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BIOLOGICAL SYNTHESIS OF Au, Ag AND Au-Ag BIMETALLIC NANOPARTICLES BY A-AMYLASE

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In recent years, studies on the improvement of biological techniques for synthesis of nanoparticles have been extensively increased due to the harmful effects of chemical preparation methods. In the present work, a nontoxic, green and eco-friendly protocol for the synthesis of metal nanoparticles using α-amylase as a reducing agent was examined toward five metal ions, including Cu^{+2}, Se^{+4}, Bi^{+4}, Au^{+3}, Ag^{+}, among which gold (AuNPs), silver (AgNPs) and gold/ silver (Au/AgNPs) alloy nanoparticles were successfully synthesized and characterized. Maximum absorbance at 530, 440 and 458 nm related to formation of AuNPs, AgNPs and Au/AgNPs, respectively, were determined by UV-Vis spectroscopy. Analysis by scanning electron microscopy (SEM) equipped with energy dispersive X-ray (EDX) microanalyser confirmed biosynthesis of nanostructures by α-amylase. Two intense peaks at 1620 cm^{-1} and 3430 cm^{-1} in Fourier transform infrared (FTIR) spectra of produced nanoparticles were ascribed to carbonyl and OH/NH groups, respectively. The size of AuNPs, AgNPs and Au/Ag alloy nanoparticles, analyzed by laser light scattering method, were determined to be 89 nm, 37 nm and 63 nm, respectively.

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1. Introduction

Metal nanoparticles have different applications in chemistry, physics, biomedical and material sciences [1]. In biomedicine, gold nanoparticles (AuNPs) are used in several purposes such as leukemia therapy [2], biomolecular immobilization [3] and biosensor design [4,5]. The use of AuNPs as anti-angiogenesis, anti-malaria and anti-arthritic agents was also reported [6]. Silver nanoparticles (AgNPs) are applied as selective coating agent for solar energy absorption, intercalation material for electric batteries, catalysts in chemical reactions and antimicrobial agents [7,8]. Alloy nanoparticles have also received attentions due to optical, electronic and catalytic properties [1].

Disadvantages of physicochemical methods used for synthesis of nanoparticles such as requirement to expensive equipments and hazardous effects of byproducts encouraged researchers to study on preparation of metal nanoparticles using biological systems providing a commercially viable, clean and nontoxic technique [9]. To date, different biological resources including herbal extracts [10–12], microalgae [13], fungi [14–16] and bacteria [17–19] have been employed for synthesis of metal nanoparticles among which filamentous fungi are able to produce highly stable

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ones [15]. The secreted proteins/ enzymes and reducing agents such as amino acids, peptides and organic acids in biological entities were found to be responsible for nanoparticle production [6,10]. In the study of Wang et al. [11], the ability of polypeptide sequence (MS14) for AuNPs production was reported. Kalishwaralal et al. [6] investigated on the biological production of gold nanoparticles using *Bacillus licheniformis* and demonstrated the key role of α-amylase and their reducing moieties (e.g. free and exposed thiol groups) in such bioreduction processes. This study describes biosynthesis of Au, Ag and Au-Ag alloy nanoparticles by the enzyme of α-amylase. The characterizations of produced nanoparticles have been also reported.

2. Materials and methods

2.1. Materials

All chemicals were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemical Co. (St. Louis, MO). α-Amylase was purchased from Merck. Alternatively, extracellular α-amylase was obtained from submerged fermentation of the isolate strain of halophilic archaeon *Halorubrum xinjiangense*.

2.2. Preparation of nanoparticles

Formation of gold, silver and Au/Ag alloy nanoparticles was investigated by dissolving 2 mg of the pure enzyme (130 U/mg) in 2 mL deionized water followed by adding aqueous concentrations of $HAuCl_4$ (0.05, 0.1, 0.5 and 1 mM), $AgNO_3$ (0.05 to 10 mM) and different Au/Ag mole ratios (0.1:0.1, 0.1:0.5, 0.1:1, 0.5:0.1, 0.5:0.5, 0.5:1, 1:0.1, 1:0.5 and 1:1). The prepared mixtures were incubated at different temperatures (40–80°C) to form maximum absorbance related to gold; 520 nm [1,12], silver; 440 nm [13] and bimetallic nanoparticles; 480 nm [1,14], respectively, compared to control tubes. The control mixtures contained the above concentrations without enzyme incubated at the same conditions. To investigate on formation of copper, selenium and bismuth nanoparticles, above protocol was performed in presence of $CuSO_4$, SeO_2 and $Bi_5O(OH)_9(NO_3)_4$ solutions.

2.3. Analysis

UV-Vis spectra of the mixtures of nanoparticles were recorded by a Labomed Model UVD-2950 UV-Vis Double Beam PC Scanning spectrophotometer, with a resolution of 1 nm in the range of 300–700 nm. Scanning electron microscope (Philips, XL30, 30 KV) equipped with EDX (energy dispersive X-ray) microanalyzer was used for structural and elemental composition studies of prepared nanostructures. Physical vapor deposition (P.V.D.) method was accomplished on sputter coater instrument (BAL-TEC, SCDOOS model). The Fourier transform infrared (FTIR) spectra of dried powders were obtained on a Perkin Elmer spectrum instrument at a resolution of 4 cm^{-1} in KBr pellet. Dry powders were prepared after centrifugation of prepared nanoparticle solutions at 13000 g for 20 min followed by drying of the formed pellets. Particle size distribution patterns of produced nanoparticles were measured by laser light scattering method using a Malvern master sizer instrument (MS2000, Ver. 5.30, UK). The agarose gel electrophoresis of nanoparticles was carried out in 0.2% agarose gel immersed in 0.5 X TBE buffer pH 8. Nanoparticle solutions, 10 mL, were centrifuged at 13000 g for 10 min and then the formed pellet washed three times using deionized water followed by solubilizing nanoparticles in 0.25 X TBE buffer containing saccharose (30%) and loading on the agarose gel [15].

3. Results

3.1. Formation of Au, Ag and Au/Ag alloy nanoparticles

In the present study, synthesis of nanoparticles using α-amylase as reducing agent was reported. According to obtained results in the case of AuNPs, the best temperature and $HAuCl_4$

concentration were found to be 70°C and 0.1 mM, respectively. AuNPs were not synthesized in other mixtures and thermal conditions (data not shown). Biosynthesis of AgNPs occurred only at 70°C in 0.1 to 4 mM concentrations of $AgNO_3$ and in concentrations higher than 4 mM, aggregation of nanoparticles was observed. Optimum condition for production of Au/Ag alloy nanoparticles was found to be at 70°C with Au/Ag mole ratios of 0.1:0.1 and 0.1:1. In the mole ratio of 1:1 Au/Ag rapid formation of large and aggregated nanoparticles was observed. In other solutions [$CuSO_4$, $Bi_5O(OH)_9(NO_3)_4$ and Se_2O] even after 48 hours, no change in maximum absorbance related to nanoparticles formation were detected.

3.2. Characterization of nanoparticles

UV-Vis spectrum for Au, Ag and Au/Ag solution after incubation showed peaks at 530, 430 and 458–470 nm, respectively (Fig. 1). Scanning electron micrographs of metal and alloy nanoparticles showed well dispersed and spherical shape nanoparticles without aggregation (Fig. 2). EDX analysis confirmed the presence of Au and Ag in nanostructures (Fig. 3). The average sizes of Au, Ag and Au/Ag alloy nanoparticle analyzed by laser light scattering method were found to be 86 nm, 37 nm and 63 nm, respectively (Table 1). The FTIR spectra of Au, Ag and Au-Ag alloy nanoparticles (Fig. 4) exhibited two peaks related to OH/NH and C=O groups. The presence of OH group in Au, Ag and Au-Ag alloy could be ascribed to peaks at 3444.6, 3430.5 and 3430.4 cm^{-1}, respectively. The peaks of 1732.0, 1627.9 and 1619.2 cm^{-1} were corresponded to C=O groups in Au, Ag and Au-Ag alloy nanoparticles, respectively.

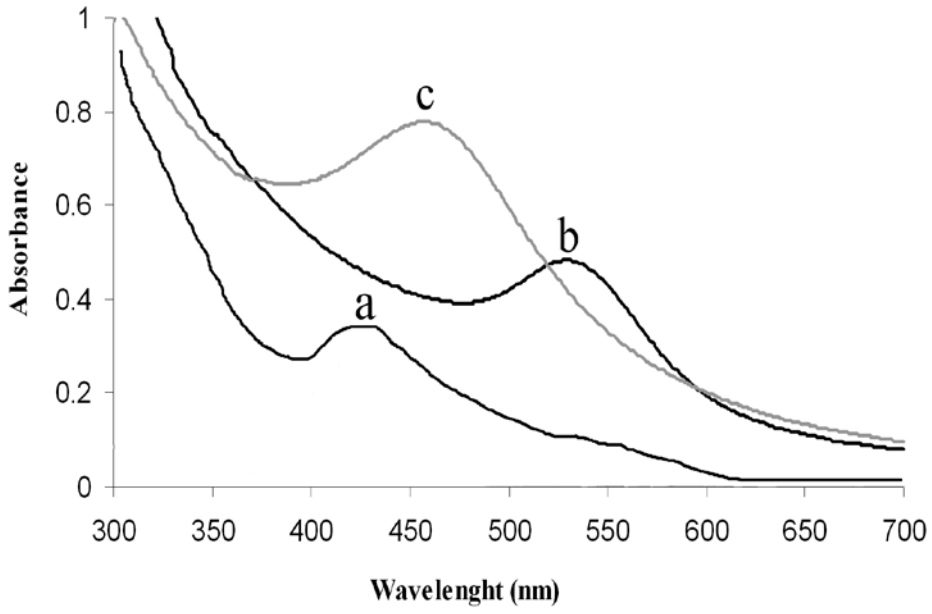
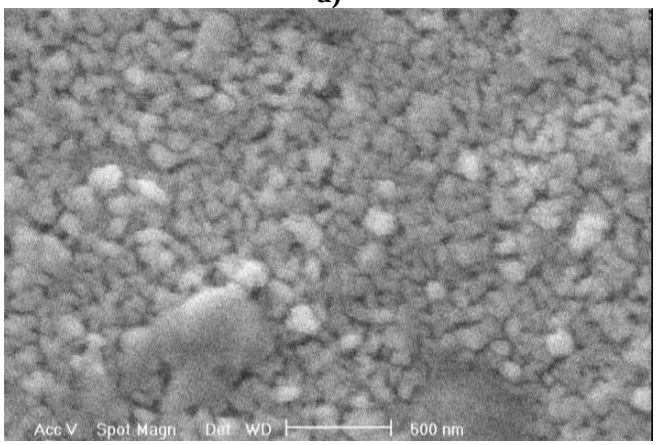
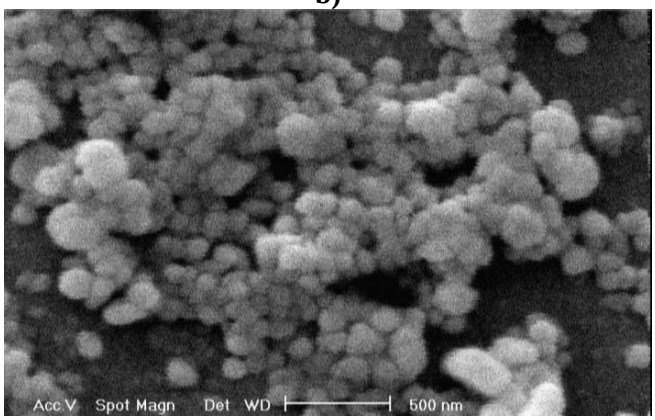


Fig. 1. Spectra of prepared nanoparticles after treatment of a) $AgNO_3$ (4 mM), b) $HAuCl_4$ (0.1 mM), and c) alloy (Au/Ag molar ration of 0.1:1 mM) by α-amylase at 70°C.

a)



b)



c)

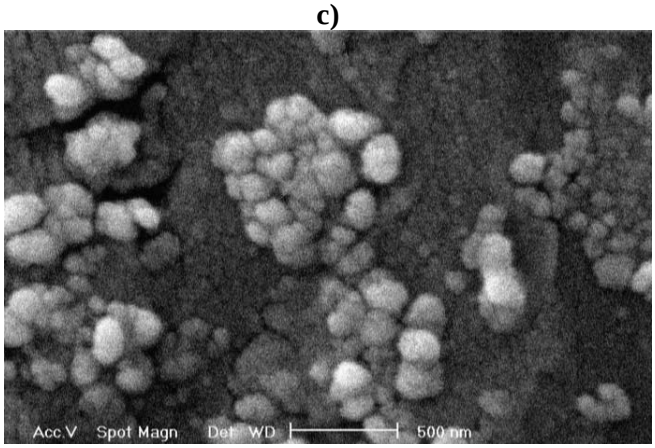


Fig. 2. Scanning electron microscopy (SEM) images of a) gold, b) silver and c) Au/Ag alloy nanoparticles prepared by α-amylase.

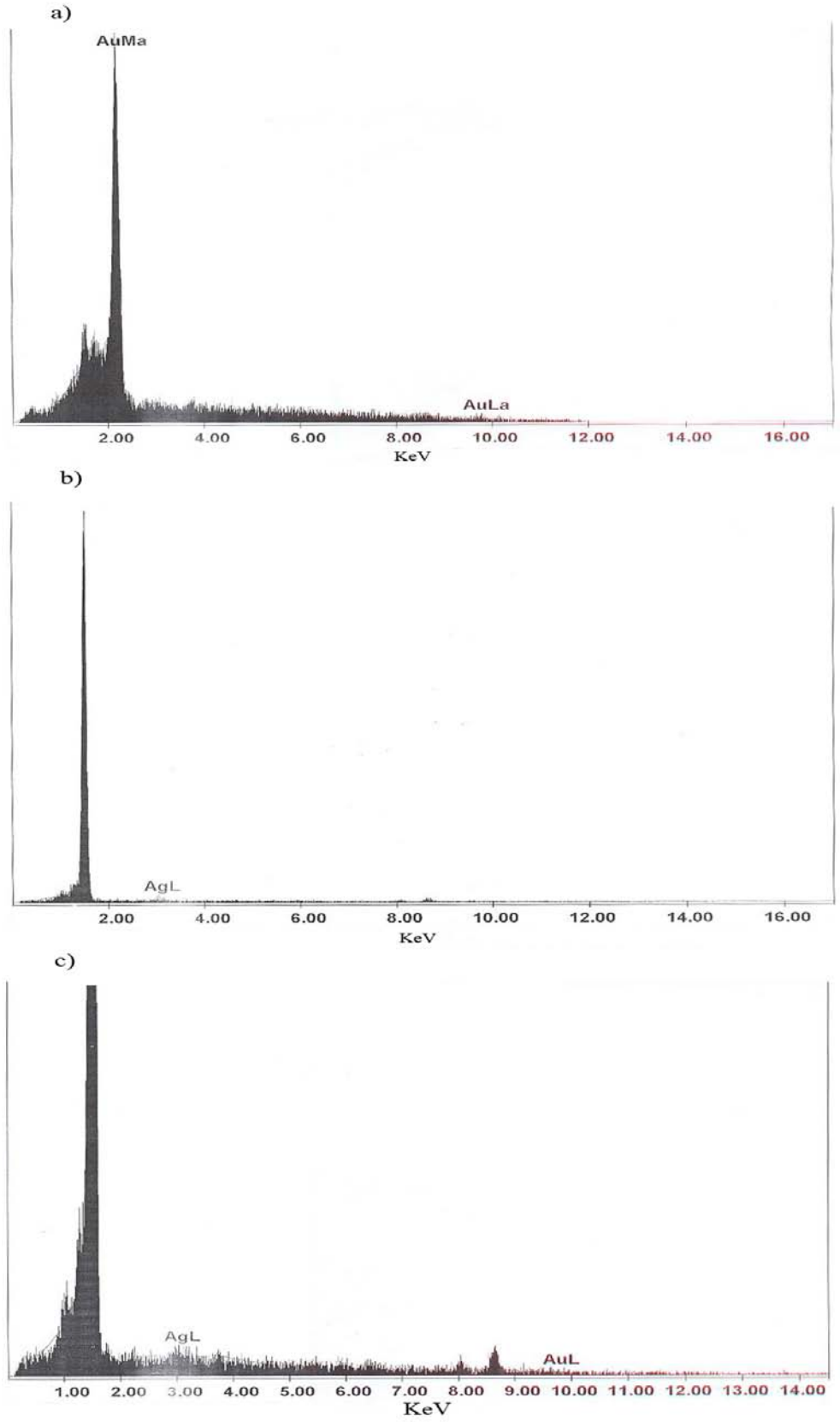


Fig. 3. Energy dispersive X-ray (EDX) profiles of a) Au, b) Ag and c) bimetallic (Au/Ag) nanoparticles.

3.3. Migration of nanoparticles in agarose gel electrophoresis

Agarose gel electrophoresis of metals (gold and silver) and bimetallic (Au/Ag) nanoparticles is illustrated in Fig. 5, among which gold and alloy moved through gel while silver nanoparticles stopped from movement.

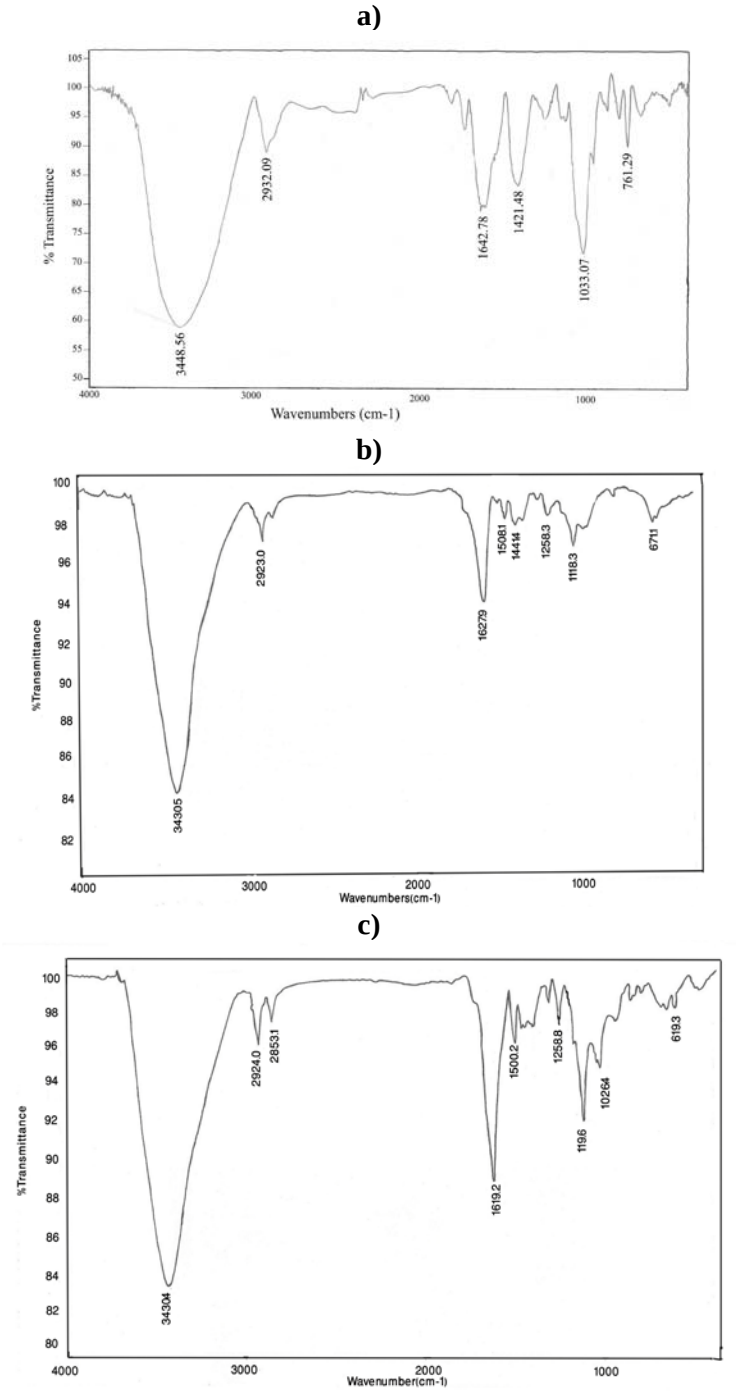


Fig. 4. FTIR spectra of a) gold, b) silver and c) gold/silver alloy nanoparticles after reduction of $HAuCl_4$ (0.1 mM), $AgNO_3$ (4 mM), and Au/Ag (molar ratio of 0.1:1 mM) in presence of α-amylase.

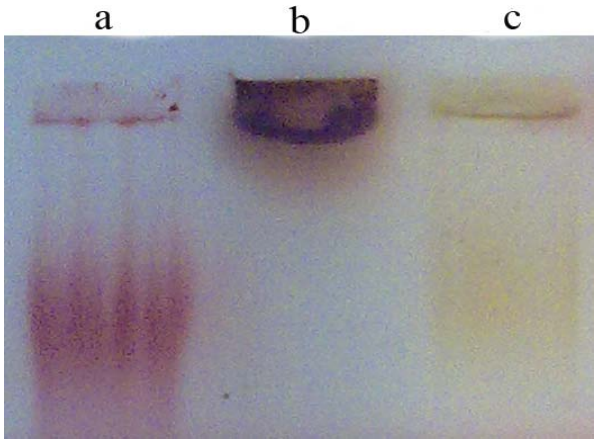


Fig. 5. Electrophoresis of formed a) gold, b) silver and c) alloy nanoparticles on agarose gel (0.2%).

4. Discussion

Preparation of nanometals using physical methods such as attrition and pyrolysis supply nanostructures with narrow and controlled size ranges, however, these methods require very expensive equipments and the final yield is low [9]. Despite the fact that chemical reduction using compounds like $NaBH_4$ and stannous chloride is the most common technique for synthesis of nanoparticles, hazardous effects of byproducts and solvents persuaded investigators to apply biological systems and their biodegradable products, e.g. protein/ enzyme, carbohydrates and chitosan, for nanoparticles preparation [11,16,17]. Raveendran et al. [1] studied on the starch-mediated production of Au, Ag and Au/Ag nanoparticles and showed the growth of nanoparticles facilitated by the hydrogen bonds in association with starch molecules. In the study of Sun et al. [18], the ability of another biological polymer (chitosan) for AuNPs production was ascribed to formation of open chain form of the biopolymer in acidic condition. Kalishwaralal et al. [6] demonstrated that α-amylase produced by *Bacillus licheniformis* has free and exposed thiol groups making it appropriate for reduction of $HAuCl_4$ and AuNPS production.

In the present study, metallic nanoparticles were synthesized by pure α-amylase. UV-visible, SEM and EDX analysis of nanoparticles confirmed the presence of Au, Ag and Au/Ag alloy in prepared spherical nanostructures. As mentioned in Results, application of higher concentrations (above 0.1 mM) of $HAuCl_4$, and $AgNO_3$ (higher than 4 mM) led to form aggregated particles. Such growth in particle size was observed in the case of alloy in different mole ratio except for 0.1:1 and 1:0.1. Incubation of reaction mixtures in lower temperatures than 70°C increased the time need for production of nanoparticles, while at temperatures above 70°C aggregation was obtained, therefore, suitable temperature for nanoparticles production was found to be 70°C. Based on the study of Kalishwaralal et al. [6], the key role of exposing thiol groups of α-amylase for AuNPs formation, such high temperature (70°C) destructs the appropriate folding of α-amylase and exposes hydrophobic and hidden groups with reductive ability and make it possible to form nanometallic structures. Agarose gel electrophoresis of charged nanoparticles using carboxylated polyethylene glycol for separation of nanometallic structures in different sizes and shapes was investigated in the study of Hanauer and colleagues [15]. In the present study, migration of Au and Au-Ag alloy nanoparticles without such coatings could be ascribed to different charge patterns of gold in applied pH. The FTIR spectra showed 3430.5 and 1620.0 cm^{-1} peaks related to OH and/ or NH and carbonyl groups capped the nanoparticle surfaces. This finding was in agreement with the study of Shakibaie and co-workers [13] characterized the AuNPs produced by marine microalgal strain of *Tetraselmis suesica*.

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