Glucose- and Citrate-capping in Plasma/Liquid Interaction Gold Nanoparticle Synthesis

Willis O'Leary[1]
[1]Department of Chemical Engineering, California Institute of Technology

ABSTRACT. Gold nanoparticles (AuNPs) can selectively deliver the bulky, water insoluble photosensitizer drugs central to photodynamic therapy into cancer cells. By exploiting the reducing environment available at a plasma/liquid interface, we synthesized monodisperse 30 nm AuNPs suitable for this application. In preliminary experiments, we found that capping agents were necessary to achieve monodisperse size distributions. Afterwards, we evaluated glucose and citrate as potential capping agents. At excess concentrations, glucose was an effective capping agent, but glucose-capped AuNPs quickly aggregated and precipitated. Citrate was an effective capping agent at lower concentrations, but citrate-capped AuNPs also aggregated and precipitated. In addition, the citrate, rather than the plasma, largely drove reduction processes. Synthesizing AuNPs at a 2:1 ratio of citrate and glucose produced high-quality, 30 nm AuNPs that were remarkably stable at room temperature. Future work will involve screening additional capping agents. With a database linking capping agents to particle size, plasma/liquid interaction syntheses could be utilized to produce AuNPs with specific sizes that are suitable for a variety of drug delivery applications.

INTRODUCTION

Gold nanoparticles (AuNPs) show promise as a drug delivery vehicle for photodynamic cancer therapy. By attaching a photosensitizer (PS) drug, such as pheophorbide a, to the outside of AuNPs, the drug can be delivered into tumor cells. When these cells are exposed to visible light, the intracellular PS drug molecules catalyze the formation of oxygen radicals. These radicals tear apart the cancer cells' DNA and induce apoptosis [1].

After introduction into the bloodstream, PS drugs attached to AuNPs have much higher specificity than PS drugs alone. This is because attaching PS drugs, which are typically water insoluble, to AuNPs, which are water soluble, discourages the accumulation of PS drugs in healthy tissue. In addition, supporting functionalities can be attached to the AuNPs via gold-thiol chemistry. A previous study attached an exciting functionality that only armed the PS drug in the abnormally high intracellular antioxidant concentrations characteristic of cancer cells [2]. This functionality further protected normal tissue and increased the effectiveness of photodynamic therapy.

We sought to synthesize AuNPs for use in photodynamic therapy. The ideal size of such AuNPs is an active area of research, so we aimed for a particle diameter of 20-40 nm in accordance with a previous study [2]. Furthermore, we aimed for a monodisperse particle size distribution since particle size heavily affects drug uptake. To make these AuNPs, we used plasma/liquid interaction (PLI) synthesis. This synthesis is driven by a plasma struck over a gold salt solution, in our case aqueous $HAuCl_4$. Free electrons in the plasma accelerate towards the liquid through the plasma sheath and enter solution. In solution, these electrons, as well as H_2O_2 produced by the interaction between water and UV radiation, reduce gold cations. The neutral gold atoms then aggregate to form a suspension of AuNPs.

During our efforts, we evaluated the use of two capping agents – D-glucose and citrate – in PLI AuNP synthesis. Capping agents coat the outside of AuNPs are thus used synthetically to control particle growth and size. As expected, use of these capping agents had marked effects on particle size, size distribution, and aging.

EXPERIMENTAL METHODS

PLI Reactor Setup

Our PLI reactor (FIGURE 1) utilized a micro-hallow cathode plasma discharge between a stainless-steel capillary tube (the cathode) hovering over the surface of a $HAuCl_4$ solution (the anode). The capillary tube had an inner diameter

0.007" and an outer diameter of 1/16". We supplied the voltage difference between the cathode and anode with a Matsusada Precision high voltage power supply (model AU-5R60) set at 0.96 kV. We grounded the liquid through an immersed graphite electrode. We measured the sustaining voltage (the voltage across the plasma) using a Cen-Tech digital multimeter (item 90899) with leads connected across the capillary tube and the graphite electrode. We measured the plasma current from the read-out of the high voltage power supply. For safety reasons, we placed a 50 kΩ resistor between the power supply and the capillary tube. That way, the current never exceeded 15 mA.

We flowed argon gas through the capillary tube. We controlled the gas flow using a Unit Instruments mass flow controller (model UFC-1100) connected to an MKS Type 247 mass flow controller power supply. We calibrated the read-out of the mass flow controller, displayed by its power supply, to the actual argon volumetric flow rate using a soap-film flowmeter.

We controlled the distance between the cathode and the solution surface using a laboratory jack, upon which the solution container sat. Rather than measuring the distance at the beginning of each experiment, we adjusted the distance on-the-fly to produce a target voltage and current. By maintaining a pre-chosen voltage and current, we could easily and consistently maintain a roughly 3 mm cathode/liquid distance throughout all our experiments.

During our experiments, we used two different solution containers. We began with a 50 mL beaker containing a large stir bar, which forced us to use 50 mL of solution per experiment. However, due to concerns with surface mixing and, to a lesser part, excessive waste, we began using the bottom half of a glass petri dish with a smaller stir bar. The petri dish setup only required 25 mL of solution per experiment.

UV-Vis Spectroscopy

We captured UV-Vis spectra with a Vernier SpectroVis Plus USB Spectrophotometer, which uses 5 mL plastic cuvettes to hold solutions. We chose this spectrophotometer for its ease of use and long path length. We recorded and viewed the spectra with Vernier's Logger *Pro* 3 software. We subtracted a reference from all spectra reported. For all experiments, the reference solution was simply the solution before the synthesis. We took spectra at the end of each synthesis.

Electron Microscopy

We captured an electron micrograph using a ZEISS 1550VP field emission scanning electron microscope operating at a voltage of 10.00 kV.

Solution Preparation

All our solutions were aqueous. We used $HAuCl_4$ as the Au(III) source. We made our solutions by diluting a concentrated $HAuCl_4$ solution from Sigma Aldrich (484385 ALDRICH). Sigma Aldrich's $HAuCl_4$ solution contained dilute HCl, but the precise concentration was unspecified. However, even after heavy dilution, our prepared solutions still contained enough HCl to be conductive. We also prepared stock solutions of D-glucose and sodium citrate by dissolving solid crystals of these two capping agents in water.

PLI Synthesis Without Capping Agent

We performed a PLI synthesis without capping agent in a 50 mL beaker. The gold solution had an initial Au(III) concentration of 5.0 mM. We carried out the synthesis at a sustaining voltage of 420 ± 30 V, a current of 10 ± 1 mA, a stir rate of 200 rpm, and an argon flow rate of 19.0 ccm. We exposed the solution to plasma for 10 minutes. We took a UV-Vis spectrum at the end of the synthesis.

PLI Synthesis with Glucose Capping

We performed a PLI synthesis with glucose capping in both a 50 mL beaker and a 25 mL petri dish. In both experiments, the gold solution had an initial Au(III) concentration of 3.0 mM and an initial glucose concentration of 24.0 mM. We carried out the synthesis at a sustaining voltage of 500 ± 20 V, a current of 9.0 ± 0.5 mA, a stir rate of 400 rpm, and an argon flow rate of 20.0 ccm. In the petri dish setup, we exposed the solution to plasma for only three

minutes. In the beaker setup, we exposed the solution to plasma for five minutes to roughly account for the larger solution volume. We took UV-Vis spectra at the end of each synthesis.

PLI Synthesis with Citrate Capping

We performed three PLI syntheses with citrate capping in a 25 mL petri dish. All solutions had an initial Au(III) concentration of 3.0 mM. We used three different citrate concentrations: 3.0 mM, 4.0 mM, and 6.0 mM. We carried out all syntheses at a sustaining voltage of 500 ± 20 V, a current of 9.0 ± 0.5 mA, a stir rate of 400 rpm, and an argon flow rate of 20.0 ccm. We exposed each solution to plasma for three minutes. We took a UV-Vis spectrum at the end of each synthesis.

We left the 6.0 mM citrate-capped AuNPs at room temperature for 14 days, at which point the solution had separated into pink and orange/brown layers.

Citrate Reduction Synthesis

We prepared a solution with an Au(III) concentration of 3.0 mM and a citrate concentration of 6.0 mM. We left the solution to sit at room temperature for two days, at which point it turned dark purple with an orange tint. We took a UV-Vis spectrum of the final solution diluted five times. We multiplied the recorded absorbance by five to attain the true absorbance, in accordance with Beer's law.

We also took a UV-Vis spectrum of 30 nm AuNPs produced by Sigma Aldrich (741973 ALDRICH). We scaled the absorbance peak of this spectrum so we could meaningfully compare size distributions between our own citrate-synthesized AuNPs and those produced by a chemical company.

PLI Synthesis with Citrate/Glucose Capping

We performed four PLI syntheses with citrate/glucose capping in a 25 mL petri dish. All gold solutions had an Au(III) concentration of 3.0 mM. We tried four citrate/glucose concentration combinations: 3.0 mM/3.0 mM, 6.0 mM/6.0 mM, 3.0 mM/6.0 mM, and 6.0 mM/3.0 mM. We carried out all syntheses at a sustaining voltage of 500 ± 20 V, a current of 9.0 ± 0.5 mA, a stir rate of 400 rpm, and an argon flow rate of 20.0 ccm. We exposed each solution to plasma for three minutes. We took UV-Vis spectra at the end of each synthesis. After seven days, all solutions precipitated except the 6.0 mM/3.0 mM solution, which had turned a very dark purple color. We took a UV-Vis spectrum of this purple solution diluted five times. We multiplied the recorded absorbance by five to attain the true absorbance, in accordance with Beer's law.

RESULTS & DISCUSSION

PLI Synthesis Without Capping Agent

We carried out a PLI synthesis without any capping agent and took a UV-Vis spectrum of the product (FIGURE 2). The reaction solution went from bright yellow, indicative of gold chloride, to a cloudy orange color. The resulting spectrum was extremely broad, indicating an extremely wide spread of particle sizes. The absorption peak was centered around 650 nm, indicating a median particle size exceeding 100 nm, far afield from our target of 20-40 nm [3].

In the absence of capping agent, there was little to inhibit particle growth. Thus, small and large AuNPs grew at comparable rates, resulting in a broad distribution of large particles. The AuNPs aggregated and precipitated within two days. Without the protection afforded by caps, incident AuNPs had a strong affinity for one another, leading to a short shelf-life.

PLI Synthesis with Glucose Capping

We carried out two near-identical PLI syntheses using glucose as a capping agent. The only major difference was choice of solution container. In one synthesis, we used a 50 mL beaker, while in the other we used a 25 mL petri dish.

We recorded UV-Vis spectra at the end of each synthesis (FIGURE 3a). The product of the petri dish synthesis appeared nearly identical to that synthesized without a capping agent. The solution turned a hazy orange. However, the spectrum was far broader and was centered at 547 nm, indicative of 70 nm particles [3]. The product from the beaker synthesis was much different. This solution turning a pinkish red color and possessed a sharp, well-defined absorbance peak at 537 nm, indicative of 50 nm particles [3].

The unexpected difference between syntheses in the two different containers stemmed from the poor mixing characteristics of the beaker. In the beaker synthesis, we observed a substantial amount of gold foil floating on top of the solution. We saw no gold foil in the petri dish following the synthesis. In the deeper beaker, surface mixing was likely not vigorous enough to carry nucleated nanoparticles down into solution, leading much of the reduced gold to aggregate into floating films. The formation of gold foil produced a drop in overall gold concentration. This drop, however, led to higher quality particles. Therefore, we conclude that glucose works well as a capping agent in PLI synthesis only at very low [Au(III)]/[glucose] ratios.

Considering glucose's probable binding mode to AuNPs (FIGURE 3b), the requirement of a low [Au(III)]/[glucose] ratio makes sense. Glucose binds to AuNP surfaces, which are slightly positive, through lone pairs on its hydroxyl oxygens. This binding is weak, so at equilibrium excess glucose concentrations are required to form robust glucose caps.

Aging of Glucose-Capped AuNPs

The glucose-capped AuNPs synthesized through PLI crashed out of solution within two days, indicating that glucose-caps are largely ineffective at preventing AuNP aggregation. This can be rationalized by examining glucose's binding mode to AuNPs (FIGURE 3b). A few of glucose's hydroxyl groups likely participate in the binding to the particle surface. However, the remaining hydroxyl groups point away from the particle surface at various orientations. This gives glucose caps a strong tendency to form hydrogen bonds with other glucose caps. Since glucose binds relatively weakly to gold, AuNP caps can easily bind then deform, letting two nearby AuNPs make contact to form a single, larger particle. This mechanism explains why the glucose-capped AuNPs aggregated and precipitated within two days.

PLI Synthesis with Citrate Capping

We carried out a PLI synthesis using 6.0 mM citrate as a capping agent in a 25 mL petri dish. We abandoned the beaker setup due to poor surface mixing. We recorded UV-Vis spectra at the end of the synthesis and found that citrate served as an excellent capping agent (FIGURE 4a). The citrate-capped AuNPs absorbed moderately sharply at 530 nm, indicative of reasonably monodisperse 40 nm AuNPs [3]. This size was just within our target of 20-40 nm. The efficacy of citrate, even at a [Au(III)]/[citrate] ratio of 0.5, can be explained by citrate's anionic charge, while allows citrate to strongly bind to gold surfaces.

We also found that, unlike glucose, citrate appreciably reduces Au(III) at room temperature. After two days (without any plasma exposure), a gold and citrate solution identical to the one above yielded a suspension of exceptionally monodisperse 30 nm particles at high yield, though the size distribution was still much wider than particles produced by Sigma Aldrich (FIGURE 4b) [3].

We performed two additional PLI syntheses at lower citrate concentrations to ascertain the degree to which citrate is responsible for the reduction of Au(III). We found that the UV-Vis peak absorption, which relates directly to particle yield, decreased with decreasing citrate concentration (FIGURE 4a). The decrease in yield indicates that citrate plays a large role in reduction during the PLI synthesis. The small region of solution directly under the plasma hotter than the bulk solution, so it is likely in this region where citrate does most reduction. Thus, using citrate (or any other reducing species) as a capping agent in PLI synthesis presents additional complications and somewhat defeats the purpose of using a plasma to reduce Au(III).

Aging of Citrate-Capped AuNPs

After 2 days, the PLI-synthesized citrate-capped AuNPs turned from a light purple color to a much deeper purple color with an orange tint. The deeper color was a result of additional citrate reduction and AuNP formation. After 14 days, these particles had further aggregated and separated into a pink layer on top of an orange/brown layer. This indicates

that the orange tint was evidence of particle aggregation. Aggregation of citrate-capped AuNPs is likely facilitated through hydrogen bonding between molecules in caps (FIGURE 4c). Given citrate's probable binding mode, however, fewer hydrogen bonds form between citrate caps than between glucose caps [4]. This structural difference explains why the citrate-capped AuNPs aggregated and precipitated much less than the glucose-capped AuNPs.

Aging of Citrate/Glucose-Capped AuNPs

We carried out multiple PLI syntheses using various mixtures of citrate and glucose in a 25 mL petri dish. UV-Vis spectra were taken immediately after each synthesis. These spectra were very like those observed with citrate-capping alone. Immediately after synthesis, the 2:1 citrate/glucose-capped AuNPs absorbed at 536 nm, indicative of 50 nm particles (FIGURE 5a) [3]. These observations agree with our hypothesis that glucose capping is relatively unimportant at appreciable [Au(III)]/[glucose] ratios.

However, the ratio of citrate to glucose strongly affected how the citrate/glucose-capped AuNPs aged. AuNPs made with a 2:1 citrate/glucose ratio did not aggregate or precipitate appreciably. After seven days, this sample's absorbance increased dramatically due to citrate reduction, and the peak shifted to 527 nm, indicative of 30 nm AuNPs [3]. The resulting particles were well within our target size of 20-40 nm (FIGURE 5a). In addition, scanning electron microscopy imaging confirmed that the particles were very nearly spherical (FIGURE 5b). Meanwhile, AuNPs made with a 1:1 and a 1:2 citrate/glucose ratio aggregated and precipitated, meeting the same fate as glucose-capped AuNPs.

We rationalize these results by considering the citrate/glucose ratio within the AuNP caps. Too much glucose in solution implies too much glucose in the AuNP caps. This likely led to aggregation though the same mechanism discussed for glucose-capped AuNPs. However, less glucose in solution implies less glucose in AuNP caps. At the 2:1 glucose/citrate ratio, apparently, there was just enough glucose to inhibit aggregation. The glucose present in the caps may have inhibited hydrogen bonding between the caps. It may have also changed the binding mode of citrate, making the citrates attached to two nearby AuNPs less inclined to hydrogen bond to one another.

CONCLUSION

We made uncapped, glucose-capped, citrate-capped, and glucose/citrate-capped AuNPs using a PLI approach. We characterized the synthesized AuNPs with UV-Vis spectroscopy. Our highest quality AuNPs were capped with a 2:1 mixture of citrate and glucose. These monodisperse 30 nm particles were a suitable size and shape for drug delivery in photodynamic therapy. Moreover, mixing the capping agents in a 2:1 ratio prevented the particles from aggregating and precipitating after seven days of room temperature storage.

We conclude that capping agents are required to synthesize small, monodisperse AuNPs using PLI. Glucose is an effective capping agent only at low [Au(III)]/[glucose] concentrations, since glucose binds very weakly to AuNP surfaces. Citrate, an anion, is an effective capping agent at higher [Au(III)]/[citrate] ratios, but the use of citrate changes the primary mechanism of gold reduction in the PLI approach. The plasma's heat can drive the reduction of Au(III) by citrate.

Uncapped AuNPs quickly aggregate and precipitate. Glucose-capped AuNPs meet the same fate due to hydrogen bonding between glucose caps. Citrate-capped AuNPs aggregate and precipitate as well, though not as dramatically due to weaker hydrogen bonding between citrate caps. We found the 2:1 citrate/glucose capped AuNPs to be unusually stable, likely due to glucose's interference in hydrogen bonding between citrate caps.

In the future, we first wish to use electron microscopy to verify our particle sizes, shapes, and size distributions. Second, we wish to further characterize the binding modes of citrate, glucose, and citrate/glucose caps. With this information, we can better understand why 2:1 citrate/glucose capping is so effective. Third, we wish to screen additional capping agents, particularly non-reducing agents with few hydrogen bonding sites, and then build a database relating capping agent and particle size. With such a database, we could use PLI syntheses to quickly and easily synthesize monodisperse AuNPs of any size for advanced drug delivery applications.

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FIGURES

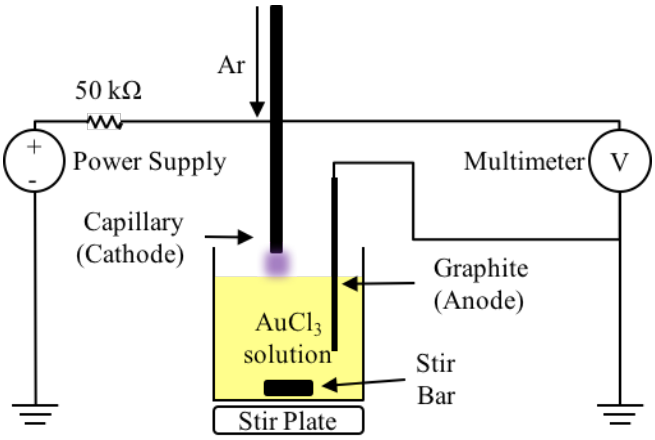

FIGURE 1. Functional schematic of our PLI reactor, which utilizes a micro-hallow cathode discharge argon plasma struck between a capillary tube and an $HAuCl_4$ solution.

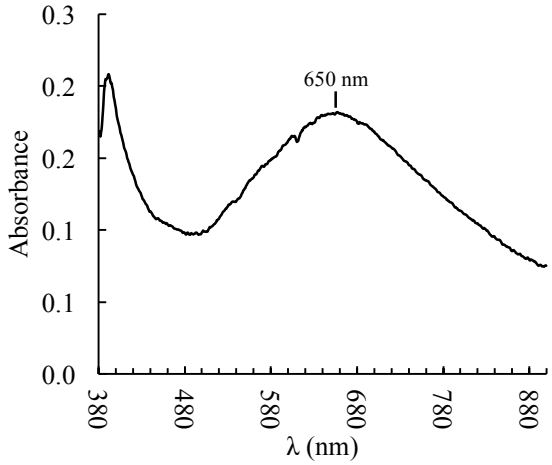

FIGURE 2. UV-Vis spectrum of PLI-synthesized AuNPs without capping agent. The peak is extremely broad and centered at 650 nm, indicating a median particle size larger than 100 nm.

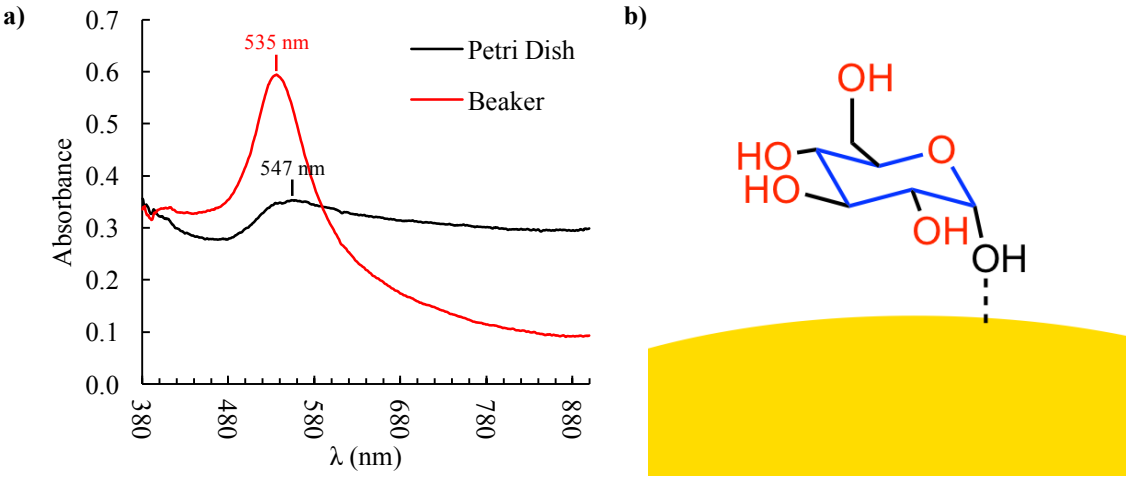

FIGURE 3. a) UV-Vis spectra of PLI-synthesized, glucose-capped AuNPs synthesized using two different reaction containers. The petri-dish synthesis gave a sharp, monodisperse peak at 536 nm, indicating a particle size of 50 nm, while the beaker synthesis resulted in a much broader peak. **b)** Probable binding mode of glucose to an AuNP surface, with available H-bond sites shaded in red.

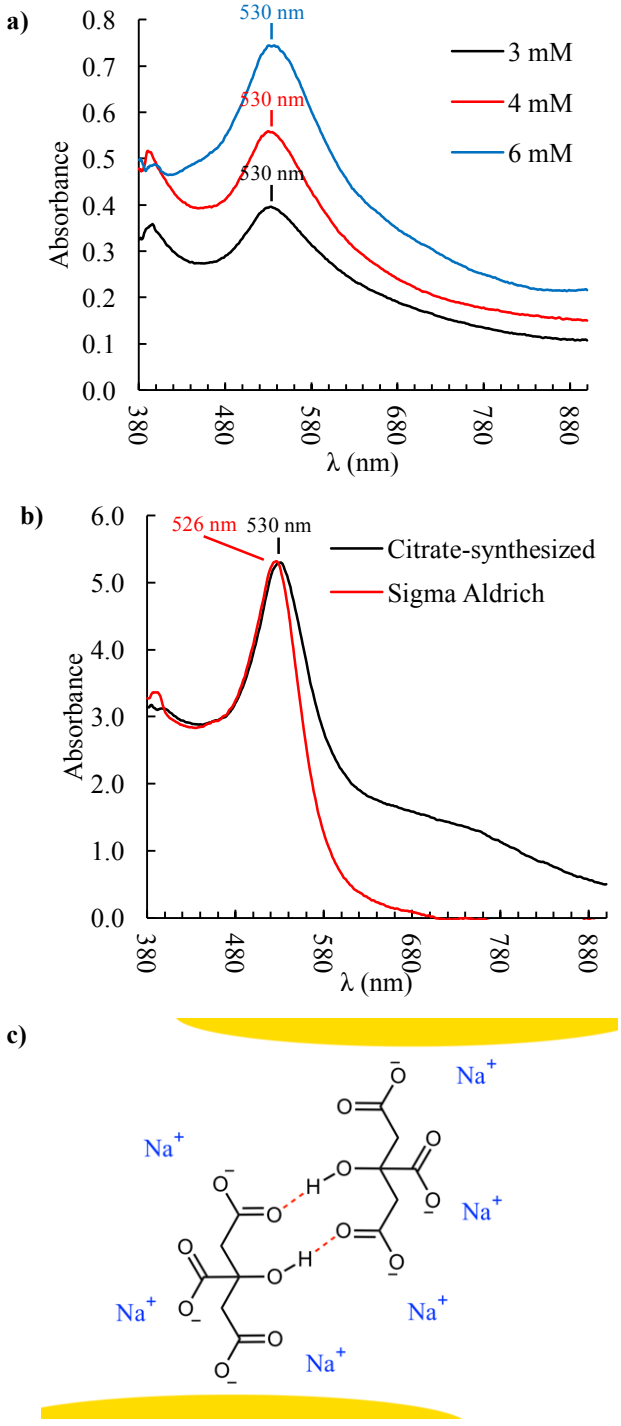

FIGURE 4. a) UV-Vis spectra of PLI-synthesized, citrate-capped AuNPs synthesized using various citrate concentrations. Higher citrate concentration resulted in higher absorption for the same exposure time. **b)** UV-Vis spectra of citrate-synthesized, citrate-capped AuNPs compared to those made by Sigma-Aldrich. **c)** Probable binding mode between two citrate caps with H-bonds shaded red.

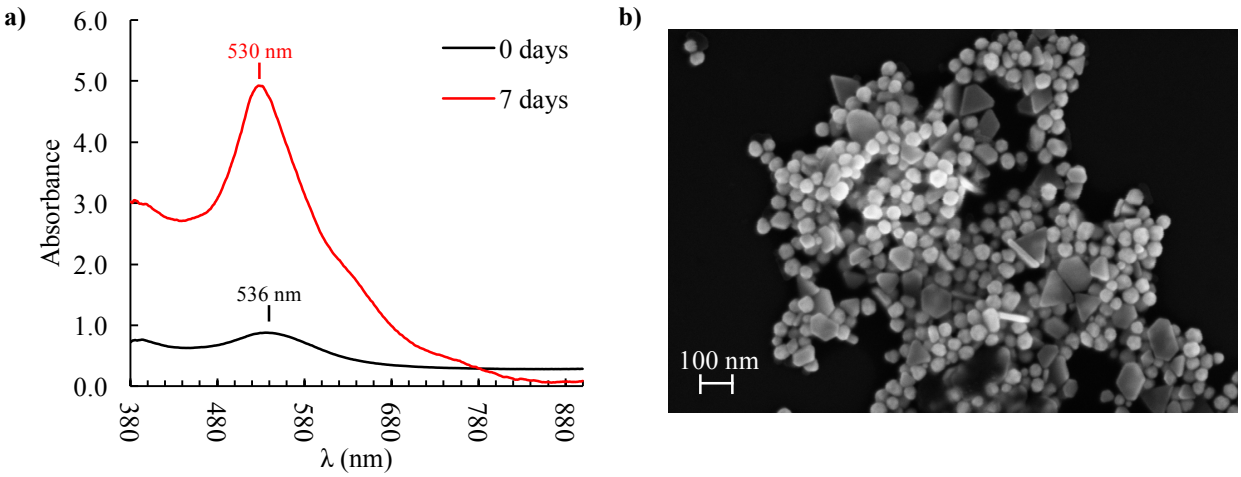

FIGURE 5. a) UV-Vis spectra of PLI-synthesized, 2:1 citrate/glucose-capped AuNPs directly after plasma exposure (zero days) and 7 days after plasma exposure. **b)** Electron micrograph of PLI-synthesized, 2:1 citrate/glucose-capped AuNPs 7 days after plasma exposure. Spherical AuNPs and irregular salt crystals are clearly visible.