Detection of mercury ions using unmodified silver nanoparticles as colorimetric indicator

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Abstract. Detection of the heavy metal presence in an environment requires a rapid and facile method. Silver nanoparticles (AgNPs) can be develop as colorimetric indicator related to their unique characteristics of its LSPR properties. In this research, biosynthetically AgNPs using Diospyros discolor leaf extract used as a colorimetric indicator for detecting Hg²⁺ ions. The solution color changes that occur when detecting the analyte was observed. The characterization was performed using a UV-Vis spectrophotometer to study changes in the LSPR spectrum of silver nanoparticles when detecting the presence of Hg²⁺ ions. In addition, the image analysis from Transmission Electron Microscope (TEM) was carried out to determine the shape and size of the AgNPs. The results obtained indicate that there was a tendency for the solution color change from brown to light-brown when AgNPs interact with Hg²⁺ ions at 500-1000 mg. L⁻¹. This result showed that without modification the biosynthesized AgNPs tend to be selective and sensitive to the presence of Hg²⁺ ions.

1. Introduction

Noble metal nanoparticles have unique optical properties that distinguish them from other types of materials. This is an interesting thing to study related to the unique characteristics of the extension values that are typical in the visible light spectrum which is not found in bulk metals [1,2]. The interaction of electromagnetic field oscillation with metal nanoparticles results in a coherent collective oscillation of metal electron conduction in the positive lattice of the nanoparticles. At certain light frequencies, this process resonates to produce a phenomenon called Localized Surface Plasmon Resonance (LSPR). The existence of LSPR plays a role in producing strong extinction bands from silver nanoparticles [1,3,4]. The changes in local environmental conditions such as acidity, temperature and interaction with other molecules can result in the LSPR shift. This advantage made noble metal nanoparticles can be used for optical sensing [5-7] and imaging applications [8].

Noble metal nanoparticles that are commonly used in sensor applications namely gold and silver nanoparticles because their advantage which can be observed in the visible light spectrum for optical sensor [8, 9] These nanoparticles can be produced through various methods of synthesis like physical, chemical and biological method, from complex to simple procedures [10-12]. During this decade, the nanoparticle synthesis through biosynthetic processes are relatively simple and environmentally friendly has been developed. The synthesis method can utilize the diversity of plants [10] in the synthesis process,
which provides the reducing agent [13-15]. The various synthesis processes of nanoparticles will produce nanoparticles with different shapes and sizes, that determine the nanoparticles LSPR characteristics for particular applications [16].

Synthesis of silver nanoparticles by biosynthetic methods is carried out by utilizing extracts from a plant species. The phytochemical content of the compounds contained in the extract will act as a reducing agent during the synthesis process. In this process, silver ions will be reduced by the reducing agents such as sugars or secondary metabolites such as alkaloids, flavonoids, saponins groups, and other unknown components [10,13]. Aside from being a reducing agent, the compounds derived from the extract also can act as capping agents that attach and surround the surface of the nanoparticles [6]. Each synthesis uses different species, so it is assumed that the compounds that act as reducing and capping agents are also different. The use of different plant extracts will show different roles and functions, which affect the properties of the silver nanoparticles produced, thus affecting its selectivity and sensitivity for sensors [17].

One of the problems of pollutants that are harmful to the environment is heavy metal contamination. So far, the method of detection and testing of heavy metals in the environment requires complex processes [7,17] and requires quite expensive equipment [9]. Therefore, developing practical, direct, and rapid detection methods in the field will significantly help to detect the presence of specific pollutants. Mercury is one of many heavy metals that need highly concern for their spread in the environment [9, 18]. Based on its ability, silver nanoparticles can be developed as colorimetric sensors to detect the presence of heavy metals by utilizing the LSPR characteristics of silver nanoparticles [1,6,9]. In this study, an unmodified silver nanoparticles (AgNPs) synthesized using D. discolor [15,19] leaf aqueous extract used to detect the presence of Hg$^{2+}$ metal ions as a colorimetric sensor.

2. Material and Methods

2.1. Silver nanoparticles biosynthesis
Synthesis of silver nanoparticles was carried out through the biosynthetic method using Diospyros discolor L. (Bisbul) aqueous extract as a reducing agent that has pH adjusted to 9 [13,15]. Nanoparticles are synthesized by adding the silver nitrate with the extract. The solution was characterized by a UV-Vis spectrophotometer [Thermo Genesys 10S]. The shape and the size of the silver nanoparticles were analyzed from the results of the Transmission Electron Microscope image (TEM FEI Tecnai G2 20S-Twin 200 kV).

2.2. Selectivity test for heavy metals detection
Silver nanoparticles produce through the biosynthesis method were tested for their selectivity against several types of metal ions at concentrations of 1000 mg. L$^{-1}$, namely Hg$^{2+}$, Fe$^{2+}$, Mn$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, and Pb$^{2+}$ (Figure 1). The solution color change significantly when detecting Fe$^{2+}$ ions [13] and Hg$^{2+}$ compared to other ion types.

![Figure 1](image-url)

**Figure 1.** Selectivity test of the unmodified silver nanoparticles to several metal’s ion showed significant color change on Hg$^{2+}$ ion detection.

2.3. Mercury ion sensitivity test
The sensitivity test was carried out using several variations of the concentration of Hg$^{2+}$ ions at concentration 0; 0.1; 1; 10; 100; 500; 1000 mg. L$^{-1}$. The change in color of the solution that occurs in
each solution were visually observed and its UV-Vis spectrum measured using a UV-Vis spectrophotometer.

3. Results and Discussion

Figure 1. shows the biosynthetically synthesized AgNPs using extract from D. discolour leaves which produced a brown solution of AgNPs colloid. The solution then measured with the UV-Vis spectrophotometer with an absorption spectrum ranging from 340 to 500 nm. The spectrum has a peak at $\lambda_{max}$ 435 nm (Fig.2a), which showed their LSPR spectrum band. The color also indicates the characteristics of LSPR from AgNPs [6,18] Based on the results of the TEM image (Fig.2b), it is known that nanoparticles had spherical shape with an average particle size of $6.7 \pm 1.6$ nm. The size distribution of the nanoparticles showed the smallest size were 3 nm and the largest size were 12 nm (Fig.2c). These results indicate that alkaline environment consistently produces AgNPs under 15 nm in size. Several studies have shown that pH at alkaline conditions the small size nanoparticles formed after the silver ion reduction and nucleation of nanoparticles [13,15,19]. Silver nanoparticles can exhibit an effective surface charge in aqueous conditions [1].

![Figure 2](image)

**Figure 2.** Silver nanoparticles colloid obtain from biosynthesis using D. discolour aqueous extract pH 9 showed brownish solution color and spectrum UV-Vis between 340-50 nm (a); TEM image showed AgNPs with size under 15 nm with spherical size; (c) size distribution from the AgNPs with average particles size $6.7 \pm 1.6$ nm.

Based on the initial detection results in Figure 1, the AgNPs obtained show the selectivity on mercury ions (Fig.1). After that, the sensitivity test of mercury ions with various concentrations was carried out (Fig. 3). The color of the AgNPs colloid was brown, and then the solution color started to change when the mercury ion added to the AgNPs. The dark brown color change into light brown color after detecting 0-100 mg. L$^{-1}$ mercury ion. Meanwhile, when sensing mercury ion above 500 mg. L$^{-1}$ the color of the
solution turns light brown and visually distinguishes from the color of other solutions. Discoloration happened at the time of detection caused by the changes in the AgNPs LSPR. This result is supported by the UV-Vis spectrum results (Figure 4a).

Figure 4a. showed the results of the UV-Vis spectrum after mercury ion detection in each concentration. The spectrum absorption intensity tends to decrease. The decrease in intensity caused by the reactions between Ag\(^0\) and Hg\(^{2+}\) ion [9, 17]. Meanwhile, Figure 4b. is a plot of absorbance at a wavelength of 435 nm. These results indicate a non-linear relationship (\(Y=0.64693+2.69084e^{-x/185.0013}\), \(R=0.99703\)) which indicates that the AgNPs indicator solution had the lowest mercury ion detection limit at 10 mg L\(^{-1}\). Those results support based on differences in the absorption spectrum besides the visually observe color. For detecting the mercury ion concentration at 0; 0.1, 1 and to 10 mg L\(^{-1}\), the visual color, and the UV-Vis spectrum also did not show any differences. Meanwhile, when detecting the 100 mg L\(^{-1}\) mercury ion, the solution color formed visually almost the same with the solution color when detecting the mercury ion from 0-10 mg L\(^{-1}\). The different for detection in those range were the spectrum intensity, which shows lower absorbance. After that, when tested for detecting the mercury ion at 500 and 1000 mg L\(^{-1}\) shows a light-brown color and lowest spectrum intensity, where there no peak found at the range of AgNPs LSPR spectrum band.

![Figure 3](image1.png)

**Figure 3.** The solution of solution when silver nanoparticles synthesize using *D. discolor* extract pH 9 (AgNPs-1) detect Hg\(^{2+}\) ions with different concentration. The brown color of the solution tend to fade when the concentration of Hg\(^{2+}\) ions increases.

![Figure 4](image2.png)

**Figure 4.** Spectrum absorbance from detection result in each concentration of Hg\(^{2+}\) ions after addition of AgNPs for detection from 0-1000 mg L\(^{-1}\) (a) and the plot of absorbance intensity at 435 nm (b).
Two possible mechanisms that cause the color of the solution to be brighter, and the absorbance spectrum intensity decreases when AgNPs used to detect the presence of Hg$^{2+}$ ions. The first is the aggregation that happened between nanoparticles. Aggregation of silver nanoparticles is caused by the bonds between the surface of AgNPs, causing the nanoparticles to cluster, and the size enlarges. This phenomenon will cause a shift in the LSPR value and occurred when we used the AgNPs to detect Fe$^{2+}$ ions [19]. Meanwhile, other phenomena studied by Farhadi et.al (2012) took TEM images from the nanoparticles before and after the detection of mercury ion, which showed there were no AgNPs found. The possible mechanism was oxidation happened. The oxidation occurred between Ag$^0$ and Hg$^{2+}$, where Hg$^{2+}$ ions bind to AgNPs and replace biomolecules from the extract attached on the surface of AgNPs. The reaction turns some Ag$^0$ into Ag$^+$. Therefore, the color of the solution after detection tends to become more transparent. It can happen when all nanoparticles have become Ag$^+$ ions and the solution become colorless [6, 9].

The change in color and spectrum band intensities when detecting different heavy metals ion concentrations are the fundament of nanoparticle-based colorimetric sensors. The differences in capping agents will affect the selectivity and sensitivity of AgNPs when detecting a specific molecule. In the biosynthetic method, the capping agent derived from extracted biomolecules which was attached on the surface of AgNPs after the reduction process occurs [9,17]. Some modifications can be done to increase the sensitivity of AgNPs for the colorimetric sensor application. Adding salts such as NaCl and KCl as the sources of Cl$^-$ ions tend to increase the nanoparticles aggregation [21] and the addition of capping agent contain thiol groups which have high affinity to metal ion can increase the selectivity and sensitivity. These capping agents can be amino acids, DNA, or polymers [1]. Furthermore, the AgNPs modification with certain capping agent will act selectively to bond with the analytes to be detected and will increase the detection limit.

4. Conclusions
Unmodified silver nanoparticles synthesized using D. discolor leaf extract turn out to be potential as a colorimetric indicator for Hg$^{2+}$ ions, where the color of the solution visually changes from brown and tend to be faded away with the increasing of the mercury ions concentration. For detection the presence of mercury ions the absorbance intensity will decrease and assumed that the existence of silver nanoparticles will decrease as the oxidation occurs which change the LSPR characteristic. To improve the sensitivity for Hg$^{2+}$ detection this method needs to be improve and optimize.

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