

**Research Article** 

# Optimizing a Novel Method for Synthesizing Gold Nanoparticles: Biophysical Studies

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### Abstract

**Background:** The properties of Gold Nanoparticles (GNPs) make them useful for cancer therapy, diagnostics and imaging. For the application of GNPs in therapy and drug delivery there is a great necessity to synthesize known particle size of GNPs with simple methods. This study aimed to optimize a novel simple method for synthesizing GNPs.

**Methods:** GNPs were synthesized by using different citrate concentrations (40, 100 and 150 mM) through reduction of hydrogen tetrachloroaurate (HAuCl<sub>4</sub>) of varying concentrations. The synthesized GNPs were characterized with transmission electron microscope, UV-Vis spectrophotometer, and size distributions with dynamic light scattering.

**Results:** At 40 mM citrate concentration, different particle sizes (18-28 nm), non-round and not homogenous GNPs were formed; at 100 mM citrate concentration, more round GNPs were formed and the particle size reduced to nearly 12 nm with very narrow particle size distribution; at 150 mM citrate concentration, the GNPs size increased up to 18 nm and the homogeneity of the particle size distribution reduced as indicted from both Transmission Electron Microscopy (TEM) image and distribution profile. When HAuCl<sub>4</sub> concentration increased from 1 to 4 mM, the particle size of GNPs increased from 12 nm to nearly 20 nm, respectively.

**Conclusions:** At 40 mM citrate concentration, the coverage of citrate is incomplete and the aggregation process leads to the formation of inhomogenous GNPs; at 100 mM citrate concentrations, the citrate behaves as a pH mediator explains the reduction of the particle size. The increase of HAuCl<sub>4</sub> concentration causes retardation of the nucleation step forming lower number of nuclei in the solution and thus increases the final particle size and polydispersity.

**Keywords:** Gold nanoparticles; Colloids; Hydrogen tetrachloroaurate; Synthesis mechanism; Sodium hydroxide

### Introduction

Gold in its bulk form has been considered an inert, noble metal with some therapeutic and medicinal value. The GNPs have found widespread applications in life sciences and attracted significant research interest. The GNPs have been widely exploited for use in Surface Enhanced Raman Scattering (SERS) [1], imaging [2], biological applications [3] and photothermal effects [4].

There are many physical and chemical processes for synthetizing GNPs. The extensively used procedures to synthesize GNPs in aqueous solutions are the reduction of ionic gold by adding the sodium citrate, sodium borohydride, ascorbic acid and tannic acid [5].

The synthesis of GNPs without reducing agents or templates has induced exciting interest for material and chemical researchers. The synthesis of extremely stable size-controlled GNPs without the use of any reducing agents in the presence of Polyvinylpyrrolidone (PVP) has been reported [6].

Polymers and/or ionic surfactants are used as capping agents in aqueous-solution systems to prepare the nanomaterials. Cationic surfactants such as Hexadecyl trimethyl ammonium bromide (CTAB) are usually used in aqueous solution to synthesize anisotropic GNPs [7,8].

Abdelhalim, 2011 and 2012 [9-11], Abdelhalim and Jarrar, 2011 and 2012 [12-16] have reported the side effects, bioaccumulation and toxicity induced by intraperitoneal administration of GNPs in several rat tissues *in vivo*.

In order to understand and categorize the mechanisms of NPs toxicity, information is needed on the response of living systems to the presence of NPs of varying size, shape, surface and bulk chemical composition. Very little information on these aspects is presently available and this implies an urgent need to find novel simple methods for synthesizing GNPs.

It is important to synthesize and optimize novel and simple high quality GNPs. In certain applications, it is also important to prepare GNPs of high concentrations. Therefore, the goal of this work is to develop a new approach to synthesize GNPs of high quality and high concentration that are appropriate for preparation of GNPs conjugates [17].

In this study, the GNPs will be synthesized and optimized by adding different citrate concentrations to hydrogen tetrachloroaurate  $(HAuCl_4)$  of varying concentrations. The synthesized GNPs will be characterized with TEM and UV-Vis spectrophotometer while the

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GNPs particle size distributions will be characterized by the dynamic light scattering.

# Synthesizing Gold Nanoparticles (GNPs)

The GNPs were prepared by citrate reduction of  $\mathrm{HAuCl}_4$  [18]. An aqueous solution of  $\mathrm{HAuCl}_4$  (100 mL: 1 and 4 mM) was refluxed for 5-10 min and warmed at (50 - 60°C) aqueous solution of sodium citrate (10 mL: 40, 100 and 150 mM) was added quickly. Reflux was continued for another 30 min until a deep red solution was observed. The solution was filtered through 0.45  $\mu\mathrm{m}$  Millipore syringe filters to remove any precipitates. The pH was adjusted to 7 using dilute NaOH solution, and the filtrate was stored at room temperature [19]. The solutions were induced dark red color with a maximum of absorbance centered at 520 nm.

# Characterization of Gold Nanoparticles (GNPs)

The synthesized GNPs were characterized with transmission electron microscopy (TEM: JEOL JSM-2100F electron microscope operated at 200 kV) and UV-Vis spectrophotometer (Spectro UVD3500: SPECTRO UV-VIS DOUBLE BEAM RESEARCH SPECTROPHOTOMETER VARIABLE BANDWIDTH; LABOMED, INC. USA). GNPs size distributions were measured by dynamic light scattering (Malvern Nanosizer ZS instrument).

# **Results and Discussions**

### Effect of sodium citrate concentration

TEM images in Figure 1 show the formation of GNPs at different sodium citrate concentrations. It is clear in this study that at low citrate concentration (40 mM), different particles size and non-round GNPs were formed. The particle size was ranged from 18-28 nm and not homogenous as shown in the particle size distribution (Figure 1a). Addition of 100 mM citrate solution resulted in gold formation of more round GNPs. The particle size was reduced to be around 12 nm with very narrow particle size distribution (Figure 1b). Further increase of citrate solution to 150 mM caused the GNPs to increase in size up to 18 nm. However, the homogeneity of the particle size distribution was reduced as indicted from both TEM image (Figure 1c) and distribution profile (inset).

In the preparation of GNPs by citrate reduction, citrate acts as both reducing and stabilizing agent. The adsorption of citrate on the particles as a stabilizer significantly affects the particle size band. The role of citrate increases the complexity of the particle preparation process. At lower citrate concentration (40 mM), the coverage of citrate is incomplete and the aggregation process leads to form inhomogenous GNPs. On the other hand, at high citrate concentrations (100 and 150 mM), the behavior of citrate as a pH mediator explains the tendency of stabile particle size.

Citrate is known as a weak base which can change the solution pH to a certain extent with its concentration. The pH of the solution increases as the citrate concentration increases. Subsequently, the less reactive complexes  $[AuCl_2(OH)_2]^-$  and  $[AuCl(OH)_3]^-$  are formed by the hydrolysis of  $AuCl_4^-$ , consequently, the nucleation rate becomes slower and the amount of Au precursor remaining in the solution becomes larger. The final particle size is determined by the diffusional growth of Au precursor on the surface of Au nuclei. It has been reported that using an excessive amount of citrate, the final particle size increases a little [20].



**Figure 1:** TEM images of gold nanoparticles (GNPs) at sodium citrate concentrations of (a) 40, (b) 100, and (c) 150 mM and 1 mM HAuCl<sub>4</sub>, (inset) their corresponding particle size distribution.



Figure 2: TEM images of gold nanoparticles (GNPs) at  $HAuCl_4$  of (a) 1 and (b) 4 mM and 100 mM sodium citrate, (inset) their corresponding particle size distribution.

# Effect of HAuCl<sub>4</sub> concentration

Variation of HAuCl<sub>4</sub> concentration was also significant in tuning the particle size and polydispersity of GNPs. The increase of HAuCl<sub>4</sub> from 1 to 4 mM (Figure 2: 2a and 2b, respectively), caused growth of particle size of GNPs from 12 nm to nearly about 20 nm, respectively. On other hand, the particle size distribution tended to become broader with increasing HAuCl<sub>4</sub> concentration which means more polydisperse particles are formed.

The increase of  $HAuCl_4$  concentration means decrease in the corresponding amount of citrate (reducing agent) which in turn might cause the retardation of the nucleation step and the reduction in the number of nuclei in the solution resulting in an increase in the final particle size and the polydispersity [21].

The synthesized GNPs are further characterized by UV-Vis spectrophotometer at the room temperature. Table 1 shows UV–VIS spectrum for different Au (1 and 4 mM) and citrate (40, 100 and 150 mM) concentrations. Table 1 indicates that when Au increases from 1 mM (at citrate concentration: 40, 100 and 150 mM)) to 4 mM (at citrate concentration 100 mM)), the peak shifted from 527, 523 and 523 to 530 nm, respectively. Table 1 also shows a typical surface absorption band ( $\lambda_{max}$ ) at about 523, 527 and 530 nm for GNPs dependent on Au and citrate concentrations. Because of the special shape and structure of GNPs, it often exhibited distinctive SPR peaks in the UV-Visible region, which was coincident with the plasmon band of the spherical GNPs [22,23] (Figure 3 and 4).

Indeed, it is well established that at 100 mM citrate concentration and with increasing Au from 1 to 4 mM concentration, the band width increased from 110 to 190 accompanied with a decrease in the absorbance from 0.60 to 0.28 and an increase in particle size of GNPs from 12 to 18 nm. Table 1 also indicates that at 100 mM citrate concentration and 4 mM Au concentration, 256, 530, 772 peaks were observed with 0.43, 0.28 and 0.29 absorbances and 50, 65 and 190 bandwidths, respectively.

The bandwidth is inversely proportional to the particle size smaller than about 20 nm and the plasmon absorption maximum is red-shifts with increasing particle size [22,23].

This study suggests that additional synthesizing methods of GNPs are needed without using reducing agents; such as the addition of different sodium hydroxide (NaOH) concentrations to  $HAuCl_4$  and Hexadecyl trimethyl ammonium bromide (CTAB). In addition to the comparison that must be done between both of the 2 methods. Moreover, additional experiments are now performed taken in

Concentration	Peak (nm)	Absorbance	Band width (nm)
1mM Au & 40 mM citrate	527	0.51	140
1mM Au & 100 mM citrate	523	0.6	110
1mM Au & 150 mM citrate	523	0.27	100
4mM Au & 100 mM citrate	256,530,772	0.43,0.28,0.29	50,65,190

 Table 1: UV–VIS spectrum for different Au and citrate concentrations.



Figure 3: The absorbance of synthesized gold nanoparticles at different citrate and gold concentrations against the wavelength (nm).



Figure 4: The absorbance of synthesized gold nanoparticles at different citrate and gold concentrations against the wavelength (nm).

consideration the use of GNPs as diagnostic and therapeutic tool for treatment of the cancer disease.

### Conclusions

This study aimed to optimize a novel simple method for synthesizing GNPs in an attempt to cover and understand the potential role of GNPs as a therapeutic and diagnostic tool.

The GNPs were synthesized and optimized by using different citrate and HAuCl<sub>4</sub> concentrations. At 40 mM citrate concentration, different particle sizes (18-28 nm), non-round and not homogenous GNPs were formed due to the incomplete citrate and the aggregation process leads to the formation of inhomogenous GNPs; at 100 mM citrate concentration, more round GNPs were formed and the particle size reduced to nearly 12 nm with very narrow particle size distribution whereas the citrate behaves as a pH mediator explaining the reduction of the particle size; at 150 mM citrate concentration, the GNPs size increased up to 18 nm and the homogeneity of the GNPs size distribution was reduced as indicted from both TEM image and distribution profile.

 $\mathrm{HAuCl}_4$  concentration was also significant in tuning the particle size and polydispersity of GNPs. When  $\mathrm{HAuCl}_4$  concentration increased from 1 to 4 mM, the particle size of GNPs increased from 12 nm to nearly 20 nm, respectively.

This study suggests the formation of less reactive complexes by hydrolysis of  $AuCl_4^-$ , consequently, the nucleation rate becomes slower and the amount of Au precursor remaining in the solution becomes larger. Because of using an excessive amount of citrate, the final particle size increases a little. The increase of HAuCl<sub>4</sub> concentration means decrease in the corresponding amount of citrate (reducing agent), resulting in an increase in the final GNPs size and the polydispersity.

#### **Competing Interests**

Author declares that he has no competing interests.

#### Author's Contributions

Mohamed Anwar K. Abdelhalim has analyzed data, interpreted and written the final draft of this manuscript. The animal model used in this study was obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University, Saudi Arabia). Abdelhalim MAK has conceived the study and its design and obtained research grants for this study. The authors have read and approved the final manuscript.

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