

A STUDY ON THE SYNTHESIS OF GOLD NANOPARTICLES USING WATER SOLUBLE CHITOSAN AS REDUCING/STABILIZING AGENT

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Received 25 March 2013

Abstract

Gold nanoparticles have been synthesized by utilizing water soluble chitosan (WSC) as reducing and stabilizing agent. The colloidal gold nanoparticles were characterized by UV-Vis spectroscopy and transmission electron microscopy (TEM). The results showed that the morphology of gold nanoparticles was spherical in shape with diameters of about 6-17 nm. The colloidal gold nanoparticles were stable and had a plasmon absorption band with maximum wavelength in the range of 520-526 nm. Effects of concentration of Au³⁺, concentration of water soluble chitosan on the size and plasmon absorption band were also investigated. The size of gold nanoparticles could be enlarged from 14 up to about 35 nm by using seed growth method.

Keywords: Gold nanoparticles, synthesis, water soluble chitosan, seed growth.

1. INTRODUCTION

The synthesis of noble metal nanoparticles, especially gold nanoparticles has been given much attention due to their diversified applications in many field include the catalysis field [1], cosmetics [1-3], electrochemistry [4], sensitive bio-sensor [5] and cancer diagnostic and therapy [6]. A number of different methods have been reported for the synthesis of gold nanoparticles (Au-NPs). Most common is chemical reduction [4-8] and irradiation method [2, 3].

Recently, some works have introduced a green method using natural polymers to synthesis Au-NPs for purpose of minimizing or eliminating pollution to the environment. Raveendran et al. [3] used β -D-glucose as a reducing agent and soluble starch as a stabilizing agent for synthesizing gold and silver nanoparticles. Vidneshwaran et al. [4] used soluble starch as a reducing and stabilizing agent for synthesizing silver nanoparticles. Pal et al. [5] prepared Au-NPs by UV photoactivation in the presence of sodium alginate as a stabilizer. Among natural polymers, chitosan is used widely because of its biocompatibility, biodegradability, nontoxicity, and adsorption properties [4]. However, common-used chitosan is insoluble in water and can only be dissolved in acid. Therefore, the applications of Au-

NPs solution are restricted.

In this work, the utilization of water soluble chitosan (denoted as WSC) instead of chitosan to synthesis gold nanoparticles was performed. The effects of concentration of Au³⁺ solution and WSC on the morphology as well as plasmon properties of Au-NPs were studied. The enlargement of particle size of Au-NPs by using the seed growth method was also discussed.

2. EXPERIMENTAL

2.1. Chemicals

Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄.3H₂O) (Merck) and water soluble chitosan (WSC) with molecular mass (M_w) $\sim 1.8 \times 10^4$ and degree of deacetylation (DDA) about 52 % was prepared by reacetylation of chitosan with acetic anhydride according to the process of Nguyen Ngoc Duy et al. [3] with some modifications. Briefly, 5 g chitosan was dissolved in 100 ml of 3% aqueous lactic acid solution, and then 50 ml ethanol was added to the chitosan solution while stirring. Reacetylation of chitosan was done by adding the mixture of 3 ml acetic anhydride in 60 ml ethanol into chitosan solution and stirred for 2 h. Then pH of the solution was adjusted

to neutral (pH 7) by 5 % NH₄OH solution. Reacetylated chitosan was precipitated by ethanol, filtered off, washed with ethanol several times and then dried at 50 °C in forced air oven to obtain WSC powder.

2.2. Methods

2.2.1. Preparation gold nanoparticles

In a typical experiment, 2.5 ml of 10 mM HAuCl₄ was added to 25 ml of 1 % (w/v) aqueous WSC solution, then the mixture was filled with distilled water to the final volume of 50 ml for obtaining solution of 0.5 mM Au³⁺/0.5 % WSC, stirring and heating the mixture at 85 °C. After the color of the suspension changed gradually from yellow to purple, the temperature of this mixture was maintained at 85 °C by using a thermoregulator for 8 hours.

2.2.2. Enlargement of seed particles

The obtained Au-NPs solution from 1mM Au³⁺ sample was used as seeds for further particles enlargement. The composition of solutions were prepared as following: one part of the 1 mM gold nanoparticles solution (Au⁰) was mixed with two, four, six, eight and ten parts of a solution containing 1 mM Au³⁺/0.5% WSC. The overall gold concentration ([Au⁰]+[Au³⁺]) in this mixture was remained again 1 mM, but the Au³⁺ concentration was from two to ten times larger than that of Au-NPs.

2.2.3. Characterization of Au-NPs

The absorption spectra of Au-NPs solutions were taken on an UV-vis spectrophotometer model UV-2401PC (Shimadzu, Japan). Morphology of obtained Au-NPs was observed by a transmission electron microscope (TEM), JEM1010. The size and size distribution calculated statistically from size of 200 particles which were estimated from TEM images.

2.2.4. Statistics analysis

To estimate the difference of particle size of Au-NPs prepared in various conditions one-way analysis of variance (ANOVA) was made. The statistics analysis was conducted using Statistical Package for Social Sciences version 19 (SPSS-19) with significance level of 0.001.

3. RESULTS AND DISCUSSION

3.1. Effect of Au³⁺ concentration on the size of Au-NPs

In this experiment, Au-NPs were prepared from samples containing 0.5 % (w/v) WSC with different Au³⁺ concentrations particularly 0.25, 0.5 and 1 mM. The TEM images of three colloidal Au-NPs samples are shown in Fig. 1. As can be seen, the morphology of obtained Au-NPs consisted of spherical nanoparticles with a high dispersity.

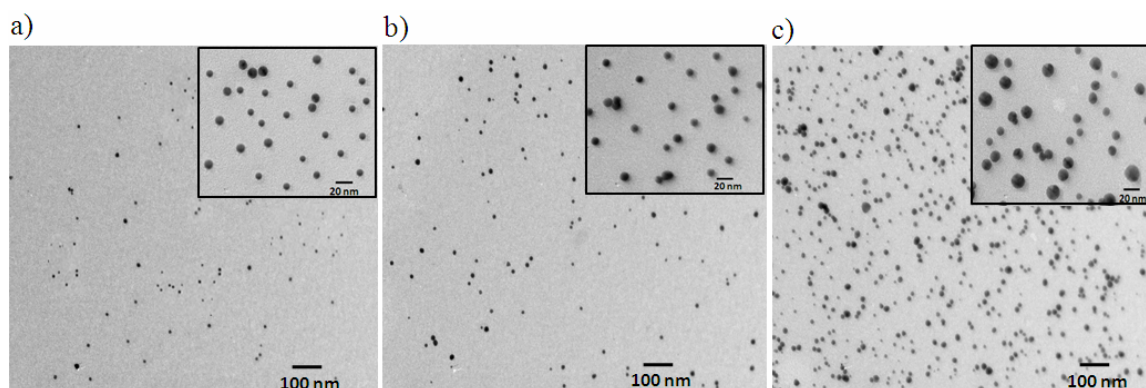


Fig. 1: TEM images of Au-NPs stabilized by 0.5% (w/v) WSC with Au³⁺ concentrations: 0.25 mM (a); 0.5 mM (b) and 1 mM (c)

Focusing on the size of Au-NPs produced by the process using three Au³⁺ concentrations, the one way-ANOVA analysis was made among all three groups. The descriptive statistics for the groups were: 0.25 mM Au³⁺ solution (0.25Au), M = 6.70 nm, sd = 2.20, n = 200; 0.5 mM Au³⁺ solution

(0.5Au), M = 8.76 nm, sd = 2.40, n = 200; 1 mM Au³⁺ solution (1Au), M = 13.87 nm, sd = 3.19, n = 200. It is clear that the mean size of Au-NPs increases with the increasing of the Au³⁺ concentration as shown in Fig. 2.

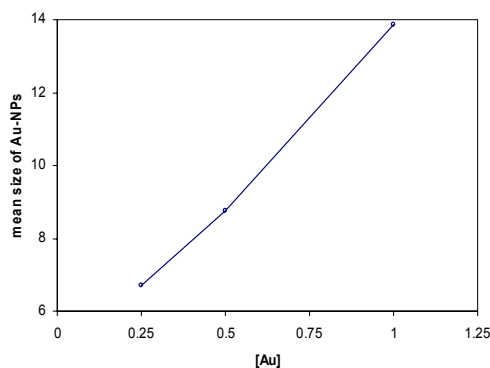


Fig. 2: Effect of Au^{3+} concentration on the mean size of Au-NPs

The one-way ANOVA comparison revealed the statistically difference among all three groups ($F(2) = 392.1, p < 0.001$). Since Levene test indicated a statistical difference of variances among groups by the Levene statistic = 35.17, $p < 0.001$, follow-up comparison using Tamhane's test found a statistical difference between the Au0.25 and Au0.5 (mean difference = -2.054, $p < 0.001$) or between the Au0.5 and Au1 (mean difference = 2.054, $p < 0.001$). This indicates that Au^{3+} concentration had a noticeable effect on the size of particles in which the size of Au-NPs increases with the increasing of the Au^{3+} concentration. The initial Au^{3+} concentration provides an important driving force to overcome all the mass transfer resistance and increase the rate of Au^{3+} reduction reaction. As results, the higher Au^{3+} concentration will form the Au-NPs with the larger size.

The plasmon absorption of colloidal gold solution was investigated by the UV-vis absorption spectra as shown in Fig. 3. The UV-vis absorption spectra of Au-NPs solutions shows the maximum absorption wavelengths (λ_{max}) at 520, 522 and 526 nm for Au^{3+} concentrations of 0.25, 0.5 and 1 mM, respectively. The red band of colloidal gold solution

tends to shift to higher wavelength as its size of Au-NPs increases.

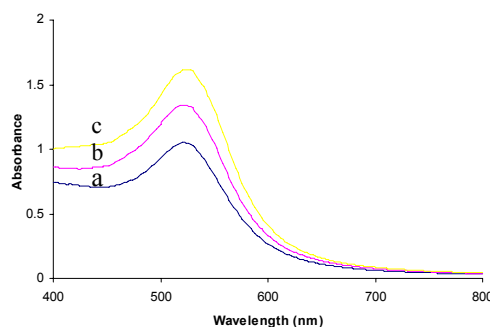


Fig. 3: UV-vis spectra of Au-NPs/0.5% (w/v) WSC with various Au^{3+} concentrations: (a) 0.25 mM, (b) 0.5 mM and (c) 1 mM

3.2. Effect of WSC concentration to the size of Au-NPs

Au-NPs was prepared by the procedure describe at 2.2.1. In this experiment, Au^{3+} concentration was kept constant at 0.5 mM, WSC was changed 0.25; 0.5 and 1%, respectively. The TEM images of Au-NPs prepared with various concentrations of WSC showed that the morphology of Au-NPs contains the spherical nanoparticles without significant agglomerations.

The size of Au-NPs prepared from 0.25, 0.5 and 1 mM of WSC solution was calculated from 200 particles observed from TEM images. The descriptive statistics for three groups were: 0.25 mM WSC solution (0.25 WSC): $M = 16.45$ nm, $sd = 3.89$, $n = 200$; 0.5 mM WSC solution (0.5WSC): $M = 8.76$ nm, $sd = 2.40$, $n = 200$; 1 mM Au^{3+} solution (1WSC): $M = 7.27$ nm, $sd = 3.23$, $n = 200$. The mean particle size of Au-NPs seemed to decrease as the WSC increases as illustrated in Fig. 5.

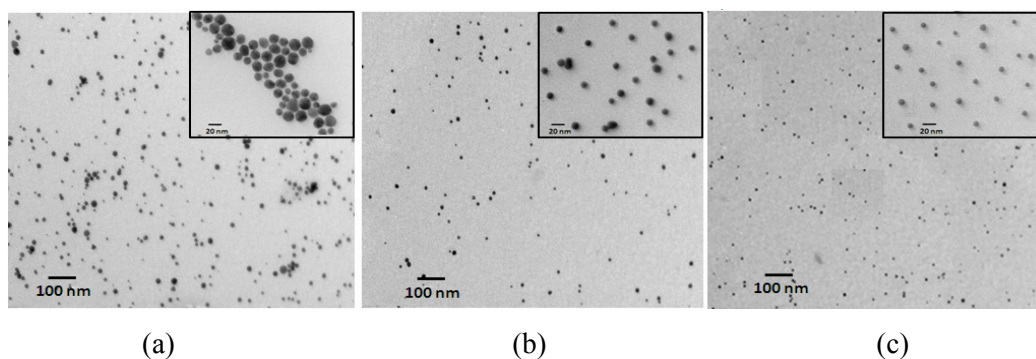


Fig. 4: TEM images of Au-NPs with WSC concentrations: 0.25 mM (a); 0.5 mM (b); 1 mM (c)

For elucidating the effect of WSC concentration on the size of Au-NPs, an one way-ANOVA

analysis was also made among all three groups. There was a statistical difference among all three

groups ($F(2) = 465.53$, $p < 0.001$). Since Levene test indicated a statistically difference of variances among groups by a Levene statistic = 15.721, $p = 0.000$, follow-up comparison using Tamhane's test found a statistical difference between the WSC0.25 and WSC0.5 (mean difference = 7.695, $p < 0.001$) or between the WSC0.5 and WSC1 (mean difference = 1.492, $p = 0.000$). The chitosan might form the complex with ion Au^{3+} and it will confine the nanoparticles of Au to limit growing Au particles. Consequently, the size of Au-NPs will be restricted as the WSC concentration increases.

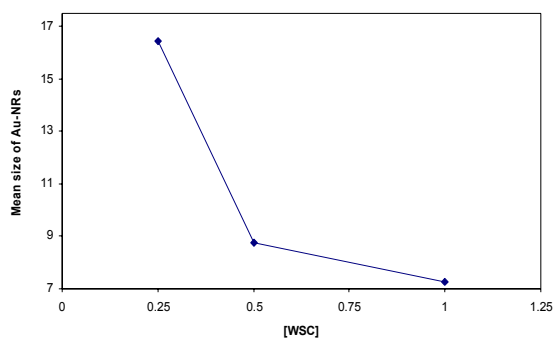


Fig. 5: Effect of WSC concentration on the mean size of Au-NPs

The UV-vis absorption spectra of Au-NPs solutions are shown in Fig. 6. The maximum absorption wavelengths (λ_{max}) were observed at 528, 522 and 518 nm for WSC concentrations of 0.25, 0.5 and 1%, respectively. Therefore, the maximum absorption wavelengths (λ_{max}) shifted to shorter wavelengths as the WSC concentration increased. Similarly, the red band shifted to shorter

wavelengths as the size of particles was reduced. These results are consistent with those of previous works in which gold nanoparticles are prepared using chitosan suspensions as reducing and stabilizing agents [5] and Au-NPs are prepared by Gamma Co-60 irradiation method, using alginate as stabilizing agent [2].

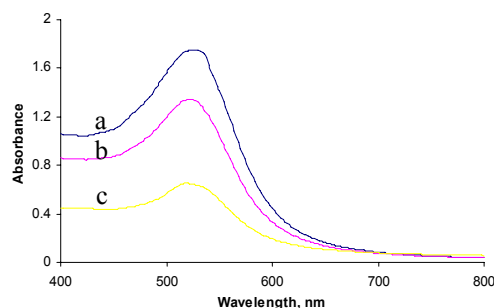


Fig. 6: UV-vis spectra of Au-NPs with WSC concentrations: 0.25 mM (a); 0.5 mM (b); 1 mM (c)

3.3. Controlled growth of seed particles

The Au-NPs with diameter of about 15 nm as prepared from the solution of Au^{3+} 1 mM/0.5% WSC were used as seeds for preparation of particles with larger diameter. The descriptive statistics for the particle sizes of Au-NPs prepared using the seed growth method with the various ratios of $\text{Au}^{3+}/\text{Au}^0$ were: $\text{Au}^{3+}/\text{Au}^0$ of 2 (LC1), $M = 24.59$, $sd = 3.64$, $n = 200$; $\text{Au}^{3+}/\text{Au}^0$ of 4 (LC2), $M = 27.23$, $sd = 4.77$, $n = 200$; $\text{Au}^{3+}/\text{Au}^0$ of 6 (LC3), $M = 30.56$, $sd = 3.81$, $n = 200$; $\text{Au}^{3+}/\text{Au}^0$ of 8 (LC4), $M = 34.83$, $sd = 6.11$, $n = 200$; $\text{Au}^{3+}/\text{Au}^0$ of 10 (LC5), $M = 31.79$, $sd = 6.30$, $n = 200$.

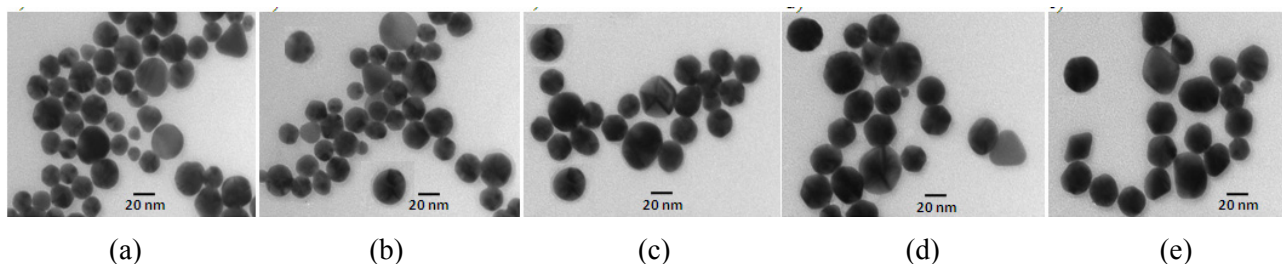


Fig. 7: TEM images of Au-NPs obtained by enlargement of seed particles for $[\text{Au}^{3+}]/[\text{Au}^0] = 2$ (a), 4 (b), 6 (c), 8 (d) and 10 (e)

The mean particle sizes of all five groups are illustrated in Fig. 8.

As shown in Fig. 8, the mean size of Au-NPs increases with the ratio of $\text{Au}^{3+}/\text{Au}^0$ increases and reaches the maximum size at the ratio of 8. The mean size of Au-NPs seems to decrease with a further increase in the ratio of $\text{Au}^{3+}/\text{Au}^0$.

The one-way ANOVA analysis showed the statistically difference among all five groups ($F(4) = 108.65$, $p < 0.001$). Since Levene test indicated a statistically difference of variances among groups (Levene statistic = 12.705, $p = 0.000$), follow-up comparison using Tamhane's test found a statistical difference between the LC1 and LC2 (mean

difference = -2.638, $p < 0.001$), between the LC2 and LC3 (mean difference = -3.332, $p < 0.001$), the LC3 and LC4 (mean difference = -4.2623, $p < 0.001$) or the LC4 and LC5 (mean difference = -3.035, $p < 0.001$). The similar results also observed by Nguyen et al. [2] in which they enlarge the size of Au-NPs by seed growth method, using alginate as stabilizing agent. In the growing step, the initial seeds act as nucleation centers. When Au^{3+} solution is added, their sizes increase due to the oxidation of the gold ions into gold on surface of the seeds. It is known as the Ostwald ripening mechanism. This thermodynamically driven spontaneous process occurs because larger particles are more energetically favored than smaller particles. This originates from the fact, that molecules on the surface of a particle are energetically less stable than the ones in the interior. However, the surface of particle growing up a certain degree will be stable and will not develop further.

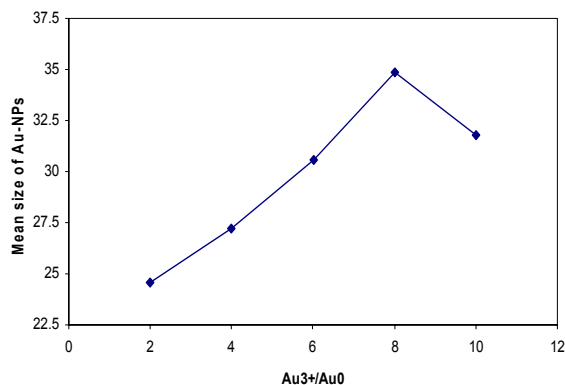


Fig. 8: The effect of ratio of $\text{Au}^{3+}/\text{Au}^0$ on the size of Au-NPs using the growth seed method

The UV-vis analysis also showed that, the maximum absorption wavelengths (λ_{max}) of gold nanoparticles solution shifted to longer wavelengths as shown in Fig. 9. The result of the UV-vis absorption spectra showed that after 3 months of

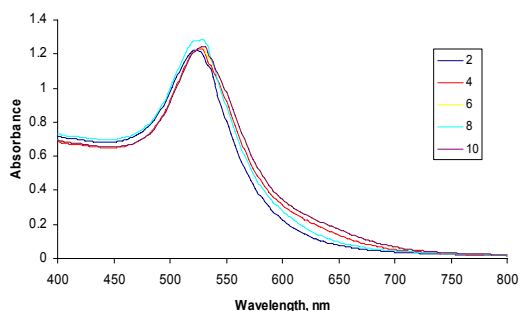


Fig. 9: UV-vis spectra of Au-NPs obtained by enlargement of seed particles for $[\text{Au}^{3+}]/[\text{Au}^0] = 2$; 4; 6; 8 and 10

storing, the absorbance of gold nanoparticles solution were almost unchanged. It proved that Au-NPs prepared by using WSC as reducing and stabilizing agent has excellent stability.

4. CONCLUSION

This paper reports a simple method for preparation of spherical gold nanoparticles by using water soluble chitosan as reducing and stabilizing agents. The average diameters increase when Au^{3+} concentrations increase or WSC concentrations decrease. The sizes of Au-NPs are about 6-17 nm and they are enlarged up to 35 nm by using seed growth method. The obtained colloidal gold nanoparticles solutions are stable and have plasmon properties.

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