# A Novel High Yield Process for Gold Sulfide Nanoparticle Synthesis via Shifting Equilibrium of Self-Assembly Reaction

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## ABSTRACT

This project is focused on a novel process for gold-gold sulfide (GGS) nanoparticle synthesis. GGS nanoparticles have a strong near infrared (nIR) absorbing property, allowing their use in a variety of biomedical applications including cancer therapy. By combining a 1-step self-assembly process and simultaneously performing dialysis, the equilibrium of the reaction is shifted to favor the formation of the nIR-absorbing fraction. This hybrid dialysis based synthesis process, DiaSynth, results in removal of smaller ions and particles during the reaction leading to minimal formation of pure small gold particles within the system. The result is a suspension of nIR absorbing nanoparticles with very little gold colloid contamination, which require minimum further processing for therapeutic or other applications. The purity and properties of these particles match that of GGS nanoparticles produced by the self-assembly process followed by multi-step centrifugation.

Keywords: gold nanoparticles, dialysis, near infrared peak (nIR) nanoparticles, gold/gold-sulfide nanoparticles

## 1. INTRODUCTION

Gold nanoparticles (AuNPs) have attracted enormous attention in the field of nanotechnology for applications such as, immunoassays, drug delivery, contrast enhancement, ex/in-vivo imaging, in-vitro assays, and cancer therapy (theranostic applications) [1, 2]. Specifically, gold/gold sulfide (GGS) nanoparticles have been introduced for many applications in addition to gold nanoshells [3] and nanorods [4], for their smaller size, higher absorptive properties, and ease of fabrication [1, 5]. Theranostic applications have surfaced as a novel approach to combine diagnosis, treatment, and imaging in cancer research, not only decreasing time, but improving efficacy of therapy and patient compliance. The ideal theranostic approach system for cancer therapy should have multiple tunable factors, which can be adjusted for imaging or treatment, as well as the ability to specifically target certain cancer cells [6]. The therapeutic effect of GGS nanoparticles is the result of surface plasmon oscillations, converting light into heat, which then causes an increase in local temperature to ablate specifically targeted cells. This phenomenon occurs when the frequency of light used matches the peak resonance of nanoparticles [7-12].

Traditionally, GGS nanoparticles are synthesized by reacting sodium sulfide ($Na_2S$) or sodium thiosulfate ($Na_2S_2O_3$) with chloroauric acid ($HAuCl_4$) forming a core-shell structure [13, 14]. However, there is considerable debate as to the true structure of these particles [5]. Regardless, these particles display a gold surface that allows for increased biocompatibility and conjugation potential for their use as a therapeutic or theranostic agent. The self-assembly process leads to formation of a variety of species of particles including: colloidal gold, GGS (circular/spherical), triangular plates, and rods [1, 5, 15].

The absorbance spectrum of the synthesized particles contains two predominant peaks at wavelengths of 520–540 nm (colloidal gold peak) and a near infrared (nIR) peak between 650–1000 nm (GGS, triangular plates, and rods peak) [1, 5, 15-17]. Although the full mechanism of the nanoparticle growth is not yet fully understood, researchers have developed theories on the self-assembly process. Equation (1) – shows one proposed reduction mechanism leading to a gold-sulfide core as gold and sulfur simultaneously reduce, then coating with gold when excess gold is reduced, concurrently with gold colloid formation [14].

$$2AuCl_4^- + 3HS^- \rightarrow 2Au + 3S + 3H^+ + 8Cl^- \quad (1)$$

The current synthesis requires $Na_2S$ to be aged for 2-3 days, and depending on the concentration or volume of $Na_2S$ being added, it can affect the nIR peak. Upon further investigation of the reaction when $Na_2S$ is aging, Schwartzberg et al determined two key points: (1) because of the chemical change during the aging process of $Na_2S$ solution, there is an increase in nIR absorption, and (2) Na2S2O3 is the likely product from $Na_2S$ aging. It was also reported the $Na_2S_2O_3$ solution is more stable than $Na_2S$ solution, but can still produce the same results (colloidal gold, GGS, triangular plates, and rods), it is not known whether the same by- products are being formed [5].

The current technique for producing nIR GGS nanoparticles using $Na_2S_2O_3$, based on a single step procedure, will result in a low yield of nIR particles compared to colloidal gold, based on the relative absorbance at those wavelengths. We define a **quality ratio** of the absorbance of peaks to describe the nanoparticle purity, $R^{nIR/Au}$; this is a ratio of the nIR region (650 – 1000

nm) relative to the colloidal gold peak (~530 nm). We use this as a quality guide based on previous experiments, for which minimal contamination of colloidal gold lead to product that was usable for *in vitro* and *in vivo* testing. GGS nanoparticles previously formed using $Na_2S$ resulted in large amounts of colloidal gold (low $R^{nIR/Au}$), generally in the range of 0.4 – 0.8, before any separation steps. However, in therapeutic applications such as nIR photothermal therapy [1] or drug delivery via nIR irradiation [18], Gobin et al. reported colloidal gold formation during the self-assembly process is considered a contaminant, and must be removed via multistep centrifugation. Not only does this reduce the total amount of therapeutic nIR absorbing nanoparticles being delivered, but also reduces exposure to non-therapeutic particles, and could thus limit immune response reaction [1, 19]. By combining published methods for $Na_2S_2O_3$ instead of $Na_2S$, and including results published by Schwartzberg et al., with equation (1), we hypothesize dialysis performed concurrently with the GGS nanoparticle self-assembly process will force ion-exchange, allowing the equilibrium of the reaction to be shifted to favor the formation of nIR absorbing particles; we call this hybrid process DiaSynth for Dialysis-Synthesis. This technique will minimize production of AuNPs having resonance peak at 530 nm (colloidal gold), resulting in an increase of the quality of the as-made product. This would then require minimum further processing for use as a therapeutic agent. The DiaSynth method reported here can increase the quality ratio of the synthesized particles to 1.7 – 2.0; thus, centrifugation processes may not be required to match the quality of previously produced therapeutic particles.

## 2. METHODS

### 2.1 GGS Synthesis (1-step method)

Hydrogen tetrachloroaurate (III) trihydrate (chloroauric acid, $HAuCl_{4*}3H_2O$) was purchased from Alfa Aesar and diluted to a concentration of 2mM. Sodium Thiosulfate ($Na_2S_2O_3$) was prepared at a concentration of 3mM. The ratio of $HAuCl_{4*}3H_2O$ to $Na_2S_2O_3$ can be varied from 2.2:1 up to 5.5:1 by volume. Next, spectral scans were obtained after an hour of the self-assembly synthesis at room temperature (RT), using a UV/Vis spectrophotometer (Carey 50 Varian). A Malvern Zetasizer (ZS90) and TEM (FEI Tecnai F20) were used to characterize the GGS nanoparticles surface charge, size and morphology respectively.

### 2.2 GGS Synthesis (DiaSynth)

GGS nanoparticles are synthesized by mixing 2 mM $HAuCl_{4*}3H_2O$ and 3 mM $Na_2S_2O_3$, at various volume ratios, inside assembled dialysis tubing with different molecular weight cut-off (MWCO) dialysis membrane ranging between 2 - 12 KDa. These are dialyzed against 8 L of DI water, during the self-assembly process. The reaction is considered complete after 1 hour. Spectral profile, size, and zeta data was obtained using the instruments mentioned in the previous section.

## 3. RESULTS AND DISCUSSION

### 3.1 Purification of GGS nanoparticles

Theranostic applications have emerged as a technique to combine diagnosis, treatment, and imaging in cancer research. The ideal theranostic approach for cancer therapy has several tunable factors, mostly imaging and treatment, with a display of specifically targeting cancer cells. In order to use GGS nanoparticles for therapeutic applications, the smaller gold-colloid contaminants formed during the self-assembly process must be removed. Traditionally, colloidal gold particles are removed via multi-step (3X) centrifugation, which is not only time consuming but inefficient. The removal of colloidal gold will reduce the total number of particles delivered to the body and reduce the impact on the reticulo-endothelial system (RES). Once the GGS particles have been synthesized, using 2 mM $HAuCl_{4*}3H_2O$ and 3 mM $Na_2S_2O_3$, they undergo a multistep centrifugation, Figure 1 shows the spectra of the samples, "as made" and after "3X centrifugation". There is a significant increase in the quality ratio, as seen in Figure 1, due to the removal of the colloidal nanoparticles.

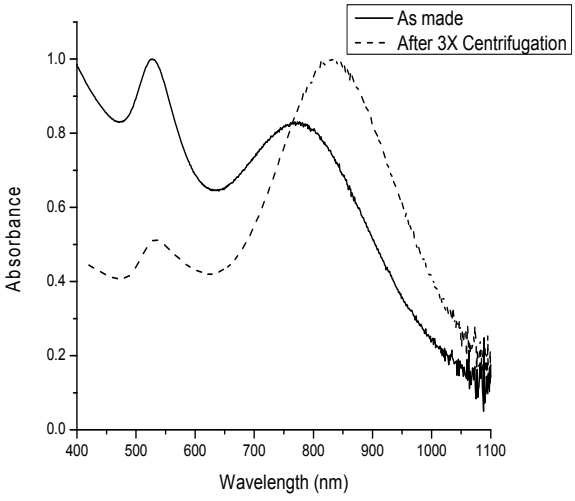


Figure 1: Normalized absorbance of GGS nanoparticles with traditional single step self-assembly process, solid-line represents particles as made with $R^{nIR/Au} = 0.83$, while the dashed-line is the particles after multi-step centrifugation with $R^{nIR/Au} = 1.95$.

### 3.2 DiaSynth

We modified the synthesis of GGS nanoparticles to use $Na_2S_2O_3$ as an alternative to Na2S, based on results published by Schwartzberg et al. in the context of equation (1).

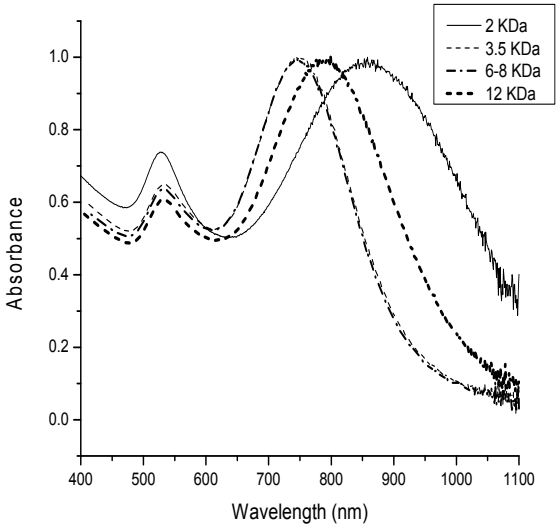


Figure 2: Normalized Spectral profiles after DiaSynth method using different MWCO membranes and fixed volume ratios of reactants: MWCO = 2KDa, 3.5KDa, 6-8KDa, and 12 KDa, resulting in $R^{nIR/Au}$ of 1.35, 1.54, 1.57, and 1.64, respectively.

Using dialysis during the production of GGS nanoparticles not only forces ion exchange through the dialysis membrane, but, as a consequence of the ion exchange the reaction has shifted to favor the formation of nIR absorbing particles within the dialysis tubing and formation of gold colloid outside. Several dialysis MWCO membranes were used including: 2KDa, 3.5KDa, 6-8KDa, and 12KDa, samples were then reacted for 1 hr in 8 L of DI water at RT. From the spectral profile, given in Figure 2, dialysis with a 12KDa MWCO dialysis membrane shows the highest quality ratio.

Next, the experiment was repeated using a 12 MWCO dialysis membrane, varying the amount of $Na_2S_2O_3$ added. As evidenced in Figure 3, using the 2.5 mLs of $Na_2S_2O_3$, further improved the quality ratio, compared to the one in Figure 2. These results indicate the larger pore sizes of the membrane facilitate the diffusion and removal of certain species, during the reaction, which favors the production of nIR absorbing particles within the membrane. Additionally, increasing $HAuCl_4/Na_2S_2O_3$ ratio shows an increase in purity at a fixed pore size.

## 4. CONCLUSION

The DiaSynth method can increase the quality of the synthesis of GGS nanoparticles to a ratio of 1.7 – 2.0 or greater, significantly improved from the quality ratios realized during centrifugation. During the synthesis of GGS nanoparticles, colloidal gold has been the predominant by-product, and it must be removed for therapeutic applications.

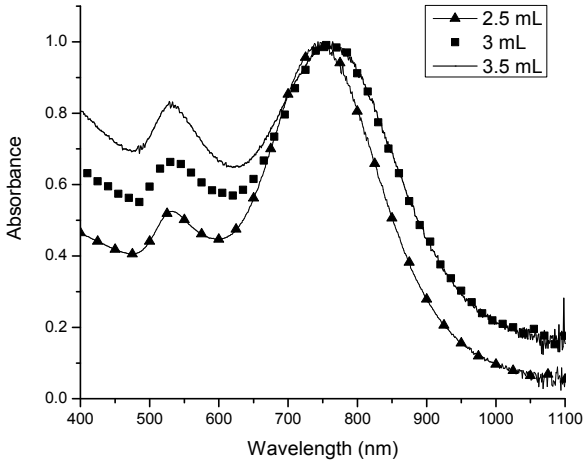


Figure 3: Using the 12KDa MWCO membrane the experiment was repeated with HAuCl4 constant at 11 mL and varying ratios of $Na_2S_2O_3$, 2.5, 3, and 3.5 mLs. From the normalized graph using 2.5 mL the particles yield in a 1.93 ratio of the as made product.

In this work, we have demonstrated that nIR absorbing GGS nanoparticles can be synthesized, with high purity and without centrifugation, Figure 4. Not only is this critical on minimizing the particles being delivered, to the body, but DiaSynth process is more efficient and economical.

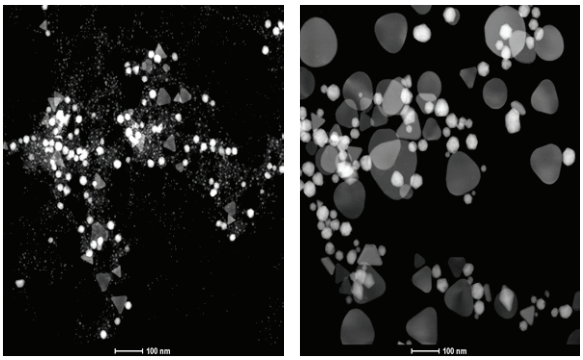

Figure 4: STEM of as made particles (left) without centrifugation, and DiaSynth method (right).

The use of $Na_2S_2O_3$ as an alternative to $Na_2S$ has allowed a robust process to be developed where the nIR-absorbing peak can be precisely controlled. By varying the volumes of reactants, this nIR peak can be reproduced time and again with great accuracy and precision (+/- 5 nm), in direct contrast with the method used where the aging of $Na_2S$ had to be within a precise range, and still resulted in particles with nIR peaks that could vary as much as +/- 50 nm each time. In addition, the centrifugation process selects various components of the population, thus resulting in a shift of the peak absorbance wavelength of the final product may be away from the desired peak for the application.

Detailed analysis of the dialysate and product must be performed to understand the exact mechanism leading to increased yields, high quality ratio. However, this process represents a dramatic shift in production and scale up strategy, which could enable wider use of these nIR-absorbing particles in a variety of applications.

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