の出願登録状況　(2002～2003年)

| 内容 | 発明者 | 権利者 | 知的財産権の種 | 番号 | 出願年月日 | 取得年月日 |
|---|---|---|---|---|---|---|
| 多重粒子及びその製造方法 | 川添良幸<br>粕谷厚生 | 川添良幸<br>粕谷厚生<br>佐竹正延<br>大内憲明<br>東レ・ダウコーニング株 | 特許 | 100064908 | 2002/12/26 | 2002/12/26 |
| センチネルリンパ節検出剤及びその検出方法 | 武田元博<br>大内憲明 | 武田元博<br>大内憲明<br>川添良幸<br>粕谷厚生<br>佐竹正延<br>東レ・ダウコーニング株 | 特許 | 100064908 | 2003/3/10 | 2003/3/10 |

# Ⅳ. 研究成果の刊行物・別刷

ELSEVIER

Colloids and Surfaces A: Physicochem. Eng. Aspects 251 (2004) 197–201

COLLOIDS AND SURFACES A

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# Silica-coating of AgI semiconductor nanoparticles

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Received 29 July 2004; accepted 7 October 2004

**Abstract**

A method for silica-coating of AgI nanoparticles is proposed, which applies Stöber method in the presence of a silane coupling agent, 3-mercaptopropyltrimethoxysilane (MPS), with the use of dimethylamine (DMA) catalyst for alkoxide hydrolysis. The AgI nanoparticles were prepared from $AgClO_4$ and KI. The silica-coating was performed with 0–$2.3 \times 10^{-5}$ M MPS, 11–20 M water, 0–0.1 M DMA and 0.0004–0.15 M tetraethyl orthosilicate (TEOS). The addition of MPS suppressed generation of free silica particles and improved uniformity of shell thickness. Silica shells were formed at water concentrations of 11–15 M, but excess water (20 M) caused aggregation of free silica particles, and resulted in formation of gel network. The silica shell thickness could be varied from 3 to 33.0 nm as the TEOS concentration was increased from 0.0004 to 0.04 M at $4.5 \times 10^{-6}$ M MPS under the condition of 11 M water and 0.01 M DMA.


*Keywords:* AgI; Nanoparticle; Core-shell; Silica coating; Sol–gel; Stöber method

## 1. Introduction

Nano-sized semiconductor particles such as CdS, CdSe and AgI attract special interest because they are in an intermediate state between atoms or molecules and bulk material, and can be expected to exhibit excellent properties different from bulk material [1–3].

A common technique for stabilizing nanoparticles is the use of surface active agents or macromolecular substances that are adsorbed to particle surface to form a physical barrier against other approaching particles. The coating of the particles with inert silica shells can also be used as a stabilizing technique [4–9]. Role of the silica shell is two-fold, since it not only provides a greatly enhanced colloidal stability in water, but also can be used to control distance between core particles within assemblies through shell thickness.

From this view point, extensive studies on silica-coated nanoparticles have been made [5,7]. The method of the silica-coating was composed of three steps: (1) modification of the nanoparticle surface to make it vitreophilic by using silane coupling agents with an amino or thiol group such as 3-aminopropyltrimethoxysilnae and 3-mercaptopropyltrimethoxysilane (MPS), (2) slow silica deposition in water from a sodium silicate solution, and (3) extensive growth of the silica shells through sol–gel reaction of silicon alkoxide in ethanol/ammonia mixtures [5]. The silica deposition in the sodium silicate solution requires long-time if silica shells are to be grown to certain thickness. In addition, the sodium silicate possibly introduces impurities to the particles. Therefore, a simplified and more rapid method with no sodium silicate is desirable.

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doi:10.1016/j.colsurfa.2004.10.007

A study on preparing silica-coated AgI nanoparticles was performed by Giersig et al. [10]. They showed that silica-coated Ag nanoparticles that were prepared according to the ref. [7] were mixed with $I_2$ solution, in which $I_2$ molecules diffused in silica shell layer, and eventually transforming the Ag nanoparticles inside to AgI nanoparticles. However, long-time processes with sodium silicate were required, and generation of AgI nanoparticles also took place on external silica surface. We have recently developed [11,12] a technique for direct silica-coating of metal nanoparticles in one single step without need of sodium silicate, which is based on Stöber method. In the present work, the direct silica-coating method was extended to silica-coating of AgI nanoparticles in the presence of MPS.

## 2. Experimental

### 2.1. Chemicals

Silver perchlorate ($AgClO_4$) (Kanto Chemical Co. Inc., 99%) and potassium iodide (KI) (Wako Pure Chemicals Ltd., 99.5%) were used as silver precursors. Tetraethyl orthosilicate (TEOS) (Wako Pure Chemicals Ltd., 95%), MPS (Aldrich, 97%) and ethanol (Wako Pure Chemicals Ltd., 99.5%) were used for silica-coating, and DMA (Wako Pure Chemicals Ltd., 50%) was used as catalysts for a sol–gel reaction of TEOS and MPS. All chemicals were used as received. Ultrapure deionized water (resistivity higher than 18 MΩ cm) was used in all the preparations.

### 2.2. Preparation of materials

#### 2.2.1. AgI nanoparticles

Colloids of AgI nanoparticles were prepared by mixing of $AgClO_4$ and KI. Freshly prepared 0.015 ml of 0.2 M $AgClO_4$ in $H_2O$ was added to 6 ml of 0.001 M KI under vigorous stirring at room temperature. Color of the mixture turned yellow immediately. Fig. 1 gives absorption spectrum of AgI nanoparticle colloid. A sharp peak around 421 nm and a shoulder peak around 330 nm were attributed to exciton peaks of AgI [13–16], which provided an evidence for generation of AgI particles. Typically, spherical AgI nanoparticles with an average size of 23.8 nm were observed in TEM (see inset of Fig. 1).

#### 2.2.2. Silica-coating

Stöber method with TEOS was applied to silica-coating of the AgI nanoparticles. Six millilitres of the AgI colloid was added to 0.1 ml of 0.00135 M MPS in $H_2O$. After 15 min, 24 ml of ethanol and successively 0.0266 ml of TEOS were added to the colloid. Then, the silica-coating was initiated by rapidly injecting an aqueous DMA solution into the AgI/TEOS colloid. The concentrations of TEOS and water were ranged from 0.0004 to 0.15 M and from 11 to 20 M, respectively.

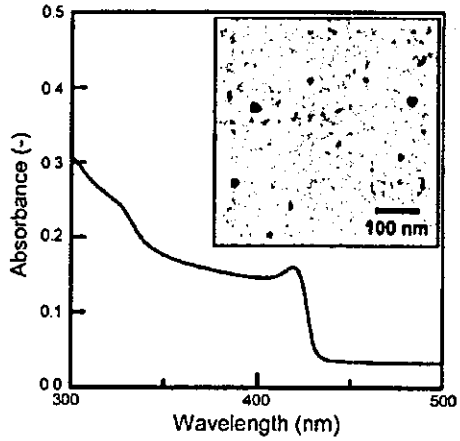


Fig. 1. TEM image and UV–vis absorption spectrum of AgI colloid in ethanol/water.

### 2.3. Characterization

The silica-coated AgI (AgI–$SiO_2$) nanoparticles were characterized by transmission electron microscopy (TEM) and ultraviolet (UV)–visible (vis) spectroscopy. TEM was performed with a Zeiss LEO 912 OMEGA microscope operating at 100 kV. Samples for TEM were prepared by dropping and evaporating the nanoparticle suspensions on a collodion-coated copper grid. Silica shell thickness was estimated as the difference between silver particle and composite particle sizes. UV–vis extinction spectra were measured with a Hitachi UV-3010 spectrophotometer.

## 3. Results and discussion

### 3.1. Effect of MPS concentration

Fig. 2 shows TEM micrographs of AgI–$SiO_2$ nanoparticles prepared at various MPS concentrations. In Fig. 2a and b, many core-free silica particles and shell-free AgI nanoparticles were observed, though a few core-shell particles were present. Probably, silica did not have a strong affinity for the AgI nanoparticle surfaces during growth from silica nuclei to silica nanoparticle. Particles in Fig. 2c and d had core-shell structures composed of the AgI core with a size of 13.5 nm and the silica shell with a thickness of 15.1 nm. MPS molecules possibly had strong affinity for AgI surface so that condensation reaction between MPS and TEOS was initiated on the surface.

### 3.2. Effect of water concentration

Fig. 3 shows TEM micrographs of AgI–$SiO_2$ nanoparticles prepared at water concentrations from 11 to 20 M. All the DMA concentrations were 0.01 M in a series of the experiments. The water concentration of 11 M was the lower limit in the present experimental, because the water

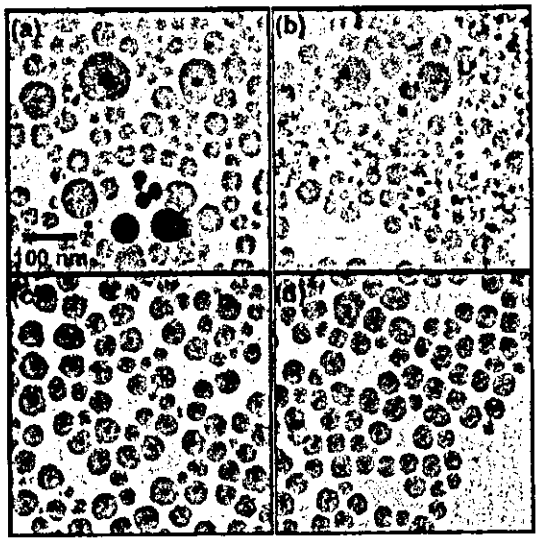


Fig. 2. TEM images of AgI–$SiO_2$ prepared at MPS concentrations of 0 (a), $4.5 \times 10^{-7}$ (b), $4.5 \times 10^{-6}$ (c) and $2.3 \times 10^{-5}$ M (d). Initial concentrations of water, DMA and TEOS were 11, 0.01 and 0.004 M, respectively.

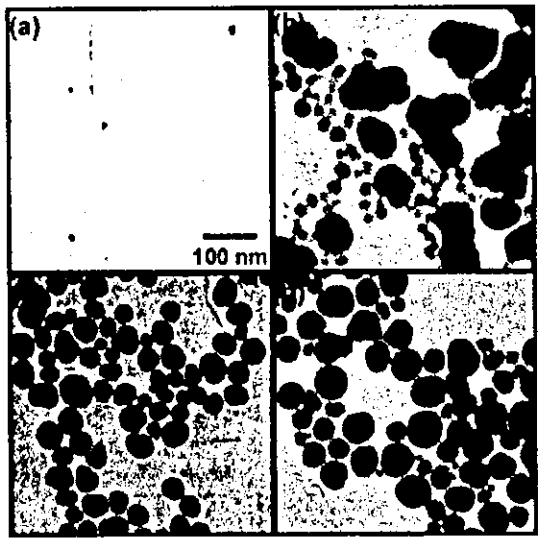


Fig. 4. TEM images of AgI–$SiO_2$ prepared at DMA concentrations of 0 (a), 0.005 (b), 0.01(c) and 0.1 M (d). Initial concentrations of MPS, water and TEOS were $4.5 \times 10^{-6}$, 11 and 0.004 M, respectively.

concentration in the commercial DMA solution is 50% and adjusting the DMA concentration to 0.01 M provided the water concentration of 11 M at lowest. AgI–$SiO_2$ core-shell particles were observed in Fig. 3a and b. The AgI cores that were observed in Fig. 3b were large, compared to Fig. 3a. As the water concentration rises, DMA is dissociated and consequently ionic strength increases in turn [17]. Since the increase in ionic strength reduces electrostatic repulsion between the AgI nanoparticles, the reduction of electrostatic repulsion probably promoted the aggregation and growth of AgI nanoparticles. It was also observed in Fig. 3b that many core-free silica particles with a size of 22.1 nm were generated. The increase in ionic strength due to the dissociation of DMA reduces electrostatic repulsion not only between AgI nanoparticles but also between silica nuclei generated with hydrolysis of TEOS. Consequently, the silica nuclei aggregated and grew as the silica nanoparticles with the increase in water concentration. In Fig. 3c, network structures were formed, though some AgI–$SiO_2$ core-shell particles were still observed. In Fig. 3d, no core-shell particles were formed. Further increase in ionic strength at high water concentration probably promoted aggregation of silica nanoparticles and succeeding formation of gel network structures.

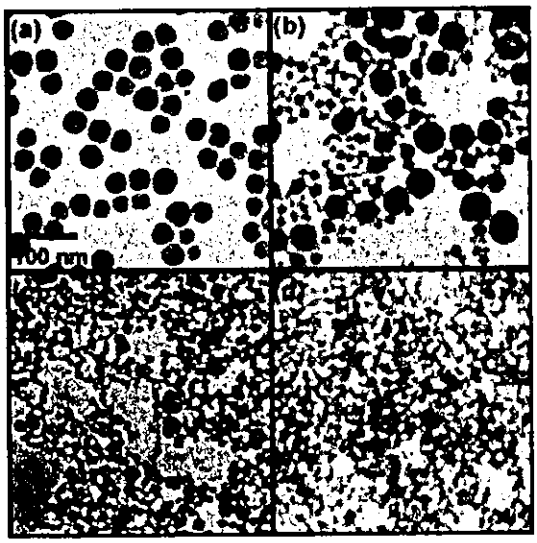


Fig. 3. TEM images of AgI–$SiO_2$ prepared at water concentrations of 11 (a), 12 (b), 15 (c) and 20 M (d). Initial concentrations of MPS, DMA and TEOS were $4.5 \times 10^{-6}$, 0.01 and 0.004 M, respectively.

### 3.3. Effect of DMA concentration

Fig. 4 shows TEM micrographs of AgI–$SiO_2$ nanoparticles prepared at different DMA concentrations. At a DMA concentration of 0 M (Fig. 4a), no silica shell and no silica particle was observed because of a shortage of the catalyst. At 0.005 M (Fig. 4b), particles that contained multiple cores and core-free silica particles were obtained. At 0.01 M, core-free silica particles were obtained a little and the AgI nanoparticles were coated with silica with a thickness of 15.1 nm (Fig. 4c). An increase in the concentration to 0.1 M increased silica shell thickness to 16.6 nm (Fig. 4d). Addition of DMA is considered to increase the ionic strength of the solution due to the dissociation and catalyzes the hydrolysis and condensation of the alkoxysilanes [18]. Thus, the high DMA concentration should reduce the double layer repulsion between the AgI nanoparticles and the silica nuclei. As a result, the silica nuclei were deposited on the AgI particle surfaces and then the silica shells grew.

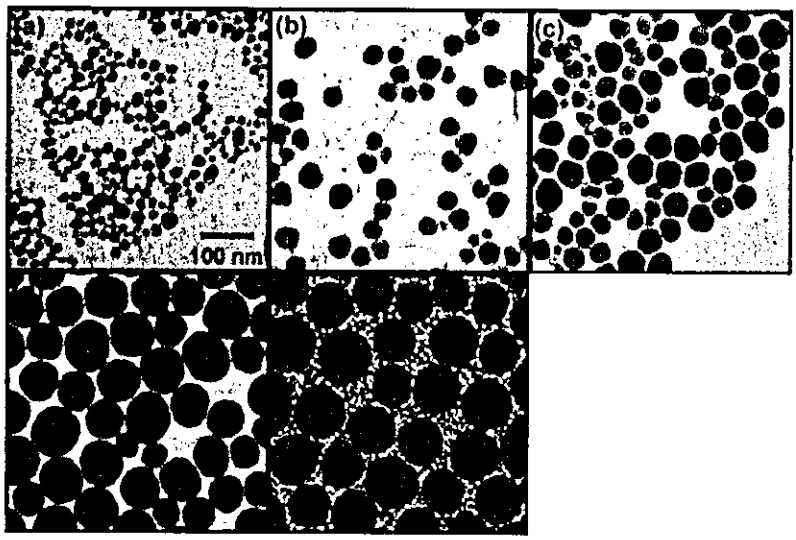


Fig. 5. TEM images of AgI–$SiO_2$ prepared at TEOS concentrations of 0.0004 (a), 0.002 (b), 0.004 (c), 0.04 (d) and 0.15 M (e). Initial concentrations of MPS, DMA and water were $4.5 \times 10^{-6}$, 0.01 and 11 M, respectively.

### 3.4. Effect of TEOS concentration

For the control of shell thickness, TEOS concentration was varied in the experiments of Fig. 5 a–e. At [TEOS] = 0.0004–0.04 M (Fig. 5a–d), most of the particles were quasi-perfect core-shells with just one AgI core and the shell thickness increased from 3.0 to 33.0 nm. Thus, the TEOS concentration was found to control the silica shell thickness within a certain threshold. However, further addition of TEOS ([TEOS] = 0.15 M) did not increase the shell thickness, and generated a large amount of core free silica particles, as shown in Fig. 5e.

### 3.5. UV–vis spectroscopy

Fig. 6 gives absorption spectra of AgI–$SiO_2$ colloid. In the spectra in Fig. 6a through c, a sharp peak around 421 nm and a shoulder peak around 330 nm were observed, which were the typical absorption spectra of the AgI nanoparticles. As the shell thickness increased, these peaks seemed to be screened by the strong scattering from the silica shell.

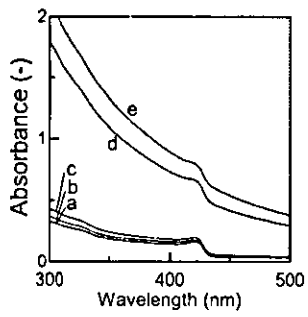


Fig. 6. UV–vis absorption spectra of AgI–$SiO_2$. Refer to Fig. 5 for symbols.

Although the shoulder peak was not clearly seen in Fig. 6d and e, the absorption peak of the AgI nanoparticles appeared around 421 nm in these figures. Thus, the existence of AgI nanoparticles was confirmed from the UV–vis spectroscopy.

## 4. Conclusion

A synthetic method for AgI–$SiO_2$ core-shell particles was developed. The method was based on the deposition of a silica shell on the AgI cores. The silica-coating was performed with a sol–gel reaction of TEOS in the presence of the AgI nanoparticles. At high water 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 the difference in ionic strength of the solution.

## Acknowledgements

This research was partially supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for the COE project, Giant Molecules and Complex Systems), and by the Ministry of Health, Labor and Welfare of Japan.

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COLLOIDS AND SURFACES A

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

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Received 30 October 2003; accepted 20 April 2004

Available online 15 June 2004

**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 singlet 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

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doi:10.1016/j.colsurfa.2004.04.052

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%), $NH_4OH$ (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

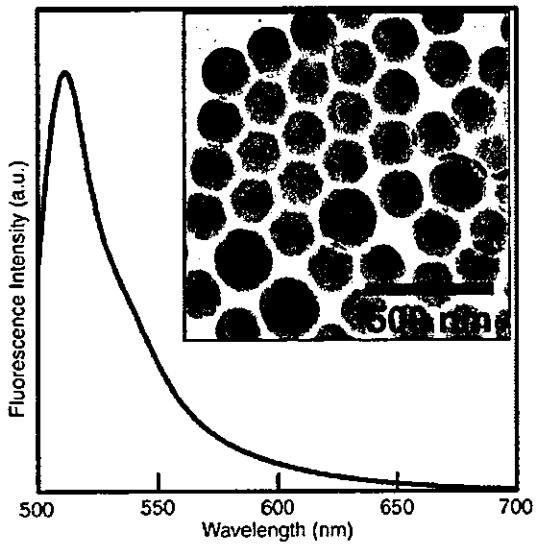


Fig. 1. Fluorescence spectrum of FluoSpheres® (F-8811) and their TEM image shown in the inset. The excitation wavelength was 488 nm.

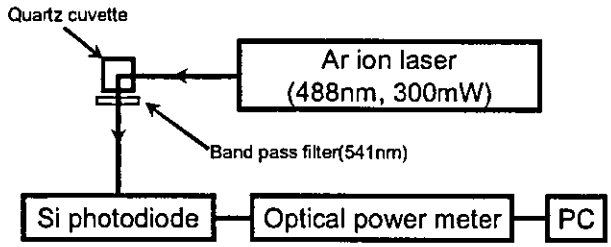


Fig. 2. Experimental set-up for measurements of photo-bleaching.

was reacted for 24 h under stirring. Concentrations of PVP, water, ammonia and TEOS and 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(a) 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