NIR-Sensitive Au-Au$_2$S Nanoparticles for Drug Delivery

L. Ren$^a$ and G.M. Chow$^{a,b}$

$^a$Molecular Engineering of Biological and Chemical Systems, Singapore-Massachusetts Institute of Technology Alliance (SMA), $^b$Department of Materials Science, National University of Singapore

Abstract—Near IR (NIR) sensitive Au-Au$_2$S nanoparticles were prepared by mixing HAuCl$_4$ and Na$_2$S in aqueous solutions. An anti-tumor drug, cis-platin, was adsorbed onto Au-Au$_2$S nanoparticle surface via the 11-mercaptoundecanoic acid layers. The results showed that the degree of adsorption of cis-platin onto Au-Au$_2$S nanoparticles was controlled by the pH value of solution, and the drug release was sensitive to NIR irradiation. The cis-platin loaded Au-Au$_2$S nanoparticles can be potentially applied as NIR activated drug delivery carrier.

Index Terms—Drug delivery, Near-infrared, Au-Au$_2$S Nanoparticles, cis-Platin

I. INTRODUCTION

Chemotherapy for the cancer treatment cannot be used to its full potential since it involves saturating the body with toxic drugs that produce harmful side effects such as lowered immune response to infection [1-3]. There are several approaches to achieve dose limitation, one of which is to use controlled drug delivery system. Drug delivery systems (DDS), in which the carriers incorporate the drug either by chemical bonding or passive adsorption, can deliver the drug to specific cells or release drugs optimally at predetermined rates [4-8]. The DDS approach offers advantages such as improved efficacy, reduced toxicity, and improved patient compliance and convenience.

The near-infrared (NIR) radiation, which is non-destructive to human tissues, has been employed for a number of medical applications. An example is the optical coherence tomography (OCT) that involves the reflections of a near-IR laser source to image tissues that lie beneath the skin [9]. Another example involving the use of NIR irradiation is the photodynamic therapy (PDT), where a photosensitizing drug is introduced into the tissue of interest, and is subsequently activated when irradiated by the NIR light during treatment [10-11]. Moreover, tissue hyperthermia, induced by NIR light, can be synergized for the release of photosensitizing drug. However, the traditional NIR photo-sensors are organic dyes that are harmful to human tissue, limiting the use of NIR radiation for cancer therapy.

The goal of the current study is to investigate the potential of NIR sensitive Au-Au$_2$S nanoparticles as a novel kind of drug delivery carriers. Au-Au$_2$S nanoparticles are a new class of optically active nanoparticles that consist of Au$_2$S dielectric core encapsulated by a thin gold shell [12-13]. Gold is essentially a bio-inert material and has been found to be useful in fields ranging from dental surgery to arthritis treatments [14-15]. The gold shell (surrounding the Au$_2$S core) of the nanoparticles can take advantage of the inherent biocompatibility of gold, without requiring further surface functionalization or protective layer. The most interesting feature of the Au (shell)-Au$_2$S (core) nanoparticles is that their plasmon resonance (wavelength of optimal optical extinction) can be tailored in the NIR region by varying the particle geometry. For our purposes, the Au-Au$_2$S nanoparticles can be “tuned” to absorb NIR light, particularly in a spectral range known as the “water window”. This window represents a gap in the absorption spectrum of tissue that exists between the absorption spectra of the chromophores (<800 nm) and that of water (>1200 nm) [16].

In addition, when using nanoparticles as drug carriers, the reduction of particle size to nanoscale not only enables intravenous injection and minimize possible irritant reactions at the injection site [17], but also allows the carriers to penetrate the membranes of the diseased cells, and deliver drugs to cancerous tumors [18].

In this paper, we described a recently reported study [19] of the adsorption of cis-platin, a common drug applied in treatment of a broad range of solid cancers and lymphomas [20], to NIR sensitive Au-Au$_2$S nanoparticles. In order to functionalize the surface of NIR sensitive Au-Au$_2$S nanoparticles, 11-mercaptopoundecanoic acid (MUA) was immobilized on the colloidal carriers before drug adsorption. MUA molecules can adsorb onto Au-Au$_2$S nanoparticles since
the thiol (-SH) group is active to gold surface [21]. Another terminal -COOH group of MUA may provide further reactivity with cis-platin. The release of cis-platin from the Au-Au₂S nanoparticles under NIR light is also addressed.

II. EXPERIMENTAL

A. Chemical

Chloroauric acid (HAuCl₄·4H₂O) was obtained from Acros Organics. Sodium sulfide (Na₂S·9H₂O), 11-mercaptopoundecanoic acid (HS(CH₂)₁₀COOH), and cis-platin (cis-[Pt(NH₃)₂Cl₂]) were obtained from Sigma. All chemical reagents were used as purchased without further purification. The glassware were thoroughly cleaned and rinsed with deionized water.

B. Synthesis

The growth of Au-Au₂S nanoparticles occurred when the aqueous solutions of HAuCl₄ and Na₂S were mixed. Briefly, 20 ml of 2 mM HAuCl₄ was respectively mixed with 16ml, 20 ml, and 40ml of 1 mM Na₂S, and stored at 25°C for 1 day. The reaction was monitored using a UV-visible spectrophotometer at a range of 400 - 1100 nm. After centrifuging at 15,000 r.p.m, the Au-Au₂S nanoparticles were re-dispersed in a 100 mM 11-mercaptopoundecanoic acid (MUA) solution in ethanol for 3 days at 40°C. Excess MUA was removed from solution by at least 3 repeated cycles of centrifuging at 15,000 r.p.m, and subsequent re-dispersing in water. Finally, 10 mg of cis-platin was mixed with 10 ml MUA-modified Au-Au₂S nanoparticles by sonication. Afterwards the flask containing the drug carriers was capped and left to stand for 2 days. Determination of the degree of loading of cis-platin onto nanoparticles was carried out by separating the free cis-platin molecules from the supernatant fraction after centrifuging at 15,000 r.p.m for 30 min. The concentration of cis-platin in supernatant was measured by high performance liquid chromatography (HPLC) method.

C. Drug Release under NIR light irradiation

After centrifuging and rinsing, the cis-platin loaded Au-Au₂S nanoparticles were transferred to glass vials containing 2 ml of water. Each vial was then irradiated along its long axis with a pulsed Nd:YAG laser (1064 nm, 100 mJ/pulse, 7 nsec per pulse length, 10 Hz repetition rate, Surelite II, Continuum). The entire colloidal solution was exposed to the cross-sectional area of the beam. During the irradiation of 1 hr, samples were moved from the vials at different time intervals. The amount of released cis-platin was subsequently determined using HPLC. Control experiments without laser irradiation were also performed.

D. Characterization

Samples for UV-Vis study were placed in quartz crystal cuvettes (path length 1 cm) and the absorption spectra were acquired at room temperature using a UV-1601 spectrophotometer (Shimadzu). Samples for the TEM measurements were prepared by placing a drop of solution onto carbon-coated copper grids (200 mesh; 3 mm, Pelco Ltd.). The TEM samples were dried prior to investigation using a JEOL 100CX microscope (100 kV accelerating voltage). For Fourier transform infrared (FTIR) spectroscopy, nanoparticles were recovered from solution by centrifugation at 15000 r.p.m and completely dried by freeze-drying. The FTIR spectra were obtained by forming thin (~100 µm) transparent KBr pellets containing the materials of interest. The transmission spectra were recorded by using a Bio-red spectrometer (FTS 135) at a resolution of 4 cm⁻¹, and 256 scans were taken in the range of 400 - 4000 cm⁻¹. HPLC measurements were carried out in a liquid chromatograph instrument with a constant-flow-rate pump and diode array detector (model HP 1050, Hewlett Packard, USA). Samples were chromatographed using an analytical APS-Hypersyl column (Hewlett Packard of 20 cm length, 4.6 mm internal diameter). The mobile phase had a flow rate of 1.0 ml/min under isocratic conditions of acetonitrile-water (90:10). The UV detector was set at 210 nm. Each sample was injected 3 times and the results were averaged to obtain the value of the concentration.

III. RESULTS AND DISCUSSION

The optical properties of the nanoparticles strongly depended on the mixing compositions. As the molar ratio of S/Au (denoted as Mₛ/ₐᵤ) ≥ 0.4, clear and stable nanoparticles were obtained. Figure 1 shows the UV-Vis spectra of a series of Au-Au₂S nanoparticles as a function of Mₛ/ₐᵤ. As Mₛ/ₐᵤ = 0.4, nanoparticles A only displays band I with a maximum around 520 nm. With further increase of Mₛ/ₐᵤ to 0.5, the UV-Vis spectrum of nanoparticles B consisted of two absorption bands, i.e. band I at 520 nm and band II at 790nm. The band I at 520 nm was assigned to the surface plasmon resonance of the Au nanoparticles, whereas Zhou and Halas have assigned the band II at 790 nm to the Au-coated Au₂S nanoparticles [12-13]. Figure 1 also shows that both UV-Vis absorption bands I and II disappeared for nanoparticles C (Mₛ/ₐᵤ = 2), indicating that neither pure Au nor Au coated Au₂S nanoparticles were formed in sample C. The effects of the composition, structure, size, and interface on the optical properties of Au-Au₂S nanoparticles are currently investigated and the results will be published elsewhere.

Since gold nanoparticles can bind the thiol groups [21], mixing the Au-Au₂S nanoparticles with 100 mM solution of 11-mercaptopoundecanoic acid (MUA) resulted in immobilization of a MUA layer onto the Au-Au₂S nanoparticle surfaces. Moreover, the MUA molecule has a free terminal -COOH group, which provides a means for further reaction. Figure 2 shows the UV-Vis absorption of MUA-modified nanoparticles B varied as a function of the solution pH. When the pH was adjusted to 2.5, the absorption band of MUA-modified Au-Au₂S nanoparticles shifted from 790 nm to ~1000 nm. This behavior is attributed to the tendency of flocculation of the nanoparticles in acidic medium. However, adjusting the pH to > 8 only caused an
increase in the intensity of the absorption bands, but no band shifting. Note that Au-Au$_2$S nanoparticles were stable against aggregation even after 3 months when coated with MUA at pH 6, whereas the uncoated Au-Au$_2$S nanoparticles or MUA-coated Au-Au$_2$S nanoparticles in acidic conditions (pH $\leq$ 5) aggregated in 1 week (data not shown). The long-term stability of Au-Au$_2$S nanoparticles against aggregation is important for applications.

Figure 3 shows the FTIR spectra of Au-Au$_2$S, MUA-modified Au-Au$_2$S, and cis-platin loaded Au-Au$_2$S nanoparticles, respectively. Pure Au-Au$_2$S nanoparticles did not have the characteristic stretching vibration band of organic groups, whereas most of the IR bands of MUA were observed for MUA modified Au-Au$_2$S nanoparticles. The strongest bands at 2920 cm$^{-1}$ and 1700 cm$^{-1}$ were assigned to asymmetric and symmetric stretching vibrations $\nu_{\text{CH}}$ of the methylene groups and stretching vibration $\nu_{\text{C=O}}$ of the carboxylic acid groups of MUA, respectively, indicating that MUA was adsorbed on the Au-Au$_2$S nanoparticles. The FTIR spectra for the cis-platin loaded, MUA-functionalized Au-Au$_2$S nanoparticles showed the appearance of new bands (indicated by closed circles) at 3280 cm$^{-1}$, 3200 cm$^{-1}$, 1614 cm$^{-1}$, and 1530 cm$^{-1}$, which were readily identified as the amine group. This indicates that a substantial amount of cis-platin was bound to Au-Au$_2$S nanoparticles through the MUA layer. It is suggested that MUA was adsorbed to the Au-Au$_2$S nanoparticles via its -SH end group. The -COOH end group of MUA may be ionized to -COO$^{-}$ group, which may subsequently provide attachment sites for the protonated NH$_3$ (NH$_4^+$) group of cis-platin. This resulted in the adsorption of cis-platin to Au-Au$_2$S nanoparticles via the MUA layer. A schematic illustration of such processing is shown in Fig. 4.
Figure 5 shows a typical TEM bright field image of the cis-platin loaded, MUA-modified Au-Au₂S nanoparticles. The drug loaded spherical particles were about 40 – 50 nm in diameter. A dense core of Au-Au₂S nanoparticles (dark contrast) was surrounded by a coating (light contrast). The coating presumably consisted of MUA and cis-platin.

Figure 6 shows the UV-Vis spectra of Au-Au₂S, MUA-modified Au-Au₂S, and cis-platin loaded, MUA-modified Au-Au₂S nanoparticles. All UV-Vis spectra consisted of two absorption bands. The band II for both of MUA-modified Au-Au₂S and cis-platin loaded Au-Au₂S nanoparticles shifted to a longer wavelength, which may due to the coating of MUA and cis-platin on Au-Au₂S nanoparticles.
Figure 7 shows the effect of pH of solution on adsorption of cis-platin to the MUA-modified Au-Au₂S nanoparticles. The maximum cis-platin adsorption value (~80%) was obtained at pH 6.0 - 7.0. However, below or above this pH, a smaller amount of cis-platin was absorbed. At pH ≤ 2.5 or ≥ 8.5, the cis-platin was almost not adsorbed onto the MUA-modified Au-Au₂S nanoparticles. This pH-dependent behavior can be interpreted as follows: when the pH was decreased, the COO⁻ groups of the MUA layer became protonated; when the pH was increased, the NH₃⁺ group of cis-platin was not protonated to NH₄⁺. As a result, ionic bond between COO⁻ groups of MUA layer and NH₄⁺ groups of cis-platin could not readily form in either acidic or basic solutions.

The release of cis-platin from MUA modified Au-Au₂S nanoparticles over the observed period (≤ 1 hr) under NIR light irradiation and by heating (40°C) without NIR irradiation is shown in Fig. 8. The NIR irradiation was performed at room temperature. The temperature of the solution during irradiation was not monitored. About 90% of cis-platin was released at the initial period (≤ 1 min) under NIR irradiation, and then the rate of release decreased with increasing time. For heating without NIR irradiation, the release of cis-platin started after heating at 40°C for 10 min, and only 40% of cis-platin was released from Au-Au₂S nanoparticles during the period of 1 hr. This shows that more cis-platin was released under NIR irradiation compared to heating without laser irradiation. The control experiments without laser irradiation or heating showed that only a little amount (1.2%) of cis-platin was released. It is therefore suggested that the release of cis-platin from Au-Au₂S nanoparticles is sensitive to both of heating and NIR irradiation. However, such dissociative reaction is more readily initiated by the absorption of NIR light compared to heating without laser irradiation. The cis-platin loaded Au-Au₂S nanoparticles may absorb the NIR photons, and this absorption with high energy may lead to photochemical interaction, thermal interaction, photo ablation, plasma induced ablation, and photo disruption among nanoparticles [22-23]. These interactions may also occur to cause the cleavage of the ionic bond between COO⁻ and NH₄⁺, and may finally release cis-platin from Au-Au₂S nanoparticles. However, the mechanism of the interactions between NIR light and NIR sensitive Au-Au₂S nanoparticles is presently unclear, and future work is in progress. The thermal effects in NIR irradiation also need further investigations. Based on the results shown in Fig. 7, it is suggested that the adsorption of cis-platin on Au-Au₂S nanoparticles is stable in physiological conditions where pH is about 7.2-7.4. When NIR light is applied in therapy, cis-platin will be released from the nanoparticles to destroy the cancerous cells. Our preliminary study of drug release under NIR irradiation shows that cis-platin loaded Au-Au₂S nanoparticles can be a potential drug delivery system for cancer treatment.

IV. SUMMARY

A preliminary study of preparing a drug carrier system involved the use of MUA as a linker between the drug and the NIR sensitive Au-Au₂S nanoparticles. The degree of adsorption of cis-platin onto Au-Au₂S nanoparticles was controlled by the solution pH value, and cis-platin was released from nanoparticles under NIR irradiation at a greater rate than thermal activation (without laser irradiation). The potential of this drug delivery system for cancer therapy warrants further investigation.
ACKNOWLEDGMENT

We thank Dr. G. Chen in NTU for his help on using Nd:YAG laser. We also thank Dr. C.L Ho and Dr. S.C Li of the Department of Pharmacy, NUS, for their advice and discussion.

REFERENCES


