Photoacoustic investigation of gold nanoshells for bioimaging applications

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ABSTRACT

In this study, we report photoacoustic (PA) measurements of gold-covered polystyrene nanoparticles (Au nanoshells). Two types of Au nanoshells were examined: 1) polystyrene core with sparsely covered Au nanoparticles, and 2) polystyrene core which were fully covered by Au nanoparticles. The fully covered Au nanoshell exhibited a broad extinction cross section (500 nm – 850 nm), which is in the first infrared optical window where light transmission is optimal for optical based studies in tissues. The optical properties were compared to numerical simulations using Mie scattering theory. Using a photoacoustic microscope, the PA signal measured from fully covered Au nanoshells was 1.27 ± 0.18 mV per fluence (mJ/cm2), which was 10x greater than the PA signal from sparsely covered Au nanoshells (0.12 ± 0.14 mV). These novel Au nanoshell nanoparticles can be used for multispectral optical and PA imaging.

Keywords: Surface plasmon, nanoshell, Mie scattering, photoacoustic, multispectral

1. INTRODUCTION

Surface plasmon (SP) represents a collective oscillation of free electrons[1-2]. SP is observed only in materials having a free charge carrier density of about ~ 10^{21}/cm^3. Nobel metals such as gold and silver are well known SP materials. Metal nanoparticles have a wide range of biomedical applications due to their superior absorption cross section and wide tunability of excitation of surface plasmon resonance. Gold (Au) nanoparticles are commonly studied due to their excellent stability and biocompatibility. Additionally, polyethylene glycol (PEG) can be grafted onto the nanoparticles to help avoid the immune system[3]. Gold nanoparticles of various sizes and shapes have been investigated; these include gold nanorods, cages, stars and shells as they exhibit an absorption peak in the near-infrared (NIR) optical window (650 nm to 1100 nm) where light transmission is optimal for biomedical optical imaging[3-6]. Au nanoshells have been developed as their core can be loaded with drugs with higher capacity for therapeutic applications[7]. In this study, we investigate possible applications of Au nanoshells for PA studies. Recently we have used numerical simulations to investigate the optical properties of Au nanoshells on polystyrene spheres, and reported that two plasmonic peaks were due to long range surface plasmon polariton (LSPR) excitation on either side of the dielectric interfaces of the nanoshell[8].

PA studies on Au nanoshells are not common[3,7,9]. Most studies used Au shells with silica as the core[3,7,9]. So far, there have not been any studies on using polystyrene cores with Au nanoshells, Polystyrene Au nanoshells exhibit a plasmonic peak further in NIR regime than silica Au nanoshells. The SP excitation depends on the surrounding dielectric medium of the Au layer, which is described by the following dispersion relation of SP excitation

$$k_{sp} = \frac{\omega}{c}\left(\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}\right)^{1/2} = k_o\left(\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}\right)^{1/2},$$

where ω is the angular frequency of the incident radiation, k_o is the free space wavevector, and ε_m and ε_d are the dielectric functions of the metal and the dielectric medium, respectively.

2. MATERIALS AND METHODS

2.1 Materials

The following chemicals were used to synthesis Au nanoshell:

(1) 2-Aminoethanethiol hydrochloride (AET) (Sigma-Aldrich, USA), (2) N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) (Sigma-Aldrich), (3) 2-(N-Morpholino)ethanesulfonic acid hydrate (MES) (Sigma-Aldrich), (4) gold(III) chloride hydrate (chloroauric acid) (Sigma-Aldrich), (5) sodium hydroxide (NaOH) (Sigma-Aldrich), (6) tetrakis(hydroxymethyl)phosphonium chloride (THPC) (Sigma-Aldrich), (7) potassium carbonate (Sigma-Aldrich), (8) formaldyde (HCHO) (Sigma-Aldrich), (9) ammonium hydroxide (Sigma-Aldrich), (10) Phosphate-buffered saline (PBS), (11) HPLC water, (12) 3-Aminopropyltriethoxysilane (APTES) (Sigma-Aldrich) and (13) carboxylated polystyrene microspheres (Polyscience Inc, Warrington, USA).

2.2 Synthesis of the Au nanoshell nanoparticles

Au nanoshells nanoparticles were synthesized as described in literature[10-11]. Polystyrene spheres (PS) with a 350 μm diameter were used for these studies. PS with a carboxylic acid modified surface (carboxylated microspheres) were used for Au nanoshell synthesis, as they can be thiolated, forming transition metal thiolate complexes with metal ions. Carboxylated microspheres were procured from Polyscience Inc. The microspheres were available in aqueous suspension with concentration 2.5% (w/v) and surface charge between 0.1 and 2.0 milliequivalent/gram. 1 mL of microsphere solution was used to prepare Au nanoshells.

Au nanoshells were synthesized through two steps using two types of Au nanoparticles to achieve complete coverage of PS spheres. First, the carboxylic acid surface of PS was thiolated using (AET) in the presence of EDAX. Then it was treated with Au nanoparticles (Au(THPC) nanoparticles) which were synthesized by the reduction of hydrogen tetrachloroaurate(III) trihydrate using (THPC). The Au nanoparticles obtained using this method were 1-2 nm in size. This step resulted in partial /sparse coverage of Au nanoparticles over the PS sphere.

Complete coverage of Au nanoparticles over the PS sphere was achieved by treating this partially/sparsely covered PS with a second type of Au nanoparticles. These nanoparticles were synthesized by reducing tetrachloroaurate(III)trihydrate in the presence of potassium carbonate (K_2CO_3). The reaction was carried out at PH 3. The Au nanoparticles used in this reaction were 2-5 nm in size. The synthesized Au nanoshell was centrifuged three times using DI water and stored as stock solution in 20 mL water for further studies.

2.3 Protocol to prepare monodispersed Au nanoshells on glass slides

The glass slide was ultrasonically cleaned using isopropyl alcohol followed by water to remove any trace of oil or dirt. The glass slide was then air dried and plasma cleaned. The glass surface was silylated using silane containing amino group APTES by soaking the glass slide in ethanol containing APTES in the ratio 1:50. The silane group of APTES reacts covalently with the OH group on the glass substrate. The amide bond of the silane group that was grafted on the glass substrate was then ready to react with the Au nanoparticles. The silylated glass substrate was immersed in water containing Au nanoshells, where the nanoshells self-assembled as monolayers on the glass substrate.

2.4 Photoacoustic microscope

A scanning acoustic/photoacoustic microscope (SASAM, Kibero GmbH, Germany) was used for the PA measurements[9-10]. An IX81 inverted optical microscope (Olympus, Japan) was modified to include a transducer that was positioned above the sample holder, where it was co-aligned with the microscope optics. PA measurements were made using a 532 nm laser (Teem Photonics, France) collimated through the side port of the microscope, which was then focused onto the sample using a 10x optical objective. The laser had a pulse width of 330 ps, and a pulse repetition rate of 4 kHz. A schematic of the system is shown in figure 1. All measurements were made with a transducer that had a center frequency of 375 MHz (60° aperture, 42% bandwidth). All signals were averaged 100 times, amplified by a 40 dB amplifier (Miteq, USA) and digitized at 8 GS/s.
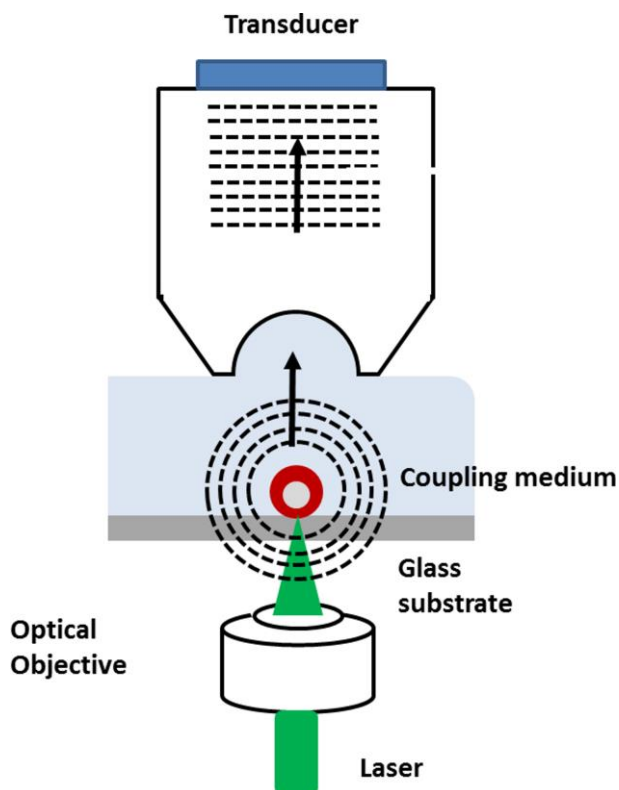
Figure 1. A schematic of the photoacoustic microscope (SASAM) used in this study. The 375 MHz transducer was positioned above the sample, and was co-aligned with the laser focused by the optics below the sample.

2.5 Characterization of Au nanoshells

All optical absorption spectra were acquired with a spectrophotometer (PerkinElmer, USA), and electron microscopy images were made using a Tecnai G2 transmission electron microscope (TEM). A Mie scattering simulation using Comsol Multiphysics (Burlington, MA, USA.) was performed by assuming polystyrene as the core and Au as the shell[8]. The simulation used a core of size 350 nm with a Au shell thickness of 15 nm. The PS core refractive index (RI) was assigned a value of 1.59, whereas the refractive index of gold is obtained from the Handbook of Optical Constants of Solids by Edward D. Palik[11].

3. RESULT AND DISCUSSION

Figure 2 shows TEM images of Au nanoshells. Figure 2(a) shows a representative image of a PS sphere sparsely covered with Au nanoparticles (Au(THPC)), whereas figure 2(b) shows representative image of a PS sphere densely covered with Au nanoshells.
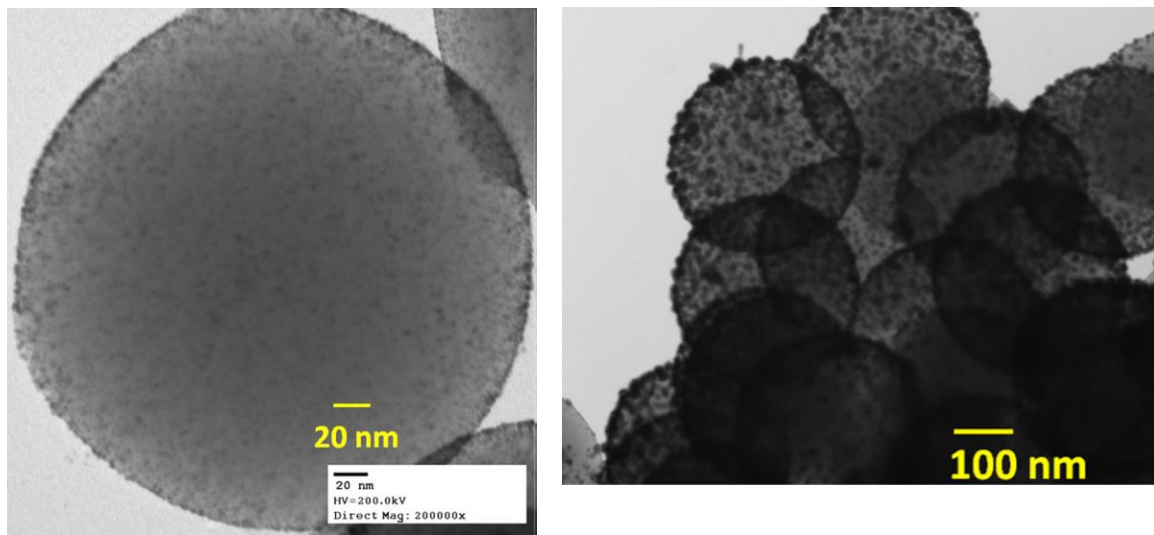
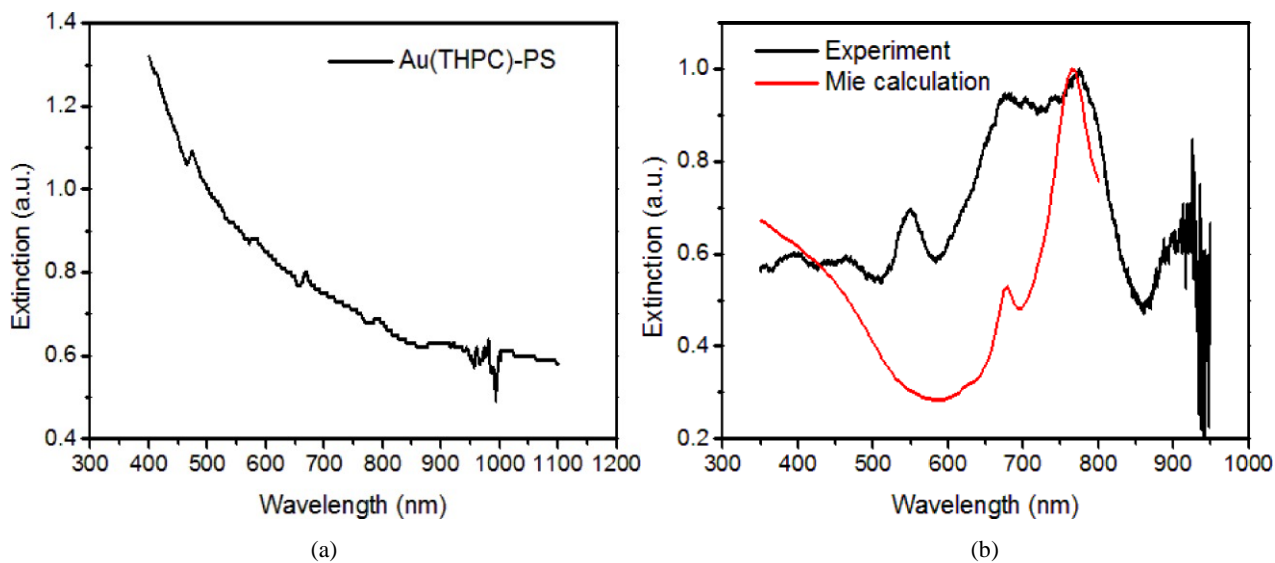(a)   (b)

Figure 2. TEM images of (a) the sparsely and (b) the completely covered Au nanoshell.

Figure 3(a) and (b) shows optical extinction spectra of the sparsely and the completely covered Au nanoshells The solid dark and red lines in figure 3(b) represent the extinction spectrum of the completely covered Au nanoshells and those calculated using Mie scattering theory assuming a PS sphere of size 350 nm and a Au shell with a 15 nm thickness. The spectroscopy measurement was made with water as reference. The extinction spectrum of the completely covered Au nanoshells shows three plasmonic peaks at 548.2 nm, 675.4 and 774.4 nm, whereas the Mie scattering analysis shows only two plasmonic peaks at 679 nm and 764 nm with a slight wavelength shift compared to the experimental measurements.

(a)   (b)

Figure 3. Optical extinction spectrum (a) the sparsely and (b) the densely covered Au nanoshells. The solid black and red lines represent optical extinction spectra of the completely covered Au nanoshell and those calculated using Mie scattering analysis with Au nanoshell of thickness 15 nm.

The peak at 764 nm was low in amplitude as compared to the Mie scattering calculation possibly due to the rapid precipitation of the Au nanoshells during the UV-vis spectroscopy measurement. The rapid precipitation during UV-vis spectroscopy measurement resulted in a continuous decrease in the concentration of the particles in the sampling volume

which eventually resulted in decrease in the absorption amplitude. These plasmonic peaks are due to LSPR excitations on both sides of the dielectric-metal interfaces of the Au nanoshells[12]. The additional plasmonic peak that observed at 548.2 nm was likely due to the formation of Au nanoparticles aggregates around the PS core.

(a)   (b)
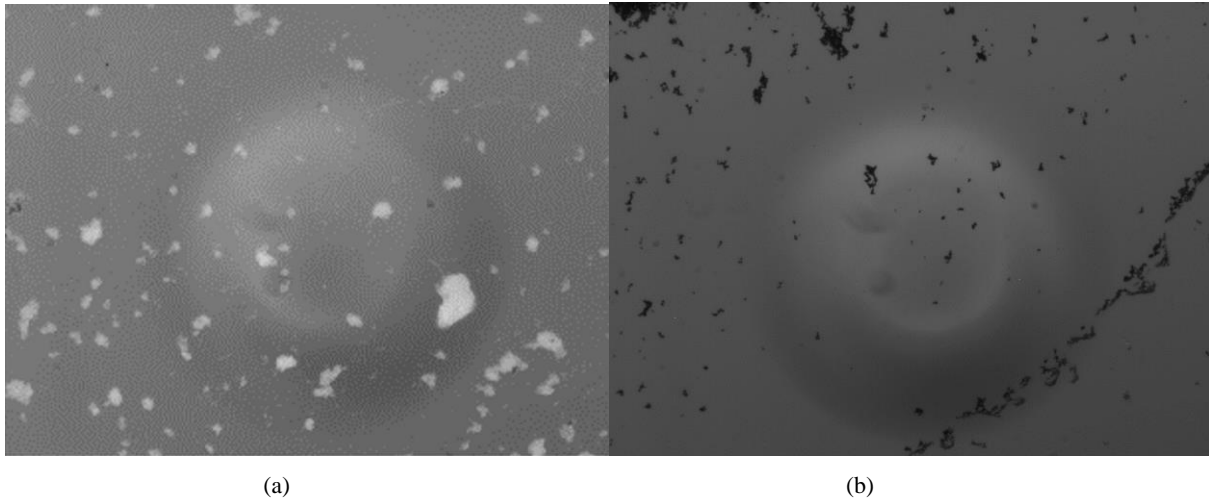
Figure 4. Optical microscope images of monodispersed nanoshell on the glass substrate (a) the sparsely and (b) the completely covered Au nanoshell.

Figure 4 (a) and (b) shows the monodispersed sparsely and completely covered Au nanoshells on the glass substrate as viewed optically in the acoustic microscope. The large spherical projection centered in the optical image is the out of focus acoustic transducer tip. PA studies on monodispersed sparsely and densely covered Au nanoshells show PA amplitudes of about 0.12 ± 0.14 mV and 1.27 ± 0.18 mV per fluence (mJ/cm^2), respectively. The PS particle with the densely covered Au nanoshell exhibited a 10x increase in PA signal as compared to the sparsely covered PS particle. This could be due to inter and intra plasmonic coupling between Au nanoparticles within and neighboring PS spheres, and also could be due to dense packing of Au nanoparticles in the Au shell. These measurements agree with other studies, which showed similar intensity dependent PA signals using silica coated Au particle aggregates[13]. These measurements show that full coverage of the polystyrene core by the Au nanoparticles is important for increasing the PA amplitude by an order of magnitude over the sparsely coverage polystyrene core, and ensuring the presence of strong spectral features for multispectral photoacoustic imaging.

4. CONCLUSION

Gold covered polystyrene nanospheres 350 μm in diameter were synthesized using a seed and growth technique. The Au nanoshell exhibits a broad extinction cross section in the near IR regime which is ideal for multispectral PA imaging. PA studies on Au shell shows that it exhibits PA amplitude of about 1.27 ± 0.18 mV per fluence (mJ/cm^2) due to inter and intra plasmonic coupling between Au nanoparticles within and between neighboring PS spheres, which is 10x larger than a sparsely covered PS sphere.

5. ACKNOWLEDGEMENTS

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