GREEN FABRICATION OF GOLD NANOBRANCHES BY HEPES BUFFER

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Abstract

In recent years, asymmetric gold nanoparticles have attracted a lot of attention from researchers owing to their unique properties and varied applications in many fields. In this study, gold nanobranches were prepared using a one-step, green reducing method, with the HEPES buffer acting as both a reducing agent and surfactant. The formation of gold nanoparticles was evaluated using UV-Vis spectroscopy by controlling several practical factors, including the volume of gold salt precursor, the concentration of HEPES buffer, and the solution pH. The morphologies and crystallization of the gold nanobranches were characterized by Scanning electron microscopy (SEM) and X-ray diffraction (XRD). The results indicated that under the optimal synthesis conditions, namely 250 µL of 5 mM $HAuCl_4$, 0.10 M HEPES, and a pH of 7.5, most of the gold particles in the colloidal solution exhibited multiple branches, with an average size ranging from 20 to 35 nm and high crystal density. This study presented a simple synthesis method utilizing eco-friendly substances to replace conventional reducing agents, contributing to the sustainable development of nanotechnology.

Keywords: gold nanobranches, green fabrication, one-step synthesis, HEPES

1. Introduction

Gold nanoparticles (AuNPs) are noble nanoparticles known for their unique properties. The physicochemical and optical characteristics of AuNPs are significantly influenced by their shape and size, and these can be easily adjusted by controlling the morphology of the nanoparticle structure (Rad et al., 2011). Gold nanobranches have attracted great attention due to their optical, electronic, and magnetic properties, often superior to spherical gold nanoparticles (Grzelczak et al., 2008). The attractive feature of gold nanobranches is the presence of a plasmon band in the near-infrared region spanning the wavelength range from 650 to 800nm, which may promote many potential applications in biosensing, catalysis, imaging, and photothermal therapy (Sharifi et al., 2019; Li et al., 2019).

The development of synthetic methods has resulted in a diverse range of morphologies and anisotropies in AuNPs. Chemical synthesis methods for gold nanobranches commonly include one-step reduction (Bakr et al., 2004; Minati et al., 2014) and the seeding-growth method (Kawamura et al., 2008; Zhai et al., 2019). The one-step reduction is considered a simple, easy-to-implement, high-yield method; however, controlling the desired branch length of nanoparticles is more difficult than the seeding-growth method. On the other hand, conventional reduction methods mainly use reducing agents and surfactants, which are more or less toxic and require multiple steps to purify nanoparticles after synthesis (Souza et al., 2019; Mulder et al., 2019).

The report of Habib et al. (2005) on the fabrication of AuNPs in the presence of Good's buffers opened up a green synthesis process for AuNPs without using reducing agents and surfactants as in previous conventional processes. Some typical Good's buffers used in the synthesis of AuNPs included Tris base (Luo et al., 2011), 4-(2-hydroxyethyl)-1-piperazinepropanesulphonic acid (EPPS) (Lu et al., 2016), or 3-N-morpholino propanesulfonic acid (MOPS) (Chandra et al., 2016). One of them, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), was a useful stabilizer that was commonly employed in the one-step synthesis of mbAuNPs. As a zwitterionic ion, the piperazine moiety in HEPES generated nitrogen-centered free radicals that converted Au^{3+} ions in $HAuCl_4$ to Au^0 atoms even at low concentrations (about 6 ppm) (Mulder et al., 2019). HEPES was initially reported by Xie et al. (2007) for the synthesis of mbAuNPs, achieving a high yield of nanoparticles with 1-8 branches. Subsequent investigations were followed on the process with certain modifications; for example, the synthesis of spherical AuNPs utilizing Cys-terminal peptides (Serizawa et al., 2008), the production of monodisperse star-shaped AuNPs with silver nitrate salt serving as an assistance for shape directing (Mulder et al., 2019), and the pretreatment of star-shaped AuNPs with acid for SERS applications in benzene detection (Xi et al., 2019).

In this study, gold nanobranches were green synthesized using a one-step reduction method, in which HEPES served as both a reducing agent and surfactant. This synthesis method controlled the formation and morphology of the gold particles through a simple procedure performed at room temperature by adjusting the precursor concentration, HEPES concentration, and solution pH. According to the results, we found the optimum synthesis conditions to gain gold nanobranches with a high yield.

2. Materials and methods

2.1. Materials

Tetrachloroauric (III) acid trihydrate ($HAuCl_4.3H_2O$ 99.9%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES 99.5%), sodium hydroxide (NaOH 99.5%), and Millipore water were purchased from Sigma-Aldrich. The chemicals were used directly without further purification.

2.2. Synthesis of gold nanobranches by the one-step reduction

Gold nanobranches were synthesized through a one-step reduction using HEPES, following the procedure of Xie et al. with some adjustments to optimize the experimental conditions. HEPES served as both a reducing agent and a surfactant. Initially, a 0.10M solution of HEPES was prepared with Millipore water, and the pH was adjusted to 7.5 using a 1M NaOH solution. Slowly, 500µL of a 5 mM $HAuCl_4$ solution was added to a

20mL glass container containing 10mL of 0.1M HEPES. With magnetic stirring at a rate of 600rpm, the solution's color gradually changed from pink-purple to blue within 5 minutes. The reaction was conducted under light-protective conditions and stored away from light at 4°C. Several factors affecting the synthesis of mbAuNPs, including the amount of $HAuCl_4$ precursor, HEPES concentration, and solution pH, were investigated as detailed in Table 1.

Gold nanoparticles were separated from the fresh colloids before further analysis. By centrifugating the colloidal solution at 6000rpm for 10 minutes, the precipitation was collected after removing the upper fluid. Subsequently, the precipitation was rinsed three times with Millipore water to remove the residual reagents.

TABLE 1. Investigating factors on the synthesis of gold nanobranches

Samples	The volume of $HAuCl_4$ 5mM (µL)	Concentration of HEPES (M)	pH
HE/Au50	50	0.10	7.5
HE/Au100	100		
HE/Au150	150		
HE/Au200	200		
HE/Au250	250		
HE/Au300	300		
HE/Au350	350		
HE/Au400	400		
HE/Au450	450		
HE/Au500	500		
HE/HE0.05	500	0.05	7.5
HE/HE0.10		0.10	
HE/HE0.15		0.15	
HE/HE0.20		0.20	
HE/HE0.25		0.25	
HE/HE0.30		0.30	
HE/HE0.35		0.35	
HE/HE0.40		0.40	
HE/pH6.5	500	0.10	6.5
HE/pH7.5			7.5
HE/pH8.5			8.5
HE/pH9.5			9.5
HE/pH10.5			10.5
HE/pH11.5			11.5
HE/pH12.5			12.5

2.3. Methods

The formation of the gold nanobranches was evaluated by UV-Vis spectroscopy on the UV-Vis Jasco V670 spectrometer (Japan) with a testing wavelength of 400-800nm and a scanning rate of 200nm/min. X-ray diffraction (XRD) was conducted on the D8 Advance Bruker (Germany) with the 2θ degrees varying from 30° to 80° to analyze the crystal structure of AuNPs. The morphology of nanoparticles was obtained from Scanning electron microscopy (SEM) collected on the S–4800 HITACHI (Japan). Using ImageJ software to measure a minimum of 30 particles on SEM images with the respective scale to determine the average size of formed nanoparticles.

3. Results and discussions

Effect of $HAuCl_4$ volume

To evaluate the effect of the amount of precursor on gold nanoparticle formation, the volume of 5mM $HAuCl_4$ changed from 50 to 500µL, corresponding to the color of the colloidal solution changing from blue to purple. As Figure 1, UV-Vis results showed that the intensity of an absorbance peak increased from 0.187 to 1.104 with the increase of $HAuCl_4$ volume from 50 to 250µL. This proved that the quantity of gold nanoparticles formed in the colloidal solution progressively increased. The presence of an absorbance peak of the HE/Au 250 sample at 650nm predicted the gold nanobranches' formation in the colloidal solution (Luo et al., 2011). However, when the volume of $HAuCl_4$ increased by an excess of 250µL, the absorbance peak gradually moved to the blueshift, from 628nm (HE/Au300) to 577nm (HE/Au500). It was observed that the color of the colloidal solution changed to red as a specific characteristic of spherical gold nanoparticles. It could be explained that the increasing volume of $HAuCl_4$ corresponded to the higher amount of Au^{3+} ions that existed in the solution, resulting in the reduction of the activity of HEPES. Consequently, the formed AuNPs would be tented to agglomerate into a spherical shape (Serizawa et al., 2009).

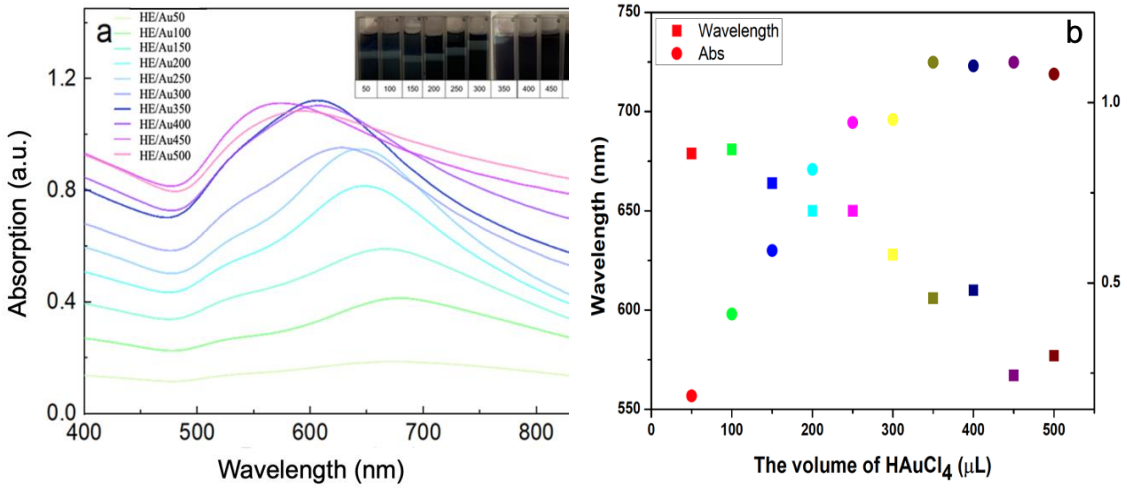

Figure 1. (a) UV-Vis spectrum of the gold colloidal solution AuNPs with different 5mM $HAuCl_4$ volumes from 50 to 500µL; (b) the corresponding graph between the wavelength and intensity of absorption peaks

Effect of HEPES concentration

The formation of gold nanobranches in a variety of HEPES concentrations was investigated by UV-Vis as shown in Figure 2. The color of the colloidal solution changes from slight green to slight purple. When the HEPES concentration increased from 0.05M to 0.10M, the absorption intensity gradually increased from 1.045 to 1.079, and the absorption peak shifted from 663nm to 673nm, which indicated the formation of gold nanobranches in the colloidal solution. As the HEPES concentration exceeded 0.10M, the absorption peak moved from 670nm to 640nm, corresponding to the HEPES concentration ranging from 0.15 to 0.40M. It was explained that the Au^{3+} ion from $HAuCl_4$ was reduced by free radicals generated from HEPES. The reaction was stopped as the reduction of the Au^{3+} ion was completed, at this point, the residual HEPES in the solution acted as a surfactant agent covering gold particles, leading to the absorption intensity decrease.

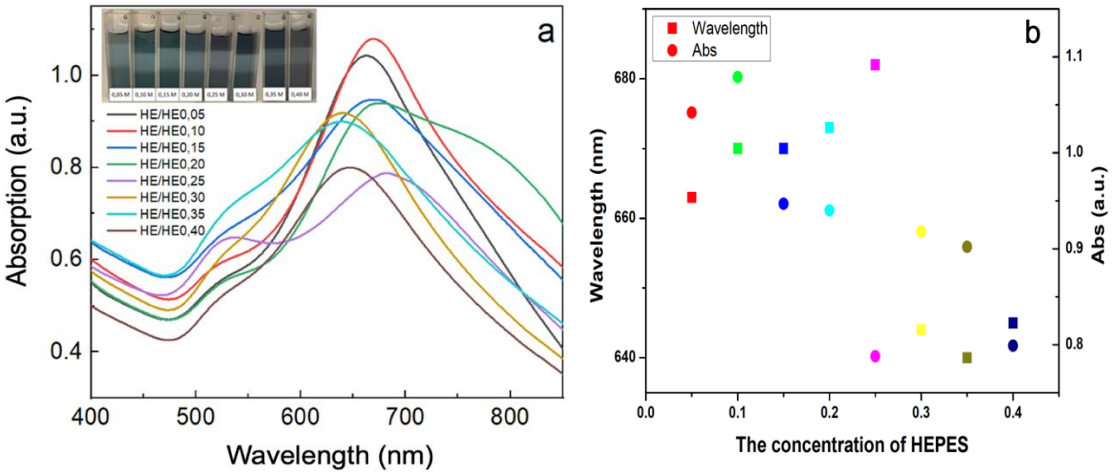

Figure 2. (a) UV-Vis spectrum of the gold colloidal solution AuNPs with different HEPES concentrations from 0.05 to 0.40M; (b) the corresponding graph between the wavelength and intensity of absorption peaks

Effect of solution pH

The UV-Vis spectrum illustrated the role of pH in the formation of gold nanobranches, as shown in Figure 3. The absorption peak shifted from 607nm to 674nm when the pH was adjusted from 6.5 to 7.5. The absorption intensity reached a maximum of 0.84 at a wavelength of 674nm at pH 7.5. However, the absorption intensity decreased sharply when the pH increased from 8.5 to 12.5. HEPES, a zwitterion, at a pH of 7.4, allowed the piperazine group in the HEPES moiety to generate nitrogen-centered free radicals, which play a role in reducing Au^{3+} ions to Au^{0} due to the aggregation of gold atoms (Xie et al., 2007; Mulder et al., 2019).

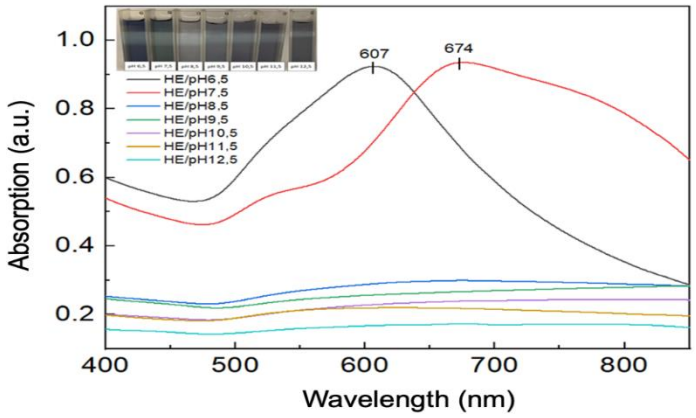

Figure 3. UV-Vis spectrum of the gold colloidal solution AuNPs with different pHs from 6.5 to 12.5

The morphologies of gold nanobranches

The morphologies of the nanoparticles were characterized using SEM, as shown in Figure 4. It was observed that a large quantity of gold nanoparticles formed in the colloidal solution. Most of the particles were multi-branched, with an average size of approximately 20 to 35nm. Furthermore, due to the substantial amount of nanoparticles generated, the particles tended to decrease their distance of independent existence while still preserving the shape of gold particles. The SEM images were consistent with the

aforementioned UV-Vis results. The crystallization of AuNPs was further confirmed by XRD analysis in Figure 5, revealing five Bragg diffraction peaks at 2θ degrees of 38.2°, 44.7°, 64.7°, and 82.0°, which correspond to the (111), (200), (220), and (311) crystal planes of the Au atom. This indicated the characteristic of the face-centered cubic (fcc) gold crystal structure.

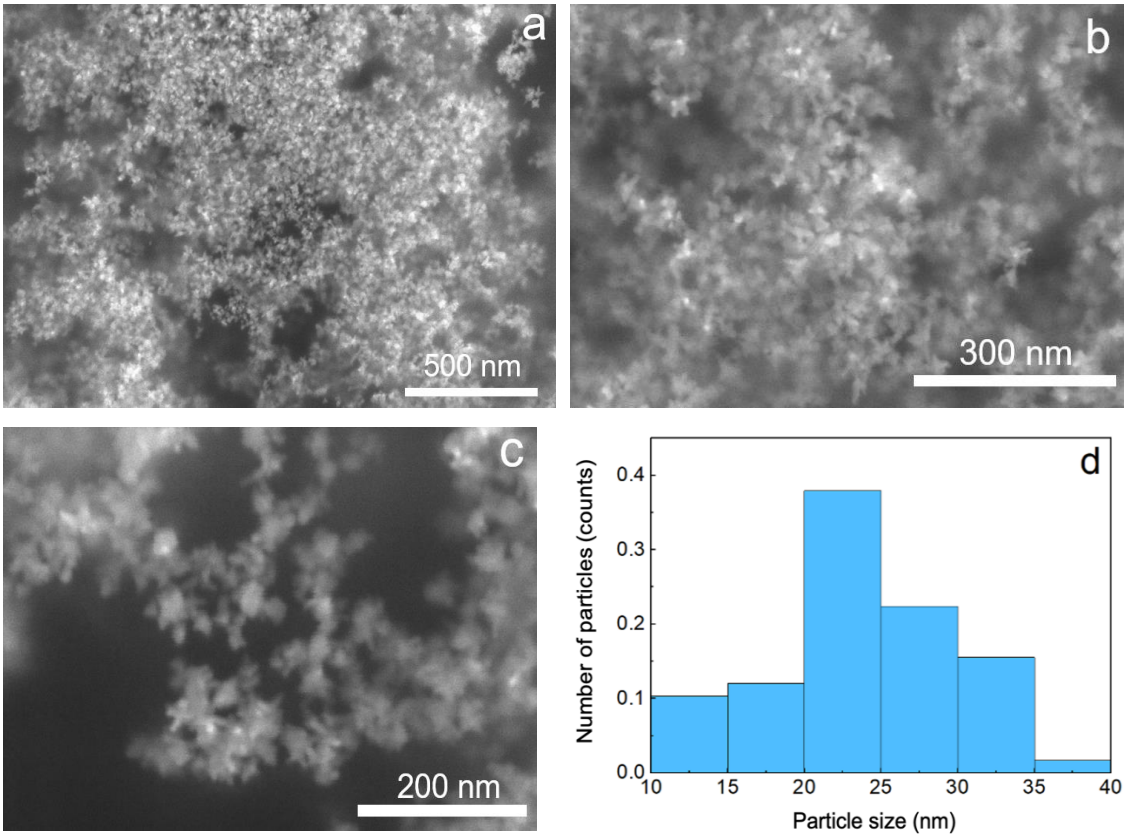

Figure 4. SEM images of synthesized gold nanoparticles under a pH of 7.5 at different scales: (a) 500nm, (b) 300nm, (c) 200nm, and (d) the corresponding distribution of the average particle size

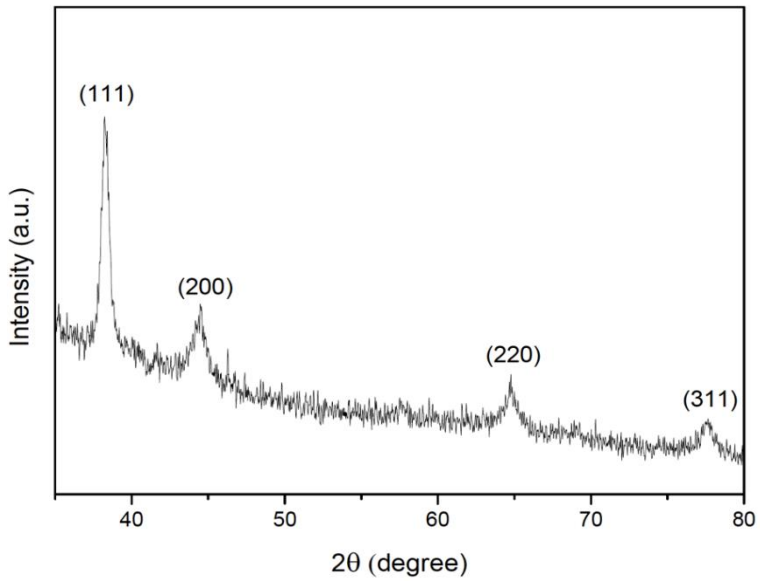

Figure 5. XRD pattern of synthesized gold nanobranches

4. Conclusions

In summary, gold nanobranches were successfully synthesized through a one-step method using HEPES as both a reducing agent and a surfactant. As determined by UV-Vis spectrum analysis, the volumes of $HAuCl_4$, the concentration of HEPES, and the pH were controlled for the formation of the gold nanoparticles. At a ratio of 250µL of 5mM $HAuCl_4$, 0.10M HEPES, and a pH of 7.5, most of the multi-branched nanoparticles formed in the gold colloidal solution with an average size of approximately 20 to 35nm. The synthesized gold nanobranches exhibited high density and crystallization, characterized by a face-centered cubic (fcc) crystal structure. This study could potentially extend green synthesis procedures to other noble metal particles.

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