# Research Article

# **Gold Nanocomposites: Kinetic, Mechanistic and Structural Effects of Reducing Agents on the Morphology**

Shaeel Ahmed AL-Thabaiti<sup>1\*</sup>, Abdullah Yousif Obaid<sup>1</sup>, Zaheer Khan<sup>1,2\*</sup>

<sup>1</sup>Department of Chemistry, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah,, 21589, Saudi Arabia

<sup>2</sup>Nanoscience Research Laboratory, Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi-110025, India

\*Corresponding Author, E-mail: <u>sthabiti@hotmail.com</u> (S. Thabaiti), <u>drkhanchem@yahoo.co.in</u> (Z. Khan)

Received: September 03, 2014 Revised: November 29, 2014 Accepted: November 29, 2014 Published: December 3, 2014

**Abstract:** Different reducing agents, namely, adipic, ascorbic, benzoic, oxalic (organic acids) and tyrosine (amino acid) have been used to determine the morphology of gold nanoparticles in presence of cetyltrimethylammonium bromide (CTAB). The kinetic data suggests that the nature of the reducing agents and presence of phenolic hydroxyl group have great influence on the size and the size distribution of gold nanocomposites. The particles are poly-dispersed with an approximate size of 24 (spherical), 30 (spherical), 83 (irregular), 60 (irregular) and 40 nm (nano flower) for adipic, ascorbic, benzoic, oxalic and tyrosine, respectively. The formation rates of nano flower was higher in comparison the other nanoparticles. In case of tyrosine, tiny particles aggregated in an unsymmetrical mode, leads to the formation of beautiful branched-like gold nano flower. The reaction follows first-order kinetics with respect to [tyrosine]. Probable mechanism has been proposed and discussed to the formation of nano flower gold.

Keywords: Gold nanoparticles; Organic acids; Morphology; Tyrosine

# **1. INTRODUCTION**

Turkevitch and his coworkers reported a conventional and simple chemical reduction method to the synthesis of gold nanoparticles (AuNPs; size = 20 nm) using citrate and HAuCl<sub>4</sub> in water for the first time [1]. In a typical experiment, Frens varied the ratio of reducing agent ( trisodium citrate ) and oxidizinig agent ( gold derivatives ) and obtained the AuNPs having the size between 16 and 147 nm [2]. Generally, citric acid, ascorbic acid, amino acids, and/or their salts and a stabilizer, namely,





(A)





60

80

(B)







**Figure 1.** TEM images (left panel) and particle size distribution histogram (right panel) of AuNPs prepared by the reduction of organic acids with HAuCl<sub>4</sub> in presence of CTAB. *Reaction conditions*: [HAuCl<sub>4</sub>] =  $2.0 \times 10^{-4}$  mol dm<sup>-3</sup>, [CTAB] =  $20.0 \times 10^{-4}$  mol dm<sup>-3</sup>, [adipic acid] = 40.0 (A), [oxalic acid] = 40.0 (B), [ascorbic acid] = 40.0 (C), and [benzoic acid] = 40.0  $\times 10^{-4}$  mol dm<sup>-3</sup> (D).



**Figure 2.** TEM images of AuNPs and selected area diffraction electron ring patterns prepared by the reduction of tyrosine with HAuCl<sub>4</sub> in presence of CTAB. *Reaction conditions*: [HAuCl<sub>4</sub>] =  $2.0 \times 10^{-4}$  mol dm<sup>-3</sup>, [CTAB] =  $20.0 \times 10^{-4}$  mol dm<sup>-3</sup>, [tyrosine] =  $40.0 \times 10^{-4}$  mol dm<sup>-3</sup>.

cetyltrimethlyammonium bromide have been employed to the morphological evaluation (from spherical to multi branched included flowers) of advanced nano materials of gold and silver in seed growth and chemical reduction methods from past decades [3-12]. Henglein used radiation method to the formation of long-time stable colloidal silver nanoparticles and suggested that citrate was a spectator and capping agent to the ongoing redox processes [13]. It has been established that the nucleation, growth, adsorption, stabilization, and morphology of nano particles strongly depended on the methods, nature of reducing agents, presence of stabilizers, pH of the reaction media and rates of reactants addition [3, 6, 14-20]. Au nanoflowers with tailored surface textures have been synthesized by Yamauchi et al. with the redox reaction of KAuBr<sub>4</sub> - ascorbic acid in presence of gum Arabic [21]. They demonstrated that Au nanoflowers exhibited excellent biocompatibility to human bladder cancer cells T-24. Zhang et al. synthesized flower-like Au nano-, and multiple tips with dendritic like- structures through a one-step reduction of HAuCl<sub>4</sub> with dopamine at room temperature and discussed their biocompatibility with cancer cells [22]. Feng and his coworkers reported a simple method to the synthesis of network-like Au nanochains and Au nano-flowers by using ascorbic acid, sodium borohydride, and cytosine as reducing-, and capping agents, respectively and the resulting particles has been exploited for the amperometric detection of  $H_2O_2$  [23]. Xiao and Qi in their pioneering feature article discussed the general strategies of various methods (adsorbate-directed, seed-mediated, and template-assisted) which controlled the growth kinetics of Au-nanocrystals [24]. Due to their unique plasmonic properties, shape-controlled AuNPs have wide potential applications in light concentration, manipulation, photovoltaic devices, biosensors, and fano resonance related applications [21-25].

CTAB is a cationic surfactant serves as a soft template, soluble in water, readily soluble in alcohol and one of the components of the topical antiseptic cetrimide. It has been widely used in synthesis of AuNPs (e.g., spheres, rods, bipyramids, platonic, nanoplates, and branched nano stuctures) and acted as a shape-directing, capping and stabilizing agent [26, 27]. Adipic acid is one of the most important compounds among all aliphatic dicarboxylic acids. It has been used as a raw material in the production of synthetic fibers, nylon 6,6, polyurethanes, plasticizers, low temperature synthetic lubricants e.t.c. [28, 29]. Benzoic acid ( aromatic carboxylic acid ) and its salts used as a food preservatives, which inhibit the growth of mold, yeast and some bacteria. Bal et al. developed a new synthetic strategy to prepare metallic silver nanoparticles supported on tungsten oxide nanorods with diameters between 40 and 60 nm in the presence of CTAB [30]. Recently we have used tyrosine (polar side chain aromatic amino acid) to the preparation of silver nanoparticles and discussed the shape directing role of CTAB [19]. To the best of our knowledge, the synthesis and morphology evaluation of AuNPs by the chemical reduction of  $HAuCl_4$ with organic acids in presence of CTAB is limited in literature. Our goal is to use the different organic acids (monobasic, dibasic, tribasic, and vitamic) for the preparation of AuNPs. For this purpose, we have chosen monobasic (benzoic acid), dibasic (adipic, and oxalic acid ), tribasic ( tyrosine; α-amino acid play an important role in determining the morphologies of the noble metal nanocomposites) and vitamin C ( ascorbic acid ) to see the structural effects on the morphology of AuNPs. We performed a systematic study with the following aims: (1) to see the structural effects of reducing agents on the nucleation and growth processes; (2) to establish the role of ionization constant on the morphology; and (3) to determine the effects of shape-directing CTAB on the stability of AuNPs. The mechanism to the formation of nanoflower by tyrosine-HAuCl<sub>4</sub> redox reaction has also been proposed and discussed for the first time in presence of CTAB.



# Table 1. Structural effects of reducing agents on the morphology of gold nanoparticles<sup>a</sup>

<sup>a</sup>[HAuCl<sub>4</sub>] =  $2.0 \times 10^{-4}$  mol dm<sup>-3</sup>, [CTAB] =  $20.0 \times 10^{-4}$  mol dm<sup>-3</sup>, [oxalic acid] = 40.0, [ascorbic acid] = 40.0, [adipic acid] = 40.0, [tyrosine] = 40.0, and [benzoic acid] =  $40.0 \times 10^{-4}$  mol dm<sup>-3</sup>.

#### **2. EXPERIMENTAL**

#### 2.1. Chemicals and instruments

Double distilled, deionized and CO<sub>2</sub> free water was used as solvent. All the glass wares were washed with aqua regia solution (HCl / HNO<sub>3</sub>, 3:1), then rinsed thoroughly with water prior to use. Adipic acid (HOOC(CH<sub>2</sub>)<sub>4</sub>COOH), benzoic acid ( $C_6H_5COOH$ ), oxalic acid (HOOCCOOH), tyrosine (HOC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>CH(NH<sub>2</sub>)COOH), ascorbic acid ( $C_6H_8O_6$ ), chloroauric acid (HAuCl<sub>4</sub>), and cetyltrimethylammonium bromide were purchased from Sigma Aldrich, and used as received. Tyrosine and benzoic acid are slightly soluble in water, solubility increases in hydrochloric acid and in sodium hydroxide solutions at 25 <sup>0</sup>C. Therefore, stock solutions of these reducing agents (0.01 mol dm<sup>-3</sup>) were prepared by adding the required amount of these acids in 25 ml double distilled water and diluted with 0.01 mol dm<sup>-3</sup> HCl and NaOH solutions to the required volume and stored overnight. The Perkin Elmer (Lambda 25) UV-Vis spectrophotometer, Accumet, Fisher Scientific digital pH meter 910 fitted with a

combination electrode and Transmission electron microscope (Hitachi 7600 with an accelerating voltage of 120 kV) were used to record the spectra, pH and morphology of AuNPs. The size distributions were determined by image analysis using the Image J software package. All TEM images samples were prepared by drop casting of nanoparticles solution onto a carbon-coated copper TEM grid.



**Figure 3.** Spectra of AuNPs prepared by the reduction of organic acids with HAuCl<sub>4</sub> in presence of CTAB. *Reaction conditions*: [HAuCl<sub>4</sub>] =  $2.0 \times 10^{-4}$  mol dm<sup>-3</sup>, [CTAB] =  $20.0 \times 10^{-4}$  mol dm<sup>-3</sup>.



**Figure 4.** Effects of [CTAB] on the nucleation and growth of AuNPs. *Reaction conditions*: [HAuCl<sub>4</sub>] =  $4.0 \times 10^{-4} \mod \text{dm}^{-3}$ , [tyrosine] =  $20.0 \times 10^{-4} \mod \text{dm}^{-3}$ .

Wavelength	Reducing agents			
(nm)	Oxalic acid	Benzoic acid	Tyrosine	Ascorbic acid
350	0.548	0.580	0.550	0.430
375	0.578	0.592	0.580	0.420
400	0.590	0.596	0.590	0.412
425	0.586	0.596	0.588	0.402
450	0.572	0.592	0.576	0.386
475	0.558	0.584	0.562	0.380
500	0.468	0.54	0.468	0.408
525	0.334	0.432	0.327	0.468
550	0.243	0.336	0.235	0.504
575	0.165	0.25	0.165	0.516
600	0.098	0.166	0.104	0.508
625	0.061	0.117	0.070	0.498
650	0.046	0.096	0.051	0.482
675	0.039	0.080	0.045	0.462
700	0.031	0.074	0.048	0.432

Table 2. Wavelength and absorbance data to the formation of AuNPs for different reductants

# 2.2. Preparation and Kinetics of AuNPs

In a typical experiment, reducing agent(s)  $(5 \text{ cm}^3; 0.01 \text{ mol } \text{dm}^{-3})$  was added to CTAB solution (5 cm<sup>3</sup>; 0.01 mol dm<sup>-3</sup>) and diluted with distilled water (total volume = 50 cm<sup>3</sup>). After complete mixing, HAuCl<sub>4</sub> solution (5 cm<sup>3</sup>; 0.01 mol dm<sup>-3</sup>) solution was added into the reaction mixture and stirred well. Surprisingly, the colorless reaction mixture became orange as the reaction time increases, which might be

due to the reduction of HAuCl<sub>4</sub> into  $Au^0$ , suggesting the formation of AuNPs [19, 21, 22]. It is to be noted that we did not observed the appearance of any color in absence of CTAB for a short reaction time, i.e. 20 min. Controls experiment also did not show the formation of any color in the absence of either HAuCl<sub>4</sub> and/or reducing agent(s). For the measurement of reaction rates, the two necked reaction vessel containing required concentrations of HAuCl<sub>4</sub> (  $2.0 \times 10^{-4} \text{ mol dm}^{-3}$ ) and CTAB (  $20.0 \times 10^{-4} \text{ mol dm}^{-3}$ ) equipped with a double-surface condenser to prevent evaporation was kept immersed in the water bath thermo stated at room temperature and the solution was left to stand for 5 min to attain equilibrium. The reduction of HAuCl<sub>4</sub> was initiated with the addition of required volume of reducing agent(s). The formation of AuNPs was followed spectrophotometrically by pipetting out aliquots at definite time intervals and measuring the surface Plasmon resonance absorbance of AuNPs gold at definite time intervals. Apparent rate constants were calculated from the initial part of the slopes of the plots of ln (a / (1 - a) versus time, where  $a = A_t / A_{\alpha}$  (absorbance  $A_t$  at time t and  $A_{\alpha}$  is the final absorbance) with a fixed time method (vide infra). The results were reproducible to within  $\pm 5$  % with average linear regression coefficient,  $r \ge 0.998$  for each kinetic run. The pH of the reaction mixture was also measured at the end of each kinetic experiment and observed that pH drift during the course of the reaction is very small (with in 0.05 unit). Preliminary observations showed that CTAB play a crucial role in the preparation of different colored gold sols. We did not observed the appearance of perfect transparent colored of AuNPs in absence of CTAB. Surprisingly, the different color was developed with different reducing agents and HAuCl<sub>4</sub> as the reaction time increases, suggesting the formation of nanoparticles [19].

#### **3. RESULTS AND DISCUSSION**

#### 3.1. General consideration and TEM images

It has been established that electron-donating and electron-withdrawing substituents seem to increases and decrease the reactivity of the molecule to gain or loss the electrons [5, 13]. In order to compare the reactivity of HAuCl<sub>4</sub> towards different reducing-agents, a series of experiments were carried out. In the first set of experiments, adipic, oxalic, benzoic acids were used to the reduction of  $HAuCl_4$  in absence and presence of CTAB. In the second set of experiments, tyrosine and ascorbic acid were used for the same purposes under similar experimental conditions. In order to establish the role of [HAuCl<sub>4</sub>], [CTAB], and [tyrosine], a series of experiments were performed under different experimental conditions, i.e.,  $[HAuCl_4] = 2.0$  to  $10.0 \times mol dm^{-3}$ , [CTAB] = 5.0 to  $40.0 \times 10^{-4} mol dm^{-3}$  and [tyrosine] = 10.0 to  $40.0 \times 10^{-4} mol dm^{-3}$  $10^{-4}$  mol dm<sup>-3</sup>. Figures 1 and 2 show the TEM images of AuNPs prepared by using the above mentioned reductants. The corresponding histogram of the particles size distribution for the respective samples is presented along with the TEM images. The variation of the average particle sizes have been observed due to different electrons losing abilities of reducing agents (adipic acid, oxalic acid, ascorbic acid, and benzoic acid) towards HAuCl<sub>4</sub> salt reduction in the CTAB micellar system. The size of the resulting AuNPs were also calculated from the TEM images and found to be for adipic acid = 8, 13, 24, 30, 32 nm, oxalic acid = 47, 62, 78, 93, 109 nm, [ascorbic acid] = 12, 17, 23, 82 nm, and [benzoic acid] = 54, 72, 90, 109 nm with standard deviation = 8.2, 21.9, 33.5 and 20.4, respectively. In case of adjoint acid, we did not observe the formation of any color under normal experimental conditions because -COOH groups could not oxidized by HAuCl<sub>4</sub>. Presence of 4 methylene (CH<sub>2</sub>) groups decreases the escaping tendency of -COOH (electron losing properties) in adipic acid. The morphology (size, shape and the size distribution) of advanced nano-materials strongly depend on the nature of the reducing agents, reduction potentials of

Wavelength	$10^{4}$ [CTAB] (mol dm <sup>-3</sup> )			
(nm)	10.0	20.0	40.0	
350	0.532	0.584	0.552	
375	0.558	0.586	0.576	
400	0.584	0.586	0.592	
425	0.578	0.580	0.588	
450	0.558	0.554	0.574	
475	0.536	0.504	0.560	
500	0.424	0.384	0.464	
525	0.278	0.276	0.316	
550	0.182	0.207	0.222	
575	0.119	0.152	0.144	
600	0.060	0.107	0.074	
625	0.035	0.089	0.041	
650	0.030	0.079	0.027	

Table 3. Wavelength and absorbance data to the formation of AuNPs at different [CTAB]

reactants, presence and nature of stabilizer(s). It is been established that transition metals are involved in the formation of solute complexes and/or compounds, which in turn decreases the standard redox potential of the metal [31]. The decrease in the potential depends on the stability of the complexes. The consequence of this effect is a drastic limitation in the choice of reducing agents capable of reducing a metal and the need for harsher conditions as the stability of its complex increases. Oxalic acid is a sacrificial electron donor and relatively strong acid, despite being a carboxylic acid (reduction potential = -0.48V for the couple HOOC-COOH / CO<sub>2</sub>). With regard to its chemical structure (HOOCCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>COOH) adipic acid is not expected to hydrolyze under environmental conditions and it is a very weak reducing agent. We can stated confidently that numbers of AuNPs would be are higher with oxalic acid which might be due to the higher reactivity of oxalic acid towards HAuCl<sub>4</sub> (Table 1). Velegraki et al. extensively studied the oxidation of benzoic acid in presence of a catalyst at higher temperature (range 150-180 ° C) with oxygen partial pressure in the range 10-30 bar [32]. On the other hand, Au-nano flower and/or aggregated nano-rods were observed in case of tyrosine (Fig. 2). Interestingly, TEM images from other areas of the grid also showing the formation of single nano flower morphology (Fig. 2 A-C). They also suggested that the oxidation proceeds through the hydroxylation of the aromatic ring. Tyrosine has a strong driving force to eject the phenolic proton. Thus, presence of – OH group is responsible for the higher reactivity of tyrosine in comparison to benzoic acid which transfers the proton to CTA-HAuCl<sub>4</sub> complex (*vide infra*) leading to the formation of stable perfect transparent AuNPs. Formation of tiny particles and their unsymmetrical aggregation depends on the fast nucleation step. We also observed the same effect of –OH group on the reactivity of tyrosine, paracetamol and acetanilide towards MnO<sub>4</sub><sup>-</sup>[33].

#### 2.2. U.-V. visible spectra and kinetics

It is well known that UV-visible spectroscopy is one of the best widely used techniques for the structural determination of metal nanoparticles. Visual observation showed that the appearance of typical color was due formation of AuNPs, which indicated the nanoparticle morphology altering with the structure of each reducing agents (Table 1). The UV-visible spectra of AuNPs were recorded for benzoic acid, ascorbic acid, oxalic acid, and tyrosine. The observed results (wavelength and absorbance) are summarized in Table 2 and depicted graphically in Fig. 3. The most characteristic part of the AuNPs is the sharp surface resonance plasmon band observable in the visible range (400- 650 nm). The absorption spectra have a broad band at 450 nm for tyrosine, benzoic acid ascorbic acid. The shape of the spectra gives preliminary information about the morphology of the advanced metal nanoparticles [34] and appearance of a band at longer wavelength (bathochromic shift) can be attributed to the increase in size, aggregation, polydispersity, and the size distribution to the multiplasmon excitation of facted and anisotropic nanoparticles [35]. The appearance of colour, absorbance in a colloidal solution of gold sols might be due to the excitation of a SPR, according to the Mie theory, which, in a dipole approximation of the oscillating conduction electrons (Eq. 1).

$$\alpha = \frac{9(V_{\varepsilon_m})^{3/2} \cdot \omega \varepsilon_2(\omega)}{c \cdot (\varepsilon_1(\omega) + 2\varepsilon_m)^2 + \varepsilon_2(\omega)^2}$$
(1)

where all the symbols have their usual significance (*vide infra*). The SPR (a resonance) is expected, when the denominator of Eq. 1 becomes small ( $\varepsilon_2$  ( $\omega$ ) = - 2  $\varepsilon_m$ . The absorption peak position is thus sizedependent within the dipole approximation. The intensity and position of wavelength changed with the structure of used reducing agent. The wave length shift and observed line broadening with reaction-time might be due to an increase in particle size and shape anisotropy. These results are in concord with TEM images (Fig. 1B). Higher reactivity of tyrosine might be due to the presence of phenolic –OH group. Tyrosine reduced the gold ions into nanoparticles through -OH group. The –NH<sub>2</sub> and -COOH groups remain with the tyrosine molecule [19]. The absorption peak is all at around 350 nm corresponding to ascorbic acid and its oxidation product, i.e., dehydroascorbic acid [36]. At the end of the reaction, the concentration of ascorbic acid decreases, this, in turn decreases the absorbance (Fig. 3).



Figure 5. Plots between  $\ln [a / (1-a)]$  versus time: *Reaction conditions*:  $[HAuCl_4] = 4.0 \times 10^{-4}$ mol dm<sup>-3</sup>, [CTAB] = 10.0 (•), and  $20.0 \times 10^{-4}$  mol dm<sup>-3</sup> ( $\circ$ ,  $\blacksquare$ ,  $\Box$ ), [tyrosine] = 10.0 (•,  $\blacksquare$ ), 20.0 ( $\circ$ ) and 40.0 × 10<sup>-4</sup> mol dm<sup>-3</sup> ( $\Box$ ).



**Figure 6.** X-ray diffraction patterns of the Au-nanoflowers and/or nanorods. *Reaction conditions*:  $[HAuCl_4] = 2.0 \times 10^{-4} \text{ mol dm}^{-3}$ ,  $[CTAB] = 20.0 \times 10^{-4} \text{ mol dm}^{-3}$ ,  $[tyrosine] = 40.0 \times 10^{-4} \text{ mol dm}^{-3}$ .

Borderless Science Publishing

# 3.3. Role of CTAB and mechanism

The morphology of metalic nano materials of different metals (Au, Ag, Cu, Pt, Fe, Pd, ect.) depend on the reduction potentials and structure of reactants [31]. It is well known that  $AuCl_4$  ions are involved in the formation of complexes with reducing agents and CTAB, which in turn decreases the standard redox potential of  $AuCl_4$  [12, 26]. The consequence of this effect is a drastic limitation in the choice of reducing agents capable of reducing a metal and the need for harsher conditions as the stability of its complex increases. Among the various reducing agents used in this study (vide supra), we observed the formation of nano-flower of AuNPs with tyrosine. Therefore, tyrosine selected for the detailed kinetic experiments that we address here. UV-visible spectra of AuNPs were recorded at different [CTAB] by reducing HAuCl<sub>4</sub> with tyrosine. The effects of [CTAB] on the spectra of AuNPs formation were carried out at three different [CTAB] and the observed results are given in Fig.4 and their respective wavelengthabsorbance data are also summarized in Table 3. We did not observed considerable changes in the position of the surface Plasmon resonance band of these nanoparticles with [CTAB]. There was a slight batho-chromic shift of the plasmon absorption which is mainly due to the solubilization and/or incorporation of tyrosine into the micellar pseudo-phase of cationic CTAB micelles through electroistatic and hydrophobic interactions, which helps to aggregates the tiny AuNPs in an unsymmetrical manner. Since the [CTAB] is above its critical micellar concentration, CTAB molecules can form bilayer micelles forming complexes with the AuCl<sub>4</sub> ions. Therefore, the reaction between AuCl<sub>4</sub> and AuNPs only takes place in the presence of CTAB. These observations are in good agreement to our previous results regarding the solubilization of tyrosine and Ag-nanoparticles into the cationic CTAB micelles [19, 34]. The apparent rate constants were determined from the slopes of the initial tangents to the plots of  $\ln(a/(1$ a)) versus time (Fig. 5). The effect of [tyrosine] was studied at fixed [HAuCl<sub>4</sub>] =  $2.0 \times 10^{-4}$  mol dm<sup>-3</sup>,  $[CTAB] = 20.0 \times 10^{-4} \text{ mol dm}^{-3}$ , and temperature = 27 <sup>o</sup>C. The reaction follows first-order kinetics with respect to [tyrosine] ( $10^4$  k = 3.9, 3.8, 3.9, 3.7 and 3.9 s<sup>-1</sup> for 10.0, 20.0, 30.0, 40.0 and 50.0 ×10<sup>-4</sup> mol  $dm^{-3}$  [tyrosine], respectively). It is known that tyrosine has three ionization constants (  $pK_{a1} = 2.2$  (COOH) ;  $pK_{a2} = 9.1$  (NH<sub>3</sub>);  $pK_{a3} = 10.0$  (OH)) [37] and exists in different forms (cationic, zwitter ionic, and anionic) in an aqueous solution. The concentration of these species strongly depends on the pH of the working reaction mixture. Therefore, pH of the working solutions was measured under different experimental conditions. It was observed that pH values were found to be constant (pH =  $5.5 \pm 0.2$ ) with increasing [tyrosine] from 10.0 to  $50.0 \times 10^{-4}$  mol dm<sup>-3</sup> in presence of CTAB ( $20.0 \times 10^{-4}$  mol dm<sup>-3</sup>). It is not surprising judges from the fact that tyrosine is a weak acid and ionic micelles show a marked difference in the effective local pH to exist at its micellar surface over that in bulk aqueous solvent [38]. The different pH in the micellar pseudo phase of ionic micelles is understandable because most of the acidic and/or basic species should be in the micellar phase and in addition pKs in micellar systems are all most of time different from the pKs measured in water. The fractions of various forms ( $\alpha$ ) of tyrosine in solution were calculated by using the relation [39]:

$$\alpha_{1} = \left[H^{+}\right]^{3} / B \; ; \; \alpha_{2} = K_{a1} \left[H^{+}\right]^{2} / B \; ; \; \alpha_{3} = K_{a1} \; K_{a2} \; \left[H^{+}\right] / B \; ; \; \alpha_{4} = K_{a1} \; K_{a2} \; K_{a3} \; / \; B \; ; \; \alpha_{4} = K_{a1} \; K_{a2} \; K_{a3} \; / \; B \; ; \; \alpha_{5} \; K_{a3} \; K_{$$

where  $B = [H^+]^3 + K_{a1} [H^+]^2 + K_{a1} K_{a2} [H^+] + K_{a1} K_{a2} K_{a3.}$ 

In the vicinity of pH = 5.5, the values of  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_4$  were found to be  $9.09 \times 10^{-4}$ , 0.909,  $9.09 \times 10^{-6}$ , and  $9.09 \times 10^{-12}$ , respectively. Under our experimental conditions, zwitter ionic form is the major existing species of tyrosine, which is responsible to the reduction of HAuCl<sub>4</sub> to Au<sup>0</sup>.



Carbon dioxide, ammonium ions and corresponding aldehyde had not been identified as the oxidation products of tyrosine under our experimental conditions.  $AuCl_4^-$  ions also formed the stable complex with positive head group of cetyltrimethylammonium ions (CTA) [26]. All these considerations, along with the above results, lead to the proposal of the following mechanism for the formation of AuNPs (Scheme 1).

$$HAuCl_{4} \longrightarrow AuCl_{4}^{-} + H^{+}$$
(1)



Scheme 1. Mechanism to the reduction of HAuCl<sub>4</sub> by tyrosine and rate-law for the formation of AuNPs.

In Scheme 1, Eq. (1) is the complete dissociation of  $HAuCl_4$  into  $AuCl_4^-$  (square planer geometry) and  $H^+$  ions in aqueous solution. Eq.(2) shows the complex formation (CTA-AuCl\_4) between ionized CTA and  $AuCl_4^-$  ions. It is well known that amino acids with aromatic side chain were oxidized more

Borderless Science Publishing

rapidly than the alkyl side chain amino acids [40]. Tyrosine is involved in many protein oxidations, being one of the most easily oxidized amino acids. Oxidation of tyrosine and nature of the oxidation products depend upon the pH, nature of oxidizing agent and the condition of the reaction mixtures [41, 42]. In the rate determining step (Eq. (3)), the AuCl<sub>4</sub>-CTA complex undergoes one-electron oxidation–reduction mechanism leading to the formation AuCl<sub>3</sub> -CTA complex and tyrosyl radical [43, 44]. On the other hand, tyrosyl radical immediately gets converted into the stable reaction products in excess of tyrosine (Eqs 4 to 7). Gedanken et al. discussed the surface chemistry of colloidal gold which was dominated by electrodynamic factors related to its (negative) surface charge [45].

# 3.4. Structure characterization

In order to confirm the crystalline nature of AuNPs, the resulting products were collected by centrifugation, thoroughly washed with double distilled water, and re-dispersed in water for use. XRD spectra were monitored on a Rigaku Dmax-2000 diffractometer with a Cu Ka radiation source  $(\lambda=0.15418 \text{ nm})$ . These results are depicted graphically in Fig. 6. XRD pattern showed orientation at 39.3 plane parallel to the surface and a second orientation at 44.4, and other orientations at 64.6, 77.6. These might be attributed to the diffractions from the (111), (200), (220), and (311) planes of an fcc gold, respectively. These observations are in good agreement to the results of Niu and his coworkers regarding the synthesis of CTAB bilayer-encapsulated gold nanosheets and nanocrystals [46]. Fig. 2D gives an electron micro-diffraction ring pattern of the single crystalline AuNPs with inter-planar spacing and clear lattice fringes in the high-resolution TEM images. Mandal and his coworkers reported that the average particle size of AuNPs and reaction rate depend on the number of tyrosine moieties present in the peptide molecules [47]. TEM images of Fig. 2 A-C are in accord with the previous observations that tyrosine might be responsible to the number of nanoparticles formation [16, 47, 48]. In case of tyrosine, nanoflower is clearly composed of many branched subunits (Fig. 2) and AuNPs display polycrystalline nature from the SAED patterns (Fig. 2D), similar to the observations of Feng et al. and Sau and Murphy for the sunthesis of flower-like Au- nanochains and star-like outlines to branch [23, 49, 50].

It is well known that the metallic properties of gold result from the lone valence electron in the half-filled s-subshells (6s). The band structure of gold displays five comparatively flat d-bands, lying 1-3 eV below the Fermi energy,  $E_F$ , in which the ten d-electrons are located. The optical excitations exist both in gold nanoparticles as well as in bulk gold and begin at the inter-band gap E = 1.7 eV. The optical band gap was calculated by using the following relation:

$$E_{a}$$
 (eV) = 1240 / (wavelength in nm)

Under our experimental conditions, the value of band gap for tyrosine assisted AuNPs is calculated from the visible spectra and found to be 2 eV, which is in good agreement with the theoretical value.

# 4. CONCLUSIONS

Kinetic and TEM images data clearly shows that structure and nature of reducing agents play an important role to reduce the HAuCl<sub>4</sub> into gold nanocomposites of different morphology. This method provides highly stable water soluble, cetyltrimethylammonium bromide functionalized nanoparticles with

different ratios. Tyrosine synthesized gold nanocomposites were found to have nano-flower like morphology, which was due to the fast reduction of gold salt into the metallic gold by the presence of phenolic -OH [21, 47]. Reaction proceeds through the formation of stable AuCl<sub>4</sub>-CTA complex. UV-vis, and TEM studies were used to characterize the nanoparticles and their relation with the observed structural effects of reducing agents.

#### ACKNOWLEDGEMENT

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant no.(MS / 15 / 301 / 1434). The authors, therefore, acknowledge with thanks DSR for technical and financial support.

#### **REFERENCES AND NOTES**

- [1] Turkevitch, J.; Stevenson, P. C.; Hillier, J. A study of the nucleation and growth processes in the synthesis of colloidal gold, *Discuss. Faraday Soc.* **1951**, *11*, 55-75.
- [2] Frens, G. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions, *Nature: Phys. Sci.* **1973**, *241*, 20-22.
- [3] Chow, M. K.; Zukoski, C. F. Gold sol formation mechanisms: Role of colloidal stability, J. Colloid Interface Sci. 1994, 165, 97-109.
- [4] Nikoobakht, B.; El-Sayed, M. A. Evidence for bilayer assembly of cationic surfactants on the surface of gold nanorods, *Langmuir*. 2001, 17, 6368-6374.
- [5] Daniel, M.-C.; Astruc, D. Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology, *Chem. Rev.* **2004**, *104*, 293-346.
- [6] Eustis, S.; El-Sayed, M. A. Why gold nanoparticles are more precious than pretty gold: Noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes, *Chem. Soc. Rev.* 2006, *35*, 209-217.
- [7] Ji, X.; Song, X.; Li, J.; Bai, Y.; Yang, W.; Peng, X. Size control of gold nanocrystals in citrate reduction: The third role of citrate, *J. Am. Chem. Soc.* **2007**, *129*, 13939-13948.
- [8] Al-Thabaiti, S. A.; Al-Nowaiser, F.M.; Obaid, A.Y.; Al-Youbi, A.O.; Khan, Z. Formation and characterization of surfactant stabilized silver nanoparticles: A kinetic study, *Colloids Surfs. B: Biointerfaces.* 2008, 67, 230-237.
- [9] Bakshi, M. S. A simple method of superlattice formation: step-by-step evaluation of crystal growth of gold nanoparticles through seed-growth method, *Langmuir*. **2009**, *25*, 12697-12705.
- [10] Polte, J.; Ahner, T. T.; Delissen, F.; Sokolov, S.; Emmerling, F.; Thunemann, A. F.;Kraehnert, R. Mechanism of gold nanoparticle formation in the classical citrate synthesis method derived from coupled in situ XANES and SAXS evaluation, *J. Am. Chem. Soc.* **2010**, *132*, 1296-1301.
- [11] Pallavicini, P.; Chirico, G.; Collini, M.; Dacarro, G.; Dona, A.; D'Alfonso, L.; Falqui, A.; Diaz-Fernandez, Y.; Freddi, S.; Garofalo, B.; Genovese, A.; Sironi, L.; Taglietti, A. Synthesis of branched Au nanoparticles with tunable near-infrared LSPR using a zwitterionic surfactant, *Chem. Commun.* **2011**, *47*, 1315-1317.
- [12] Khan, Z.; Singh, T.; Hussain, J. I.; Hashmi, A. A. Au(III)–CTAB reduction by ascorbic acid: Preparation and characterization of gold nanoparticles, *Colloids Surfs. B: Biointerfaces*, **2013**, *104*, 11-17.
- [13] Hanglein, A. Colloidal silver nanoparticles: Photochemical preparation and interaction with O<sub>2</sub>, CCl<sub>4</sub>, and some metal ions, *Chem. Mater.* **1998**, *10*, 444–450.

- [14] De, S.; Pal, A.; Jana, N. R.; Pal, T. Anion effect in linear silver nanoparticle aggregation as evidenced by efficient fluorescence quenching and SERS enhancement, J. Photochem. Photobio. A: Chem. 2000, 131, 111-123.
- [15] El-Sayed, M. A. Some interesting properties of metals confined in time and nanometer space of different shapes, *Acc. Chem. Res.* **2001**, *34*, 257-264.
- [16] Huang, Y.F.; Lin, Y.W.; Chang, H.T. Growth of various Au-Ag nanocomposites from gold seeds in amino acid solutions, Nanotechnology. 2006, 17, 4885-4894.
- [17] Khullar, P.; Mahal, A.; Singh, V.; Banipal, T. S.; Kaur, G.; Bakshi, M. S. How PEO-PPO-PEO triblock polymer micelles control the synthesis of gold nanoparticles: Temperature and hydrophobic effects, *Langmuir*. **2010**, *26*, 11363-11371.
- [18] Rafey, A.; Shrivastav, K. B. L.; Iqbal, S. A.; Khan, Z. Growth of Ag-nanoparticles using aspartic acid in aqueous solutions, J. Colloid Interface Sci. 2011, 354, 190-195..
- [19] Khan, Z.; Al-Thabaiti, S. A.; Obaid, A. Y.; Khan, Z. A.; Al-Youbi, A. A. O. Shape-directing role of cetyltrimethylammonium bromide in the preparation of silver nanoparticles, *J. Colloid Interface Sci.* 2012, 367, 101-108.
- [20] Bashir, O.; Hussain, S.; Khan, Z.; Al-Thabaiti, S. A. Encapsulation of silver nanocomposites and effects of stabilizers, *Carbohydrate Polymers*. **2014**, 107, 167-173.
- [21] Wang, L.; Liu, C.-H.; Nemoto, Y.; Fukata, N.; Wu, K. C.-W.; Yamauchi, Y. Rapid synthesis of biocompatible gold nanoflowers with tailored surface textures with the assistance of amino acid molecules, *RSC Advances.* 2012, 2, 4608-4611.
- [22] [22] Yi, S.; Sun, L.; Lenaghan, S. C.; Wang, Y.; Chong, X.; Zhanga, Z.; Zhang, M. One-step synthesis of dendritic gold nanoflowers with high surface-enhanced Raman scattering (SERS) properties, *RSC Advances.* 2013, *3*, 10139-10144.
- [23] Zhang, Q.-L.; Zhou, D.-L.; Li, Y.-F.; Wang. A.-J.; Qin, S.-F.; Feng, J.-J. Cytosine-assisted synthesis of gold nanochains and gold nanoflowers for the construction of a microperoxidase-11 based amperometric biosensor for hydrogen peroxide, *Microchim Acta.* **2014**, *181*, 1239-1247.
- [24] Xiao, J.; Qi, L. Surfactant-assisted, shape-controlled synthesis of gold nanocrystals, *Nanoscale*. **2011**, *3*, 1383-1396.
- [25] Atwater, H. A.; Polman, A. Plasmonics for improved photovoltaic devices, *Nat. Mater.* **2010**, 9, 205-213.
- [26] Perez-Juste, J.; Pastoriza-Santos, I.; Liz-Marzan, L. M.; Mulvaney, P. Gold nanorods: Synthesis, characterization and applications, *Coordination Chem. Revs.* **2005**, 249, 1870-1901.
- [27] Grzelczak, M.; Perez-Juste, J.; Mulvaney, P.; Liz-Marzan, L. M. Shape control in gold nanoparticle synthesis, *Chem. Soc. Rev.* 2008, *37*, 1783-1791.
- [28] Sato, K.; Aoki, M.; Noyori, R. For a green route to adipic acid from cyclohexene and H2O2, Science. **1998**, *281*, 1646-1647.
- [29] Wen, Y.; Wang, X.; Wei, H.; Li, B.; Jin, P.; Li, L. A large-scale continuous-flow process for the production of adipic acid via catalytic oxidation of cyclohexene with H<sub>2</sub>O<sub>2</sub>, *Green Chem.* **2012**, 14, 2868-2875.
- [30] Ghosh, S.; Acharyya, S. S.; Adak, S.; Konathala, L. N. S. Sasaki, T.; Bal, R. Selective oxidation of cyclohexene to adipic acid over silver supported tungsten oxide nanostructured catalysts, *Green Chem.* 2014, 16, 2826-2834.
- [31] Goia, D. V.; Matijevic, E. Preparation of monodispersed metal particles, *New J. Chem.* **1998**, *22*, 1203-1215.
- [32] Velegraki, T.; Nouli, E.; Katsoni, A.; Yentekakis, I. V.; Mantzavinos, D. Wet oxidation of benzoic acid catalyzed by cupric ions: Key parameters affecting induction period and conversion, *Applied Catalysis B: Environmental.* **2011**, *101*, 479-485.
- [33] Malik, M. A.; Basahel, S. N.; Obaid, A. Y.; Khan, Z. Oxidation of tyrosine by permanganate in presence of cetyltrimethylammonium bromide, *Colloids and Surfaces B: Biointerfaces.* **2010**, *76*, 346-353.

Borderless Science Publishing

- [34] Kim, D.-W.; Shah, S.-I.; Oh, S.-G. Preparation and stabilization of silver colloids in aqueous surfactant solutions. In: Mittal, K. L.; Shah D. O. *Surfactants, science series*, Ed.; Marcel Dekker: New York, 2003; vol. 109; p. 255.
- [35] Krenn, J. R.; Schider, G.; Rechberger, W.; Lamprecht, B.; Leitner, A.; Aussenegg, F. R.; Weeber, J. C. Design of multipolar plasmon excitations in silver nanoparticles, *Appl. Phys. Lett.* **2000**, *77*, 3379-3381.
- [36] Xiong, J.; Wang, Y.; Xue, Q.; Wu, X. Synthesis of highly stable dispersions of nanosized copper particles using L-ascorbic acid, *Green Chem.* **2011**, 13, 900-904.
- [37] Haynes, W. M. CRC hand book of chemistry and physics, 95th Edition, Haynes W. M. Taylor and Francis group 2014-2015.
- [38] Bunton, C. A.; Rivera, F.; Sepulveda, L. Micellar effects upon the hydrogen ion and general acid catalyzed hydration of 1,4-dihydropyridines, *J. Org. Chem.* **1978**, *43*,1166-1173.
- [39] Laitinen, H. A. Chemical Analysis, McGraw-Hill Book CO., New York, p.36, 1960.
- [40] Laloo, D.; Mahanti, M. K. Kinetics of oxidation of amino acids by alkaline hexacyanoferrate(III), J. Chem. Soc. Dalton Trans. 1990, 311-313. DOI: 10.1039/DT9900000311.
- [41] Giulivi, C.; Davies, K. J. A. Mechanism of the formation and proteolytic release of H<sub>2</sub>O<sub>2</sub>-induced dityrosine and tyrosine oxidation products in hemoglobin and red blood cells, *J. Biol. Chem.* **2001**, *276*, 24129-24136.
- [42] Stubbe, J.; Van Der Donk, W. A. Protein radicals in enzyme catalysis, Chem. Rev., 1998, 98, 705-762.
- [43] Hammes-Schiffer, S. Theoretical perspectives on proton-coupled electron transfer reactions, *Acc. Chem. Res.* **2001**, *34*, 273-281.
- [44] [44] Box, H.C.; Budzinski, E. E.; Freund, H. G. Effects of ionizing radiation on tyrosine, *J. Chem. Phys.* **1974**, *61*, 2222-2226.
- [45] Zhong, Z.; Patskovskyy, S.; Bouvrette, P.; Luong, J. H. T.; Gedanken, A. The surface chemistry of Au colloids and their interactions with functional amino acids, *J. Phys. Chem. B.* **2004**, *108*, 4046-4052.
- [46] Wang, Z.; Yuan, J.; Zhou, M.; Niu, L.; Ivaska, A. Synthesis, characterization and mechanism of cetyltrimethylammonium bromide bilayer-encapsulated gold nanosheets and nanocrystals, *Appl. Surface Sci.* 2008, 254, 6289-6293.
- [47] Si, S.; Bhattacharjee, R. R.; Banerjee, A.; Mandal, T. K. A mechanistic and kinetic study of the formation of metal nanoparticles by using synthetic tyrosine-based oligopeptides, Chem.-Eur. J. **2006**, *12*, 1256-1265.
- [48] Daima, H. K.; Selvakannan, P. R.; Homan, Z.; Bhargava, S. K.; Bansal, V. Tyrosine mediated gold, silver and their alloy nanoparticles synthesis: Antibacterial activity toward gram positive and gram negative bacterial, *Nanoscience, Technology and Societal Implications (NSTSI), 2011 International Conference on.* 2011, 1-6.
- [49] Wang, A.-J.; Qin, S.-F.; Zhou, D.-L.; Cai, L.-Y.; Chen, J.-R.; Feng, J.-J. Caffeine assisted one-step synthesis of flower-like gold nanochains and their catalytic behaviors, *RSC Adv.* **2013**, *11*, 14766-14773.
- [50] Sau, T. K.; Murphy, C. J. Room temperature, high-yield synthesis of multiple shapes of gold nanoparticles in aqueous solution, *J. Am. Chem. Soc.* **2004**, *126*, 8648-8649.

#### The authors declare no conflict of interest

© 2015 By the Authors; Licensee Borderless Science Publishing, Canada. This is an open access article distributed under the terms and conditions of the Creative Commons Attribution license http://creativecommons.org/licenses/by/3.0