NIR-Sensitive Au-Au_2S Nanoparticles for Drug Delivery

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Abstract—**Near IR (NIR) sensitive Au-Au_2S nanoparticles were prepared by mixing $HAuCl_4$ and Na_2S in aqueous solutions. An anti-tumor drug, *cis*-platin, was adsorbed onto Au-Au_2S nanoparticle surface via the 11-mercaptoundecanoic acid layers. The results showed that the degree of adsorption of *cis*-platin onto Au-Au_2S nanoparticles was controlled by the pH value of solution, and the drug release was sensitive to NIR irradiation. The *cis*-platin loaded Au-Au_2S nanoparticles can be potentially applied as NIR activated drug delivery carrier.**

***Index Terms*—Drug delivery, Near-infrared, Au-Au_2S, 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_2S nanoparticles as a novel kind of drug delivery carriers. Au-Au_2S nanoparticles are a new class of optically active nanoparticles that consist of Au_2S 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_2S 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_2S (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_2S 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_2S nanoparticles. In order to functionalize the surface of NIR sensitive Au-Au_2S nanoparticles, 11-mercaptoundecanoic acid (MUA) was immobilized on the colloidal carriers before drug adsorption. MUA molecules can adsorb onto Au-Au_2S nanoparticles since

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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-Au_2S nanoparticles under NIR light is also addressed.

II. Experimental

A. Chemical

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Acros Organics. Sodium sulfide ($Na_2S \cdot 9H_2O$), 11-mercaptoundecanoic acid ($HS(CH_2)_{10}COOH$), and *cis*-platin (*cis*-$[Pt(NH_3)_2Cl_2]$) 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-Au_2S nanoparticles occurred when the aqueous solutions of $HAuCl_4$ and Na_2S were mixed. Briefly, 20 ml of 2 mM $HAuCl_4$ was respectively mixed with 16ml, 20 ml, and 40ml of 1 mM Na_2S, and stored at 25^{o}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-Au_2S nanoparticles were re-dispersed in a 100 mM 11-mercaptoundecanoic acid (MUA) solution in ethanol for 3 days at 40^{o}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-Au_2S 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-Au_2S 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 $M_{S/Au}$) ≥ 0.4, clear and stable nanoparticles were obtained. Figure 1 shows the UV-Vis spectra of a series of Au-Au_2S nanoparticles as a function of $M_{S/Au}$. As $M_{S/Au}$ = 0.4, nanoparticles A only displays band I with a maximum around 520 nm. With further increase of $M_{S/Au}$ 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 Au_2S nanoparticles [12-13]. Figure 1 also shows that both UV-Vis absorption bands I and II disappeared for nanoparticles C ($M_{S/Au}$ = 2), indicating that neither pure Au nor Au coated Au_2S nanoparticles were formed in sample C. The effects of the composition, structure, size, and interface on the optical properties of Au-Au_2S nanoparticles are currently investigated and the results will be published elsewhere.

Since gold nanoparticles can bind the thiol groups [21], mixing the Au-Au_2S nanoparticles with 100 mM solution of 11-mercaptoundecanoic acid (MUA) resulted in immobilization of a MUA layer onto the Au-Au_2S 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-Au_2S 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_2S nanoparticles were stable against aggregation even after 3 months when coated with MUA at pH 6, whereas the uncoated Au-Au_2S nanoparticles or MUA-coated Au-Au_2S nanoparticles in acidic conditions (pH ≤ 5) aggregated in 1 week (data not shown). The long-term stability of Au-Au_2S nanoparticles against aggregation is important for applications.

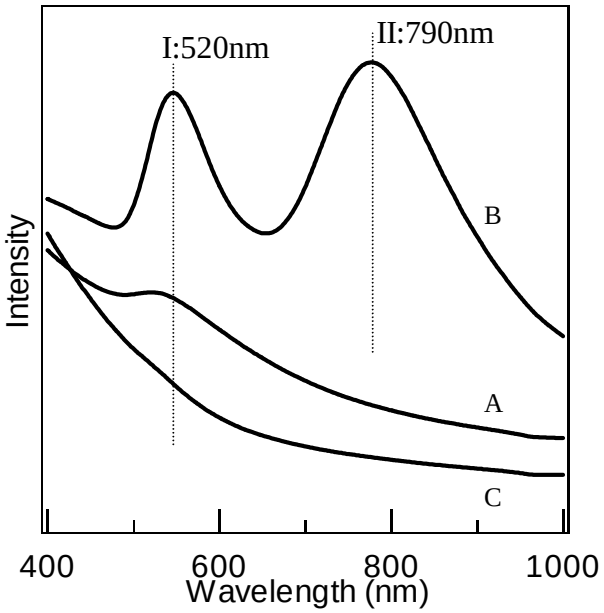

Fig. 1 UV-Vis spectra of a series of Au-Au_2S nanoparticles as a function of the molar ratio of S/Au ($M_{S/Au}$). $M_{S/Au}$ = 0.4, 0.5, and 2 for samples A, B, and C, respectively.

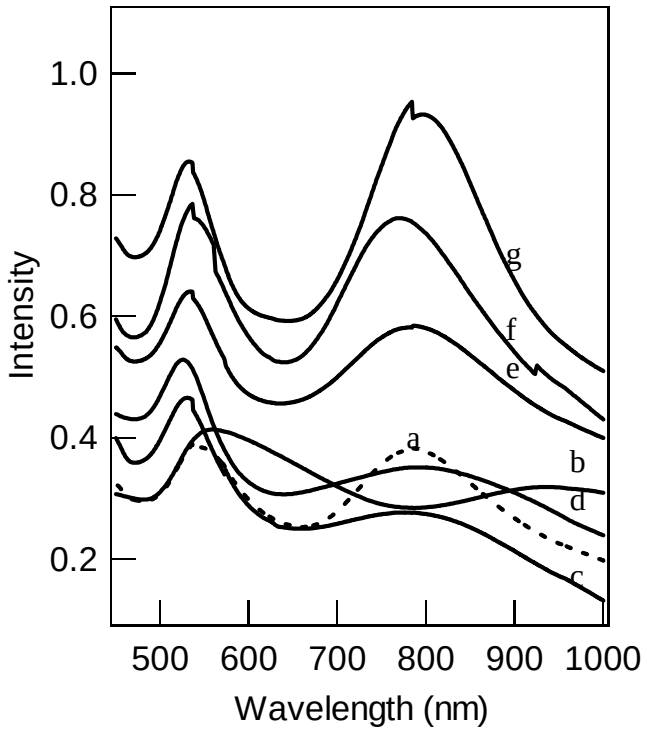

Figure 3 shows the FTIR spectra of Au-Au_2S, MUA-modified Au-Au_2S, and *cis*-platin loaded Au-Au_2S nanoparticles, respectively. Pure Au-Au_2S nanoparticles did not have the characteristic stretching vibration band of organic groups, whereas most of the IR bands of MUA were observed for MUA modified Au-Au_2S nanoparticles. The strongest bands at 2920 cm^{-1} and 1700 cm^{-1} were assigned to asymmetric and symmetric stretching vibrations ν_{CH} of the methylene groups and stretching vibration $\nu_{C=O}$ of the carboxylic acid groups of MUA, respectively, indicating that MUA was adsorbed on the Au-Au_2S nanoparticles. The FTIR spectra for the *cis*-platin loaded, MUA-functionalized Au-Au_2S 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_2S nanoparticles through the MUA layer. It is suggested that MUA was adsorbed to the Au-Au_2S 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_2S nanoparticles via the MUA layer. A schematic illustration of such processing is shown in Fig. 4.

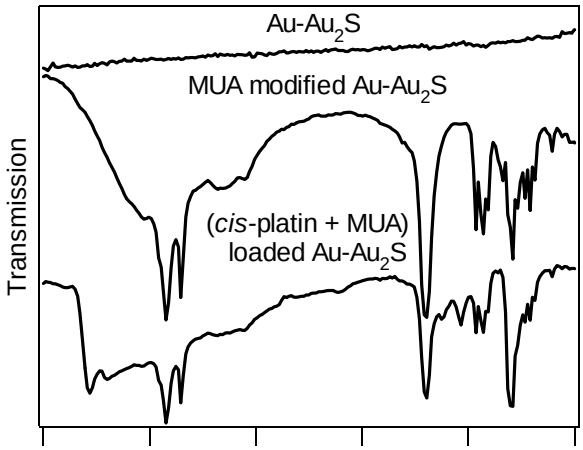

Fig. 2 UV-Vis absorption of MUA-modified nanoparticles B as a function of the solution pH. a: nanoparticle B, without MUA;
b: with MUA, without pH adjust (pH = 6.5)
c: with MUA, pH = 2.5; d: with MUA, pH = 5.0;
e: with MUA, pH = 8.0; f: with MUA, pH = 9.0
g: with MUA, pH = 11.0

Fig. 3 FTIR spectra of Au-Au_2S nanoparticles, MUA-modified Au-Au_2S nanoparticles, and cis-platin-loaded, MUA-modified Au-Au_2S nanoparticles.

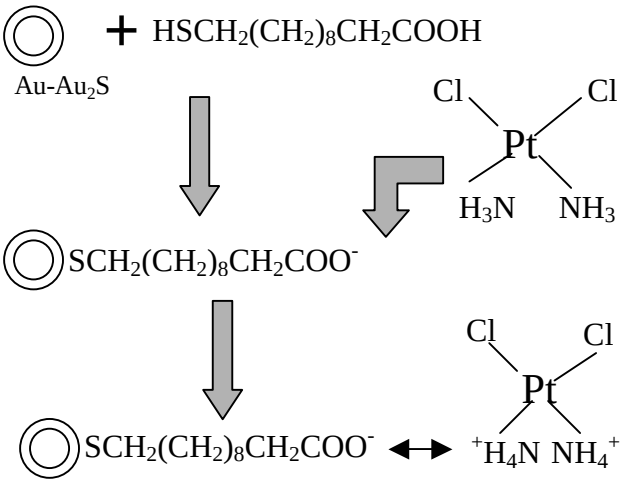

Fig. 4 Schematic illustration of the adsorption of *cis*-platin to Au-Au_2S nanoparticles via the MUA layer.

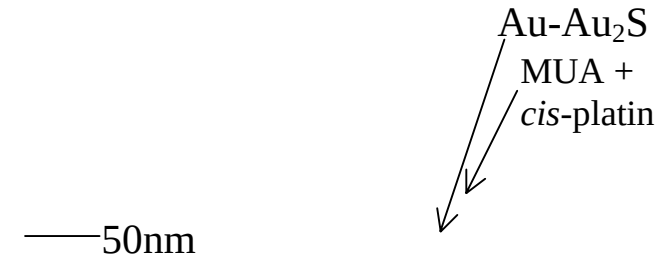

Fig. 5 TEM image of cis-platin-loaded MUA-modified Au-Au_2S nanoparticles. Core: Au-Au_2S; Shell: MUA-*cis*-platin.

Figure 5 shows a typical TEM bright field image of the *cis*-platin loaded, MUA-modified Au-Au_2S nanoparticles. The drug loaded spherical particles were about 40 – 50 nm in diameter. A dense core of Au-Au_2S 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-Au_2S, MUA-modified Au-Au_2S, and *cis*-platin loaded, MUA-modified Au-Au_2S nanoparticles. All UV-Vis spectra consisted of two absorption bands. The band II for both of MUA-modified Au-Au_2S and *cis*-platin loaded Au-Au_2S nanoparticles shifted to a longer wavelength, which may due to the coating of MUA and *cis*-platin on Au-Au_2S nanoparticles.

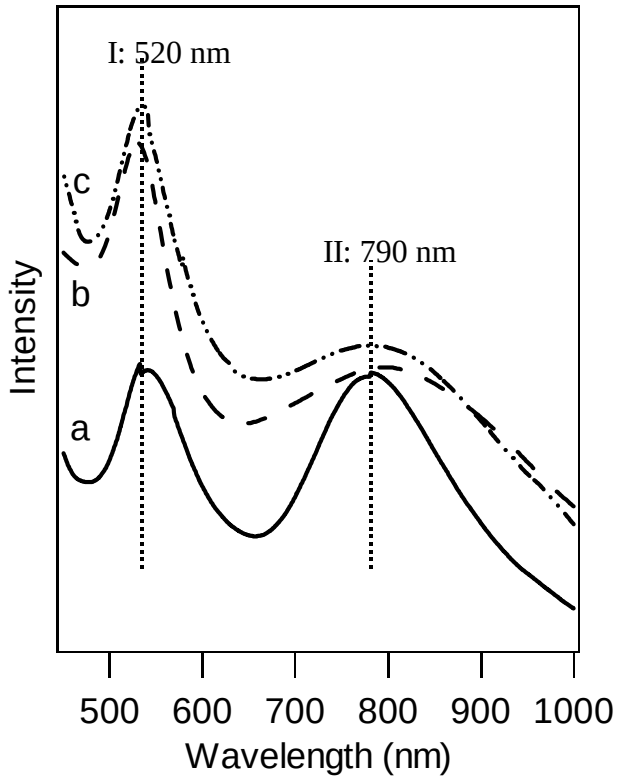

Fig. 6 UV-vis spectra of (a) Au-Au_2S nanoparticles, (b) MUA-modified Au-Au_2S nanoparticles, and (c) *cis*-platin-loaded Au-Au_2S nanoparticles.

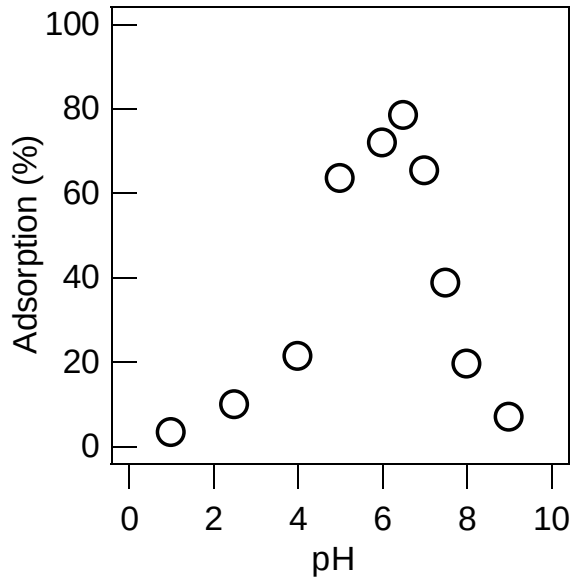

Fig. 7 Adsorption percentage of *cis*-platin onto the MUA-modifed Au-Au_2S nanoparticles as a function of pH.

Figure 7 shows the effect of pH of solution on adsorption of *cis*-platin to the MUA-modified Au-Au_2S 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-Au_2S 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 NH_3 group of *cis*-platin was not protonated to NH_4^+. As a result, ionic bond between COO^- groups of MUA layer and NH_4^+ groups of *cis*-platin could not readily form in either acidic or basic solutions.

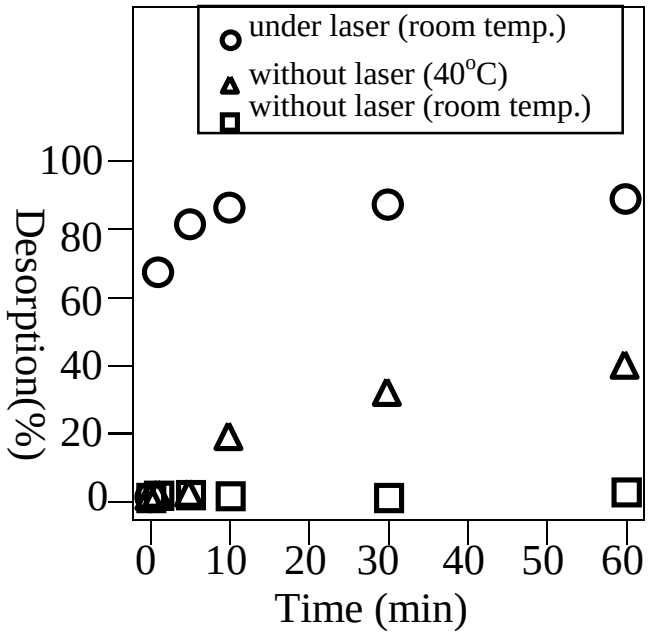

Fig. 8 Release of *cis*-platin from the Au-Au_2S nanoparticles under Nd: YAG laser (room temperature) and heating (40°C, without laser irradiation).

The release of *cis*-platin from MUA modified Au-Au_2S 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-Au_2S 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-Au_2S 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-Au_2S 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 NH_4^+, and may finally release *cis*-platin from Au-Au_2S nanoparticles. However, the mechanism of the interactions between NIR light and NIR sensitive Au-Au_2S 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-Au_2S 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-Au_2S 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-Au_2S nanoparticles. The degree of adsorption of *cis*-platin onto Au-Au_2S 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.

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