

## PREPARATION OF CHITOSAN-GOLD NANOPARTICLES: PART 1 (OF 2). EFFECT OF REDUCING TECHNIQUE

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Received 27 February 2008; Accepted 24 March 2008

### ABSTRACT

Chitosan-stabilized gold nanoparticles were synthesized in aqueous formic acid, citric, or acetic acid with and without chitosan as the stabilizer. Refluxing in methanol, addition of hydrazine or sodium borohydride, photo-irradiation were employed as the reducing agents & reduction technique of gold ions. Dispersed particles of chitosan-stabilized gold were obtained in aqueous acetic acid-methanol solution. The chitosan-stabilized gold colloids (chi-Au) were dispersed and the particle size were in range of 9-30 nm, some of which were crystalline with various shapes. Chi-Au prepared with hydrazine as the reducing agent resulted in large and aggregated particles. The finest and dispersed chi-Au particles were obtained if  $\text{NaBH}_4$  was used as the reducing agent and the diameters could be controlled within 2.2-2.3 nm.

**Keywords:** Gold nanoparticles, chitosan, size control

### INTRODUCTION

Chitosan or poly[ $\beta$ -(1-4)-2-amino-2-deoxy-D-glucon] is a natural polymer isolable from crustacean shell and available commercially. It is biodegradable and has been used in numerous applications in industrial and manufacturing processes including waste water treatment, pharmaceutical, medical, food, agriculture and textile industries [1]. The use of chitosan in catalyst formulations have been explored. The preparation of chitosan-stabilized nanosized metal colloid catalysts including Pd, Rh, Ru, Pt, Ag and Au has been reported relatively large average diameters [2-3]. The amino groups of chitosan were base Lewis for the metal ions and dispersing the metal ions in chitosan matrix. Metal nanoparticles have interesting and unique properties compared to the larger corresponding metal particles [4]. Metal particles with nano and uniform sizes have been intensively investigated recently because they have applications in optics, electronics, magnetic devices, catalysts, photo catalysts, adsorbents and sensors [5]. Gold, which is known as inactive metal for catalysis became an active catalyst when it was in nanoparticle size [6]. Other studies on gold nanoparticles stabilized with other synthetic polymers and alkanethiol have also been much published [7-11]. Studies on the control of the gold particle growth using synthetic polymer have been reported [12-18] but there is barely any report on size control of chitosan-stabilized Au nanoparticles. Furthermore, the effect of the reducing agent and the respective techniques on chitosan-stabilized gold nanoparticle preparation have not been much studied [19]. Employing chitosan as stabilizer for gold metal nanoparticles will inspire some potential uses in biological system.

### EXPERIMENTAL SECTION

#### Material

The following commercially available materials were used without further purification: sodium borohydride 95 % (Reidel de Haen), chitosan of medium molecular weight ~400000 (Fluka, Switzerland), hydrazine 99 %, formic acid 98 % and acetic acid 99.8 % (BDH, England),  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  99.5 % and citric acid monohydrate (Merck, U.S.A) and methanol (System, A.C.S certified grade, Malaysia).

#### Equipment

Philip CM 12 transmission electron microscope (TEM) was employed to obtain micrographs and to determine of particle size and size distribution. A drop of the colloid solution was placed between two thin plastic films supported on a carbon grid and the solvent was evaporated off. The particle diameters were measured using a computer program "analySIS Docu 2.11" (SIS GmbH, Germany, 1986-1997). The average particle size and size distribution were obtained from approximately > 300 particles.

Gold ions were reduced in aqueous formic acid (for) and citric acid (cit) or acetic acid with and without chitosan as stabilizer. Refluxing in methanol, addition of  $\text{NaBH}_4$  or photo-irradiation/microwave irradiation were employed as reducing agent and reducing technique for the gold ions. Formic chitosan-stabilized gold particles prepared by  $\text{NaBH}_4$  and  $\text{N}_2\text{H}_4$  as the reducing agent in aqueous acetic acid- methanol solution are abbreviated as Au-nb and Au-hz respectively. Au-me stands for chitosan-stabilized

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colloidal gold prepared by reflux in aqueous acetic acid-methanol solution. Molar ratio of chitosan:metal was designated as 5:1, 10:1 or 15:1 *etc.* For example, Au-me 5:1 means that Au was reduced by methanol with molar ratio of chitosan: Au of 5:1. Au-for, Au-cit, Au-ir, and Au-mw denote gold (III) reduced by formic acid, citric acid, photo-irradiation methods respectively. Solution of 1.5 % aqueous acetic acid was used as chitosan solvent and for most preparations the solvent was (1:1 v/v) aqueous acetic acid- methanol except for Au-for and Au-cit for which acetic acid was excluded. The formation of the colloidal Au nanoparticles with each of the reducing agent was followed by using a UV-Vis spectrophotometer.

### Preparation of chitosan-stabilized gold colloids (chi-Au)

The chitosan-stabilized metal colloids were prepared with modification of the previously reported methods [3] as follows:

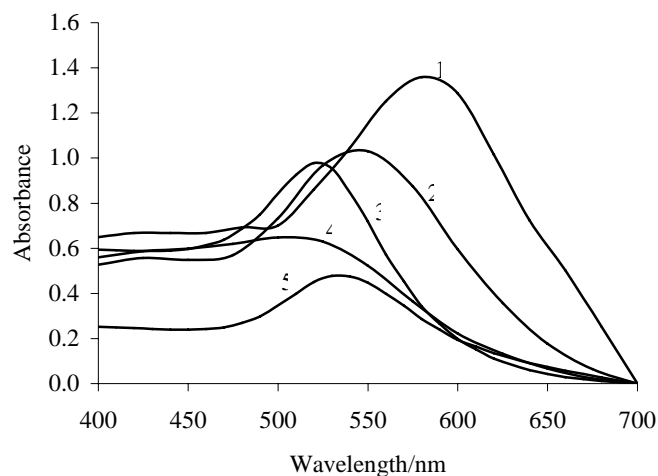
Stock solution of a  $1 \times 10^{-4}$  mol mL<sup>-1</sup> of HAuCl<sub>4</sub> was freshly prepared. The concentration of gold was adjusted to  $6.22 \times 10^{-4}$  M in all preparations with various chitosan: Au molar ratios of 2:1, 5:1, 7:1, 10:1, 20:1, 30:1, and 40:1. All glassware was carefully cleaned with *aqua regia* before use.

Typically, chitosan: Au of molar ratio 5:1 was prepared by dissolving  $5.6 \times 10^{-4}$  mol (0.0900 g) of chitosan in 88.8 mL of aqueous acetic acid solution and diluted with addition of 90 mL of methanol with vigorous stirring. Then, 1.12 mL ( $1.12 \times 10^{-4}$  mol) of gold stock solution was very slowly added to the above solution (total volume of 180 mL) and kept stirring for 1 h. The chitosan-gold solution was refluxed in an oil bath or photo-irradiated with a 100 W tungsten lamp located 20 cm from the flask at 30 °C under stirring. Another series of experiments were also done using sodium borohydride or hydrazine as the reducing agent. The reducing agents were added portion-wise until molar ratio of NaBH<sub>4</sub> or hydrazine to gold (III) ion was 25:1 and 30:1, respectively. About 4 mL of the solution was sampled every 30 min, cooled in an ice bath and the absorbance recorded with a UV-Vis spectrophotometer in the wavelength range of 700-200 nm. The reflux was stopped when the absorbance reached a maximum.

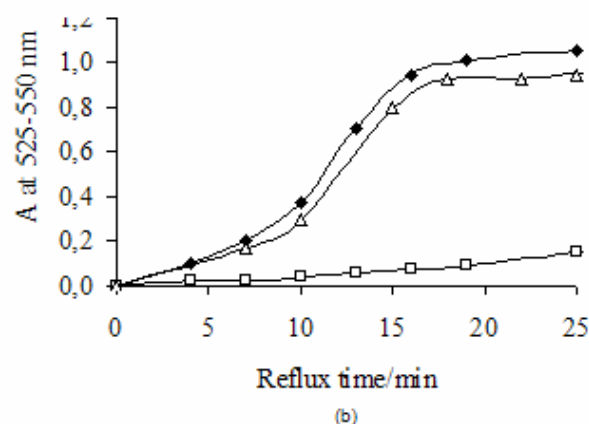
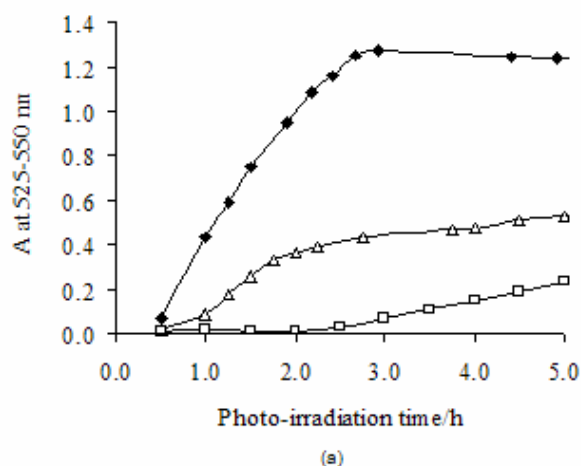
## RESULT AND DISCUSSION

### Effect of solvent and the reduction method

The UV-Vis spectra of gold nanoparticles is good indicator for their size and shape (20). The plasmon band of Chi-Au colloid was observed at 525-550 nm which is consistent with other reports on different stabilizers [21-22]. In formic acid, the Chi-Au-for band



**Fig 1.** Representative UV-Vis spectra of chitosan stabilized gold (7:1) prepared With various methods. 1) Chi-Au-for (reflux or irradiation) ; 2) Chi-Au-hz 3) Chi-Au-me (reflux or irradiation); 4) Chi-Au-nb 5) Chi-Au-cit (reflux or irradiation)



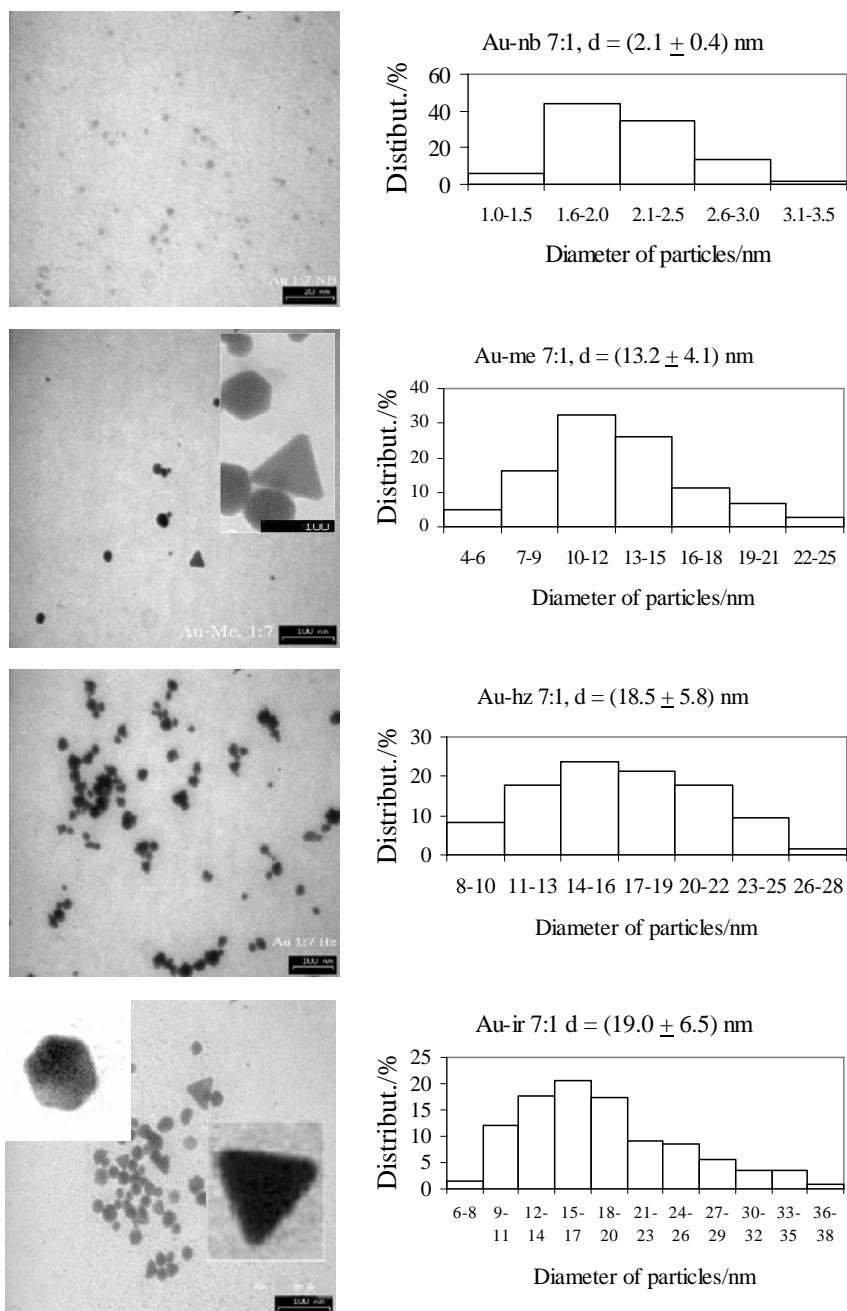
**Fig 2.** UV-Vis absorption intensity of Chi-Au as a function of time prepared with (a) reflux, (b) photo-irradiation (100 W tungsten lamp) at 30 °C in ; ♦ : formic acid, Δ : citric acid, □ : acetic acid.

### The effect of reducing agent

Gold particles prepared by using most of reducing agents including formic and citric acids, were relatively large with the diameter range of 7-34 nm depending on the preparation technique (Table 1 and Fig 3). The size and distribution of gold particles prepared by using hydrazine were large and very broad. Aggregation in the hydrazine reduced sample was very likely due to

chemical interaction between chitosan and hydrazine since hydrazine is slightly reactive towards hydroxyl and carbonyl groups especially in a cyclic ring which also presents in chitosan structure [26].

Chitosan-stabilized gold nanoparticles prepared using  $\text{NaBH}_4$  as the reducing agent was the best methods since it gave fine gold particles with diameter of 2.2-2.7 nm with very narrow size distribution. Consistently, the average particle size was relatively



**Fig 3.** TEM images of chi-Au prepared with various methods ; (a)  $\text{NaBH}_4$ , (b) reflux, (c) hydrazine, (d) irradiation (100 W tungsten lamp). Insert ; some geometric shaped Au crystals (the scale bar = 100 nm).

**Table 1.** Size and distribution of chitosan-stabilized gold nanoparticles

Reducing agents/ techniques	Chi: Au, (solvents)	Average Diameter/nm	Dispersion, shape
Photo irradiation, 30°C	7:1 (HCOOH)	22.9 ± 2.6	Dispersed, some are crystalline
	7:1 (H <sub>5</sub> C <sub>3</sub> (COOH) <sub>3</sub> )	13.1 ± 4.7	Aggregated some are crystalline
	7:1 (HOAc)	14.2 ± 5.0	Aggregated, some are crystalline
	10:1 (HOAc)	13.1 ± 5.0	Aggregated, some are crystalline
	10:1 (HOAc-MeOH)	19.1 ± 6.9	Dispersed, some are crystalline
reflux	7:1 (HCOOH)	22.7 ± 14.4	Aggregated, precipitate out (5 days)
	10:1 (HCOOH)	11.6 ± 3.8	Aggregated, precipitate out (5 days)
	7:1 (H <sub>5</sub> C <sub>3</sub> (COOH) <sub>3</sub> )	11.2 ± 3.9	Aggregated
	10:1 (H <sub>5</sub> C <sub>3</sub> (COOH) <sub>3</sub> )	14.6 ± 5.6	Aggregated
	7:1 (HOAc-MeOH)	13.2 ± 4.1	Dispersed, some are crystalline
NaBH <sub>4</sub>	5:1 (HOAc-MeOH)	2.4 ± 0.5	Dispersed
	7:1 (HOAc)	2.3 ± 0.4	Dispersed
	7:1 (HOAc-MeOH)	2.1 ± 0.4	Dispersed
	10:1 (HOAc-MeOH)	2.6 ± 0.5	Dispersed
	20:1 (HOAc-MeOH)	2.2 ± 0.4	Dispersed
	30:1 (HOAc-MeOH)	2.3 ± 0.5	Dispersed
	40:1 (HOAc-MeOH)	2.7 ± 0.7	Dispersed
N <sub>2</sub> H <sub>4</sub>	5:1 (HOAc-MeOH)	9.0 ± 3.5	Aggregated
	7:1 (HOAc-MeOH)	18.5 ± 5.8	Less aggregated
	10:1 (HOAc-MeOH)	17.1 ± 4.4	Less aggregated
	20:1 (HOAc-MeOH)	16.2 ± 3.9	Less aggregated
	30:1 (HOAc-MeOH)	14.5 ± 4.6	Less aggregated
40:1 (HOAc-MeOH)	13.9 ± 4.1	Less aggregated	

constant in all chitosan: Au molar ratios with standard deviation of 0.4-0.7. This suggests that the ability of chitosan to control the particle size of Au almost uniformly to around 2 nm if Au was reduced by NaBH<sub>4</sub>. These gold nanoparticles were smaller with narrower size distribution compared with those reported using different protective polymers and techniques which were relatively more complicated [4,7,12]. However, the particle size of chi-Au-nb was slightly larger than that previously reported [27] using KBH<sub>4</sub> as the reducing agent with various protective synthetic polymers including poly(1-vinylpyrrolidone-vinyl acetate) copolymer, poly(1-vinylpyrrolidone-co-acrylic acid), poly(2-ethyl-2-oxazoline), poly(4-vinylpyridine) and poly(4-vinylpyridine-co-styrene).

## CONCLUSION

Chitosan-stabilized gold colloids (chi-Au) prepared in aqueous acetic acid-methanol and reduced by reflux or irradiation gave dispersed particles (9-30 nm) and some of the particles were crystalline with various identifiable shapes. Chitosan-stabilized gold colloids

obtained by hydrazine reduction afford aggregated particles with very broad size distribution. Chitosan-stabilized gold colloids prepared by reduction with NaBH<sub>4</sub> gave fine gold particles (2.1-2.4 nm) with narrow size distribution.

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