

Fabrication and characterization of gold nanostructures for cancer therapy

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INTRODUCTION

Gold nanostructures have already proved their usefulness in nanomedicine technologies with applications in both disease diagnosis and treatment. As of now, many studies are underway which explore the application of gold nanoshells and nanoparticles in photothermal therapy and imaging, a great majority of them being in the field of combating cancer. Gold reigns among the metallic nanoparticles for biomedical applications due to its inert nature and thus, better biocompatibility (Hirsch L., 2006).

Nanoshells aim to exploit the biocompatibility of gold towards the development of a thermally responsive therapeutic agent. Nanoshells are found to give plasmon peaks in the NIR range which is biologically ideal as it falls in the water window of biological tissues (Loo C., 2005). The present study aims to fabricate different types of gold nanoshells and study the effectiveness of each in a temperature responsive drug delivery carrier like thermosensitive liposomes.

Two popular gold nanoshells, viz. gold-gold sulfide nanoshells and silica-gold nanoshells have been fabricated for these experiments. The synthesis of gold-gold sulfide nanoshells is a single step process wherein aurochloric acid is reduced to gold sulfide using sodium sulfide and then a second addition of sodium sulfide results in the formation of gold-gold sulfide nanoshells (Raschke G., 2004). Synthesis of silica-gold nanoshells is a two step process wherein silica cores are first prepared by the Stober process. The silica nanoparticles are then surface functionalized, attached with gold seed nanoparticles and finally reduced with aurochloric acid to form a thin continuous layer of gold (Rossi L., 2005). The synthesized nanoshells can be further encapsulated in thermosensitive liposomes and photothermal therapy can be performed.

MATERIALS AND METHODS

Chemicals: Tetrachloroauric acid (HAuCl_4) and 3-aminopropyltriethoxysilane (APTES) were purchased from Acros, Sodium Hydroxide (NaOH), Sodium Sulfide (Na_2S) and Ammonia (30% NH_3 , 0.5 to 3 M) from Merck, Sodium Citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) from Ranbaxy, Ethanol (99.9%) from Changshu Yangyuan and Tetraethylorthosilicate ($\text{Si}(\text{OC}_2\text{H}_5)_4$, 0.1 to 0.5 M) from Ottokemi. All chemicals were reagent grade and used as received. All glasswares were cleaned with freshly prepared aqua-regia and rinsed with water before use. The water used in the experiment was of Millipore Milli-Q quality.

Instrumentation: Gold nanostructures were characterized for particle size, morphology, surface analysis, absorption spectra and zeta potential using Transmission electron microscope (CM200, Philips), Fourier transform infrared analysis (Nicolet Instruments Corporation, USA), Absorption spectra (U2900, HITACHI), Zeta plus (Brookhaven Instruments, USA) and DLS(Dynamic Light Scattering Device, Brookhaven Instruments, USA).

Synthesis of gold nanoparticles: 100ml 0.25 mM HAuCl_4 was brought to boil in a conical flask with continuous stirring. 0.5 ml 5% $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ was added to the boiling solution and continued refluxing till the colour changed from pale yellow to wine red. The initial molar ratio of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$: HAuCl_4 was maintained at 3.5:1 (sample 1). A second experiment with an initial molar ratio of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$: HAuCl_4 at 7:1 (sample 2) and an initial pH of 6.5 was also performed (Ji X., 2007).

Synthesis of gold-gold sulfide nanoshells: 10 ml of 1mM Na_2S was added to 10 ml of 2mM HAuCl_4 and stirred. After 5 min 5 ml of 1mM Na_2S was added to the above solution and the reaction was continued. When the solution was found to give peak at desired wavelength it was subjected to centrifugation at 3000 rpm for 20min. The pellet was washed 3 times and stored at 4°C (Raschke G., 2004).

Synthesis of functionalized silica nanoparticles: Functionalized silica nanoparticles were synthesized which can be further used for fabrication of silica-gold nanoshells. Approximately 50 ml of ethanol and 4 ml of NH_3 were stirred in a glass beaker. To this solution, 2.2 ml of TEOS was added and allowed to stir for at least 8 hours and further sonicated for 10 min. Silica nanoparticles with different sizes were obtained by varying the concentrations of used reagents (Pham T., 2002). The nanoparticles were isolated by centrifugation (5000 rpm, 10 min). Functionalization of silica nanoparticles were done by grafting with 12 mM APTES in the volume ratio of 3:7 under constant vigorous stirring at 80°C for 1 h to give a terminal amine group on their surface. The obtained silica particles were then cooled to room temperature and washed with at least 7 cycles of centrifugation and redispersion in absolute ethanol at 8000 rpm for 30 min each. (Kah J., 2008).

Characterization: The morphology of the gold nanoparticles and gold-gold sulfide nanoshells were characterized using transmission electron microscopy and the optical properties were determined by UV-Vis Spectrophotometry. The morphology and size of the bare and conjugated silica nanoparticles were characterized by transmission electron microscopy. Dynamic Light Scattering (DLS) and Zeta potential study were also performed to find the particle size distribution, polydispersity. FTIR analysis was performed to confirm the attachment of APTES.

RESULTS AND DISCUSSION

Characterization of gold nanoparticles: The UV-Vis spectrum of the prepared gold nanoparticles show a strong absorption at ~ 519.5 nm for the sample 1 (Figure 1) and ~526nm for the sample 2 (Figure 2). This is typical of gold nanoparticles and corresponds to the excitation of surface plasmon vibrations in the gold nanoparticles.

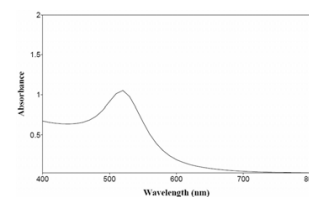


Figure 1: UV-Vis spectrum of gold nanoparticles prepared with 3.5:1 ratio of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$: HAuCl_4

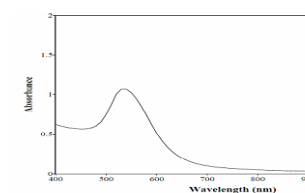


Figure 2: UV-Vis spectrum of gold nanoparticles prepared with 7:1 ratio of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$: HAuCl_4

TEM of sample 1 shows monodisperse, discrete spherical gold nanoparticles with size of ~14nm (Figure 3). However sample 2 was found to give highly polydisperse and polymorphic particles (Figure 4).

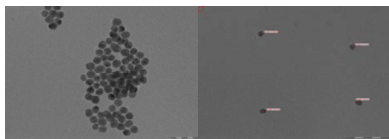


Figure 3: TEM of gold nanoparticles with initial $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$: HAuCl_4 of 3.5:1

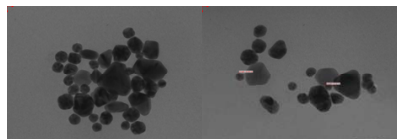


Figure 4: TEM of gold nanoparticles with initial $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$: HAuCl_4 of 7:1

Characterization of gold-gold sulfide nanoshells: UV-Vis spectrum of the sample shows a peak at ~524 nm which correspond to pure gold nanoparticles and a second peak which comes from the coated particles shifts across the visible region to the near infra red at ~867 nm (Figure 5).

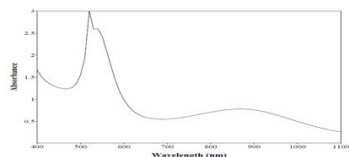


Figure 5: UV-Vis spectrum of gold-gold sulfide nanoshells

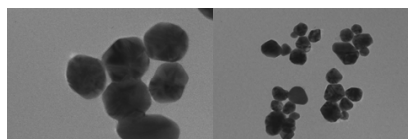


Figure 6: TEM of gold-gold sulfide nanoshells

Characterization of silica nanoparticles:

Analysis by TEM shows monodisperse, discrete silica nanoparticles. The sizes of the nanoparticles were found to be ~15-20 nm and they were spherical in shape (Figure 7). Also TEM analysis of APTES conjugated silica nanoparticles were performed but no difference is seen.

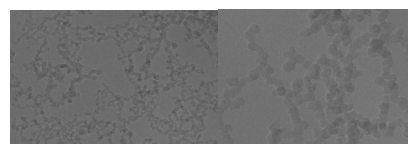


Figure 7: TEM of silica nanoparticles

DLS study and Zeta Potential: DLS instrument was operated at a light source wavelength of 630 nm and a fixed scattering angle of 90°. The average hydrodynamic diameter matches with the TEM results and the dependence of the size of silica nanoparticles on the amount of aqueous ammonia (ml) added was found. The diameter of the silica nanoparticles was found to increase with increase in amount of ammonia. DLS data showed fixed value (around 0.3) for polydispersity when the experiments were conducted in triplicates suggesting that the particles are monodisperse in nature.

FTIR analysis (for APTES conjugated silica nanoparticles): Fig 8 shows the FT-IR spectrum of the samples, SiO_2 (a) and APTES modified SiO_2 nanoparticles (b). Spectra displays the characteristic features of the Si–O–Si unit: asymmetric at 1092.5 cm^{-1} and symmetric stretching at 802.3 cm^{-1} . The broad absorption around 3400 cm^{-1} was assigned to the Si–OH residue. The band at 1637.3 cm^{-1} was the bending vibrations of water molecules adsorbed on the surface of the SiO_2 . Fig 8(b) displayed the characteristic features of APTES modified SiO_2 nanoparticles. Compared with Fig8 (a) the weak C–H stretching vibrations for methylene and methyl of APTES were observed at 2940 and 2986 cm^{-1} and the band around 3400 cm^{-1} was wider than the band of Si–OH in Fig. 8(b). The wider band consists of the asymmetric stretching vibration for N–H bonds of APTES and the Si–OH of SiO_2 . These findings indicate that NH_2 groups were introduced to the SiO_2 surface.

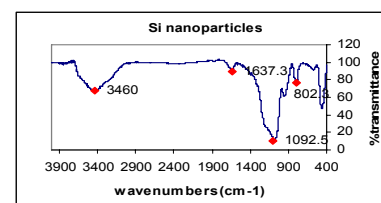


Figure 8(a): FT-IR spectrum of Si nanoparticles.

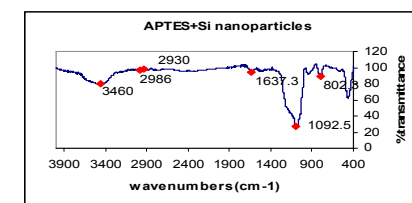


Figure 8(b): FT-IR spectrum of APTES modified Si nanoparticles.

CONCLUSIONS

Gold nanoparticles, gold-gold sulfide nanoshells and APTES functionalized silica nanoparticles were synthesized and well characterized. All the particles synthesized were well below 100nm which is ideal for drug delivery applications. Future work aims to encapsulate these gold nanostructures within thermosensitive liposomes for drug delivery applications.

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