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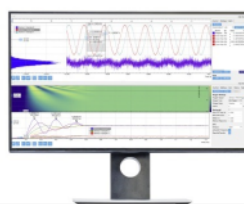
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# Synthesis Silver Nanoparticles Using Trisodium Citrate and Development in Analysis Method

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**Abstract.** Synthesis of silver nanoparticles (AgNPs) and analytical development of silver nanoparticles has been done. AgNPs were making by chemical reduction method using trisodium citrate as a reducing agent. AgNPS was Prepared with a variant of silver nitrate concentration, trisodium citrate concentration, and stirring time to result in the best silver nanoparticles. AgNPs were characterized using UV-Vis Spectrophotometer, Particle Size Analyzer (PSA), Transmission Electron Microscopy (TEM). AgNPs was applied as the colorimetric sensor of histamine. The histamine presence can induce the aggregation of silver nanoparticles by a color change from yellow to brown.

## INTRODUCTION

Nanotechnology is an important field of modern research dealing with the design, synthesis, and manipulation of particle structures ranging from approximately 1-100 nm [1]. Silver nanoparticles (AgNPs) show unique optical features in well-dispersed solutions, depending on their level of aggregation which is mostly determined by their specific surface plasmon resonance (SPR) profile [2]. As a visual element providing broad applications in chemistry, medicine, biology and material science [3]. Some of the Silver Nanoparticles synthesis methods are photochemical, ultrasonic radiation, solvothermal synthesis, chemical reduction [4] reverse micelles systems, hydroxyl functionalized cationic surfactants, and *Fusarium oxysporum* [5]. The reduction method has been used several times by researchers, using silver nitrate and trisodium citrate [4],[6]. However, trisodium citrate is used here as a reducing agent as well as a stabilizing agent. Similarly, silver nitrate as a bulk, hydrazine hydrate as a reducing agent, and two stabilizing agents, namely trisodium citrate and SDS [4].

AgNPs have attracted great interest as a colorimetric probe, which can directly detect analytes by monitoring the color change, using UV-vis spectroscopy, or naked eyes. AgNPs have been used for colorimetric sensors. They have potential applications for the detection of metal ions [7,8], Proteins [9], sensing [10], labelling [11].

Colorimetry provides a straightforward and convenient strategy for developing low-cost biosensors. The existence and concentration of the sample are read-outs according to the visual color changes [12]. The silver nanoparticles mechanism as a colorimetric detection is generally based on aggregation mechanisms, which leads to changes in color from yellow to red in the case of AgNPs [13]. In the colorimetric sensor application, the function of changing the chemical and physical of surface silver nanoparticles is based on the changes of the plasmonic resonance wavelength at maximum absorption [14]. AgNPs are a selective and sensitive analyte detection method that can be developed as a sensor method, namely colorimetry.

In this study, a histamine analyte was used to determine the ability of silver nanoparticles as a colorimetric sensor. Histamine is a relevant biological substance in medicine and food science. It is a biogenic amine that transmits signals from cell to cell in the skin, intestines, and organs of the immune system [15]. Biogenic amines with secondary amine groups can produce nitrosamines when reacting with nitrites and hence have cancer-producing ability. In another study, histamine was followed to detect the freshness of fish that was rich in histidine [16].

## EXPERIMENTAL

### Instruments and Materials

The instruments used in this research was the UV-Vis spectrophotometer (Shimadzu-1800) for measurements the absorption spectra of AgNPs. The particle size was observed by using Particle size analyzer (Horiba SZ-100). The shape and their distribution of silver nanoparticles were confirmed through a transmission electron microscope (HT7700), and the spectra were recorded at room temperature using a Thermo Scientific Nicolet iS10 ratio recording infrared spectrophotometer. The materials used in this study were AgNO<sub>3</sub>, Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, NaCl, all reagents were of analytical-reagent grade (E. Merck), and aquabidest.

### Solution Preparation

AgNO<sub>3</sub> (0.17 g) was weighed quantitatively and dissolved in aquadem. The solution is transferred to a 100 mL volumetric flask and prepared with the marked solvent. Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (0.5 g) was weighed quantitatively and dissolved in aquadem. The solution is transferred to a 50 mL volumetric flask and prepared with the marked solvent.

### Preparation of Histamine Working Solutions

Histamine standard solutions were made by dissolving quantity histamine in 50 mL using 0.9% saline solution (NaCl) to acquire solutions with concentrations of 2.0, 4.0, 6.0, 8.0, and 10.0 μM correspondingly. Experiments were performed at room temperature.

### Synthesis of AgNPs

AgNPs were synthesized using a facile approach by trisodium citrate (Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>) reduction of AgNO<sub>3</sub> according to the previously reported method [6]. Briefly, 50 mL AgNO<sub>3</sub> was poured into flask and heated until boiling, then 4 mL of 1% trisodium citrate was gradually added with continuous stirring and heating till the color of the solution changed from clear to yellow at about 9 min indicating the formation of AgNPs. The AgNPs were gradually formed as the citrate reduced Ag<sup>+</sup> to Ag<sup>0</sup> as indicated by the change color to yellow. AgNPs solution was cooled down at room temperature and stored at 4 °C.

### Preparation of AgNPS-Histamine

AgNPs-Histamine solutions were prepared by mixing AgNPs solution with histamine standard solution of concentrations 2.0, 4.0, 6.0, 8.0 dan 10.0 μM each mixture was stirred for 5 min at room temperature. The color was changed from yellow to brown. Then, the absorbance spectra were recorded. The calibration graph was obtained by plotting the absorption against the concentration of histamine.

## RESULTS AND DISCUSSION

### Synthesis of AgNPs (Silver Nanoparticles)

Synthesis of AgNPs using a chemical reduction method where AgNO<sub>3</sub> is reduced with trisodium citrate and produces AgNPs. The formation of AgNPs is indicated by a change in the color of the solution from yellow to brown. In the AgNPs synthesis process, several optimizations were carried out, namely determining the concentration of AgNO<sub>3</sub>, determining the stirring time, and determining the volume of 1% trisodium citrate.

### Effect of Concentration

Synthesis of AgNPs uses a chemical reduction method, namely AgNO<sub>3</sub> solution which reduces Ag<sup>+</sup> ions to Ag, and trisodium citrate as a reducing and stabilizing agent that produces AgNPs [6]. In the synthesis of AgNPs,

different  $\text{AgNO}_3$  concentrations were used. This is done to determine the optimum concentration required for the formation of AgNPs. The chemical reaction of silver nitrate with trisodium citrate is:

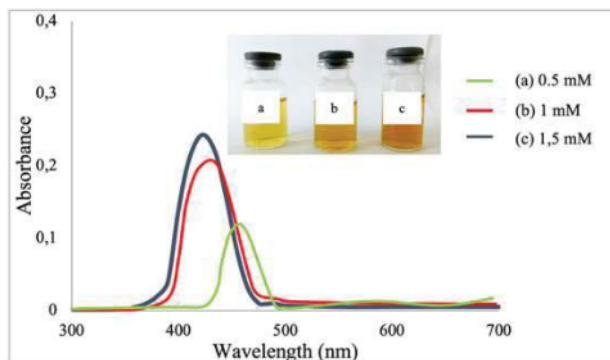
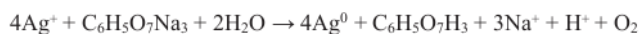


FIGURE 1. The spectra of variation in the concentration of  $\text{AgNO}_3$

The formation of AgNPs through a chemical reaction is carried out by adding 1% trisodium citrate dropwise. The synthesis of AgNPs produces different colors, such as pale yellow, golden yellow, and brownish yellow as shown in Fig. 1. Recent reports have proven that the resulting color of silver nanoparticles is caused by the collective oscillations of electrons in the conduction band, known as oscillations surface plasmon [17]-[18]. Fig. 1 shows the colloidal color of the AgNPs produced depending on the concentration of the added  $\text{AgNO}_3$  solution and shows the typical UV-vis spectrum of silver nanoparticles with different  $\text{AgNO}_3$  concentrations (0.5 mM, 1.0 mM, and 1.5 mM). At low concentrations of  $\text{AgNO}_3$ , the absorbance of the plasma surface is low with the maximum wavelength of 468 nm.

### Effect of Stirring Time

The stirring time can control the formation and growth of synthetic silver nanoparticles to produce stable colloidal yellow silver nanoparticles. If stirring is continued after all the silver nitrate has been added, it can cause an aggregation that produces colloidal yellow first and turns dark yellow, then purple, and finally gray. Aggregation of silver nanoparticles can also cause colloids to break down and particles to precipitate [14]. So, the stirring time must be controlled so that the colloidal silver nanoparticles are stable.

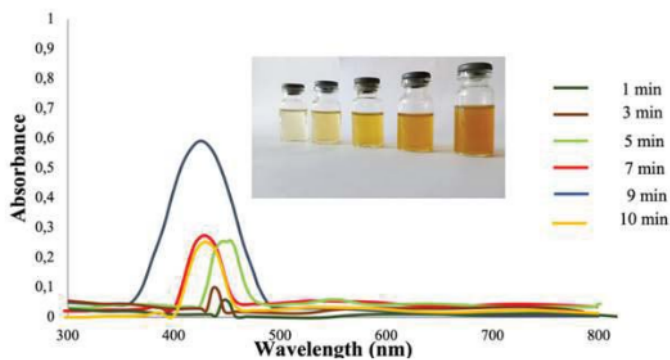


FIGURE 2. Stirring Time Variation Spectra

Fig. 2 shows that the increasing time of 5 minutes and 7 minutes produces an increasing absorbance with a maximum wavelength and begins to form a faded yellow color at 5 minutes and yellow color at 7 minutes. However, at 9 minutes it produces high absorbance with a maximum wavelength and changes its color to yellow. So that the stirring time of 9 minutes, the AgNPs synthesis has reached the optimum stirring time.

### Effect of 1% Trisodium Citrate Volume

The variation of trisodium citrate volume aims for the growth and formation of AgNPs and to find the optimum trisodium citrate volume. Figure 3. shows that the 4 mL and 5 mL volumes have the same maximum length of 423 nm and the formation of a stable yellow AgNPs color. However, a volume of 5 mL had decreased absorbance. The higher the absorbance value and the maximum wavelength of 420 nm, the more AgNPs which is formed [19].

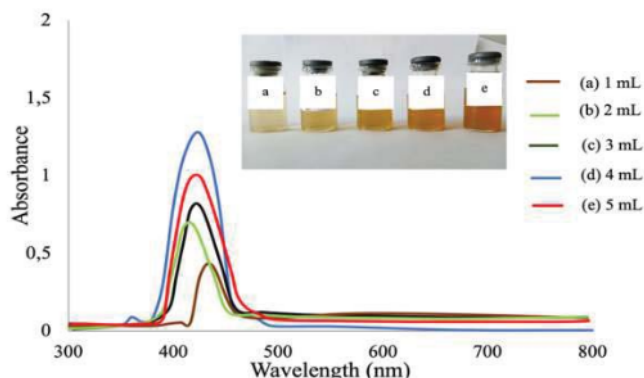


FIGURE 3. The Spectra of Sodium Citrate Volume Variation

### Characterization of AgNPs with Particle Size Analyzer (PSA) and Transmission Electron Microscopy (TEM)

AgNPs were characterized by a Particle Size Analyzer (PSA) to determine the particle size of the AgNPs. In the characterization of AgNPs, AgNPs suspension was used from the concentration variation, stirring time variation and volume variation of Trisodium Citrate, namely AgNPs 1 mM, and the maximum wavelength of AgNPs was 423 nm. The results of characterization in the Particle Size Analyzer (PSA) test showed that the 1 mM AgNPs size was 40.6 nm. AgNPs with a maximum wavelength of 420 nm has a size range of 35–50 nm [19].

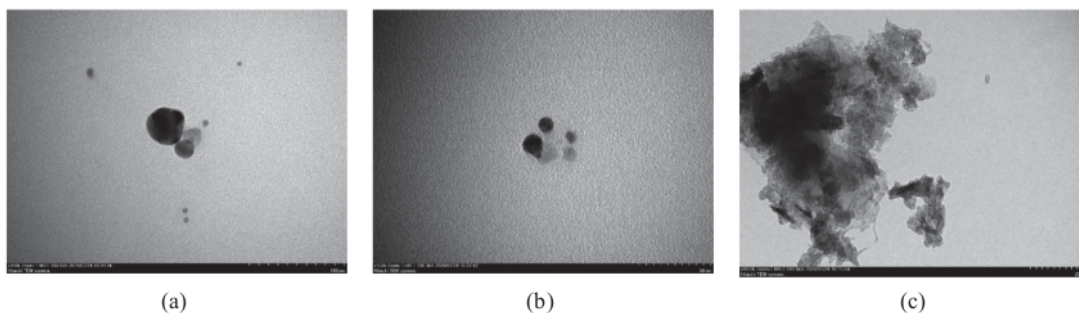


FIGURE 4. TEM photos on the AgNPs characterization (a) magnification x100k with a scale of 100 nm, (b) at a magnification of x150k on a scale of 50 nm, and (c) at a magnification of x30k with a scale of 200 nm.

The characterization of the 1 mM AgNPs suspension by Transmission Electron Microscopy (TEM) shows the morphology of the spheres' size randomly distributed at 100k magnification on a 100 nm scale. Whereas at 150k magnification with a scale of 50 nm (Figure 4.) shows a dominant spherical morphology.

### Application Silver Nanoparticles as Colorimetric Sensor of Histamine

The detection of histamine with silver nanoparticles is based on the color change of the silver nanoparticles solution from yellow to brown when silver nanoparticles interacting with histamine. The results of the UV-Vis absorbance can be seen in Figure 5.

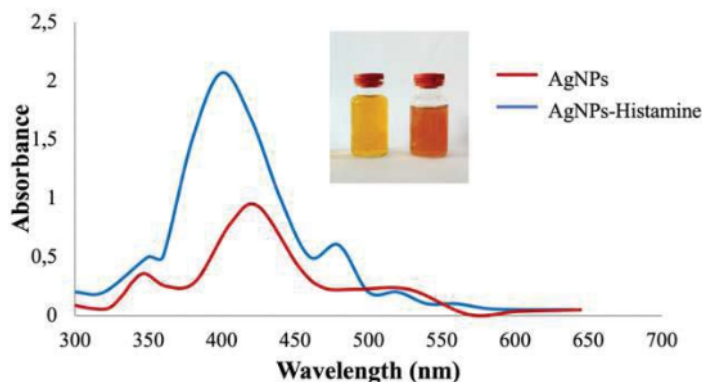


FIGURE 5. The absorbance of colorimetric of Histamine

The aggregation AgNPs-Histamine is also due to a shift in the maximum wavelength from 423 nm to 408 nm. This results in a hypochromic, where the shift in lower wavelengths is thought to be the peak of the formation of AgNPs and Histamine bonds.

### CONCLUSION

We have successfully developed the colorimetric method based on AgNPs that use histamine detection with a change in color from yellow to brownish and with a shift in hypochromic wavelength, with a change color from yellow to brown and with a shift in hypochromic wavelengths.

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