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Original Article

ANTIOXIDANT ACTIVITY OF GOLD NANOPARTICLES USING GUM ARABIC AS A STABILIZING AGENT

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ABSTRACT

Objectives: The aim of this study is to investigate the effect of gum arabic (GA) as reducing agent and stabilizer in gold nanoparticle (AuNP) formulation and the antioxidant activity of GA-AuNP.

Methods: Gold nanoparticles (AuNPs) were synthesized by reducing hydrogen tetrachloroaurate ($HAuCl_4$) via three techniques: using gum arabic (GA-AuNP), using sodium borohydride (NaBH4) with GA as a stabilizing agent (GA-AuNP), and using NaBH4 (AuNP). These nanoparticles were characterized via UV-VIS spectroscopy, TEM, AFM, and PSA. The antioxidant activity of the AuNPs was measured via the DPPH method. Their physical stability was determined using a UV-VIS spectrophotometer over 5 weeks during storage at room temperature.

Results: The AuNPs formed using gum arabic as both the reducing agent and stabilizer, using sodium borohydride as the reducing agent with gum arabic as the stabilizer, and using sodium borohydride alone had particle sizes of 6.52 ± 0.66 nm, 4.045 ± 0.99 nm, and 55.82 ± 39.87 nm, respectively. The percentages oxidation inhibition for these particles were **74.39 ± 0.255%, 73.51 ± 0,251%, and 72.63 ± 0.567%,** respectively.

Conclusion: Gum arabic (GA) was successfully acted as reducing and stabilizing agent to the gold nanoparticle (AuNP) and increase the percentage oxidation inhibition of AuNP. The stability test indicated that the GA-AuNP were physically stable for at least 5 weeks.

Keywords: Antioxidant activity, Gold, Nanoparticle, Gum arabic, Sodium borohydride.

INTRODUCTION

Nanotechnology has been applied to topical and transdermal applications such as preparing gold nanoparticles (AuNPs). Gold has been used as a cosmetic material because it is poorly oxidized, inert, and nontoxic and has antioxidant and anti-aging properties [1]. Several studies have shown that AuNPs have antioxidant activity that significantly decreased the oxidative stresses that form reactive oxygen species (ROS) [2].

AuNPs are generally unstable because of their high surface energy. A suitable stabilizer should be added to prevent aggregation [3]. Preparing AuNPs requires a stabilizer to control growth and prevent aggregation. One commonly used stabilizer is gum arabic (GA).

GA can be used as both a stabilizing and reducing agent. This property has been used to synthesize both nonradioactive and radioactive AuNPs with steric stability. Based on the information above, this study manufactured and characterized AuNPs using GA as a stabilizer.

MATERIALS AND METHODS

Materials

Au foil (Sigma-Aldrich, Germany), gum arabic (Sigma-Aldrich), sodium borohydride (Merck, Germany), aqua bidestilata (IPHA, Indonesia), 1,1-diphenyl-2-picril-hydrazil (DPPH; Sigma-Aldrich), bovine serum albumin (Sigma-Aldrich), methanol (Sigma-Aldrich), phosphate buffer saline (IPHA), PD-10 column (GE Health Care, England), sodium dibasic phosphate (Merck), and sodium phosphate monobasic (Merck) were obtained.

Preparation of the HAuCl, $NaBH_4$ and GA solutions

A 0.002 M HAuCl solution was prepared by dissolving 20.1 mg of Au foil in hot aqua regia (HCl (aq):HNO3(aq) = 3:1). This solution was heated, and 10 mL of an aqua pro injection was added.

The heating process and aqua pro injection were performed three times. The final solution was added to a 0.01 M HCl solution to obtain the 0.002 M HAuCl solution.

A 0.1 M $NaBH_4$ reduction solution was prepared by dissolving 0.038 g of solid NaBH4 in 10 mL of the aqua pro injection. This solution needed to be freshly prepared before use. A concentration of 0.02 M was reached by diluting with aqua pro injection. The gum arabic solution was prepared by dispersing 0.6088 g of GA into up to 50.0 mL of aqua pro injection.

Preparation of AuNPs using GA as both the stabilizer and reductor

An 8.20 mL aliquot of the GA solution and 0.3 mL aliquot of the aqua pro injection were heated to 55°C and continuously stirred. To this hot GA solution, 1.5 mL of the 0.002 M HAuCl solution was added with continuous stirring. Once the solution had become reddish purple, the stirring was continued for 1 minute without heating.

Preparation of AuNPs using GA as the stabilizer and NaBH4 as the reductor

An 8.20 mL aliquot of the GA solution and 0.25 mL aliquot of the aqua pro injection were heated to 55°C with continuous stirring. To this hot GA solution, 1.5 mL of 0.002 M HAuCl was added with continuous stirring for 1 minute. Next, 0.05 mL of 0.02 M NaBH4 was added with continuous stirring. Once the solution had become reddish purple, the stirring was continued for 1 minute without heating.

Preparation of AuNPs without stabilizer

An 8.20 mL aqua pro injection was heated to 55°C and continuously stirred. To the warm aqua pro injection, 1.5 mL of 0.002 M HAuCl was added with continuous stirring for 1 minute. Next, 0.05 mL of 0.02 M NaBH4 was added with continuous stirring. Once the solution had become reddish purple, the stirring was continued for 1 minute without heating.

Purification of AuNPs

The purification process used size exclusion chromatography (SEC) with a Sephadex G-25 medium (PD-10) column. Before the purification, the column was eluted using phosphate buffered saline (PBS), pH 7.5, saturated with 1.0 mL of 0.5% bovine serum albumin

(BSA), and then rinsed with 0.01 M PBS, pH 7.5. After the column was conditioned, 1.0 mL of the AuNP sample was poured into the column and eluted using 0.01 M PBS, pH 7.5. Fractions were collected every 1.0 mL.

Characterization of the AuNPs

Each fraction was collected, characterized, and analyzed. The physical appearance of the AuNPs, including their morphology, color, and odor, was characterized. The UV-Vis absorbance spectrum of GA, HAuCl, and the four preparations of AuNPs were collected and analyzed at the wavelengths from 400-800 nm.

Transmission electron microscopy (TEM) was used to characterize the morphology of the AuNPs. The zeta potentials of the AuNPs were measured via electrophoretic light scattering (ELS) using a Delsa Nano C particle size analyzer (PSA) at scattering angles of 15°, 30°, and 60° to measure the electrophoretic mobility. The polydispersity index was measured via photon correlation spectroscopy (PCS) using a Delsa Nano C PSA via the same procedure.The AuNP surface topographies were observed using atomic force microscopy (AFM). The optimum characterization conditions for the AuNPs were 2000 rpm for 30 seconds.

Table 1: Reaction Conditions for Preparing the Gold Nanoparticles

Preparation	GA (ml)	Precursor 0.002 M $HAuCl_4$ (mL)	Reductor 0.02 M $NaBH_4$ (mL)	Reaction Time (hours)	Solution Color	Ratio
GA-$HAuCl_4$	8.2	1.5	-	4	Reddish purple	0.13: 1
GA-$HAuCl_4$-$NaBH_4$	8.2	1.5	0.05	4	Reddish purple	0.13: 1: 0.3
GA-$HAuCl_4$-$NaBH_4$	0.5	2.5	0.05	4	Reddish purple	0.005: 1: 0.3
$HAuCl_4$-$NaBH_4$	-	1.5	0.05	4	Reddish purple	1: 0.3

Antioxidant activity of the AuNPs

DPPH was prepared as a control solution by dissolving DPPH in methanol to obtain a 40 ppm DPPH solution. A 2.0 mL aliquot of methanol was added to 2.0 mL of this solution and placed into a container protected from light. This mixture was homogenized by vortexing for 10 seconds before incubating at 37°C for 30 minutes. Its absorbance spectrum was measured using a UV-Vis spectrophotometer across the wavelengths of 400-800 nm. The maximum wavelength was then determined.

The antioxidant activities of the sample AuNP solutions were determined using the above procedure at the maximum wavelength. Vitamin C was used as a positive control [4,5].

The absorbance of the sample solution was used to calculate the inhibition percentage via the formula below:

$$\text{Inhibition percentage} = \frac{\text{absorbance of control solution} - \text{absorbance of sample}}{\text{absorbance of control solution}} x\ 100\%$$

RESULTS AND DISCUSSION

Preparation and purification of the gold nanoparticles

The first step in the preparation of the AuNPs was to dissolve the Au foil in aqua regia (HCl (p):HNO3 (p) = 3:1) because Au only dissolves in this solution. Nitric acid, a strong oxidizer, oxidizes the Au foil to Au^{3+}. The resulting Au^{3+} reacts with Cl^- to form $AuCl_3$. The $HAuCl_4$ AuNPs were prepared using GA as both the reductor and stabilizer, sodium borohydride as the reductor with gum arabic as the stabilizer and sodium borohydride as the reductor without a stabilizer. The UV-Vis spectra showed the Au absorbance at wavelength 290 nm disappeared, which indicated that the Au was reduced [6]. The AuNPs exhibited absorbance at wavelengths from 500 – 550 nm. Higher AuNP solution concentrations had higher absorbances.

The purification process was performed via SEC with a Sephadex G-25 medium (PD-10) column. A total of 10 fractions were obtained. Fractions 4 and 5 showed the highest absorbances, 0.63574 at a wavelength of 535 nm and 0.52857 at a wavelength of 536 nm, respectively. This purification method is based on separation by molecular size. The molecular weights of HAuCl4, GA, and NaBH4 are 339.7865, ~250,000, and 37.8325, respectively.

Larger molecules elute before smaller molecules. This purification was suitable for separating the AuNP-GA complexes, free AuNPs, and sodium borohydride.

Characterization of the gold nanoparticles

The obtained nanoparticle solutions were reddish purple and had no odor, as shown in Figure 1.

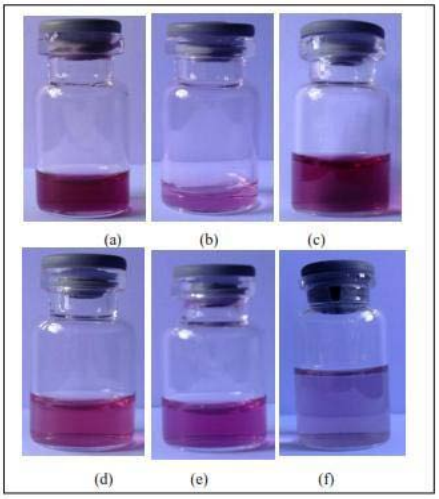

Fig. 1: Physical appearance of (a) gum arabic (GA) – gold nanoparticles (GA-$HAuCl_4$-$NaBH_4$ 0.005:1:0.3), (b) purified GA – gold nanoparticles (GA-$HAuCl_4$-$NaBH_4$ 0.005:1:0.3) with GA as a stabilizer, (c) GA – gold nanoparticles (GA-AuNP 0.13:1:0.3) with GA as a stabilizer, (d) purified GA – gold nanoparticles (GA-HAuNP 0.13:1:0.3) with GA as a stabilizer, (e) GA – gold nanoparticles (GA-AuNP) with GA as both a stabilizer and reductor, and (f) unstabilized gold nanoparticles (AuNP).

All the AuNPs were prepared as clear solutions, which indicates that the Au was homogenously dispersed with very small particle sizes. Therefore, the particles cannot be observed by the naked eye. When Au^{3+} was perfectly reduced to Au^0, the clear yellow solution became reddish purple. This color developed due to surface plasmon resonance (SPR), which is a specific property of AuNPs. The maximum AuNP absorbance occurred at wavelengths from 520-565 nm, which correspond to green and purple.

Figure 1 shows that the AuNPs using the GA stabilizer had a more intense reddish purple color. The solution turned clear after purification. This result indicates that the purification method successfully separated the AuNPs from the other compounds. The AuNPs with GA as both a stabilizer and reductor had a less intense reddish purple color. This result was caused by the lower reductive activity of GA relative to a chemical reductor. The unstabilized AuNPs were purple because fewer AuNPs were produced in the solution without a stabilizer. The varying solution colors indicated that the different protocols produced AuNPs with different sizes.

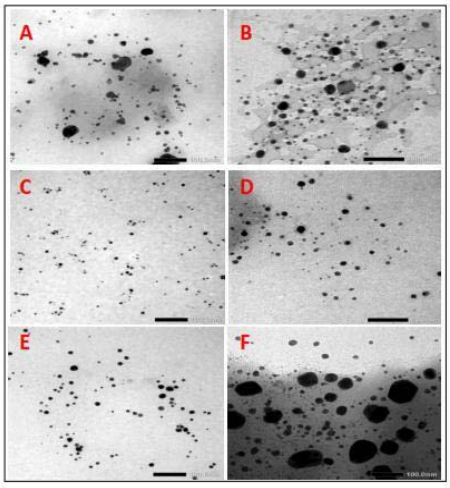

Fig. 2: Morphology of (a) gum arabic (GA) – gold nanoparticles (GA-AuNP 0.005:1:0.3) with GA as a stabilizer, (b) purified GA – gold nanoparticles (GA-AuNP 0.005:1:0.3) with GA as a stabilizer, (c) GA – gold nanoparticles (GA-AuNP 0.13:1:0.3) with GA as a stabilizer, (d) purified GA – gold nanoparticles (GA-AuNP 0.13:1:0.3) with GA as a stabilizer, (e) GA – gold nanoparticle (GA-AuNP 0.13:1) with GA as both a stabilizer and reductor, and (f) unstabilized gold nanoparticles (AuNP) using transmission electron microscopy (TEM) with 40,000× magnification.

Figure 2 shows a TEM micrograph of the AuNPs. The GA-$HAuCl_4$-$NaBH_4$ ratio of 0.005:1:0.3 with GA as the stabilizer produced spherical particles with nonhomogeneous sizes. Due to the low stabilizer concentration, the nanoparticles tended to aggregate. Unstabilized AuNPs (Fig 2.f) were larger than the other prepared AuNPs. The absence of a stabilizer resulted in particle aggregation. This result demonstrates that there is a correlation between the solution color and the AuNP size.

The AuNP size distribution was determined using a particle size analyzer. The particle size distributions for the GA-AuNPs using the GA stabilizer (0.005:1:0.3), the purified GA-AuNPs using the GA stabilizer (0.005:1:0.3), GA-AuNPs using GA as a stabilizer (0.13:1:0.3), the purified GA-AuNPs using GA as a stabilizer (0.13:1:0.3), GA-AuNPs using GA as a stabilizer and reductor, and unstabilized AuNPs were 4.045 ± 0.99 nm, 4.83 ± 1.03 nm, 4.87 ± 1.68 nm, 12.12 ± 3.36 nm, 6.52 ± 0.66 nm, and 55.82 ± 39.87 nm, respectively. These values indicate that the formed AuNPs were less than 100 nm in size. GA effectively reduced the AuNP size. These results show that the GA-AuNP sample using GA as both the stabilizer and reducing agent still formed AuNPs smaller than 100 nm without using a chemical reductant.

The zeta potential of the AuNPs was determined using an electrophoretic particle analyzer with ELS. The zeta potentials of the GA-AuNPs using GA as a stabilizer (0.005:1:0.3), purified GA-AuNPs with GA as a stabilizer (0.005:1:0.3), GA-AuNP using GA as a stabilizer (0.13:1:0.3), purified GA-AuNPs using GA as a stabilizer (0.13:1:0.3), GA-AuNPs using GA as both a stabilizer and reductant, and unstabilized AuNPs were -26.4 ± 1.62, -28.4 ± 1.30, -30.1 ± 2.16, 25.8 ± 1.53, -31.9 ± 1.88, and -39.3 ± 0.46, respectively.

The surface characteristics affect the stability and cell membrane interactions of nanoparticles. The nanoparticle zeta potentials are commonly used to characterize the charge of a nanoparticle surface. In the electric double layer theory, the zeta potential predicts the stability of a colloid system. High zeta potentials (either positive or negative) indicate a stable colloidal system that prevents particles from aggregating due to electrostatic repulsion between the particles. In general, particles with zeta potentials above +30 mV or below -30 mV are predicted to be stable during storage and prevented from undergoing particle aggregation.

The polydispersity indexes for GA-AuNPs using GA as a stabilizer (0.005:1:0.3), purified GA-AuNPs using GA as a stabilizer (0.005:1:0.3), GA-AuNPs using GA as a stabilizer (0.13:1:0.3), purified GA-AuNPs using GA as a stabilizer (0.13:1:0.3), GA-AuNPs using GA as both a stabilizer and reductant, and unstabilized AuNPs were 0.511, 0.505, 0.742, 0.703, 0.507, and 0.708, respectively. The GA-AuNP samples using GA as both a stabilizer and reductant exhibited the lowest polydispersity index. This result indicated that the AuNPs formed were monodisperse without particle aggregation. In addition, the SEC purification process made the GA-AuNP system more monodisperse than the unpurified samples. The polydispersity index is a parameter detailing the particle size distribution of the nanoparticle system with values of 0.01 to 0.7 indicating monodisperse particles and values above 0.7 indicating a wide particle size distribution. The polydispersity index reflects the stability of a nanoparticle system, with increasing values indicating increasing particle aggregation. A monodisperse nanoparticle system has a narrow particle size and contains stable nanoparticles with fewer aggregates. A qualitative analysis of the infrared spectrum of the GA-AuNP samples searched for interactions between the HAuCl and GA and aimed to prove that GA protects the AuNPs. The infrared spectrum for GA exhibits primary peaks at 1049 and 1413 cm^{-1} (bound C-O), 1060 cm^{-1} (C-O), 1616 cm^{-1} (C-O and N-H), and 3000-3600 cm^{-1} (free O-H).

The AuNP spectra were identical to the GA spectrum. This result suggests that GA is present on the AuNP surface. Thus, the high AuNP stability may be due to protection by GA.

Metal nanoparticles tend to aggregate due to strong interparticular forces pulling the particles closer together to form larger particle clusters over time. Although gold nanoparticles are more stable than other nanoparticles, they still tend to aggregate.

The absorbance and maximum wavelength measurements showed a relationship over time. The stability of the AuNPs was evaluated using the change in wavelength and maximum absorbance 5 weeks after synthesis.

The λ max measurements shifted with increasing storage time. This condition was caused by the large AuNP surface energy, which is unstable and easily leads to aggregation. Therefore, increasing the time resulted in the formation of larger particles, and thus the λ max underwent a bathochromic shift. The absorbance showed a similar trend with the absorbance values increasing to a maximum within 4 hours before decreasing again. This increased absorbance indicated that more AuNPs were formed, whereas a decreased absorbance would indicate that the AuNPs agglomerated into larger particles, decreasing the transmitted light.

The AFM analysis of the AuNPs indicated that the particle size was ~ 20 nm. The observed AuNP surface profile of the AuNPs using GA as a stabilizer is shown in Figure 3. The sample surface area was 500 nm × 500 nm and 1 µm × 1 µm. The AFM particle size analysis for the AuNPs is slightly different from the results measured using PSA. This difference may result from a textural change smaller than the tip size, which would prevent properly recording small positional shifts of the cantilever toward the normal (on the order of tens of nanometers).

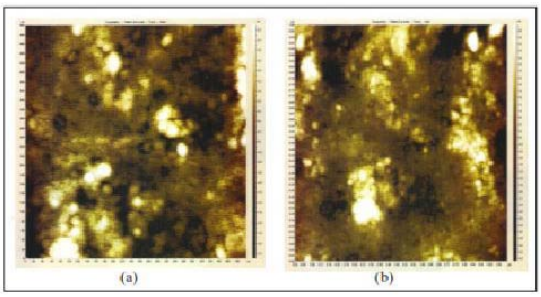

Fig. 3: Topography of Formula E gold nanoparticles using atomic force microscopy (AFM) with scan area of (a) 500 nm × 500 nm and (b) 1 µm × 1 µm.

Table 2: Inhibition Activity of Gold Nanoparticles

Preparation	Concentration (µg/ mL)	Absorbance at 516 nm			Inhibition (%)
		Control	Sample	Sample + DPPH	
GA-$HAuCl_4$-$NaBH_4$	59.1	0.453	1.136	1.256	**73.51±0.221**
GA-$HAuCl_4$	59.1	0.453	0.683	0.799	**74.39±0.255**
$HAuCl_4$-$NaBH_4$	59.1	0.453	0.202	0.326	**72.63±0.567**

Antioxidant activity test of the AuNPs

The scavenging of free radicals by DPPH was used in this study to measure the antioxidant activity. During this process, the test substance donates a hydrogen atom to the DPPH free radical, thus reducing DPPH to the stable non-radical compound 1,1-diphenyl-2-picril-hydrazine. This transition is characterized by a color change from purple to pale yellow or colorless. Testing the antioxidant activity of the AuNPs revealed that the percent inhibition was **73.51 ± 0.221%** for AuNPs using GA as a stabilizer, **74.39 ± 0.255%** for AuNPs using GA as both a stabilizer and reductant, and **72.63 ± 0.567%** for AuNPs without any stabilizer. These results suggest that the AuNPs had good radical scavenging capabilities.The percent inhibitions obtained for the three nanoparticle variations were similar. However, the unstabilized AuNPs had the lowest percent inhibition. This reduced inhibition likely results from the reduced particle surface area available to scavenge the DPPH radicals due to the presence of large clusters in this sample.

CONCLUSIONS

The above results lead to the following conclusions:

1. Three AuNP variations were prepared using GA as a stabilizer, GA as both a stabilizer and reductant, and as an unstabilized solution, which yielded spherical AuNPs of 4.045 ± 0.99 nm, 6.52 ± 0.66 nm, and 55.82 ± 39.87 nm, respectively, in size that were stable for up to 5 weeks.

2. The percentage oxidation inhibition was **73.51 ± 0.221%** for the GA-stabilized AuNPs, **74.39 ± 0.255%** for the AuNPs using GA as both a stabilizer and reductant, and **72.63 ± 0.567%** for the unstabilized AuNPs.

CONFLICT OF INTERESTS

Declared None

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