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Silver Nanoparticle Produced by Using *Abelmoschus esculentus* (L.) Moench Leaves Extract Bioreduction Processs as Blood Glucose Nanosensor

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Abstract

Silver nanoparticles were produced through bioreduction using Abelmoschus esculentus leaves extract and applied as a blood glucose nano sensor. The biosynthetic reaction produced silver nanoparticles by mixing *Abelmoschus esculentus* leaves extract and Ag^{+}. The formation of silver nanoparticles was characterized by a color change in the solution from yellow to brown. Silver nanoparticles were analyzed using UV-Vis, FT-IR, PSA, and TEM. Furthermore, the design and testing of blood glucose nanosensors was carried out. The UV-Vis test results showed that the best silver nanoparticles were produced during an incubation period of 6 days with a band gap energy of 2.096 eV. The FT-IR spectrum showed that there had been a bioreduction process as indicated by a decrease in the intensity of the functional groups which were bioreductors. TEM and PSA results showed that silver nanoparticles were spherical and oval in shape with a size of less than 50 nm. The design and application results for the nano sensor showed that silver nanoparticles were in the range of 0.5 mM - 8 mM with a Regression (R) of 0.9494. The detection limit of the silver nanoparticle sensor was at a concentration of 1.68 mM with a sensitivity of 3.27 A. mM^{-1}. mm^{-2}. The glucose level contained in blood samples with silver nanoparticle sensors was 93.05 mg / dL with a measurement difference of 1.95% when compared to Automated Analyzed Clinical Chemistry as gold standard.

***Keywords*:** silver nanoparticles, bioreduction, nanosensors, blood glucose, *Abelmoschus esculentus*, detection.

1. Introduction

Nanoparticles are one part of nanotechnology that is attracting attention, because it is related to the production of nanoparticles of various sizes, shapes, and chemical compositions, which can be applied and utilized in various fields [1]. Nanoparticles are defined as the dispersion of particles or solid particles with sizes in the 1–100 nm range. Nanoparticles can occur naturally or through the process of synthesis by humans [2]. Metal nanoparticles are a widely studied nanomaterial. One of them is silver nanoparticles because these nanoparticles have a very high exclusion coefficient and optical properties that depend on the size and shape of the particles, the medium dielectric constant, composition, and the distance between particles [3].

Silver nanoparticle biosynthesis is another viable option besides physical and chemical methods. In addition, in the bottom up method, metal nanoparticles can be synthesized by utilizing living things as biological agents in the nanoparticle synthesis process [4]. The principle of metal nanoparticle biosynthesis is the use of plants or microorganisms as reducing agents. The microorganisms used can be bacteria, yeast, and fungi. The biosynthesis of metal nanoparticles using microorganisms has disadvantages, such as difficult culture maintenance and long synthesis times [4]. Nanoparticle biosynthesis using plants provides several advantages, such as environmentally friendly, compatible for pharmaceutical and biomedical applications, low cost, low pressure, energy and temperature, and no toxic chemicals [5].

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Various plants have been used such as *Cucurbitha maxima* [6], *Elaeis quineensis* [7], *Ziziphus zizyphus* [8], *Syzigium polyanthum* [9]. These plants contain antioxidant compounds such as flavonoids, coumarin, alkaloids, and tannins. One of the plants that can be used as a reducing agent is the *Abelmoschus esculentus* plant, better known as okra [10]. Okra contains flavonoid compounds that function as antioxidants. The content of flavonoid compounds in okra leaves allows it to be used as a bioreductor which is the basis for sensors of blood sugar levels. Based on the results of research by [11], okra fruit extract can be used to synthesize silver nanoparticles. Research on okra (*Abelmoschus esculentus* (L.) Moench) has been widely carried out, but the use of okra leaf extract in synthesizing silver nanoparticles for biosensor applications has never been carried out. Therefore in this study silver nanoparticles were synthesized using *Abelmoschus esculentus* (L.) Moench as a bioreductor for application as a blood glucose nanosensor.

2. Materials and Methods

2.1. Materials

The materials used were okra leaves, distilled water, aquabides, glucose anhydrid, methanol, Whatman No. paper. 42, buffer, $AgNO_3$, polyacrylic acid, 0.1 M NaOH, blood serum, filter paper, tissue, label paper. The instruments used erlenmeyer, beaker, volumetric flask, stirring rod, spray bottle, pipette, volume pipette, scissors, analytical balance (Acculab), power cut, shaker, sample bottle, aluminum foil, Magnetic Stirrer (VWR Scientific) UV-Vis Spectrophotometer (Shimadzu UV-2600), FTIR, Vasco DLS Particles Size Analyzer (PSA), Spray Dray (Buchi 190), Transmission Electron Microscopy (TEM) HR TEM H9500, oven, silver electrode, voltameter (ED410), platinum electrodes, Ag / AgCl electrodes, platinum cables.

2.2. Methods

2.2.1. Making Abelmoschus esculentus *(L.) Moench leaves extract*

Abelmoschus esculentus (L.) Moench was obtained from Moncongloe Bulu Village, Moncongloe District, Maros Regency, South Sulawesi-Indonesia. The leaves were taken and then washed with distilled water and then drained. After that the leaves were cut into small pieces and weighed 10 grams, then boiled with 100 mL of distilled water in 500 mL of erlenmeyer. Then the stew was allowed to boil for 5 minutes. After reaching room temperature, the cooking water was poured and filtered using Whatman No.1 paper. The boiled water can then be used directly for biosynthesis of silver nanoparticles. Okra leaves cooking water that had not been used was stored in the refrigerator.

2.2.2. Biosyntesis of silver nanoparticles with okra leaf extract

The biosynthesis of silver nanoparticles was carried out by mixing the Ag^+ solution and boiling water from the okra leaves. A total of 2 mL of boiled water from okra leaves was mixed into a 40 mL Ag^+ solution, then incubated with time variations of 1, 2, 3, 4, 5, 6, and 7 days. The formation of nanoparticles was characterized by a change in the solution from yellow to brown. Furthermore, the nanoparticles were tested using UV-Vis, FT-IR, PSA and TEM.

2.2.3. Silver electrodes preparation and silver nanoparticle drilling

Silver nanoparticles were deposited using a layer-by-layer technique (LBL), namely the silver electrode was immersed in a 0.2% polyacrylic acid solution (pH 10) for 30 minutes, then rinsed with distilled water, and dipped in silver nanoparticle suspension for 15 minutes, and rinsed with distilled water. This treatment was repeated 3 times.

2.2.4. Electrochemical measurement

Measurement of standard sugar solution was carried out using the voltammetric method. The electrode assembly consists of a silver electrode as a working electrode, platinum wire as an auxiliary electrode, and an Ag / AgCl electrode as a comparison electrode. The electrochemical measurements were carried out at 0.1 M NaOH. The electrodes used were immersed in a glucose solution and then measured with a potentiometer at a potential of -0.5 eV to +9 eV. Furthermore, the calculation of the sensor measurement range, detection limit and sensitivity is carried out. Blood sample measurements were also carried out.

3. Result and Discussion

3.1. Silver nanoparticle analysis using UV-Vis

This study observed the effect of incubation time on changes in Surface Plasmon Resonance (SPR) and the resulting nanoparticles. Silver nanoparticles that were synthesized at various incubation times were analyzed by UV spectra to observe changes in SPR, changes in the size of silver nanoparticles (Fig. 1). The change in color of the yellowish solution to brown and UV-Vis light absorption in the 446-448 nm range is a feature of SPR excitation and the formation of silver nanoparticles as previously reported [4]. The absorbance intensity value which increased from the first day to the sixth day of the incubation time indicated that in the solution an increase in the number of silver nanoparticles formed. After increasing the incubation time until the 7^{th} day there was a decrease in the absorbance intensity, this phenomenon shows that the bio-reduction process of Ag^{+} into silver nanoparticles in solution has been reduced due to the reduced number of reducing compounds in the solution. The highest UV-Vis absorption intensity was observed after the sixth day of incubation, these results indicate that the silver nanoparticle formation process has been completed.

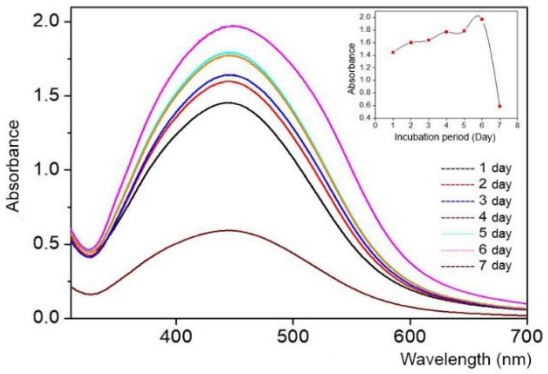


Fig. 1. UV-Vis spectrum of silver nanoparticles, incubation time of 1, 2, 3, 4, 5, 6, and 7 days

Fig. 1 also shows the shift in the resulting peak wavelength of silver nanoparticles to a higher wavelength or bathochromic shift during the incubation period of the first day to the sixth day. The bathochromic shift from the first day to the sixth day indicated that along with the addition of the incubation period there has been an increase in the average diameter size of the silver nanoparticles produced. However, the addition of the incubation time for the sixth day to the seventh day turned out to be a shift in the bathochromic type into a hypochromic shift. The shift indicates that the average diameter size of the silver nanoparticles produced decreases with increasing incubation time.

Table 1. Band gap energy of silver nanoparticles

Incubation time (day)	Band gap energy (eV)
1	2.263
2	2.226
3	2.225
4	2.193
5	2.191
6	**2.096**
7	2.227

The data in Table 1 showed that the bandgap energy of the silver nanoparticles decreased with the increase in the average particle size of the resulting nanoparticles. This related to quantum mechanics if the size of materials was larger (especially nanoscale materials), the number of orbitals they overlap or their energy levels increase. This condition causes the distance between the valence band and the conduction band to get closer. The results of calculating the bandgap energy value for silver nanoparticles obtained the lowest band gap energy (incubation time of six days) shown in Fig. 2.

Based on the calculation of the band gap energy value of silver nanoparticles, it was known that the smallest bandgap energy value of silver nanoparticles during the six-day incubation period was 2.096 eV. Therefore, for further analysis the process of forming silver nanoparticles using an incubation period of six days.

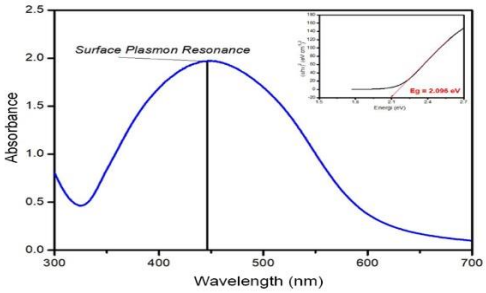


Fig. 2. The lowest bandgap energy of silver nanoparticles (incubation time 6 days)

3.2. Silver nanoparticle analysis with FT-IR

The results of analysis using FT-IR on silver nanoparticles of okra leaves extract were shown in Fig. 3. Fig. 3 (a) showed the widening peaks at 3446.79 cm^{-1} and 667.32 cm^{-1} which indicates the strain vibration of OH and alcohol. Deformation outside the plane. The appearance of a weak band at wave number 2958.60 cm^{-1} came from the strain vibration of the $–CH_3$ group. The bands with weak intensities at 2924.09 cm^{-1} and 2746.63 cm^{-1} were derived from vibrations from the aldehyde group (-CHO). The vibrations that occurred in the $-CH_2-$ group were indicated by the appearance of the band at 2852.72 cm^{-1}. The band at 2398.94 cm^{-1} originated from the vibration of the acetaldehyde group binding while the C-C binding vibration of the R-CH = CH_2 bond appeared at 1805.37 cm^{-1}. The strike vibration (C = C) of the aliphatic compound, the C = C vibration in the cyclic compound and the C-N vibration of the amine group respectively appeared at wave numbers 1651.07 cm^{-1}; 1541.12 cm^{-1}; 1083.39 cm^{-1}. Based on the results of the analysis, it showed the number of groups that can function as bioreductors (undergoing oxidation) which was owned by the extract of *Abelmoschus esculentus*

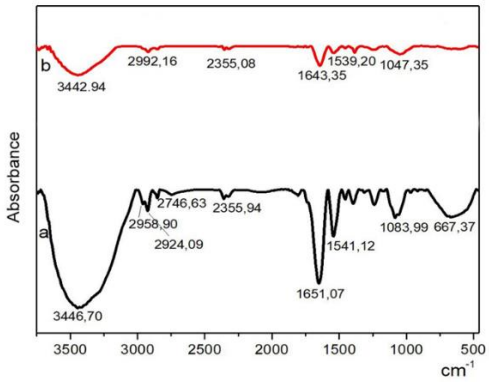


Fig. 3. (a) FT-IR of okra leaf extract (b) silver nanoparticles

The results of previous studies indicated that the functional groups responsible for the bioreduction process using plant extracts were –CN, -CHO, and –OH. Fig. 3 (b) showed a slight shift in the FT-IR band of Abelmoschus esculentus leaves extract after the formation of silver nanoparticles. For the FT-IR spectrum produced by silver nanoparticles (Fig. 3b) there was a decrease in the band intensity of 3442.94 cm^{-1}; 1643,35, cm^{-1}; 1049.28 cm^{-1}; 609.81 cm^{-1} and the loss of the band at 2746.63 cm-1. The bands at 3442.94 cm^{-1} and 609.81 cm^{-1} originate from the alcohol group, the band 1047.35 cm^{-1} came from the -C-N group of protein compounds and the band 2746.63 cm^{-1} is a characteristic of aldehyde compounds. The reduced intensity and loss of bands in the wavelength after the silver nanoparticles were formed suggest that this group was responsible for bioreduction of silver ions. The results of this FT-IR analysis were similar to those obtained by previous researchers [12,13,14].

3.3. Silver nanoparticle analysis with Particle Surface Analyzer (PSA)

The results of testing the size distribution of silver nanoparticles using PSA are shown in Fig. 4. The average size of silver nanoparticles obtained was 89.5 nm. These results provide an overview of the silver nanoparticles tested that have met the nano-scale particle size (<100 nm). The PSA test results showed the polydispersity index of the nanoparticles produced. The polydispersity index provided an overview of the homogeneity of the size distribution of the nanoparticles scattered in the solution phase. The polydispersity index value was calculated by dividing the average weight of the nanoparticles by the average weight of the nanoparticles. The greater the polydispersity index value, the smaller the nanoparticle size uniformity.

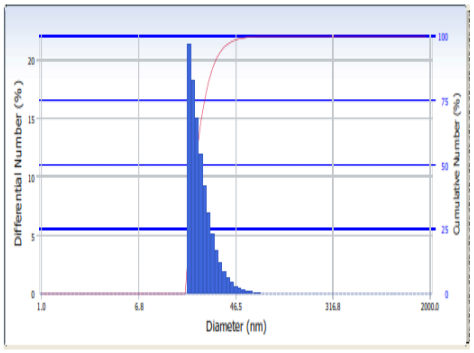


Fig. 4. Distribution of polydispersity index of silver nanoparticles

The ideal nanoparticle polydispersity index value was less than 0.5. The existence of a significant difference in the size of the nanoparticles (getting smaller and bigger) will affect the characteristics of these nanoparticles. Nanoparticles with a larger size tend to easily experience deposition. Bioreduction silver nanoparticles using *Abelmoschus esculentus*

leaf extract had a polydispersity index of 0.381. These results indicate that the resulting nanoparticle size is more homogeneous because it has a polydispersity index value of less than 0.5.

3.4. Transmission Characterization of Electron Microscopy (Tem) of Silver Nanoparticles

TEM results on silver nanoparticles showed that the morphology of silver nanoparticles analyzed was spherical and oval. This is in accordance with the results of research conducted by [15], which states that the silver nanoparticles produced have a surface structure with non-uniform grain shapes. The results of research using TEM can be seen in Fig. 5.

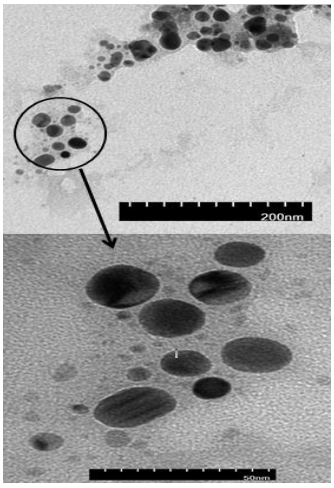


Fig. 5. Silver nanoparticle TEM test results.

Fig. 6 showed the results of the analysis of the diameter distribution of silver nanoparticles particles. The analysis showed that the diameter of the silver nanoparticles formed was mostly in the range of 16-18 nm.

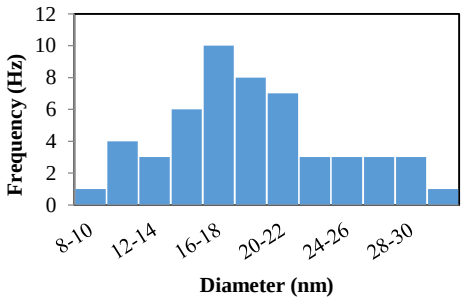


Fig. 6. Diameter distribution of silver nanoparticles

Fig. 7 showed the results of the analysis of the surface area distribution of silver nanoparticles. The analysis showed that the silver nanoparticles produced were dominated by particles with a surface area of 600-1400 nm^2.

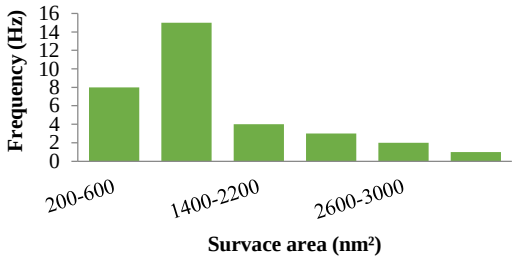
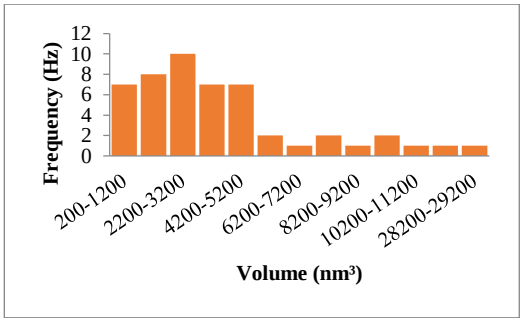


Fig. 7. Distribution of surface area of silver nanoparticles.

Fig. 8 showed the results of the volume distribution analysis of the resulting silver nanoparticles. These results indicated that the largest volume distribution range possessed by silver nanoparticles is 2200-3200 nm^3.



3.5. Silver nanoparticle based sensor application

In this study, the application of silver nanoparticles in measuring glucose levels was carried out using a voltameter. Observations were made by comparing the three working electrodes of the voltammogram used in the 0.5 mM - 9 mM glucose measurement, namely the working electrodes that were not coated with silver nanoparticles and the working electrodes coated with silver nanoparticles. The relationship between the voltage and glucose concentration in the working electrode that was not coated with silver nanoparticles showed an irregularity in the measured current pattern at various glucose concentrations as shown in Fig. 9A. Working electrodes that are not coated with silver nanoparticles are less sensitive to glucose, so they cannot be used for glucose analysis. The relationship between voltage and glucose concentration on the working electrode coated with silver nanoparticles can be seen in Fig. 9B, which showed the regularity of the measured current patterns at various glucose concentrations. This indicated that the working electrode coated with silver nanoparticles was

sensitive to glucose and can be used in glucose analysis.

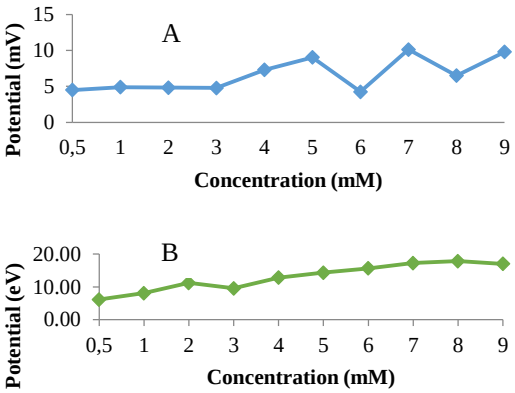


Fig. 9. Curve of the potential and concentration relationship, (a) working electrode that is not coated with nanoparticles (b) working electrode coated with silver nanoparticles.

The results of the working electrode potential test coated with silver nanoparticles indicated an oxidation reaction. This showed that glucose undergoes an oxidation reaction to gluconolactone as in the following equation:

It was because the use of silver nanoparticles on the sensor could increase the direct electron transfer between the biomolecular compound (glucose) and the electrode surface. In addition, the presence of silver nanoparticles attached to polyacrylic acid could expand the surface of the electrode with the analyte so that the resulting voltage was higher.

3.6. Sensor measurement range based on silver nanoparticles

The potential and concentration relationship curves showed that the measuring range of the working electrode coated with silver nanoparticles was in the range of 0.5 mM – 8.0 mM. It was shown from the linear concentration vs potential glucose curve (Fig. 10) and the range of measurements of the working electrode coated with silver nanoparticles (Table 2).

Table 2. Measurement ranges of working electrodes coated with nanoparticles silver

Concentration (mM)	Potential (V)
0.5	6.14
1.0	8.08
2.0	11.17
3.0	9.54
4.0	12.79
5.0	14.28
6.0	15.59
7.0	17.24
8.0	17.80
Measuring range	0.5 mM – 8.0 mM
R	0.9494
Linear regression equation	y = 1.5217x + 5.0074

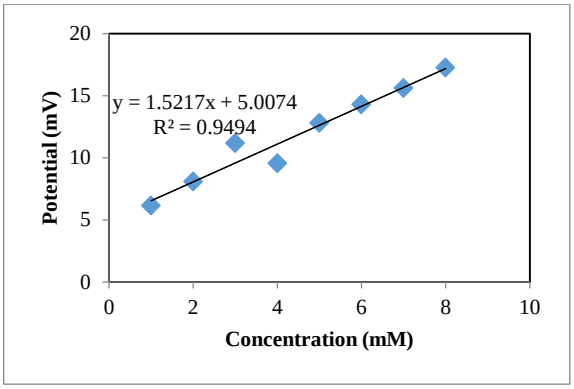


Fig. 10. Linear Regression Curve for Concentration vs Flow of Silver Nanoparticles

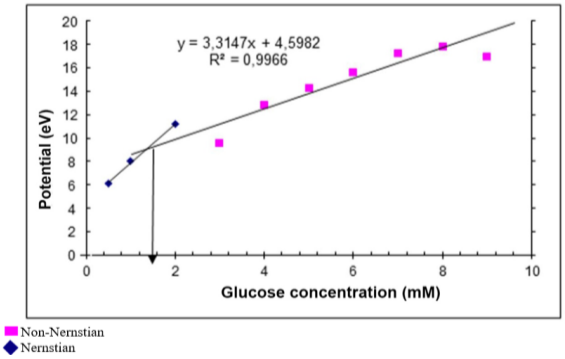


Fig. 11. Potential of a silver nanoparticle-based sensor as a function of glucose concentration

Measuring range 0.5 mM – 8.0 mM obtained a linear regression equation y = 1.5217x + 5.0074 with R = 0.9494 indicating that the working electrode coated with silver nanoparticles had a good ability to measure glucose levels. Detection limits and sensitivity measurements were also carried out to determine the ability and performance of silver nanoparticle-based sensors.

3.7. Detection limit

The detection limit of the working electrode coated with silver nanoparticles (Fig. 11) was determined by making a tangent to a significant and non significant linear function. The intersection of the two lines was extrapolated to the x-axis to obtain the concentration of the detection limit.

The results of extrapolation between the meaningful and non-essential linear curves, the detection limit was obtained at a concentration of 1.68 mM.

3.8. Sensitivity

The sensitivity test was determined by dividing the slope of the linearity curve by the surface area of the working electrode used. Sensitivity test was performed to determine the sensitivity of a sensor to the analyte.

Sensitivity = Slope / A

Sensitivity = 1.5217 / (3.14 x 0.4 x 0.4)

= 3.02 A. mM^{-1}. mm^{-2}

The sensor sensitivity value showed that the silver nanoparticle-based sensor had a sensitivity of 3.02 A. mM^{-1}. mm^{-2}.

3.9. Measurement of blood glucose samples

The resulting stress value is then entered into the linear regression equation to obtain a blood glucose concentration of 93.05 mg / dL. The results of these measurements were compared with the results of sample measurements using Automated Analyzed Clinical Chemistry .0, namely 91.27 mg / dL. From the two measurement results, the range of difference in concentration values is not too far away, namely 1.95%.

Table 3. Measurement results of silver nanoparticles in blood samples

Measurement No.	Sensor		*Automated Analyzed Clinical Chemistry*
	Potential (V)	**Concentration (mg/dL)**	**Concentration (mg/dL)**
1	12.904	93.41	92.01
2	12.814	92.34	90.20
3	12.903	93.40	91.60
Mean	12.874	93.05	91.27

4. Conclusions

Based on the results of the research that has been done, it shows that okra leaf extract can be used in the synthesis of silver nanoparticles. Based on the results of UV-Vis analysis, the smallest band gap energy of silver nanoparticles is 2.096 eV. The results of FT-IR analysis show that there has been a bioreduction process characterized by shifts and decreases in the intensity of the –CN, -CHO, and –OH spectra before and after the nanoparticle synthesis process. The results of characterization of silver nanoparticles with the Particle Size Analyzer

showed a high homogeneity of the silver nanoparticles produced. The TEM test results showed that the silver nanoparticles were perfectly formed which was characterized by the morphological shape and particle size that matched the silver nanoparticles. Silver nanoparticle based sensors are excellent for measuring glucose levels. The measurement range of the silver nanoparticle sensor is in the range of 0.5 – 8.0 mM with Regression (R) 0.9494, the sensor detection limit is at a concentration of 1.68 mM with a sensor sensitivity of 3.02 A. mM^{-1}. mm^{-2}. The glucose level contained in blood samples with silver nanoparticle sensors was 93.05 mg / dL with a measurement difference of 1.95% when compared to Automated Analyzed Clinical Chemistry.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

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