SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING WATER EXTRACT OF SARANG SEMUT (Myrmecodia pendans) FOR BLOOD GLUCOSE SENSORS

Rosalinda Zeniona Maarebia^{1,2*}, Abdul Wahid Wahab³, Paulina Taba³

¹ Study Program of Chemistry Education, Faculty of Teacher Training and Education, Musamus University, Merauke, Indonesia

²Graduate Student Magister Program of Chemistry, Mathematics and Natural Science Faculty, Hasanuddin University, Makassar, Indonesia

³Department of Chemistry, Mathematics and Natural Science Faculty, Hasanuddin University, Makassar,

Indonesia

*Corresponding author: rosalindamaarebia@yahoo.co.id

Abstrak. Prinsip-prinsip kimia hijau dalam nanoteknologi adalah salah satu isu utama dalam penelitian nanosains. Biosintesis nanopartikel menggunakan ekstrak tumbuhan adalah pendekatan ramah lingkungan yang menghilangkan kebutuhan untuk menggunakan teknik fisik dan kimia. Sebuah rute sintetik hijau untuk produksi nanopartikel perak stabil (AgNPs) dengan menggunakan larutan perak nitrat sebagai prekursor logam dan ekstrak air Myrmecodia pendans sebagai bioreduktor dan PVA sebagai stabilisator sedang dilaporkan untuk pertama kalinya. Dalam hal ini, sintesis nanopartikel perak (AgNPs) dilakukan dengan metode reduksi. Bio-reduksi dan stabilisasi nanopartikel perak yang terbentuk dianalisis menggunakan spektrofotometri UV-Vis. Penyerapan maksimum AgNPs menggunakan Polyvinyl Alcohol (PVA) berada pada panjang gelombang 408.50 nm sedangkan AgNP tanpa menggunakan PVA berada pada panjang gelombang 408 nm. Gambar SEM menunjukkan bahwa morfologi AgNPs adalah globular. Ukuran rata-rata nanopartikel perak menggunakan PVA dan tanpa menggunakan PVA adalah 78,3 nm dan 76,1 nm. Hasil X-Ray Diffraction (XRD) menampilkan pola puncak yang sama dengan yang ada pada logam perak standar yang menunjukkan bahwa partikel yang dihasilkan adalah nanopartikel perak yang memiliki struktur FCC. Analisis kadar glukosa dalam darah menggunakan sensor berbasis nanopartikel perak menunjukkan konsentrasi glukosa yaitu 71,71 mg/dL.

Kata kunci : Nanopartikel perak, bioreduktor, polivinil alkohol, sarang semut

Abstract. The principles of green chemistry in nanotechnology is one of the key issues in nanoscience research. Biosynthesis of nanoparticles using plant extract is an eco- friendly approach which eliminates the need for using physical and chemical techniques. A green synthetic route for the production of stable silver nanoparticles (AgNPs) by using aqueous silver nitrate as metal precursor and *Myrmecodia pendans* extract as bioreductor and PVA as stabilizer is being reported for the first time. In this case, the synthesis of silver nanoparticles (AgNPs) was carried out by reduction method. The bio-reduction and stabilization of so formed silver nanoparticles was monitored by UV-Vis spectrophotometry. Maximum absorption of AgNPs using Polyvinyl Alcohol (PVA) was at wavelength 408.50 nm, whereas that of AgNPs without using PVA was at wavelength of 408 nm. SEM images showed that the morphology of AgNPs was globular. An average size of silver nanoparticles using PVA and without using PVA was 78.3 and 76.1 nm, respectively. The results of X-Ray Diffraction (XRD) displayed the same peak pattern with that of

the standard silver metal showing that the resultant particles are silver nanoparticles having FCC structure. Analysis of glucose levels in blood using silver nanoparticles based sensors showed glucose concentration of 71.71 mg / dL.

Keywords: silver nanoparticles, bioreductor, polyvinyl alcohol, sarang semut

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder caused by the pancreas not producing enough insulin or the body cannot use insulin produced effectively. The number of people with DM from year to year continues to increase. Based on data from the International Diabetes Federation (IDF, 2015), Indonesia is ranked 7th in the world for the highest prevalence of diabetics in the world along with China, India, the United States, Brazil, Russia and Mexico with an estimated 10 million people suffering from diabetes. Various methods have been developed for blood glucose level sensors such as traditional qualitative analysis (silver methods. mirror reaction), polarometry, and IR spectroscopy. One of the interests of many researchers is the development of sensors using nanoparticles.

The used of metal nanoparticles for chemical sensors is a very interesting topic at this time. One of the metal nanoparticles used for synthesis is silver nanoparticles. Silver nanoparticles are attractive because of their unique properties (for example, sizes and shapes that depend on optical, electrical and magnetic properties) which can be incorporated into biosensor materials.

Nanoparticle synthesis using plant extracts is an environmentally friendly method and also has special advantages that plants can be widely distributed, a few second ago available, safer to handle and act as a source of several metabolites. and have various metabolite compounds that can help reduce ions silver, and the process is faster than synthesis using microbes. In the last decade of microbial based nanoparticle synthesis, biosynthesis using plants has received important attention from many researchers al, 2003). (Torresdey, et Several biosynthesis of metal nanoparticles using plants as reducing agents have been reported. These plants include Aloe Vera (Chandran, et al, 2006), Hibiscus Rosa Sinensis (Philip, 2010) leaf extract of Dalbergia sisso (Singh, et al, 2012).

The used of plant extracts as metal ion reducing agents is relatively shorter compared to microbial use to produce metal nanoparticles. Nanoparticles can be synthesized in minutes or hours whereas microbial based synthesis methods require longer time (Rai, et al, 2008). Plant extracts can act as reducing agents and stabilizing agents in the synthesis of Various nanoparticles. chemical compounds contained in plant extracts are used to reduce and stabilize nanoparticles (Ahmed & Ikram, 2015). The nature of extracts affects the plant type of nanoparticles synthesized. In addition, the source of plant extracts is the most important factor affecting the morphology of synthesized nanoparticles (Mukunthan & Balaji, 2012). Plant extracts used as reducing agents are known to contain secondary metabolites which have antioxidant **Bioactive** activity. compounds contained in plants such as antioxidant compounds and certain secondary metabolites such as terpenoids, phenolics, flavonoids, and tannins.

The utilization of biological been resources has developed to synthesize nanoparticles. One of the plants used to synthesize nanoparticles with high antioxidant content is the Sarang Semut (Myrmecodia pendans) which is empirically utilized by local Papuans as traditional medicine. The types of flavonoid compounds contained in ant nests were obtained, namely kaempferol (13.767 mg / g), luteolin (0.005 mg / g), rutin (0.003 mg / g), quercetin (0.030 mg / g), and apigenin (4,700 mg / g) [6]. This flavonoid compound is likely to function as a bioreductor for the synthesis of silver nanoparticles. Therefore, biosynthesis of metal nanoparticles using plant extracts is an option besides the two methods above, because this method can minimize the use of harmful inorganic materials and the waste produced. The Ag-NP obtained was characterized by UV-vis spectrophotometry, X-ray diffraction (XRD), Scanning electron microscopy

(SEM) with Energy Dispersive Spectroscopy (SEM-EDS), and Particle size analyzer (PSA).

MATERIALS AND METHODS Instrument

The instrument used include analytical scales. UV-Vis Spectrophotometer Shimadzu UV-2600, Magnetic Stirer (VWR Scientific), Scanning Electron Microscopy with Energy Dispersive Spectroscopy (MA10-14-37 ZEI SS EVO), X-ray Diffraction (Shimadzu 7000), Particle size analyzer (Delsa nano C), Voltmeter (ED410), Freeze dryer (Alpha 1-2 LD), Sentrifuge (TOMY MX-305), drop pipette, erlenmeyer, beaker, measuring flask, stirring bar, spray bottle.

Materials

Some materials that will be used in this study are sarang semut (M. *pendans*), PVA as stabilizers (Sigma Aldrich), AgNO₃ (Intraco), kaempferol, quercetin, blood serum, and PAA (Sigma Aldrich).

Methods

1. Preparation of M. Pendans

The plants used for biosynthesis are the Sarang Semut (*Myrmecodia pendans*). The plant originated from Merauke Regency, Papua. *M. pendans* that have just been obtained from the forest, cleaned of dirt (wet samples of *M. pendans*). The tip of a leafy ant nest is cut using a knife. The outer shell of the ant nest is peeled using a knife. The shelled

ant nests are divided into four parts, washed with aquabides, then thinly cut. The slices of the M. pendans are then mixed until a good M. pendans sample is obtained. Then mixed M. pendans samples are stored in a closed container. *M. pendans* samples that have been mixed are weighed as much as 10 gr. Furthermore, M. pendans sample was added with distilled water at a ratio of 1:10 (b / v) and boiled for \pm 15 minutes. And then the solution cooled and filtered using Whatman paper and filtrate is taken. The filtrate obtained is stored in a clean container and can be used as a bioreductor.

2. Biosynthesis of silver nanoparticle

Silver nanoparticle biosynthesis was carried out by mixing 20 ml of AgNO₃ solution and 2 mL of *M. pendans* extract then stirred using a multi stirrer. For 2.5 hours the sample is stirred until a color change becomes brownish yellow. This solution was then characterized using a UV-Vis spectrophotometer to see the stability formed from silver nanoparticles. Then the synthesis of silver nanoparticles was carried out using a stabilizer. In this case PVA is used as a stabilizing agent. As much as 2 ml of Sarang Semut extract was included in the 100 ml erlenmeyer, added 20 ml of AgNO₃ and 2 ml PVA solution into erlenmeyer which contained M. pendans extract. Then the sample was stirred using a multi-stirrer with the same treatment. This solution was then characterized using a UV-Vis spectrophotometer to see the stability formed from silver

nanoparticles, PSA to see the size distribution of silver nanoparticles produced. And solid samples of silver nanoparticles from the freeze dryer were taken to be characterized using SEM-EDS to determine the morphology of the silver nanoparticles produced and the quantity of silver nanoparticles, and finally XRD testing to see the purity of the silver nanoparticles produced.

3. Synthesis of silver nanoparticles with kaempferol

The synthesis of silver nanoparticles was carried out by mixing 0.1M of AgNO₃ and kaempferol solution. A total of 3 drops of kaempferol 0.1M were mixed into a 20 mL AgNO₃ solution and stirred for 2.5 hours.

4. Synthesis of silver nanoparticles with quercetin

The synthesis of silver nanoparticles was carried out by mixing AgNO₃ solution and 0.1M quercetin. A total of 3 drops of 0.1M quercetin were mixed into a 20 mL AgNO₃ solution and stirred for 2.5 hours.

5. Preparation of silver electrodes

Sedimentation of silver nanoparticles was carried out by LBL technique (layer by layer) ie silver wire dipped in 1% polyacrylic acid solution for 30 minutes, then rinsed with aquabides, and dipped in silver nanoparticle suspension for 15 minutes, and rinsed with aquabides again. This cycle is repeated three times. Furthermore, this electrode is called a working electrode with modification.

EXPERIMENT RESULTS 1. Biosynthesis of silver nanoparticle

The synthesis of silver nanoparticles in this study was carried out by using *M. Pendans* extract as a reducing agent and AgNO₃ solution as a metal precursor. The used of *M. pendans* extracts as reducing agents because plant extracts are known to contain several molecules that might have the potential to reduce silver ions to silver nanoparticles

and stabilize them (Mittal, et al, 2015). The formation of silver nanoparticles through the reduction of Ag^+ ions to Ag^0 using an extract of *M. pendans* can be observed using **UV-Vis** а spectrophotometer. The formation of silver nanoparticles was carried out by mixing *M. pendans* extract with AgNO₃ solution then stirring using a multi stirrer for 2.5 hours to homogenize the mixing of the solution. Silver nanoparticles are formed characterized by a change in color from light yellow to brownish yellow.



Figure 1. (a) Nanoparticle solution before stirring; (b). Nanoparticle solution after stirring

Figure 1, showed that the silver nanoparticle solution before stirring was vellow and after stirring and incubated gradually the silver nanoparticle solution turned dark brown. According to Hasan (2012),the presence of certain compounds in plants affects the color of the solution. The higher the compound content, the color produced will tend to be more concentrated gradually. The color change that occurs, shows the occurrence of the reduction process of silver ions by compounds contained in extracts of ant nests.

2. Variation of AgNO₃

The formation of silver nanoparticles is influenced by several factors such as temperature, pH, reducing concentration, and concentration of AgNO₃ (Khan, et al, 2013; Shankar, et al, 2004). So to find out the optimum concentration in the synthesis of silver nanoparticles, a study of variations in the concentration of AgNO₃ was carried out. Variations in the concentration of AgNO₃ used were concentrations of 0.5 mM, 1 mM, 1.5 mM, 2 mM, and 2.5 mM. Each concentration uses the same ratio of extracts and AgNO₃ solutions, namely 1:10. Furthermore, the five samples were analyzed using a UV-Vis spectrophotometer. The results of the study can be seen in Figure2.

Figure 2 shows that from each of the concentrations analyzed silver nanoparticles are formed which are characterized by the presence of

absorption peaks and wavelengths in the nm. 400-500 And the best range optimization results are formed at a concentration of 1 mM with a higher absorption peak of 1.763 and а wavelength of 469 nm compared to other variations in concentration. The large number of nanoparticles formed can be seen from the absorbance value. The higher of absorbance showed that the greater of number silver nanoparticles (Saeb, et al, 2014). So that the concentration of 1 mM AgNO₃ used as a reference in this study.



Figure 2. Variation of AgNO₃

3. Variation of stirring time

The same is done for variations in stirring time to determine the best time for the formation of silver nanoparticles. And the stirring time variations used were 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 150 minutes. Each sample uses the same extract ratio and AgNO₃ solution, which is 1:10. Furthermore, five samples were analyzed using a UV-Vis spectrophotometer.



Figure 3. Variaton of stirring time

Figure 3 shows that from each sample was analyzed using variations of stirring time formed silver nanoparticles. This is indicated by the presence of absorption peaks in the wavelength range of 400-500 nm. The optimization results showed that the best variation of stirring time was 150 minutes, the best time needed to form silver nanoparticles with absorbance of 1,250 at a wavelength of 436 nm. Decrease in particle size occurs with increasing speed and time of stirring nanoparticles (Gupta, 2011; Dangi, 2013). The longer the stirring will result in smaller particle size because more particles are split into nano-sized particles

4. Characterization of silver nanoparticle

UV-Vis is one part that can be used to prove the formation of silver nanoparticles. UV-Vis absorption is used

to see the amount of silver nanoparticles formed according to the absorbance value obtained. The measurement results of colloidal solutions of silver nanoparticles in wavelengths of 200 - 700 nm are shown in Figure 4. It appears that the colloidal of silver solution shows absorption peaks at wavelengths ranging from 397 - 408 nm. This shows compatibility with the results of previous research that the solution of colloidal nanoparticles from the synthesis results is proven as a colloidal solution with nanometer scale. Figure 4 shows an increase in absorbance after incubation for 7 days. optimization results show that the best absorbance is formed on day 7 with an absorbance value of 2.233 at a wavelength of 406.5 nm. where on this day 7 the wavelength decreases which indicates that the size of the silver nanoparticles formed is smaller with the increasing number of nanoparticles. To determine the stability of the synthesized silver nanoparticle colloid, the absorption spectrum was measured using a UV-Vis spectrophotometer based on the time function.



Figure 4. UV-Vis spectrum of silver nanoparticles without stabilizers



Figure 5. Silver nanoparticles uptake based on time function

The stability of the silver nanoparticle colloid solution can be determined from the occurrence of changes in the absorption peak. If there is a shift in the absorption peak to a larger wavelength, it indicates that the stability of the silver nanoparticle colloid solution is low due to an agglomeration event. Nanoparticles generally tend to form agglomerations which can affect the size of nanoparticles. Therefore stabilizers or protectors are needed which protect the surface of metal nanoparticles and prevent particle gathering or agglomeration (Buzea, et al, 2007; El-Nour, et al, 2010). In this study the synthesis of silver nanoparticles was carried out using PVA as a stabilizer. The synthesis of silver nanoparticles using stabilizers was carried out by mixing M.

pendans extract and AgNO₃ solution and PVA into the 100 ml erlenmeyer, stirred using a multi stirrer.



Figure 6. Spectrum of UV-Vis silver nanoparticles with stabilizers



Figure 7. Silver nanoparticles uptake based on time function

Based on UV-Vis analysis, it is known that silver nanoparticles with stabilizers do not experience significant wavelength shifts from 0 days to day 4, only ranging from 408.5 - 413 nm. Different from the results of nanoparticle formation without using stabilizers, the results from Figure 7 show that there is an increase in wavelength during 4 days incubation. Based on the results of uv-vis the best absorbance value was obtained on day 4 with an absorbance value of 2.066. It can also be seen that the hypsochromic shift that is the shift of the

maximum wavelength to the left indicates the size of the silver nanoparticles getting smaller with the increasing absorbance intensity indicating an increase in the number of Ag nanoparticles while the decrease in absorbance values indicates particle collection or agglomeration. The absorbance value in **UV-Vis** spectrophotometry shows the estimated number of silver nanoparticles formed while the wavelength of maximum absorption can indicate the size of the nanoparticles produced.

The determination of the size of silver nanoparticles and their distribution was carried out using PSA. The results of identification using PSA showed the size of the silver nanoparticles synthesized without and using PVA which is 76.1 and 78.3 nm. The particle size data obtained in the form of three distributions, namely number intensity. and volume distribution, so it can be assumed to describe the overall condition of the sample. The results of the determination of silver particle size distribution without using PVA stabilizers were distributed between 17-81.2 nm with an average size

of 76.1 nm with a value of polydispersity index (PI) of 0.324. The distribution of silver nanoparticles synthesized using PVA, where the silver particle size is distributed between 18.3-81.2 nm with an average size of 78.3 nm and the value of polydispersity index (PI) 0.303. From the results of the PSA obtained at the nanoscale the results showed that *M*. *pendans* extract has the potential as a reducing agent in the manufacture of nanoparticles and it can be seen that the use of PVA can produce small silver nanoparticles.

The results of the PSA testing are proven by the results of identification using SEM-EDS which shows that the silver nanoparticles produced have a size on the nanoscale with the smallest measured size of 50 and 64 nm. The results of SEM-EDS analysis for silver nanoparticles that not used PVA or those using PVA can be seen in Figure 8 which shows the diameter of silver nanoparticles and proves that the silver particles produced have a size on the nanoscale.





Figure 8. (a) Enlargement of silver nanoparticles 40.000x without PVA; (b) Enlargement of silver nanoparticle 40.000x with PVA

The characterization of silver nanoparticles synthesized was carried out using SEM at 40,000x enlargement. The measurement results of SEM can be seen in Figure8. Figure 8a the observation at 40,000x enlargement shows a clearer morphology of the spread of silver particles so that it is quite possible to determine the diameter of the particles formed. Silver nanoparticles without PVA stabilizers have nanoparticle sizes ranging from 50 nm to 64 nm and are globular. It can be seen from Figure8a that distributed or not assembled silver nanoparticles agglomerations in silver form as nanoparticles using PVA stabilizers. According to the manual determination of the size of the silver particles measuring less than 100 nm. The analysis was also

carried out using the Energy Dispersive X-Ray Spectrometer (EDS) device which was connected with a SEM tool. Analysis with EDS produces qualitative and quantitative information about the composition of locations in samples with a diameter of several micrometers. From the analysis using EDS to see the composition of the samples formed, it was found that Ag is an element with a percentage of mass formed at 83.70% for nanoparticles without using stabilizers (Figure 9) and mass percent Ag of 82.14% for nanoparticles using а stabilizer (Figure 10) that shows the quality of Ag elements which is best formed for the formation of silver nanoparticles.



Figure 9. Spectrum EDS of silver nanoparticle without stabilizer

XRD testing to identify the phase, structure, and size of crystals in a material. Theoretically, the crystal structure of silver nanoparticles can be determined based on hkl values. Based on the Bragg equation, if a beam of X-ray is dropped on a crystal sample, then the crystal field will refract X-rays which have wavelengths equal to the distance between the lattices in the crystal. Refracted rays will be captured by the detector and then translated as the diffraction peak. The more crystal fields contained in the sample, the stronger the intensity of refraction produced.

According to the Scherer method, ACS (Atomic Crystal Size) can be

determined by determining the Width of a Maximum (FWHM) Half with а wavelength of X-ray source from Cu (copper) of 1.5406 Å. The width of FWHM is influenced by the size of the crystal. The greater the peak width detected, the smaller the crystal size value. Very small crystals will produce diffraction peaks that are very wide because small crystals have limited x-ray fields. The smaller the crystal size of a material, the greater the FWHM and the peak intensity decreases. Data on the diffractogram of silver nanoparticles is shown in Figure 11.





Figure 11. Diffractogram of silver nanoparticles without stabilizers

No.	20		d(A)			
	Ag NP	Standard Ag	Ag NP	Standard Ag		
1.	37.80	38.12	2.378	2.359		
2.	64.24	64.48	1.448	1.4439		
3.	77.46	77.47	1.231	1.231		

Table 1 Data on XRD silver nanoparticles without PVA

It can be seen in the figure, that the XRD pattern of the sample produces 3 peaks at an angle of 2θ the three XRD peaks have

values hkl (111), (202) and (311) which approach diffractogram data from standard silver.



Figure 12. Diffractogram of silver nanoparticles without stabilizers

No.	20		d(A)	
	AgNP	Standard Ag	AgNP	Standard Ag
1.	37.77	38.12	2.379	2.359
2.	44.04	44.29	2.054	2.044
3.	64.38	64.48	1.445	1.4439
4.	77.49	77.47	1.230	1.231

 Table 2 Data on XRD silver nanoparticles with PVA

In contrast to the XRD pattern of silver nanoparticles using a stabilizer, this XRD pattern produces 4 peaks at an angle of 2θ with a miller index respectively (111), (200), (202), and (311). The diffractogram data also provides size

distribution information silver nanoparticles, from the calculation of grain size through XRD, it can be seen that the size distribution of silver nanoparticles that were successfully synthesized has varying sizes, namely 8.86-32,59 nm.

5. Synthesis of silver nanoparticles with kaempferol

Proof of flavonoid compounds (kaempferol) as reducing agents is then tested using standard kaempferol. The measurement results with standard kaempferol obtained a wavelength of 430.50 nm with an absorbance of 0.257 which is the wavelength of silver nanoparticles.



Figure 13. UV-Vis spectrum of silver nanoparticles from standard kaempferol

6. Synthesis of silver nanoparticles with quercetin

Besides kaempferol, other flavonoids contained in *M. pendans* are quercetin. Quercetin is also indicated to be a reducing agent for silver nanoparticles. And from UV-Vis data in

Figure 14 it was found that quercetin compounds could form UV-Vis spectra at wavelengths of 424.5 nm with absorbance of 0.183 which is the area of formation of silver nanoparticles, although absorbance formed from standard kaempferol was greater than quercetin.



Figure 14. UV-Vis spectrum of silver nanoparticles from standard quercetin.

7. Detection of blood samples

Silver nanoparticles based sensors that have been made are then tested by measuring how much glucose concentration is contained in human blood samples. Blood samples were taken at the South Sulawesi Public Health Laboratory Center. Analysis of glucose levels in blood samples using work electrodes coated with silver nanoparticles. The performance of working electrodes coated with silver nanoparticles was studied based on observations of voltammograms in the of form current strength. The measurement results of the sample are shown in table 3 which shows the voltammograms and the value of the current strength which is relatively stable in measurements 2 and 3.



Figure 15. Voltammogram measurements in blood samples

		1	
Measurement to-	Currents	Concentration	
1	0,60	0,218	
2	3,10	5,42	
3	3,53	5,868	
Average	2,41		

 Table 3. Glucose levels in blood samples

The average value of the current flow is then entered into the linear regression equation to obtain a glucose concentration in the blood of 71.71 mg / dL. The measurement results are results compared with the of of measurement samples using Automated Analyzed Clinical Chemistry, which is 77 mg/dL. So, glucose concentration in blood samples based on analysis with silver nanoparticle based sensors is 3.984 mM or 71.71 mg/dL.

CONCLUSION

The biosynthesis of silver nanoparticles using sarang semut extract has been synthesized by a simple, fast, cost-effective environment friendly. Analysis of UV-Vis, PSA, SEM-EDS and XRD confirm the reduction of Ag⁺ ions to Ag^0 through plant extracts which act as reducing agents and PVA as a stabilizer. From the research that has been done, the results show that M. pendans extract can be used as a bioreductor for metal nanoparticles. Bioreductor research with M. pendans using PVA stabilizer was the first study conducted. And the results obtained prove that silver nanoparticles formed with the size of nanoparticles 76.1 and 78.3 nm with the morphology of AgNPs are globular and resultant particles are silver nanoparticles having FCC structure. And the glucose content contained in blood samples is 3.984 mM or 71.71 mg / dL.

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