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# Fluorescence Quenching of Uranine on Confeito-Like Au Nanoparticles

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Effect of structure and size of Au nanoparticles (AuNPs) on fluorescence behavior of uranine was examined. Confeito-like AuNPs with different sizes (30 nm, 60 nm and 100 nm, respectively) had plasmon absorption bands at 555, 600 and 660 nm, while the band of spherical AuNP (20 nm in size) was at 525 nm. Fluorescence of uranine was significantly quenched by the small and medium confeito-like AuNPs, and the quenching effect by the large particle was less. In comparison, the spherical AuNP quenched more remarkable than the confeito-like AuNPs. A mechanism of resonance energy transfer from uranine to AuNPs via the surface plasmon was suggested, and the strong quenching effect of the small AuNPs could be explained by the energy transfer from adsorbed uranine molecules to AuNPs. These behaviors indicate that the large confeito-like AuNPs can be a preferable nano-probe and useful for plasmonic devices, which can tune or maintain the fluorescence properties of other markers.

Keywords: Gold Nanoparticle, Confeito-Like Nanoparticle, Uranine, Surface Plasmon, Fluorescence, Quenching, Energy-Transfer.

# **1. INTRODUCTION**

Nanoparticles of noble metals have been traditionally used as pigments.<sup>1</sup> Today, their optical phenomena are explained by a collective motion of free electrons on the nanoparticles (i.e., localized surface plasmon, LSP).<sup>1</sup> The LSP depends on the size and shape of nanoparticles,<sup>2-4</sup> and the resonance of LSP with incident light have been applied as optical devices.<sup>5</sup> The LSP also can influence optical properties of materials in the vicinity of nanoparticle, and a typical effect of the LSP is the surfaceenhancing on Raman scattering<sup>5,6</sup> and infrared-absorption spectra.<sup>7,8</sup> However, the LSP has a bifacial character for fluorescence: It intensify the fluorescence in some cases<sup>9–11</sup> but work as fluorescence quenchers in the other cases.<sup>12</sup> These behaviors are based on the energy-transfer between nanoparticles and fluorophors,<sup>13</sup> and it is important to study the mechanism of such energy-transfer for designing the plasmonic devices. We have developed a new class of Au nanoparticles (AuNPs) named "confeito-like AuNPs" and demonstrated that they have strong surfaceenhancing effects for Raman scattering.<sup>14, 15</sup> In the present study, the fluorescence behavior of a typical fluorophor, uranine, on confeito-like AuNPs is compared with conventional spherical AuNPs, and the behavior is discussed on the basis of LSP of AuNPs. This investigation will be useful to understand the properties of LSP of AuNPs with designed structures.

# 2. EXPERIMENTAL DETAILS

#### 2.1. Reagents

Sodium tetrachloroaurate (III) dihydrate (NaAuCl<sub>4</sub>·2H<sub>2</sub>O, 99%) were purchased from Sigma Aldrich Co. (USA). Citric acid, sodium hydroxide (NaOH), and an aqueous 35 wt% solution of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were purchased from Acros Organics Ltd. (USA). Uranine (fluorescein disodium salt) was purchased from Tokyo Chemical Industry Co. (Japan). All chemicals were of reagent grade and used without further purification. A solution of sodium citrate (1 wt%) was prepared by dissolving citric acid (1 mmol) and NaOH (3 mmol) in water (25.8 g).

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#### Ujihara et al.

Ultrapure (Millipore Milli-Q) water with a resistivity of 18 M $\Omega$  · cm was used throughout all the syntheses and measurements in the study.

## 2.2. Preparation of AuNPs and Their Dispersion

Confeito-like AuNP was synthesized at ambient temperature. Typically, 15.6 mg of citric acid was dissolved in 28 cm<sup>3</sup> of water. Then 4 cm<sup>3</sup> of an aqueous solution of NaAuCl<sub>4</sub> (1 mM) was added into the solution of citric acid. Subsequently, 80 mm<sup>3</sup> of  $H_2O_2$  (35 wt%) was added to the mixed solution. Finally, 8 cm<sup>3</sup> of an aqueous solution of NaOH (100 mM) was added with vigorous stirring. After 1 min, the stirring was slowed down, and the reaction solution was allowed to stand overnight for the reaction to go to completion. The confeito-like AuNPs with 60 and 30 nm in size were obtained by increasing the amount of citric acid to 31.2 mg and 62.4 mg, respectively. For the synthesis of spherical AuNP, 4 cm<sup>3</sup> of an aqueous solution of NaAuCl<sub>4</sub> (1 mM) was diluted by 35 cm<sup>3</sup> of water and boiled with stirring. Then a solution of sodium citrate (1 cm<sup>3</sup>, 1 wt%) was added into the solution and kept boiling for 5 min till the colour of the solution changes into red. The solution was further kept stirring for 1 min and then cooled down to the room temperature. While the pH of the prepared large confeito-like nanoparticles is originally higher than 12, the suspension of small confeito-like AuNPs (30 nm in size) was achieved pH 12 by adding the double concentration of sodium hydroxide. For spherical gold AuNPs, the buffer solution at pH of about 40 was used to keep the solution in basic condition.

## 2.3. Instruments

Transmission electron microscopic (TEM) images were taken on a Hitachi H-7000 instrument at an accelerating voltage of 100 kV. The suspension of AuNPs was dropped on a carbon-coated copper grid, air-dried, and then used for the observation. Ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectra were recorded with a JASCO V-670 spectrophotometer with a quartz cell of 1 cm light path. The fluorescence spectra were obtained with a Hitachi-7000 fluorescence spectrometer in a quart cell containing an aqueous uranine solution (50  $\mu$ M, 2 cm<sup>3</sup>) with or without AuNPs (0.1 mM). The solution (or suspension) was degassed by sonication, and then the excitation and emission spectra were measured at incident wavelengths of ( $\lambda_{em} = 515$  nm and  $\lambda_{ex} = 460$  nm, respectively).

# 3. RESULTS AND DISCUSSION

Confeito-like and spherical AuNPs were synthesized by reduction of NaAuCl<sub>4</sub> as described in experimental section. The sizes of particles used in this study were around 100, 60 and 30 nm for confeito-like AuNPs and around 20 nm for spherical AuNP, as seen in TEM images (Fig. 1). Against smooth surfaces of spherical AuNP, confeito-like AuNPs had many bosses.<sup>14, 15</sup> UV-Vis-NIR

J. Nanosci. Nanotechnol. 14, 4906–4910, 2014



**Figure 1.** Morphological structures of AuNPs observed with a TEM. (A) confeito-like AuNPs with 100 nm in size, (B) 60 nm, (C) 30 nm and (D) spherical AuNPs.

absorption spectra of these AuNPs dispersed in water are shown in Figure 2. The plasmon bands were observed at 525 nm for a spherical AuNP and at 555, 600 and 660 nm for confeito-like AuNPs of 30, 60 and 100 nm in size, respectively. Although the plasmon absorption bands of spherical AuNPs shift as a function of the particle size, the band position remains within ~ 560 nm for the diameter of ~ 100 nm.<sup>16</sup> The plasmon absorption bands of confeitolike AuNPs were obviously at longer wavelength than that of spherical ones, and their broad tails in the NIR region can come from their surface structures.<sup>2, 14, 15</sup> Theoretically, the surface plasmon of AuNPs are localized on the tips on nanoparticles, and this localization red-shifts the plasmon absorption bands.<sup>4, 17</sup>

Uranine (disodium fluorescein) is the typical fluorophore which can well perform in alkaline medium. Then, the fluorescence regulation by different sizes of confeitolike AuNPs in comparison with a spherical AuNP was compared for fluorescence of uranine. The fluorescence spectra of uranine were compared before and after adding AuNPs in an uranine solution (Fig. 3). In spectra of an uranine solution without AuNPs, an excitation band was at 490 nm as well as the absorption band (Fig. 2), and an emission band was at 512 nm. While band shifts were not observed in the all cases, both excitation and emission bands commonly suffered the hypochromic effect in the presence of AuNPs, and the degree of the effect varied with the AuNPs. When the degree of fluorescence quenching was calculated from the ratio of emission intensity at 512 nm, the spherical AuNP had the strongest effect to quench to 50% of the original solution. The confeito-like AuNPs worked as weaker quenchers than the spherical Fluorescence Quenching of Uranine on Confeito-Like Au Nanoparticles



Figure 2. UV-Vis-NIR absorption spectra of AuNPs (0.1 mM) and uranine (50  $\mu$ M) in aqueous media.

AuNP, and the emission intensity remained  $\sim 62\%$  for the small and medium particles. The large confeito-like AuNP had the least effect, and it resulted in the emission intensity of 73% of the original solution.

In the fluorescence quenching caused by AuNPs, several mechanisms have been reported.<sup>12,13</sup> Then, the quenching behaviours of confeito-like AuNPs were investigated from a viewpoint of the wavelength dependency. The ratios of the fluorescence intensity of a solution with AuNPs to a solution without AuNPs were plotted for excitation ( $\lambda_{em} = 515$  nm) and emission ( $\lambda_{ex} = 460$  nm) spectra



Figure 3. Excitation and emission spectra of uranine solutions with/without AuNPs. The spectra were measured at an emission wavelength of 515 nm and an excitation wavelength of 460 nm, respectively.



Figure 4. Ratio of fluorescence intensity in excitation and emission spectra of uranine solutions with confeito-like AuNPs (a) 100 nm, (b) 60 nm, (c) 30 nm, and (d) spherical AuNP. The small arrows on the spectra marked the Raman scattering.

(Fig. 4). Although the excitation bands were at 321, 460 and 490 nm, the excitation intensity ratio was mostly constant for the each sample in the wavelength region from 300 nm to 500 nm, except the wavelengths of surfaceenhanced Raman scattering at 360 and 396 nm<sup>14</sup> (marked by arrows in Fig. 4). It suggests that the AuNPs don't have a specific interaction with these excitation bands, because the plasmon absorption bands were not overlapped with these bands, except the weak overlap at 490 nm.<sup>12, 13, 18</sup>

In contrast, the emission intensity ratio showed different behaviours for each AuNP and had a wavelength dependency. The spherical AuNP and small confeito-like AuNP strongly depressed the fluorescence intensity in wide range (490~650 nm) and had minimums at 525 nm and 545 nm, respectively. On the other hand, the medium confeito-like AuNP indicated a strong quenching around 560~570 nm, and its quenching effect was weaker than the small confeito-like AuNPs at the both edges of emission spectrum. The large confeito-like AuNP had a mild depression of fluorescence with a minimum around 570~580 nm. The fluorescence quenching in wide range could be explained by an effective energy transfer from uranine molecules adsorbed on AuNPs.<sup>12, 19</sup> This effect was suggested by the quenching degrees at the both edges of emission spectrum, where the wavelength dependencies were relatively small (Fig. 4). Because the small particles can have larger specific surface area, the uranine molecules could adsorb on the surface of small AuNPs than the large one.<sup>20</sup>

For the quenching effects with the wavelength dependency, the masking effect of AuNPs should be considered. The emitted light could be absorbed by the AuNPs to result in an apparent quenching. The spherical AuNP had

J. Nanosci. Nanotechnol. 14, 4906-4910, 2014

### Ujihara et al.

the minimum point in the intensity ratio around 525 nm, which was very close to its plasmon absorption band (525 nm). Then the occurrence of minimum intensity ratio can be explained by the absorption of emitted light by the AuNPs. However, the other AuNPs provided the minimums of fluorescence at different wavelengths from their plasmon absorption bands, as discussed later. Although this process could also take place in the confeito-like AuNPs, it would not strongly affect the occurrence of the minimum point in their plot, as seen typically in the case of large confeito-like AuNP (Figs. 2 and 4).

As the other interaction between fluorescent molecules and AuNPs, a resonant energy transfer (RET) mediated by LSP has been reported.<sup>18</sup> For the spherical AuNP, the proximity of its plasmon absorption band to the minimum point of intensity ratio can be due to this RET. Because the efficiency of RET depends on the spectral-overlap integration between the emission band of donor (uranine) and the absorption band of accepter (AuNP), the sharp quenching by the spherical AuNP is appropriate. However, the ratio plot of the fluorescence emission intensity in the dispersion of small confeito-like AuNPs appeared a minimum around 545 nm, as the middle point between an emission band (512 nm) of uranine and a plasmon absorption band (555 nm) of AuNPs. This suggests that a new energy state was formed between the uranine and AuNP, and the RET occurred via this coupling.<sup>18, 21, 22</sup> This energy state would be affected by the band position of plasmon absorption, and then the AuNPs with longer plasmon absorption bands could resulted in the RET at the longer wavelengths. Thus, the depression of fluorescence intensity by the confeitolike AuNPs with medium and large sizes were respectively seen around 560~570 nm and 570~580 nm, although the plots for the confeito-like AuNP with were disturbed by the surface-enhanced Raman scattering at 577 nm (marked by arrow).<sup>14</sup> The quenching effect of medium confeito-like AuNP around 560~570 nm was much stronger than that of the small confeito-like AuNP at 545 nm, although the wavelength difference of its plasmon absorption band from the emission band of uranine (512 nm) was larger than that of small confeito-like AuNP. This suggests that the medium confeito-like AuNP quenched the emission from uranine mainly at longer wavelength, not the main emission band at 512 nm. The emission spectrum of uranine had a shoulder around 540~580 nm, and the sharp and strong fluorescence quenching of the medium confeitolike AuNP could be attributed to the strong coupling with this emission band due to the spectral-overlap. The large confeito-like AuNP could work mainly at this emission band, but its plasmon absorption band (660 nm) was too far to overlap well. That is, the efficiency of RET was lower than the others.<sup>12, 13, 18</sup> Incidentally, the plasmon absorption band of small confeito-like AuNP at 555 nm was very close to this emission band. However, the energytransfer from lower-energy side (at longer wavelength) to

higher-energy side (at shorter wavelength) is inefficient,<sup>18</sup> and the RET was mainly from the interaction with the emission band at 512 nm.

Thus, the fluorescence quenching should be induced by the adsorption of uranine molecules on AuNPs and the RET from uranine to AuNPs. When the AuNP is small, the larger specific surface area would cause the quenching at higher efficiency. The red-shifted plasmon absorption bands of confeito-like AuNPs could change the spectral overlap for RET. As the shift of plasmon absorption band associated with the size of confeito-like AuNPs, the larger confeito-like AuNP resulted in less quenching and could change the shape of emission spectrum.

## 4. CONCLUSIONS

It was demonstrated that the fluorescence behaviour of uranine on the confeito-like AuNPs was different from that on the spherical AuNP. For the series of confeito-like AuNPs (30, 60, 100 nm in size), their plasmon absorption band and their quenching effect indicated a size dependency. As the sizes of particles increased, the plasmon absorption band shifted toward longer wavelength (555 nm, 600 nm, and 660 nm), and the quenching effects for the emission band of uranine at 512 nm decreased. The strong quenching by small AuNPs was attributed to the energy transfer from uranine directly adsorbed on AuNPs: The larger specific surface areas, the more adsorption. On the other hand, the quenching behaviour had a wavelength dependency, and it was revealed that the RET mechanism from uranine to AuNPs functioned on the quenching process. Because of the red-shifted plasmon absorption bands of AuNPs, the medium and large particles mainly quenched the emission of uranine at the longer wavelength than the main emission band of uranine.

Thus, the fluorescence behaviour of uranine could be controlled by the AuNPs with unique shapes and sizes. Especially, characteristics of medium and large confeitolike AuNPs, namely, the red-shifted LSP and the small specific surface area, can be useful to design the plasmonic devices which can tune or maintain the luminescent properties of the coexisting fluorescent markers in the system, in addition to the strong plasmon absorption in the NIR region and the surface enhancing effects on spectroscopies.

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J. Nanosci. Nanotechnol. 14, 4906-4910, 2014

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