Synthesis of Silver Nanoparticles and the Development in Analysis Method

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Abstract. Synthesis of silver nanoparticles and the development of silver nanoparticles in analytical method has been done. Silver nanoparticles were prepared by chemical reduction method, with variant of silver nitrate concentration and stirring time to result best silver nanoparticles. The objective of this research is to determine the optimum condition for synthesis of silver nanoparticles and the ability of silver nanoparticles for a colorimetric sensor. The silver nanoparticles were characterized by UV-Visible and Particle Size Analyzer (PSA). The result of characterized by UV-visible spectrophotometer showed that the UV-vis absorption spectra of silver nanoparticles with different silver nitrate concentration gave a difference size of silver nanoparticles and the stirring time controlled the stable colloidal of silver nanoparticles. The size distribution of silver nanoparticles were confirmed by using the Particle size analyzer (PSA). We have applied colloid silver nanoparticles as colorimetric sensor of Sialic acid and Melamine, the presence of Sialic acid and Melamine induces the aggregation of silver nanoparticles, accompanied by a color change from yellow to red-purple (Sialic acid) and yellow to brown (melamine)

Keyword : Synthesis, Silver Nanoparticles, Analysis Method

1. Introduction
Nanoscience and nanotechnology have now become the topic research that many developed. Nanoparticle materials are developed in many applications because of their unique optical characteristic [1]. As a visual element providing broad applications in chemistry, medical, biology and material science [2].

Today, several nanoparticles also have been used for colorimetric sensor. Among these is silver nanoparticles (AgNPs). Silver nanoparticles (AgNPs) has been an active research area in recent years, because Silver nanoparticle have potential applications in biodiagnostics [3], imaging [4], biological labeling [5], and sensing [6]. It has been unique physicochemical properties as a size, shape, distance dependence optical properties and also exhibiting extremely large molar extinction coefficients [7]. Localized surface plasmon resonance (LSPR) of Silver nanoparticle can be charged by tuning their shape, size and aggregation state when have interaction with analytes [8].

Colorimetric sensors is simple analysis with on site and real time detection without use the compliated instrument [9]. The mechanism of silver nanoparticles as a colorimetric detection is generally based on their inherent optical or catalytic properties [10]. Silver nanoparticles has some advantages over gold nanoparticles such as a sharper extinction bands, higher extinction coefficientas and higher ratio of scattering to extinction [11].

In colorimetric sensor application, the function of changing chemical and physical of surface silver nanoparticles based on the changes of the plasmonic resonance wavelength at maximum absorption.
Silver nanoparticles has allowed the analysis development with the highly sensitive and selective detection methods that is expected to overcome deficiencies in conventional methods. With these advantages, so silver nanoparticles is suitable for development of method analysis especially in colorimetric sensor.

In this study, two analytes were used with two different sample to determine the ability of silver nanoparticles as a colorimetric sensor. These two is sialic acid and melamine. Sialic acid is a family of sugars with a nine-carbon backbone, which is often found at the outer end of glycoconjugates on the surface cells and soluble protein [12]. The increased concentration level of total sialic acid (TSA) can indicate malfunction of many organs in the human body as a brain tumor, cardiovascular, leukemia, breast and the others [13]. The TSA also can indicates an early stage of cardiovascular disease and some of cancer [14].

Melamine or 1,3,5-triazine-2,4,6-triazine (C₃H₆N₆) is a trimer of cyanamide. It is commonly used in resin manufacture for its fire retardant properties, textile industries and the product of pesticides. In recent years, melamine has been illegally added into milk products and feeds due to is low cost and contains 66% nitrogen by mass that can increase protein content measured by the standard test for food protein. Addition of 1% of melamine in food causes content to artificially boost more than 4% [15] Anyhow, melamine can be hydrolyzed to cyanuric acid which in turn cooperates with melamine to form the insoluble crystals in kidney and these crystals might cause organs failure and even death in human and animals [16, 17].

2. Experimental Method

2.1 Reagents and materials
Silver nitrate, Natrium borohydride were purchased from Sigma-Aldrich, and Melamine were obtained from Merck chemical, sialic acid were purchased from Sigma-Aldrich Co (CAS number 131-48-6, molecular weight 309.27). UV-Vis absorption spectra were performed with spectrophotometer (Thermo) in absorbance mode range 300-800 nm using a 1.0 cm quartz cell at room temperature. The particle size were observed by using Paricle size analyzer (Malvern).

2.2 Synthesis of Silver nanoparticle (Ramalingam et al., 2016)
Silver nanoparticle synthesis was carried out using AgNO₃ as a source of Ag⁺ and NaBH₄ as reducing agents. A 10 ml solution of AgNO₃ (1 mM) added drop by drop (about 20 drops per min) into 30.0 mL solution of NaBH₄ (2 mM) is prepared and placed in ice bath (±4°C) under continuous stirring. This solution is centrifuged 12000 rpm for 15 minutes and then filtered. then left at room temperature for 24 hours before use.

2.3 Effect of Stirring Time
According by Solomon et al (2007), one of some efforts to control the formation and growth of synthesis silver nanoparticles is stirring time. It must be controlled to result the stable yellow colloidal silver nanoparticles. This effect related with formation of Silver nanoparticle that reduction by NaBH₄. The stirring time that use in this experiment is 0 minutes, 1 minutes, 3 minutes, 5 minutes and 10 minutes.

2.4 The stability of silver nanoparticles
The stability of silver nanoparticles observed based on size distribution and color change from colloidal silver nanoparticles. The stage formation of silver nanoparticles is nucleation and growth. The formationa and growth of colloidal silver nanoparticles was identified by UV-Vis spectrophotometer and this stability observed up to 70 days.

2.5 Application silver nanoparticles as colorimetric sensor of sialic acid
Application silver nanoparticles as colorimetric sensor of sialic acid was based on color change of silver nanoparticles due to the aggregation after binding of silver nanoparticles with sialic acid. 0.5 mL of 1.0 mM sialic acid was added to vial that containing 2.0 mL of silver nanoparticles, and then added 1.0 mL of buffer pH 6.0. This experiment measurement with UV-Visible spectrophotometer in range of 300 – 800 nm.
2.6 Colorimetric detection of Melamine
A colorimetric analysis of melamine was achieved as follows. 2.0 mL AgNPs solution was pipetted into a vial and added 600 μL of 1.0 μM melamine and added 500 μL of buffer pH 7.0. The mixed solution was allowed to react for 5 min at room temperature. Observed the color changes that occur and absorbance measurements using UV-Visible spectrophotometer in range of 300 - 800 nm.

3. Result and Discussion
3.1 Effect of AgNO₃ Concentration
The synthesis of Silver nanoparticle carried out by chemical reduction method. Silver nitrate are a source of Ag⁺ and NaBH₄ as reducing agents that produce AgNP, the chemical reaction is the sodium borohydride reduction of silver nitrate (Solomon et al., 2007):

\[
\text{AgNO}_3 + \text{NaBH}_4 \rightarrow \text{Ag} + \frac{1}{2}\text{H}_2 + \frac{1}{2}\text{B}_2\text{H}_6 + \text{NaNO}_3
\]

The formation of Silver nanoparticle by chemical reactions is carried out by adding reactants sequentially, namely AgNO₃ compound drop by drop into NaBH₄ solution. The addition of reactants in reverse order causes the formed Silver nanoparticle to settle immediately (Song et al., 2009). The results of AgNP synthesis produce different color solutions, such as: pale yellow, golden yellow and brownish yellow shown in Fig. 1. Fig. 1 shows The color of the AgNP colloid produced depends on the concentration of the added AgNO₃ solution, and shows typical the UV-vis spectra of colloidal silver nanoparticles with prepared different initial AgNO₃ concentrations (0.5 nm, 1.0 nm, and 1.5 nm). UV-visible spectroscopy is one of the popular characterization techniques to determine particle formation and its properties (Desai et al., 2012). At low AgNO₃ concentration a weak absorption maximum of surface plasmon peaks was observed at 406 nm, with increasing the AgNO₃ concentration the intensity of the maximum plasmon peak increased and silver nanoparticles were formed.

The size distribution of nanoparticles produced at different AgNO₃ concentration by Particle size analyzer in Table 1. Table 1 show the size distribution of nanoparticles with different AgNO₃ concentration, the colloidal of Silver nanoparticle was prepared by 0.5 mM AgNO₃ gave 71.02 nm, 1.5 mM AgNO₃ gave 67.08 nm and 1.0 mM AgNO₃ gave a smallest size (34.06 nm). Therefore, molar ratio 2:1 (NaBH₄ : AgNO₃) that gave the smallest size was selected as the optimized ratio.

![Figure 1. UV-Vis spectrophotometer of Silver nanoparticle with variation AgNO₃, inset: corresponding visual color (A) AgNO₃ 0.5 mM, (B) AgNO₃ 1.0 mM dan (C) AgNO₃ 1.5 mM.](image)
Table 1. Result of effect of AgNO₃ concentration

| [AgNO₃] (mM) | λ<sub>maks</sub> (nm) | Absorbance | Size distribution (nm) |
|--------------|-----------------------|------------|------------------------|
| 0.5          | 406                   | 0.315      | 71.02                  |
| 1            | 399                   | 0.345      | 34.06                  |
| 2            | 397                   | 0.729      | 67.08                  |

3.2 Effect of Stirring Time

The stirring time can control the formation and growth of synthesis silver nanoparticles to produce a stable yellow colloidal of silver nanoparticles. If the stirring is continued once all of silver nitrate has been added, can cause aggregation that resulted yellow colloidal first and turn a dark yellow, then violet and eventually grey. Aggregation of silver nanoparticles also can cause the colloidal breaks down and particle settle out. So, the stirring time must be controlled to result the stable colloidal of silver nanoparticles. The effect of stirring time to the size distribution and absorbance of UV-Vis can find at Figure 2, Figure 3 and Table 2.

Figure 2. UV-Vis spectrophotometer of variation stirring time

Figure 3. Size distribution of effect stirring time
Table 2. Result of variation stirring time

| Stirring time (minutes) | λ_{max} (nm) | Absorbance | Size distribution (nm) |
|-------------------------|--------------|------------|------------------------|
| 0                       | 396          | 0.398      | 39.17                  |
| 1                       | 397          | 0.524      | 35.21                  |
| 3                       | 399          | 0.668      | 34.06                  |
| 5                       | 403          | 0.480      | 77.69                  |
| 10                      | 406          | 0.373      | 91.82                  |

3.3 The stability of silver nanoparticles

In general, the synthesis of metal nanoparticles is chemically carried out in the presence of precursor metals, reducing agents and stabilizers. The mechanism of metal reduction into metal nano consists of two stages, namely the nucleation and growth stage. Nucleation requires activation of energy greater than energy at the growth stage. This study observes the process through time parameters, so that it can be seen the stability of the formed silver nanoparticle.

In observing the stability of Silver nanoparticle, it can be seen that as time grows, a wavelength shift occurs in the absorbance spectra of the UV-Vis region. This situation shows that silver nanoparticle has a large enough surface tension energy, so it is less stable and not sufficiently able to fight the aggregation process. As time goes on, it shows the formation of particles with greater size. Large surface tension forces cause greater cohesion forces, making interaction between Silver nanoparticle more likely. This causes Silver nanoparticle size to tend to be larger due to cluster formation. A larger wavelength shift (batocromatic shift) shows the formation of a cluster between silver nanoparticle, so that the size of Silver nanoparticle becomes larger. The batocromatic shift of the silver particle cluster shows that the material absorbs low energy radiation compared to Silver nanoparticle in the early minutes. The absorbance value increases with increasing time until the maximum absorbance value is reached within 12 hours. This is because the growth of nanoparticles is formed more and more with time. Meanwhile, after 12 hours, the absorbance value begins to decrease and the absorption peak that forms is widening. The stability of silver nanoparticles to the size distribution and absorbance of UV-Vis can find in Figure 4 and Table 3.

![Figure 4. UV-Vis spectrophotometer of the stability of silver nanoparticles](image-url)
Table 3. The stability of silver nanoparticles

| Stability  | $\lambda_{\text{max}}$ (nm) | Absorbance | Size distribution (nm) |
|------------|-----------------------------|------------|------------------------|
| 15 min     | 397                         | 0.0447     | 45.17                  |
| 30 min     | 397                         | 0.524      | 41.47                  |
| 60 min     | 398                         | 0.531      | 40.22                  |
| 12 hour    | 399                         | 0.615      | 34.15                  |
| 24 hour    | 400                         | 0.747      | 36.74                  |
| 7 day      | 402                         | 0.548      | 49.05                  |
| 10 day     | 403                         | 0.482      | 50.46                  |
| 20 day     | 404                         | 0.382      | 63.12                  |
| 30 day     | 405                         | 0.145      | 68.51                  |
| 40 day     | 407                         | 0.128      | 79.77                  |
| 50 day     | 408                         | 0.083      | 91.80                  |
| 60 day     | 409                         | 0.041      | 136.1                  |
| 70 day     | 409                         | 0.065      | 162.7                  |

The stability of silver nanoparticles also observed by the color change. Silver nanoparticles aggregation to a larger size is marked by a change in color in the solution from yellow to dark yellow then green and finally to gray. The existence of this color change can be assumed to be the instability of the formed silver nanoparticles that show in Figure 5.

Figure 5. The color change of silver nanoparticles

3.4 Application silver nanoparticles as colorimetric sensor of sialic acid
Sialat acid detection using silver nanoparticles is based on the color change of silver nanoparticles solution from yellow to red-purplish color when interacting with sialic acid. This is due to the aggregation after the bond between silver nanoparticles and sialic acid. This aggregate also causes the peak to shift to a larger (bathochromic) wavelength and there is a new peak at 563 nm which is thought to be the peak of a new complex formed by the bond between silver nanoparticles and sialic acid. The result of absorbance of UV-Vis can find at Figure 6.

Figure 6. The absorbance of colorimetric sensor of sialic acid

3.5 Detection of melamine
Under the optimized detection conditions and at room temperature, melamine detection using silver nanoparticles was carried out. The absorption spectrum of Silver nanoparticle shows absorption peak
at 400 nm (Figure 7.). in the presence of melamine, the absorbance of Silver nanoparticle at 400 nm was decreased and accompanying the appearance of new peak at 570 nm, is seen, melamine can stimulate the aggregations of Silver nanoparticle. The color change can be observed by naked-eyes and Fig. 6 shows respective photographs of colorimetric sensor of melamine, the color of Silver nanoparticle solution was change from yellow to brown.

![Figure 7. The absorbance of colorimetric sensor of Melamine (Melamine = 1.0 mM)](image)

4. Conclusion
We have successfully developed the colorimetric sensor based on silver nanoparticles that use to detection of sialic acid and melamine. From this study can be concluded that silver nanoparticles also can use as colorimetric sensor with simple, rapid and low cost.

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