Plasmonic enhancement using core-shell nanoparticles

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ABSTRACT

In this paper, we report on a strategy, which produces enhancement of fluorescence using the so-called plasmonic effect whereby the presence of adjacent metallic nanoparticles can dramatically alter the fluorescence emission and absorption properties of a fluorophore. The effect, which is a result of the surface plasmon resonance of the metal surface, can lead to increases in quantum efficiency, radiative decay rates and photostability of the fluorophore, and depends very sensitively on parameters such as geometry of the nanoparticles, nanoparticle-fluorophore separation and fluorophore type. The work is aimed at improving the efficiency of optical biochips. Key benefits from this enhancement include lower limits of detection, reduced reagent requirements and better resolution. This study is part of a comprehensive investigation of plasmonic enhancement using a range of metal nanoparticle (NP) fabrication techniques and a range of measurement configurations. The focus here is on the fabrication of chemically prepared silver-gold alloy spherical NP with a variable thickness silica shell on the surface of which is immobilised a layer of fluorescent dye molecules. The variable thickness shell serves to control the dye-NP separation, which plays a key role in the enhancement mechanism. Transmission electron microscopy (TEM) was used to characterise the NP. The dye used here was the ruthenium polypyridyl complex [Ru(II)-tris(4,7-diphenyl-1,10-phenanthroline)], abbreviated to [Ru(dpp)₃]²⁺. This paper reports the tuning of the NP plasmon resonance via NP size and alloy composition. The wavelength of the plasmon peak as a function of NP size and composition correlated very well with theoretical predictions based on the Mie scattering theory. Preliminary fluorescence enhancement measurements on this system yielded an enhancement factor of approximately 5.

Keywords: plasmonics, enhancement, nanoparticles, fluorescence, nanoparticles

1. INTRODUCTION

Fluorescence is an important tool in the area of biomedical sensors. In particular, there is increasing interest in fluorescence-based array sensors or biochips, which consist of patterned arrays of fluorescently-labelled biorecognition elements on a planar surface, providing multianalyte detection capability. While fluorescence detection offers high sensitivity, there is generally a low level of fluorescence from the biochip due to a monolayer of fluorescent labels, hence the importance of enhancing the detected fluorescence. The work reported in this paper exploits the co-called plasmonic enhancement effect, whereby large fluorescence enhancements can be obtained from fluorophores, which are in the vicinity of metal nanoparticles. This effect is due to the surface plasmon resonance associated with the metal particle, which modifies the photonic mode density around the fluorophore and thereby increases the emitted fluorescence signal. This work aims to investigate the fundamental enhancement mechanism and its dependence on the size and shape of the metal NP as well as the distance between fluorophore and NP. This is part of a comprehensive study by the authors where a range of NP fabrication techniques are being investigated and the enhancement is being characterised for a number of different fluorophores in different experimental configurations. For example, a recent paper [1] focused on NPs (fabricated by a nanosphere lithography technique) immobilised on a planar chip with the commonly used fluorescent label Cy5. An order of magnitude enhancement in the emitted fluorescence was achieved in this investigation. This paper focuses on a parallel investigation wherein spherical gold-silver alloy particles are fabricated from solution. These particles are coated with a silica shell of variable thickness on which is immobilised a monolayer of the fluorescent dye $Ru(dpp)_3^{2+}$. The aim of the experiment is to monitor the emission of the dye-coated particles suspended in an aqueous solution, as a function of NP size, metal composition and NP-dye separation (via the silica shell thickness). Here we report preliminary results on enhancement of the excitation.. The paper describes in detail the NP core-shell fabrication process as well as the results of a theoretical model. The ability to tailor the NP size and composition to the required plasmon resonance wavelength is clearly demonstrated. Preliminary fluorescence enhancement measurements were also carried out.

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2. THEORY

The theory describing the interaction of light, fluorescent dyes and metallic nanoparticles can be divided into two parts: the interaction of the incident light with the metallic NPs and the interaction of the NPs with fluorescent dyes. Mie scattering theory predicts the exact displacement of EM-waves around spherical particles.[2],[3],[4] In the case of metallic particles smaller than the wavelength of light only the lowest surface modes can be excited. This effect is called localised surface plasmon resonance (LSPR). The LSPR can be observed by measuring the absorption spectra of the NPs. A key aspect of the LSPR effect is the associated increase in the intensity of the electric field in the proximity of NPs. Depending on the shape of the NP, the increase is approximately two orders of magnitude on the surface of the NP. The E-field decreases with increasing distance from the NP and within a few diameters of the NP the effect is negligible. In a later section it will be shown that the wavelength (λ_{res}) at which SPR occurs depends not only on the NP material, but also on the embedded medium and size of the nanoparticle [5].

A fluorescent dye (FD) in the proximity of the NP senses the altered EM-field and its fluorescence properties are changed. There are two enhancement effects: an increase in the excitation of the FD and an increase in the quantum efficiency (QE) of the FD. The first effect occurs because the excitation is directly proportional to the square of the intensity of the E-field (it is a function of the wavelength and relative position of the NP). The maximum enhancement of this type is achieved if λ_{res} equals the maximum excitation wavelength of the dye [6].

Regarding the second enhancement effect, the maximum enhancement of quantum efficiency occurs if the λ_{res} is equal to the peak emission wavelength of the FD. In parallel with the change of QE there are other changes in the emission properties of the FD; for example, a decrease in the fluorescence lifetime and an increase in photostability [7]. Furthermore, there is an additional effect, namely a metal-fluorophore quenching which occurs for very small NP-FD separations, typically within 5nm [8].

In the system studied here, a ruthenium dye Ru(dpp)₃ is used to investigate the enhancement effect. The excitation peak of the dye dissolved in water is broad and ranges from 430nm to 460nm and the emission maximum is 612nm. The Rudye was chosen for its large Stokes shift, which facilitates the separation of the two enhancement effects discussed above. The initial part of this research was focused on enhancement of excitation rate of the dye. This implies that the plasmon resonance λ_{res} of nanoparticles had to match with the excitation wavelength of the Ru- dye, which is around 440nm. Spherical nanoparticles made from pure silver or gold have plasmon resonances at 390nm and 520nm respectively and therefore are not suitable for the enhancement experiment. A strategy for tailoring the nanoparticles to the correct plasmon resonance wavelength was the preparation of gold silver alloy nanoparticles. Depending on the silver/gold ratio the resonance wavelength should change from 390nm to 520nm for a constant diameter of the nanoparticles.

The theoretical shift of the plasmon resonance of gold/silver alloy nanoparticles was calculated. Mie theory was used to compute the extinction spectra of nanoparticles, where the dielectric constant of the gold-silver alloy had to be taken into account. For example, theoretical spectra calculated with a dielectric constant of the alloy expressed as a simple summation of the dielectric constant of silver and gold $\varepsilon_{alloy} = x_{Au} \varepsilon_{Au} + (1 - x_{Au}) \varepsilon_{Ag}$, where x_{Au} the molar fraction of gold, does not correspond to experimental data as shown in ref. [9]. This is because the absorption band of d-electrons for the alloy is incorrectly calculated in the model. Better agreement was obtained by using an expression for the alloy dielectric constant, which was similar to that used in ref. [10], except that in this case the contribution of s-electrons was described using the classical Drude treatment. The comparison of theoretical curves with experiment is presented in section 4.

A theoretical estimate of the enhancement factor was also calculated. Mie theory predicts the exact distribution of the electrical field. Therefore the average enhancement of intensity of electrical field around a nanoparticle at distance \mathbf{d} can be calculated as

Average enhancement factor =
$$\frac{\oint \vec{E}(r, \varphi, \phi)\vec{E}^*(r, \varphi, \phi)dS}{E_0^2 \oint dS}$$
[1]

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The general expression for the scattered electric field by a particle is an infinite sum of vector spherical harmonics (N_{e1n} , M_{o1n})(subscripts e and o denote even and odd respectively; n denotes order) multiplied by scattering coefficients (a_n , b_n).

$$\vec{E}_{s}(r,\phi,\phi) = \sum_{n=1}^{\infty} i^{n} E_{0} \frac{2n+1}{n(n+1)} (ia_{n} \vec{N}_{e1n} - b_{n} \vec{M}_{o1n})$$
^[2]

In the case of nanoparticles smaller than wavelength of the light, only the lowest modes are excited. In the calculation presented later, the electric field was calculated only for n=1, corresponding to dipole excitation. The sum of the scattered field E_s and the incident field E_i give the final expression for electric field around the nanoparticle.

$$\vec{E} = \vec{E}_i + \vec{E}_s \tag{3}$$

where

$$\vec{E}_i = E_0 e^{ikr\cos\theta} \vec{e}_x$$
[4]

The input parameter in the calculation is the dielectric constant of the metal sphere. In the following calculation the dielectric constant of the gold/silver alloy used in computing extinction spectra was used. The resultant enhancement factor is calculated for illumination of the nanoparticle with linearly polarised light, but due to the symmetry of the system and averaging in expression (1), the result can be extended to unpolarised light. The calculation of enhancement for prepared nanoparticles was carried out and the results are discussed in section 4.

In this model some approximations were used. First, the influence of the silica shell and Ru-dye were not taken into account. But the dielectric constant of water and silica are close so there is a minimal influence. From computation of the extinction spectra of the nanoparticle alone and the nanoparticle with a silica shell in water the shift of the peak was less than 5nm.

3. MATERIALS AND METHODS

Materials

(3-amino propyl)trimethoxysilane (APS), sodium silicate solution (Na₂O(SiO₂)₃₋₅, 27 wt % SiO₂), hydrogen tetrachloroaurate (HAuCl₄), silver nitrate (AgNO₃), sodium citrate, cetyltrimethylammonium bromide (CTAB), sodium borohydride, and L ascorbic acid were all purchased from Sigma Aldrich. The Ion exchange resin, Amberjet 1200H, cationic form, was purchased from Fisher Scientific. Gold colloidal solutions (5, 10 and 15 nm diameters at 5*10¹³, 5.7 *10¹², and 1.4 *10¹² particles per ml respectively) were purchased from British BioCell International. Deionised water > 18 MΩ was used from a Millipore Academic system. All glassware was cleaned with an aqua regia solution prior to synthesis. Ruthenium (II) tris (4,7 diphenyl-1,10 phenanthroline dichloride was prepared as in ref [11].

Synthesis of gold-silver nanoparticles

Gold-silver alloys with varying plasmon absorption wavelength were prepared using Turkevich's method [12]. To prepare a pure gold sol, 2 ml of 1 wt % sodium citrate solution was added dropwise to a boiling solution of 5 mg of HAuCl₄ dissolved in 95 ml of deionised water with rapid stirring. The solution was stirred for a further 30 minutes in which time the solution changed from a light blue to a deep red colour. Gold silver alloys were prepared by substituting gold with silver nitrate according to the following equation, $\lambda_{res} = 132x_{Au} + 388nm$, where λ_{res} is the plasmon absorption wavelength and x_{Au} is the molar fraction of gold [9].

Synthesis of the silica shell around the nanoparticles

Nanoparticles were coated with a silica shell using Liz-Marzan's method [13] An aqueous solution of 0.5 ml of 1 mM was added to 100 ml of each nanoparticle sol under vigorous stirring and allowed to stand for a further thirty minutes to ensure attachment of the APTS ligand to the surface of the nanoparticles. Separately a solution of activated silica was prepared by lowering the pH of a 0.54 wt % sodium silicate solution to 10.5 with progressive additions of the cationic exchange resin. Finally 4 ml of the activated silica solution was added to the nanoparticle solution with vigorous stirring and allowed to stand for a further 24 hours. Shell thicknesses of 5 nm were achieved using this method. Incremental

changes in the thickness of the silica shell could not be achieved using the standard Stöber method because the ammonium hydroxide etched out silver from the nanoparticle alloys via an oxidation reaction [14], [15].

Attachment of Ru-dye on the silica shell

At pH 7, which was used in all the experiments, the silica coating on the NP is negatively charged and the $Ru(dpp)_3^{2+}$ is electrostatically attracted to the surface and forms an ionic bond with the surface. We proved this by performing a simple centrifugation experiment. Firstly, the fluorescence from a solution containing ruthenium dye attached to the nanoparticle surface was measured and then the nanoparticles were separated by centrifugation at 3500 rpm for two hours. Following this fluorescence from the supernatant solution was measured and showed no signal from the Ru-dye indicating that the dye was attached to the NP surface. If centrifugation was performed without nanoparticles the Ruthenium dye remained in solution.

Characterisation techniques

The UV-VIS extinction spectra of the nanoparticles in water solution were measured on a Cary 50 Scan UV-Visible Spectrophotometer (Varian Ltd) in transmission mode. TEM micrographs were obtained using a Hitachi 7000 transmission electron microscope operated at 100 kV. Images were captured digitally using a Megaview 2 ccd camera. Specimens were prepared by dropping aqueous solutions onto formvar carbon coated copper grids.

Fluorescence measurement

Fluorescence measurements were carried using a Fluoromax-2 (Jobin Yvon- Spex Instruments S.A., Inc.). The fluorescence signal was collected at 90 degrees. The solution of nanoparticles with attached Ru-dye was diluted until the absorbance of the cuvette sample, measured with UV-Vis Spectrometer, was around 0.15. The reason for this was the following: due to the high absorption of the nanoparticles the excitation beam is exponentially attenuated by propagation through the cuvette. Therefore considering samples with and without nanoparticles, the decrease of excitation intensity in the sample with nanoparticles is

$$I_{NP}/I = (1-10^{-A})(0.434/A)$$
 [4]

Where A is absorbance of the sample with nanoparticles. By keeping the absorbance less than 0.15 the decrease of intensity due to the far-field effect of nanoparticles is less than 20%.



Figure 1. Extinction spectra of prepared silver/gold nanoparticles. The peaks are around 435nm and 480nm correspond to 90% and 30% silver respectively. In the graph are also extinction spectra of pure silver (395nm) and pure gold (530nm) together with excitation and emission peaks of Ru- dye.

Enhancement experiments were performed on the Fluoromax instrument in the following way. Two aqueous samples of the same volume (usually 4 ml) were prepared: the first solution contained silica coated alloy NP with a $\lambda_{res} = 435$ nm

(sample A) and the second contained purely deionised water (sample B). In each sample 10^{-6} M of Ru-dye previously dissolved in ethanol was added in increments of 30 µl and the solution stirred for two minutes. Finally the emission spectra of the dyes was recorded with the excitation wavelength set at 435nm to maximise the enhancement effect of the nanoparticles.



Figure 2. SEM pictures of gold/silver nanoparticles. On the left is shown sample of gold/silver nanoparticles and on the right is a single nanoparticle with the silica shell. The average nanoparticle diameter is about 30nm and average thickness of silica shell is approximatelly 5nm.

4. RESULTS AND DISCUSSION

Colloidal solutions of pure gold, pure silver and gold/silver alloy nanoparticles were successfully prepared. The visible spectra of these solutions shifted in wavelength from 490 to 520 nm with increasing molar fraction of gold (see Figure 1). Figure 1 also includes the absorption and emission spectra of the Ru-dye. The colloid solution, which most closely matched the absorption of the Ru-dye at 435nm, contained nanoparticles with a molar fraction of silver at 90 %. TEM micrographs of this colloid showed that the NPs are roughly spherical in shape with an average particle diameter of 30nm (see Figure 2).



Figure 3. The theoretical calculated extinction spectra of gold/silver nanoparticles with different molar ratios. The diameter of the nanoparticles was 30nm and the embedded medium was water.

As discussed in the Theory section, the theoretical extinction curves were calculated for spherical nanoparticles with diameter of 30nm and different gold/silver compositions (see Figure 3). By comparing figures 1 and 3, it is clear that experimentally measured shift in wavelength, and also the shape of the curves, agree well with theory.

The silica coating on the NP surface can clearly be seen in the TEM micrograph, on the right of Figure 2. The silica shell provided a spacer layer between the metal surface and the absorbed dye to prevent metal-fluorophore quenching. The average thickness of the silica layer was approximately 5 nm.

The excitation enhancement factor was calculated, as described in the theory section, for NPs with 30nm diameter and silica shell thickness of 5nm. Because of the good agreement of the experimental extinction spectra with the calculated values, the dielectric constant of the silver/gold alloy used in this calculation was used. The dielectric constant was chosen such that the extinction spectra of such nanoparticles had a plasmon resonance at λ_{res} = 435nm. The predicted enhancement factor for such a system was approximately 20.

The fluorescence emission spectra of samples A (dye attached to NPs with λ_{res} = 435nm) and B (dye in solution) can be seen in Figure 4 after subtraction of the background. From a calculation of the ratio of the fluorescence peak from Rudye attached on nanoparticles and free Ru-dye the experimental enhancement factor is approximately 5. The difference between this experimental value and the predicted value of ~ 20 can be due to number of factors. Firstly, the NPs were not monodisperse in size and secondly the thickness of the silica shells was not constant and both these parameters have a significant influence on the enhancement factor. Thirdly, the dielectric constant significantly affects computation of the enhancement and although we used a sophisticated method for modelling the dielectric constant for each alloy ratio, there can be some mismatch between real and computed values. In addition experimental data of dielectric constants is only available for a small number of silver gold alloy ratios [16]. Fourthly, our assumption that all the ruthenium dye attached to the NP surface could be incorrect and there may be a residual concentration, either non bound or in equilibrium with that bound to the surface, that did not contribute to enhancement.



Figure 4. Emission spectra of Ru-dye in water solution and attached on nanoparticles with the same concentration. There is a factor of 5 increase of the Ru-emission peak (at 612nm) for the dye attached to the nanoparticles.

5. CONCLUSION

Experiments have been carried out in order to investigate the influence of nearby metal NP on the fluorescence of a Rudye. The NPs of gold/silver alloy were fabricated using wet chemistry. Good correlation was found between predicted and measured plasmon absorption as a function of alloy composition. The spherical NP were coated with a silica shell on which was coated a monolayer of the Ruthenium dye complex. Preliminary enhancement factors of approximately 5 were obtained, compared to a calculated value of ~ 20. To complete this investigation, NPs of varying diameters, with a range of shell thickness, will be fabricated and characterised, in order to determine the maximum achievable and its dependence on NP diameter and NP-dye separation.

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