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Morphology-Dependent Antioxidant Activity of Gold Nanoparticles Prepared Using Different Electrolyte Concentrations

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Abstract

Antioxidants play a crucial role in protecting the body from oxidative stress by neutralizing free radicals. The synthesis of antioxidant-active materials, such as gold nanoparticles (AuNPs), offers significant advantages due to their unique physicochemical properties. In this study, AuNPs were successfully synthesized using the electrodeposition method on indium-tin oxide substrates, with varying Na_2SO_4 electrolyte concentrations. Scanning electron microscopy analysis confirmed that the synthesized AuNPs exhibited a round to slightly irregular morphology, with an average particle size ranging from 48 to 65 nm. X-ray diffractometer characterization revealed that the nanoparticles possessed a face-centered cubic crystal structure, confirming their high purity. Antioxidant activity was evaluated using the DPPH assay, where AuNPs synthesized with 0.5 M Na_2SO_4 exhibited the highest DPPH inhibition of 72.39%. This enhanced antioxidant performance is attributed to the smaller particle size, which increases the available surface area for free radical neutralization. These findings highlight the potential of electrodeposited AuNPs as effective antioxidants and provide valuable insights into optimizing nanoparticle synthesis for biomedical and nanotechnological applications.

Keywords: gold nanoparticles, antioxidant activity, morphology, DPPH assays

1. Introduction

Free radicals are highly unstable atoms or molecules that pose potential harm to the human body due to their unpaired electrons, which make them highly reactive [1]. This reactivity enables free radicals to interact with biological molecules, potentially leading to cellular damage or the formation of abnormal compounds [2,3]. By attacking surrounding cells, free radicals can initiate further harm, contributing to oxidative stress and various health issues [4,5]. Consequently, extensive research has been conducted to explore the use of antioxidants as a means to counteract the detrimental effects of free radicals [6].

Recent advancements in antioxidant research have been closely linked to developments in nanotechnology, which enhances the active surface area of antioxidant compounds. A larger active surface area significantly increases the reactivity and effectiveness of synthesized materials [7]. Various metal oxides and metal nanoparticles (NPs) have been widely investigated for their antioxidant potential. Notable studies on ZnO nanoparticles [8,9] have demonstrated promising antioxidant activity. Additionally, other metals such as Pt [10,11], Cu_2O [12–14], Ni [15], and Co [15] have been explored for similar applications. Among these, Au nanoparticles (AuNPs) have emerged as one of the most widely studied due to their remarkable antioxidant properties [16–20].

The use of gold nanoparticles as antioxidants is primarily attributed to their low cytotoxicity, ability to inhibit reactive oxygen species, and proven efficacy in neutralizing free radicals. A study by Patra et al. [21] demonstrated the antioxidant potential of gold nanoparticles synthesized by reducing $AuCl_3$ with onion skin extract, achieving an inhibition rate of 14.44% at a concentration of 100 µg/mL. Additionally, gold nanoparticles synthesized using C. cuspidatus extract have

been shown to enhance the antioxidant activity of natural compounds. This is evident in the percentage inhibition values, where the natural extract alone exhibited 96.63% inhibition, which increased to 97.05% when adsorbed onto the surface of AuNPs. This enhancement is attributed to the synergistic effect of the hydrogen donor mechanism of natural compounds and the electron-donating properties of gold nanoparticles [22]. The electron donation process occurs at the nanoparticle surface, where interactions between the unpaired electrons of free radicals and the conduction band electrons of AuNPs facilitate their neutralization [23].

Over the years, various methods for synthesizing gold nanoparticles have been developed. Typically, Au^{3+} ions are reduced to zero-valent Au^{0} through chemical reactions with reducing agents such as citric acid, borohydride, or biological reductants derived from natural extracts, fungi, and bacteria [24–26]. However, these chemical methods can negatively impact nanoparticle purity, involve toxic and costly reagents, and pose environmental hazards [27]. An alternative and promising approach for synthesizing gold nanoparticles (AuNPs) is electrodeposition. This method offers several advantages, including a straightforward synthesis process, high purity, precise control over nanoparticle morphology, and the absence of toxic chemicals [28]. Moreover, electrodeposition enables the formation of gold nanoparticles in a solid phase, allowing for the independent evaluation of their antioxidant activity without interference from solution-based reducing agents [29,30].

One of the most effective electrodeposition techniques for gold nanoparticle synthesis is cyclic voltammetry (CV). This method is advantageous as it produces highly pure nanoparticles and allows for precise determination of optimal deposition conditions [31]. Key parameters influencing the quality of AuNPs synthesized via CV include the applied potential range, number of cycles, solution concentration, scan rate, electrolyte composition, pH, and the presence of additives [31,32]. By fine-tuning these parameters, high-quality gold nanoparticles with enhanced antioxidant properties can be successfully synthesized.

2. Materials and Method

2.1 Materials

All solutions used for electrochemical activity and deposition were prepared using deionized water. The DPPH solution was prepared in ethanol (98% purity, Sigma-Aldrich). The gold precursor, $HAuCl_4 \cdot 3H_2O$ (99.9% purity, Merck), and the supporting electrolyte, Na_2SO_4 (99.0% purity, Merck), were used for the electrodeposition process.

2.2 Methods

2.2.1 Synthesize of AuNPs

Gold nanoparticles (AuNPs) were synthesized via electrodeposition using a potentiostatic technique. A 0.5 mM $HAuCl_4$ solution was prepared by dissolving $HAuCl_4 \cdot 3H_2O$ in deionized water and diluting it with Na_2SO_4 as the supporting electrolyte. The electrodeposition was performed in a three-electrode system, where an indium tin oxide (ITO) substrate functioned as the working electrode, platinum (Pt) as the counter electrode, and Ag/AgCl as the reference electrode. The synthesis was carried out at room temperature (25°C) under controlled parameters, as listed in Table 1.

Table 1. Na_2SO_4 concentrations and CV parameters used in the AuNPs preparation.

Sample code	$[Na_2SO_4]$ (M)	Scan rate (mV/s)	$[HAuCl_4]$ (M)
AuNPs1	0.1	125	0.5
AuNPs2	0.5	125	0.5
AuNPs3	1.0	125	0.5
AuNPs4	1.5	125	0.5

2.2.2 Characterizations

The morphology of the synthesized gold nanoparticles was analyzed using Scanning Electron Microscopy (SEM, Thermo Scientific Quanta 650). Additionally, X-ray Diffraction (XRD, SMARTLAB Rigaku X-ray diffractometer with Cu $K\alpha_1$ radiation (λ = 1.540598 Å), was used to determine the crystallinity and crystal structure of the gold nanoparticles.

2.2.3 Antioxidant activity test

The antioxidant activity of gold nanoparticles was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. A DPPH solution with a concentration of 39 ppm was prepared, and changes in absorbance were measured using a microplate reader at a wavelength of 516 nm [33]. The antioxidant activity was determined using the following equation (1):

$$\%Inhibition = \frac{A_0 - A_1}{A_0} \times 100\% \quad (1)$$

where A_0 represents the control absorbance (DPPH solution without nanoparticles), and A_1 is the absorbance after the addition of gold nanoparticles. The inhibition percentage was calculated after 15 minutes to assess the effectiveness of gold nanoparticles as antioxidants.

3. Results and Discussion

AuNPs were synthesized by reducing $AuCl_4^-$ using the electrodeposition method. The reduced gold atoms were deposited on the ITO-PET substrate. The reduction of $AuCl_4^-$ during this process occurs according to the following the reaction [34]:

$$AuCl_{4(aq)}^- + 3e^- \rightarrow Au_{(s)}^0 + 4Cl^- \quad (2)$$

The X-ray diffraction (XRD) pattern of gold nanoparticles synthesized in Na_2SO_4 is presented in Fig. 1, confirming their face-centered cubic (FCC) crystal structure. The presence of distinct diffraction peaks indicates the crystalline nature of the synthesized nanoparticles. In a crystalline structure, atoms, ions, or molecules are arranged in a highly ordered, repeating pattern in three dimensions [37]. Reflections were observed at 2θ values of 38.27°, 44.48°, 64.73°, and 77.76°, corresponding to the (111), (200), (220), and (311) lattice planes, respectively. These diffraction peaks align with the reference data from the crystallography open database (COD) No. 96-110-0139 and are consistent with findings reported by Singh et al. [38]. However, a shift in the diffraction peaks was observed when comparing the synthesized nanoparticles to the COD reference. This peak shift can be attributed to variations in lattice structure within the nanoparticle clusters. Differences in lattice spacing influence crystallite size, leading to discrepancies between the synthesized nanoparticles and the reference data. Such shifts in diffraction patterns are common in nanomaterials and can result from strain, lattice distortions, or defects within the crystal structure [36]. These structural differences highlight the impact of synthesis conditions on the crystallinity and size of gold nanoparticles.

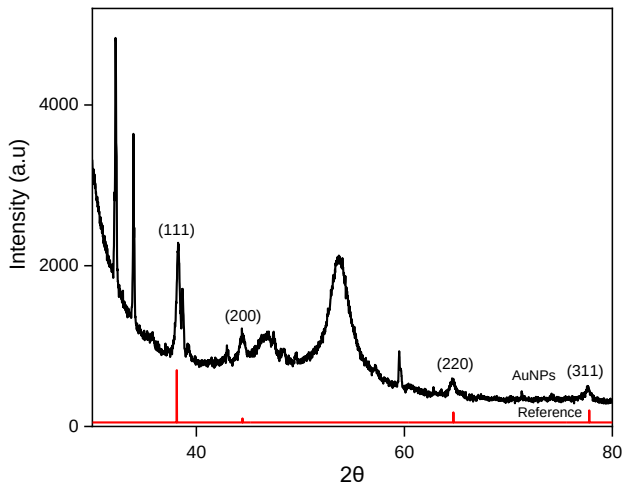

Figure 1. X-Ray diffraction pattern of AuNPs.

The morphological characteristics of AuNPs synthesized at varying electrolyte concentrations were analyzed using SEM. Figure 2 presents the morphology of gold nanoparticles prepared with different concentrations of Na_2SO_4, along with a histogram illustrating their size distribution. Na_2SO_4 is used to enhance the electrochemical activity of gold, thereby improving the synthesis of the gold film. The SEM analysis of AuNPs synthesized with a Na_2SO_4 concentration of 0.1 M (Fig. 2a) reveals that the particles are predominantly round. However, the nanoparticles are sparsely and unevenly distributed, failing to form a dense coverage on the substrate. The corresponding size distribution (Fig. 2e) indicates the presence of relatively few particles, with an average diameter of 48.29 nm. When the Na_2SO_4 concentration was increased to 0.5 M, the nanoparticles exhibited a more uniform distribution, with a round to slightly irregular shape. The particle density increased compared to the lower concentration, resulting in an average size of 54.06 nm (Fig. 2b). At a concentration of 1 M Na_2SO_4, the AuNPs formed a highly dense distribution while maintaining a predominantly round morphology, with an average particle size of 65.09 nm (Fig. 2c). This increased density led to significant particle aggregation, contributing to the formation of larger nanoparticles.

Table 2. Comparison of 2θ in this study with COD No.96-110-0139.

COD No.96-110-0139	This study
38,19	38,27
44,39	44,48
64,58	64,73
77,58	77,76

Further increasing the Na_2SO_4 concentration to 1.5 M produced larger particles with irregular shapes, Fig. 2d. This irregularity can be attributed to excessive particle density, which promotes aggregation. Aggregation occurs due to the high surface energy of smaller particles; when in close proximity, they tend to coalesce to minimize surface energy, ultimately forming bulk structures [39]. The addition of an electrolyte plays a crucial role in the electrodeposition process of AuNPs by enhancing solution conductivity, facilitating the desolvation of $AuCl_4^-$ ions, and improving electron transfer during the reduction process [40]. The SO_4^{2-} ion, classified as a kosmotropic ion, stabilizes the electrolyte solution by forming strong interactions with water molecules [41]. As the Na_2SO_4 concentration increases, it enhances the distribution of water and metal ions, as well as the interactions between the electrodes. This, in turn, influences the efficiency and

characteristics of nanoparticle deposition at the three-phase interface [42]. Consequently, the nucleation and growth of AuNPs occur more rapidly, leading to the formation of larger particles [43].

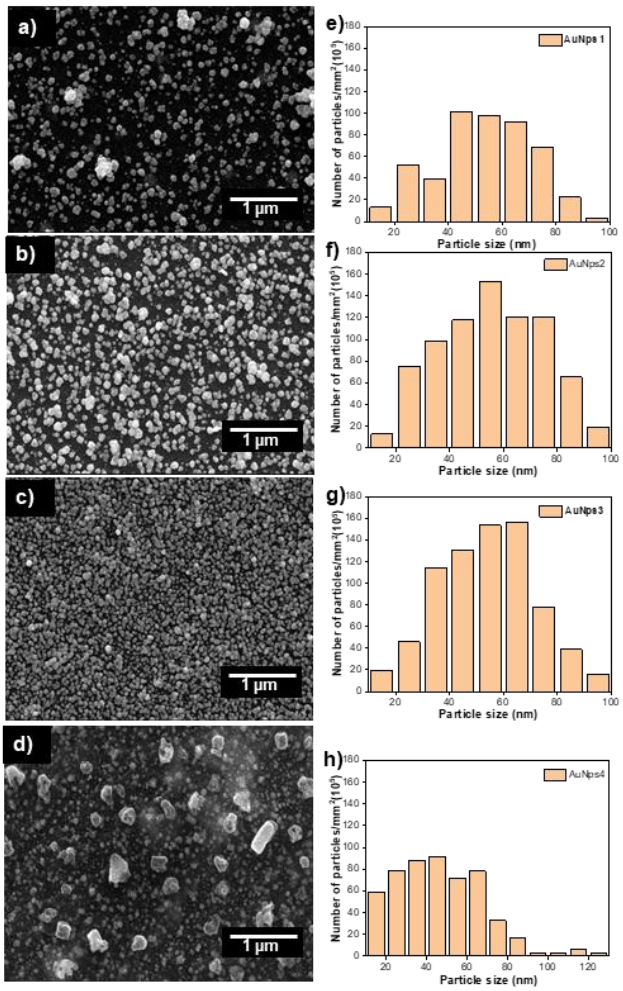

Figure 2. SEM micrograph and particle distribution of (a,e) AuNPs1, (b,f) AuNPs2, (c,g) AuNPs3, (d,h) AuNPs4.

The antioxidant activity of the synthesized AuNPs was evaluated using the DPPH assay, with the results presented in Figure 3. Based on the micrographs, AuNPs synthesized in a 0.5 M Na_2SO_4 electrolyte solution exhibited the highest inhibition percentage. Additionally, the inhibition percentage increased progressively every 15 minutes. This increase occurs because prolonged exposure allows more gold nanoparticles to interact with free radicals.

Gold nanoparticles synthesized with different Na_2SO_4 concentrations were tested to assess how electrolyte concentration influences their antioxidant properties. Figure 3 presents a comparison curve illustrating the relationship between inhibition percentage and Na_2SO_4 concentration. The results indicate that the optimal inhibition percentage was achieved at a Na_2SO_4 concentration of 0.5 M. Furthermore, the inhibition percentage increased with longer reaction times. This effect is attributed to the extended interaction between nanoparticles and free radicals, allowing for more effective scavenging activity. Additionally, prolonged contact time helps prevent the generation of new free radicals. Certain antioxidants, after neutralizing free radicals, contribute to system stabilization and inhibit further radical formation, thereby prolonging their inhibitory effect [44].

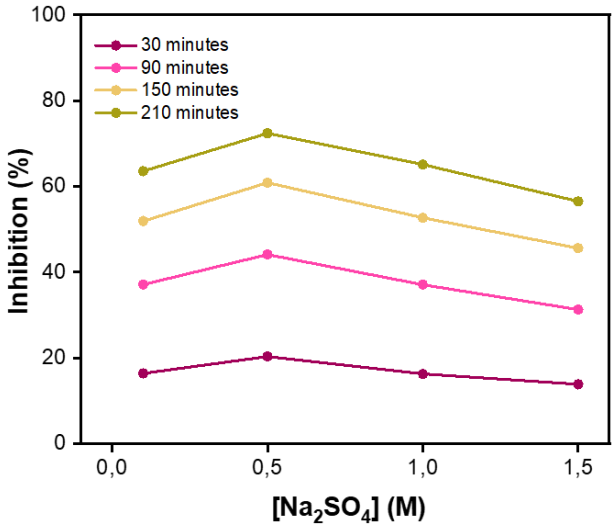

Figure 3. Curve depicting the relationship between Na_2SO_4 concentration and percent inhibition.

As shown in Table 3, when the Na_2SO_4 concentration exceeded 0.5 M, the inhibition percentage declined. Similar to the catalytic activity of AuNPs, antioxidant activity occurs on the nanoparticle surface and is significantly influenced by particle size. Smaller nanoparticles exhibit higher catalytic efficiency due to their larger surface area [45], providing more active sites for interactions with free radicals, thereby enhancing antioxidant performance [43]. This observation aligns with the SEM analysis, which showed that increasing Na_2SO_4 concentration led to larger nanoparticle sizes, reducing the overall surface area available for antioxidant interactions and subsequently lowering the inhibition efficiency.

Table 3. Percentage inhibition of AuNPs samples in 210 minutes.

Sample	Inhibition (%)
AuNPs1	63.56
AuNPs2	72.39
AuNPs3	65.11
AuNPs4	56.50

4. Conclusion

AuNPs were successfully synthesized using the electrodeposition method with varying Na_2SO_4 electrolyte concentrations. The synthesized AuNPs exhibited a predominantly round morphology with slight irregularities and an average particle size ranging from 48 to 69 nm. Antioxidant activity tests using the DPPH assay revealed that AuNPs synthesized with 0.5 M Na_2SO_4 (AuNPs3) exhibited the highest DPPH inhibition of 72.39% over 210 minutes. This enhanced antioxidant activity is attributed to smaller particle sizes, which provide a larger surface area for free radical neutralization. These findings highlight the significance of electrolyte concentration in controlling the size, morphology, and antioxidant properties of gold nanoparticles.

Author contributions

Babay Asih Suliasih: Writing – original draft, investigation, formal analysis. Azri Farhanah: writing – review & editing, formal analysis.

Conflicts of interest

There are no conflicts to declare.

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