SYNTHESIS OF GOLD NANOPARTICLES USING GLUTAMIC ACID AND ITS ANTIBACTERIAL ACTIVITY

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Abstract - The synthesis of gold nanoparticles using glutamic acid reducing agents has been successfully performed. The pH of the solution affects the formation of gold nanoparticles. Glutamic acid will be more actively to capped Au in solution when the pH is above pKa3. High pH solutions require less amount of glutamic acid to be used in the synthesis process of gold nanoparticles. The optimum reaction time is obtained within 60 minutes. The gold nanoparticles reduced by glutamic acid have antibacterial activity. However, purification of gold nanoparticles reduces its antibacterial activity. The effect of concentration occurred insignificant on the gold nanoparticles purification, but was seen more significantly on the gold nanoparticles by purification

Keywords - Antibacerial Effect, Gold Nanoparticles.

I. INTRODUCTION

Research on nanoparticles is currently growing rapidly. This nanoparticle attracts researchers because of the properties of nanoparticles that show the unique and difference with its bulk material. Differences of these properties in terms of physical, chemical and biological properties such as electronic properties, optical, magical, reactivity and other properties [1].

Metal nanoparticles have been studied intensively since 15 years ago, including the gold nanoparticles (AuNP). AuNP has a wide range of applications such as selective coatings, sensors for ion and molecular detection, hyperthermal therapy, diagnosis, medicine, biological and environmental applications [2]

The antibacterial application of AuNP has been a hot topic of recent research due to the unique physicochemical properties of AuNP as an antibacterial agent [3]. Zhang et al [2]reported that AuNP have a good antibacterial activity, especially when itsynthesized using microorganisms or plants extract as a reducing agent. Nagaraj et al [4] synthesize AuNP using Caesalpinia pulcherrima flower extract as a reducing agent and show that AuNP is effective in inhibiting bacterial growth. Mishra et al [5] synthesized AuNP and AgNP (silver nanoparticles) using a *Guida guilliermondii*as reducing agent showed the highest antibacterial activity observed in Staphylococcus aureus bacteria in both AuNP and AgNP. Other studies have been reported using Menthapiperita plant extract [6], T. decandra root extract [7], Helianthus annus flower extract [8] and Carthamus tincitorius [9].

In contrast to the results of studies on the synthesis and application of antibacterial AuNP using extract as reducing agent, research using non-extracting agents (small malecule) is still not exposed yet to do for antibacterials application. The results reported were different. Amin [10] performed AuNP synthesis using polyvinylpirrolidine (PVP) as a reducing agent with a concentration of Au 125 ppm indicating that AuNP (20-30 nm) produced did not show antibacterial activity. Hernandez-Sieraa [11] using a PVP reducing agent reported that AuNP (80 nm) only inhibited at a very high concentration of 197 g / mL. Zhou [12] synthesizes AuNP with citrate or PAH as a dyeing agent reported that AuNP (2-30 nm) can inhibit *E.Coli* at low concentrations of 0.1; 1 and 5 g / mL. Arshi [13] also synthesized AuNP with citrate as a tapering agent and CTAB as a stabilizer agent with 12 mM $HAuCl_4$and 0.2 g citrate reported that AuNP (1-22 nm) could inhibit E.coli bacteria with 22 mm inhibition zone. In the above mentioned studies, whether extracts or not, it has not been reported about where and how the antibacterial activity can be achieved. Whether it comes from its gold, its exposure agent / extract or a combination of the two

This unclear conclusion prompted to explore and conduct this research that synthesized AuNP using amino acid-capping agents. The synthesis of AuNP with amino acids as reducing agent has been widely practiced but no one has tried for antibacterial applications yet. Though amino acids are a safe substance both for the environment and for the human body.

In this study, glutamic acid is used as a reducing and capping agents in the synthesis of AuNP as an antibacterial application.

II. DETAILS EXPERIMENTAL

2.1. Materials

The materials used in this study were purchased from E-Merck such as sodium hydroxide (NaOH), nitric acid (HNO_3) 65%, hydrochloric acid (HCl) 37%, and glutamic acid. Golden stem 99.9% obtained from ANTAM. Other materials such as aquades obtained from LPPT UGM.

2.2. Synthesis of Gold Nanoparticle Using Glutamic Acid

$HAuCl_4$ was obtained by dissolving gold with aqua regia solution (mixture of HCl and HNO_3 by 3: 1). The synthesis of Gold Nanoparticles is done by mixing $HAuCl_4$solution (5 mL, 40 mg/L) with glutamic acid (5 mL, 10 mM) as reducing agent and heated in waterbath for 1 hr, followed by pH adjusment using NaOH or HCl to pH 6-12.Concentration effect were carried out by adjusting glutamic acid concentrationby 1-50 mM and $HAuCl_4$ by 10-100 mg/L. Reaction time effect were prepared by adjusting reaction time to 15-180 min. Spectra of the prepared solutions were measured using a UV/vis spectrophotometer shimadzu 1700 (LPPT UGM, Yogyakarta, Indonesia) with ~1cm quartz cell. DLS measurement were carried out usingpartcile size analyzer Horiba SZ-100 (UII, Yogyakarta, Indonesia) and the morphology and size of AuNPwere measured by using transmission electron microscope (JEM-1400 JEOL). Purification of gold nanoparticles was prepared by depositing a solution of synthesized gold nanoparticles using vacuum freeze dryer FD-10-MR (UGM, Yogyakarta, Indonesia). The precipitate was washed 5 times by using aquades. The precipitate formed was used for the antibacterial test by dispersing the precipitate of gold nanoparticles in 1 mL aquades.

2.2. Antibacterial Activity Test

The gold nanoparticle with glutamic acid as reducing agent antibacterial activity on*B. Subtillis* and *S. Thypi* was performed by the well diffusion method. The two bacterial used here was grown on MHA (Mueller Hinton Agar) medium and incubated at 35 ° C overnight up to a turbidity of 0.5 Mac Farland standard (10^8 CFU per mL). A modified sterile pasteur pipette with 5 mm of diameter is used to make 4 wells on the agar medium. 2 wells are used for gold nanoparticles without purification ($HAuCl_4$: glutamic acid concentration by 0.5 g/L : 10 g/L and 1 g/L : 20 g/L) andothers well are used for gold nanoparticles with purification (0,4 and 0,8 g/L). About 50 □l of gold nanoparticles suspension was used to inoculate petridish filled with MHA. Well were punched in the agar plates and filled with those nanoparticles solution. After the whole process is complete, all of the samples are put into incubators at 37^oC overnight. The inhibition zone of each sample order is measured using a ruler.

III. RESULTS AND DISCUSSION

3.1. Synthesis of Gold Nanoparticles Using Glutamic Acid

Gold nanoparticles synthesized by glutamic acid as reducing agent were successfully synthesized. Figure 1 shows the UV-vis spectraof AuNP-AG (gold nanoparticles reduced by glutamate acid) to show the effect of pH on the synthesis of gold nanoparticles. The synthesis of gold nanoparticles with glutamic acid as reducing agents was adjusted at pH 6-12. In that figure, it can be seen that there are two groups of spectra of AuNP-AG. the first group is the spectra group for pH 6-9 and the second is the spectra group for pH 10 and 11. In the first group spectra, the AuNP formed purple red color thatindicating the AuNP-AG has a small particles and it was well dispersed in solution. In the second group spectra, the AuNP-AG formed has a purple color that indicating it has more aggregation at that pH.At pH 6-9, the AuNP has a lower absorbance intensity than pH 10 and 11. This is indicate that more gold nanoparticles formed at pH 10 and 11. This may occur due to full deprotonation of glutamic acid above the pKa3 value of 9.67 undergoing in the -COOH group to $-COO^-$, so that glutamic acid can be more active to reduce and capped Au in solution. However, although group 1 spectra have low intensity, the wavelengths of AuNP in group 1 are lower than at pH 10 and 11 which indicate that AuNP formation at pH 6-9 is more uniform and less aggregated because aggregation can increase the wavelength of gold nanoparticle. At pH below pKa3, partially deprotonated of glutamic acid occur so that the $-COO^-$functional group does not function optimally in reducing and capping Au in solution. The more AuNP-AG is formed, the easier it is to aggregate because AuNP will get more collision by other nanoparticles.

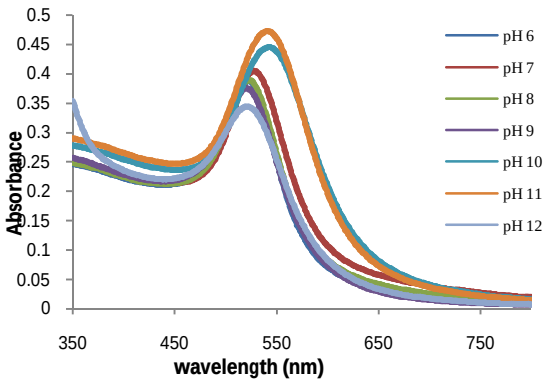


Fig.1. UV-vis spectra of Gold nanoparticle with different pH using 10 mM glutamic acid and 40 mg/L $HAuCl_4$.

The effect of glutamic acid concentration on the formation of gold nanoparticles can be seen in Figures 2 and 3. Due to the presence of 2 spectra groups on the pH effect on the formation of gold nanoparticles, the determination of the concentration effect will be choose one pH from each groups. We selected pH 11 and pH 8 for further observed how the effect of glutamic acid concentration on both pH. Figure 2 illustrates the effect of glutamic acid concentration on pH 11. At pH 11, the maximum concentration of glutamic acid is 5 mM. The figure 2shows that the greater the concentration of glutamic acid used in the AuNP-AG synthesis, the absorbance will increase indicating more AuNP-AG is formed then absorbance decrease after 5 mM glutamic acid concentration. Increased concentrations of glutamic

acid also causered shift indicating more aggregation occur. The color of the solution changes from red wine to purple as the maximum wavelength increases. In contrast, at pH 8, the maximum concentration of glutamic acid is 30 mM. The interesting thing to observe here is the fact that AuNP-AG formation requires less amount of glutamic acid to form more AuNP-AG particlesat the high pH, whereas AuNP-AG formation requires more reducing agentsat lower pH. This further reinforces the hypothesis that the -COO- groups play a dominant role in the formation of gold nanoparticles rather than in the -COOH form.

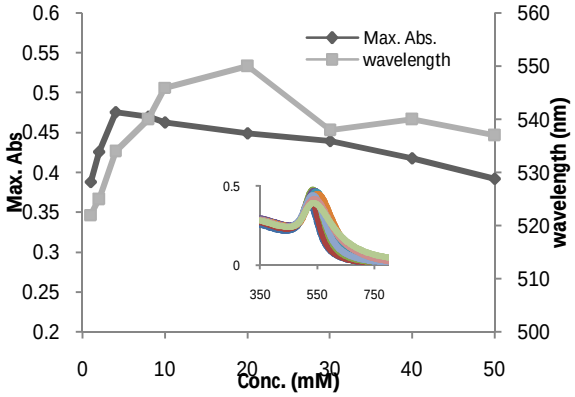


Fig.2. Effect of Glutamic acid concentration on maximum absorbance using 40 mg/L $HAuCl_4$and pH 11.

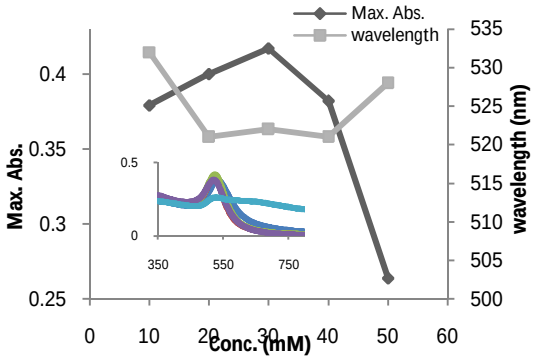
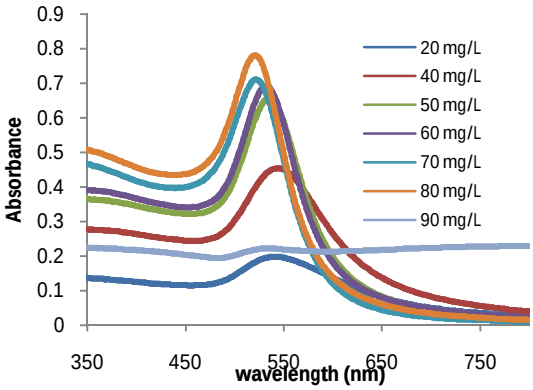


Fig.3. Effect of Glutamic acid concentration on maximum absorbance using 40 mg/L $HAuCl_4$and pH 8.

The effect of gold concentration in the formation of gold nanoparticles can be seen in figure 4. It is seen that the absorbance of AuNP-AG solution increased from the 20 mg/L concentration to the 80 mg/L concentration indicating that AuNP-AG formation increased and then decreased significantly at 90 mg/L concentration of Au. This is because when the Au excess in the solution, the glutamic acid will be difficult to reduce and cap Au as it is have more steric effect by another Au so that not many nanoparticles are formed. Increased Au concentration also decreases the maximum wavelength and narrows the Uv-vis band indicating that the uniformity of the size and shape of the nanoparticles is better at80 mg / L of Au.



Reaction time is one of the parameters that influence the synthesis of gold nanoparticles. The color change from clear to purple starts to be observed when the reaction runs for 15 minutes. The color intensity increase when reaction runs 45 minutes and turns into a purplish red when the reaction reaches the 60 minute. From the Uv-vis spectra data shows that after the reaction lasted for 60 minutes, the spectra of the formed spectra tends to be the same and the intensity of the absorbance is not significantly different. This is because glutamic acid and HAuCl4 have been reacted so that there is no significant difference in UV-vis spectra after the reaction runs 60 minutes.

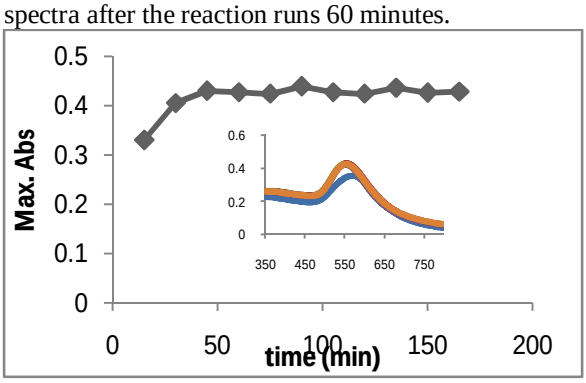


Fig.5. Effect of Reaction time on maximum absorbance using pH 11, glutamic acid 5 mM and $HAuCl_4$ 80 mg/L

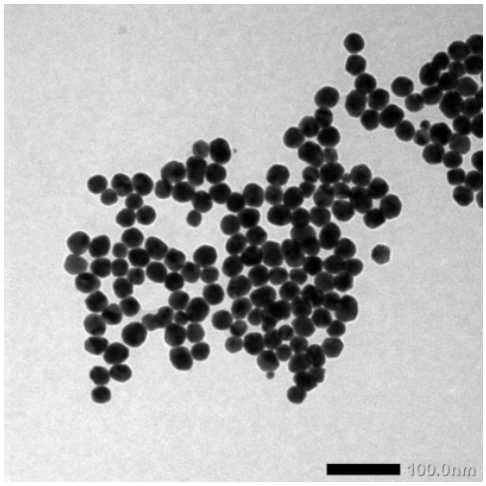


Fig.6.TEM image of AuNP-AG using pH 11, glutamic acid 5 mM and $HAuCl_4$ 80 mg/L, and reaction time 60 min

3.2. Antibacterial Activity Test

The antibacterial activity test of gold nanoparticles with glutamic acid reducing agent was performed with gram-positive bacteria (*B. subtilis*) and gram-negative bacteria (*S.Thypi*). the antibacterial test was performed in 2 ways ie by purification of AuNP-AG first and without purification. The results obtained are shown in Figure 5

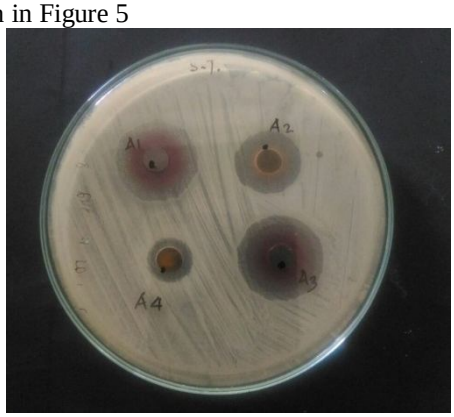


Fig.5. antibacterial activity of AuNP-AG against *Salmonella thypi* bacteria. A1 and A3 was used unpurified AuNP-AG with different concentration. A2 and A4 was used purified AuNP-AG with different concentration

Figure 5 describes the antibacterial activity of AuNP-AG against *S. thypi*bacteria. A1 ($HAuCl_4$ 0.5 g/L: Glutamic Acid 10 g/L) and A3 (HAuCl4 1 g/L: Glutamic Acid 20 g/L) are filled with AuNP-AG without purification. While A2 (0.2 mg/L) and A4 (0.5 mg/L) were filled with AuNP-AG by purification. A1 has a inhibition zone of 18 mm and A3 of 17 mm. while the A2 zone nhibition is 13 mm and A4 is 7 mm. Figure 5 shows that AuNP-AG without purification has a higher antibacterial activity than AuNP-AG by purification. This may be due to the presence of Au^{3+}or Au^{+}ions or residual $HAuCl_4$ which may also play a role in bacterial inhibition. The presence of HCl or HNO_3 in gold dissolution can also play a role in bacterial inhibition. Overall, in this case a considerable AuNP-AG concentration is required so that AuNP -AG may act as an antibacterial well



Fig.6. antibacterial activity of AuNP-AG against *Bacillus Subtillis* bacteria. A1 and A3 was used unpurified AuNP-AG with different concentration. A2 and A4 was used purified AuNP-AG with different concentration

Not much different from antibacterial activity in gram-negative bacteria, AuNP-AG also has antibacterial activity in Gram-positive *B. Subtilis* bacteria. Figure 6 is the result of an antibacterial test for gram-positive bacteria. From the figure 6.AuNP-AG without purification, A1 has an inhibition zone of 18 mm and A3 also of 18 mm. this may indicate that there is no significant effect of AuNP-AG concentration on antibacterial activity. While in AuNP-AG with purification, A2 has 12.5 mm inhibition zone and A4 has 7 mm inhibition zone.AuNP-AG has significant effect on antibacterial activityby purification. Similar to antibacterial activity in gram-negative, antibacterial activity in gram-positive by AuNP-AG without purification has a larger inhibitory zone than AuNP-AG by indicating there are other impurities such as Au^{3+} or Au^{+} ions which may play a role in inhibiting bacterial growth

CONCLUSIONS

Gold nanoparticles was successfully synthesized by glutamic acid as reducing and capping agent and its have an antibacterial activity for gram positif and gram negative bacteria.

1. pH can affect the formation of gold nanoparticles. The use of pH above the pKa3 values of glutamic acid makes glutamic acid in become total deprotonated, so that more gold nanoparticles are formed
2. The use of low pH requires greater concentration of glutamic acid to form more nanoparticles
3. the greater the $HAuCl_4$ concentration used, the more gold nanoparticles are formed and produce lower wavelength and narrow band.
4. AuNP-AG has antibacterial activity against gram-positive bacteria (*B. subtilis*) and gram negative bacteria (*S. thypi).* However, the antibacterial capacity of AuNP-AG decreases if it purified compared to without purification.
5. The antibacterial ability of non-purifying gold nanoparticles does not depend on AuNP-AG concentrations, but with purification having considerable differences.

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REFERENCES

[1] Bastus N.G., Merkoci F., Piella J.,and Puntes, V., "Synthesis of Highly Monodisperse Citrate-Stabilized Silver Nanoparticles up to 200 nm: Kinetic Control and Catalytic Properties" Chemistry Material, vol 26, pp. 2836-2846, 2014.

[2] Shareena Dasari TP, Zhang Y, Yu H,"Antibacterial Activity and Cytotoxicity of Gold (I) and (III) Ions and Gold Nanoparticles" Biochem Pharmacol, vol. 4, 2015

[3] Allaker RP, Vargas-Reus MA, Ren GG,"Nanometals as antimicrobials",John Wiley & Sons, Inc., pp. 327-350, 2012
[4] Nagaraj B, Divya TK, Barasa M, Krishnamurthy NB, Dinesh R, Negrila CC, Predoi D,"Phytosynthesis of gold nanoparticles using caesalpinia pulcherrima (peacock flower) flower extract and evaluation of their antimicrobial activities", J Optoelectron Adv Mater,vol. 15, pp. 299-304, 2013
[5] Mishra A, Tripathy SK, Yun S-I,"Biosynthesis of gold and silver nanoparticles from Candida guilliermondii and their antimicrobial effect against pathogenic bacteria", J Nanosci Nanotechnol, vol. 11(1), pp. 243-248, 2011.
[6] Mubarak A.D., Thajuddin N., JeganathanK., Gunasekaran M., "Plant Extract Mediated Synthesis of Silver and Gold Nanoparticles and Its Antibacterial Activity Against Clinically Isolated Pathogens", Coll Surface,vol. 85(2), pp. 360-365, 2011.
[7] Geethalakshmi R, Sarada DV,"Gold and silver nanoparticles from Trianthema decandra: synthesis, characterization, and antimicrobial properties", Int J Nanomedicine, vol. 7, 5375-5384, 2012.
[8] Liny P, Divya TK, Malakar B, Nagaraj B, Krishnamurthy NB, Dinesh R,"Preparation of gold nanoparticles from Helianthus annuus (sun flower) flowers and evaluation of their antimicrobial activities", Int J Pharma Bio Sci, vol. 3(1), pp. 439-446, 2012.
[9] Nagajyothi PC, Sreekanth TVM, Prasad TNVKV, Lee KD,"Harvesting Au nanoparticles from Carthamus tinctorius flower extract and evaluation of their antimicrobial activity", Adv Sci Lett,vol.5(1), pp. 124-130, 2012
[10] Amin R.M., Mohamed M.B., Ramadan M.A., Verwanger T., Krammer B., "Rapid and Sensitive Microplate Assay for Screening the Effect of Silver and Gold Nanoparticles On Bacteria",Nanomedicine, vol. 4(6), pp. 637-643, 2009.
[11] Hernández-Sierra J.F. Ruiz F., Cruz, PenaD.C., Martínez-Gutiérrez F., Martínez A.E., Guillén A., Tapia-Pérez H., Martínez, Castañón G., "The Antimicrobial Sensitivity of *Streptococcus* Mutans to Nanoparticles of Silver, Zinc Oxide, and Gold", Nanomed Nanotechnol Biol Med, vol. 4(3), pp. 237-240, 2008.
[12] Zhou, Y., Kong, Y., Kundu, S., Cirillo, J.D., Liang H., "Antibacterial Activities of Gold and Silver Nanoparticles Against Escherichia coli and Bacillus Calmette-Guerin",J. Nanobiotechnology, vol. 10, 2012.
[13] Arshi N, Ahmed F, Kumar S, Anwar MS, Lu J, Koo BH, Lee CG,"Microwave assisted synthesis of gold nanoparticles and their antibacterial activity against Escherichia coli", Curr Appl Phy, vol. 11(1), pp. 360-363, 2011.

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