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Optimization of Gold Nanoparticles and Molecular Beacon Probe for DNA Detection by Colorimetric Method

Hari Widada[1,5*] Mar'atus Sholikhah[2] , Sabtanti Harimurti[3], Dyani Primasari Sukamdi[4], Abdul Rohman[6], Riris Istighfari Jenie[7], Sismindari[8]

[1,2,3,4] *School of Pharmacy, Faculty of Medical and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia.*
[5,6,7,8] *Doctoral Program, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia.*
[*]*Corresponding author. Email: hr.widada@umy.ac.id*

ABSTRACT

Gold nanoparticles are platforms that can be immobilized with a probe; thus, they can be used as biosensors. In the health sector, biosensors are utilized for biomedical applications, such as for DNA marking and cell isolation. In addition, it can be used as a method for Halal Authentication applications for meat products. This study aims to determine the optimum conditions for the synthesis of gold nanoparticles as a platform for immobilization and application of AuNPs-MB as biosensors with the colorimetry method for Halal Authentication applications of meat product. This research optimized gold nanoparticles using 6 series of $HAuCl_4$ concentration, namely 0.0635; 0.127; 0.19; 0.254; 0.635; dan 1.27 (mM). The most optimum concentration was immobilized with probe MB and measured using Uv-Vis Spectrophotometer to identify the absorption and Scanning Electron Microscope (SEM) to identify nanoparticles' size distribution. The results showed that 0.19 mM of AuNPs gave the best results, which was stable for 3.5 months and had good results measured using SEM with an average size of 21.7 nm. The value of absorbance, before immobilization, decreased from 0.226 to 0.167 and indicated that the prose was successful. Based on the result of this study, it can be concluded that AuNPs-MB was proven to be a biosensor for Halal Authentication applications using the colorimetric method.

Keywords: *Biosensor, Colorimetry, Gold Nanoparticles, Molecular Beacon*

1. INTRODUCTION

DNA-based detection is essential and required for a wide variety of purposes and applications. Detection for diagnosis and analysis of biological contamination mostly uses DNA as the target. Compared to other biological materials, the relatively stable nature of DNA makes it feasible as a target for detection (1). DNA can also be a specific marker to identify a specific target in each individual. Specific DNA detection can be done qualitatively and quantitatively as DNA can be amplified using the PCR system. With the help of specific primers, DNA with very small levels can be measured by PCR due to the ability to multiply with the help of the polymerase enzyme (2).

Detection of DNA can be increased in sensitivity with the help of a probe (3). The probe can increase the sensitivity as it is naturally designed based on the gene originating from the intended target to identify that target specifically. Several probe types are known, and molecular beacon probes are one of the unique probe types. The peculiarity of the molecular beacon probe lies in its structure, where the two ends complement each other while the middle forms a loop. The two ends are usually modified with a reporter in the form of fluorescent and quencher (4). Both ends of the MB probe can be modified with various conjugates/ groups according to the detection system (5).

The Molecular Beacon probe modification provides the opportunity to be applied to detection platforms, increasing its sensitivity (6). One of the detection platforms that can be used is gold nanoparticles (AuNP). AuNP has unique optical and Physico-chemical characteristics that can be used to immobilize biosensors such as molecular beacon probes. (7), (8). To be immobilized on the surface of the gold nanoparticles, one end of the probe must be modified with a group that can be chemically bonded to the AuNP surface, such as a thiol group (-SH). (6). Immobilization of the probe on the AuNP surface also aims to functionalize it into a biosensor system (9). AuNP that has been functionalized with the MB probe will experience changes in its optical character. These changes in optical characteristics can be explored into detection systems against DNA targets (9).

The optical and Physico-chemical characteristics of AuNP, which have been functionalized with the MB probe, allow detection by the Colorimetric Method (10). Colorimetric detection can be carried out with the UV-Vis Spectrophotometry instrument; thus, it becomes a relatively simple detection system. This system is simpler as it does not require an amplification process such as PCR to amplify the detection signal (11). A simple and efficient system promises to be applied in DNA detection for various purposes, such as disease detection, sources of disease transmission, or even food safety applications and food product authentication (12).

Molecular beacon probes can be developed for various purposes, depending on the genes from which the probe was designed. In this study, the MB probe was designed virtually from the pig cytochrome B gene (Sus scrofa domestica). Furthermore, this probe will be developed into a detection system with a colorimetric AuNP platform to be used in authenticating food products derived from meat.

2. METHODOLOGY

2.1. Material

Glassware used pyrex garde (Iwaki), analytical scales (Metler Toledo Al 204), and Barnstead Thermolyne Cimarec. Heating used Hot Plate Magnetic Stirrer SP131325 7x7. Visualization of nanoparticles used the Hitachi SU 3500 Scanning Electron Microscope (SEM). Meanwhile, the immobilization process of the MB-AuNP probe used the Shaking Incubator FS-50B and the General Incubator LIB-030M. Preparation of buffer solutions and scanning solutions utilized the OMEGA PHH222 pH meter and UV-Vis Spectrophotometry with Jasco V-730.

The synthesis of gold nanoparticles and the immobilization process used the following materials: Tetrachloroauric acid ($HAuCl_4.4H_2O$) from Sigma-Aldrich, Co. LLC., USA, Sodium Citrate ($C_6H5Na_3O_7$), and Water For Injection (WFI). MB (Genetic IDT) probe, Tris-HCl, NaCl (Sigma-Aldrich, Co. LLC., USA), Sodium Hydroxide (NaOH), Dithiothreitol (DTT), acetate buffer (pH 5.2), Tris-acetate buffer (pH 8.2).

2.2. Research Method

2.2.1. Synthesis of Gold Nanoparticles (AuNP)

The gold nanoparticles were synthesized using $HAuCl_4$. A concentration series of 0.0635; 0.127; 0.19; 0.254; 0.635; and 1.27 (mM) were applied in 100 mL aqueous solution, and reduced with 2.5 mL sodium citrate at a concentration of 38.8 mM rapidly into HAuCl4. They were then stirred and heated using a magnetic stirrer with a speed of 270 rpm with a temperature of 150 oC until a color change was formed from pale yellow to red wine.

2.2.2. Immobilization of AuNP with Molecular Beacon Probe (MB)

A suitable environment was prepared by soaking the vial in 12 M NaOH for 1 hour. It was mixed into the vial 36 µL MB probe, 0.5 µL DTT (100 mM), and 6.4 µL of acetate buffer. The mixture was incubated for 1 hour at 25 oC (Solution 1). It was then added with 3000 µL AuNP (Solution 2). The solution was stirred using a Shaking Incubator and then incubated for 24 hours. Furthermore, 175.6 µL of tris acetate buffer and 123 µL of 100 mM of NaCl were added (Solution 3). The mixture was then re-incubated for 52 hours.

3. RESULTS AND DISCUSSION

3.1. Optimization of Gold Nanoparticles (AuNP)

A solution of 100 mL $HAuCl_4$ of various levels, namely 0.0635; 0.127; 0.19; 0.254; 0.635; and 1.27 (mM) was prepared and heated at a temperature of 150 ^oC and rotated at a speed of 270 rpm, to help reduce Au^{3+} metal ions to Au^0 metal ions (uncharged). The use of optimum temperature and speed would produce nanoparticles that were stable for more than 2 months. The addition of 2.5 mL of Na-Citrate 38.8 mM functioned to reduce Au metal ions, which then coated the uncharged Au; thus, nanoparticles of a certain size were formed. This process is described in Equation 1:

$$2HAuCl_{4(l)} + 3C_6H_8O_{7(l)\ (citric\ acid)} \rightarrow 2Au_{(s)} + 3C_5H_6O_{5(l)\ (3\text{- ketoglutaric acid})} + 8HCl_{(aq)} + 3CO_{2(g)} \ldots\ldots\ldots (1)$$

The gold (Au) ion is stable if it has a charge. However, once it is reduced, Au loses charge and is neutral. In a neutral state, the ion will not be stable and will even return to its original form. For this reason, the addition of Na-Citrate also functions as a capping agent that will stabilize and protect the finished nanoparticles from aggregating. In other words, the negative charge of the citrate ion will be absorbed by the surface of the gold nanoparticles so that the gold nanoparticles will repel due to the negative environment and prevent the aggregation of gold nanoparticles.

The formation of gold nanoparticles can be seen from the resulting color change (Figure 1). When the Au ion had not been reduced initially, the solution was yellow. Furthermore, the colors began to form, ranging from ash, purple to burgundy, according to the $HAuCl_4$ concentration. The color change stage showed the size of the resulting particles until the nanosize was obtained (1-100 nm). When the burgundy color was formed, it indicated that the process has ended and must be stopped immediately as the continuous heating process would cause the nanoparticles' size to become larger, causing aggregation, and vice versa.

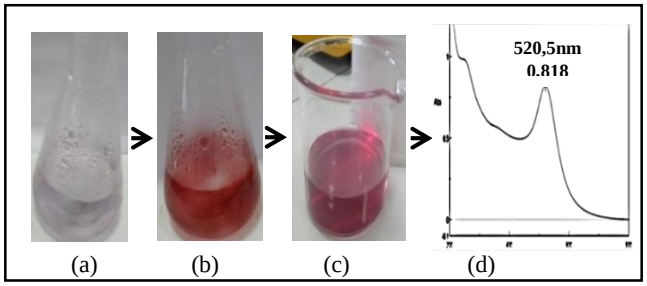

Figure 1 Synthesis of AuNP Illustration: (a) Main solution $HAuCl_4$ (b); Color change after addition of Na-citrate (c); Laser light penetrates, indicating there are nanoparticles; (d) Spectra measurement results *with a Uv-Vis Spectrophotometer.*

Table 1 Synthesis Product of Gold Nanoparticle (AuNP)

No.	Concentration (mM)	Color of the solution	Wave length (nm	Absorbanc (abs)
1.	1.27	Dark cloudy chocolate (like chocolate milk), 2 hours later clear	-	-
2.	0.635	Dark Purple	526	1.525
3.	0.254	Clear Red	520.5	0.818
4.	0.19(1)	Clear purplish red	521	0.542
5.	0.19 (2)	Clear Red	521.5	0.503
6.	0.19 (3)	Clear Red	523.5	0.229
7.	0.127(1)	Clear Bright Red	521	0.486
8.	0.127 (2)	Purple red	535	0.261
9.	0.127 (3)	Deep purple, clear	537	0.069

10.	0.0635	Light purple	529	0.037

At a concentration of 0.0635 mM, a very faint purple color was formed. It indicated that the solution was very dilute so that the number of nanoparticles formed was very small. At a concentration of 1.27 mM, the colloid suddenly turned brown (like muddy soil). However, after leaving for about 2 hours, the colloids turned clear, and there was aggregation. Some of the factors that could influence were the presence of citrate as a reducing agent, which was not enough to coat Au0; thus, Au in a neutral state tended to be unstable and caused attractive bonds between similar particles, which then formed larger particles (formed deposits). Of the six series of levels used, the concentration of 0.19 mM showed the optimum result, from the test results using the Uv-Vis spectrophotometer, and the stability of storage that lasted 3.5 months at 2 ºC (Table 1).

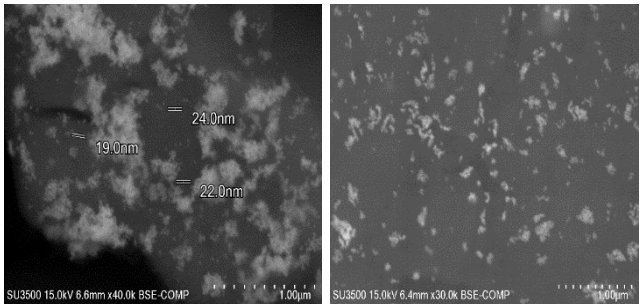

Figure 2 SEM results of 0.19 mM concentration of AuNP

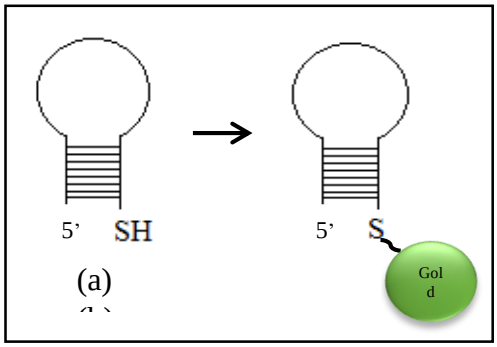

Figure 3 (a) MB Probe modified into Thiolated Oligonucleutida Probe (b) Immobilization of MB Probe with AuNP.

The size range of the resulting gold nanoparticles was 19-24 nm. According to research conducted by Ali et al. (2011) (13), the optimum size of nanoparticles to use was 20 nm. Based on these results, a concentration of 0.19 mM could provide a good color change and absorption spectra.

3.2. Immobilization of AuNP with Molecular Beacon (MB) Probe

Immobilization of DNA-based probes can be carried out if one end is modified with the addition of a thiol group or has been thiolated (Figure 3). The thiolized probe can be functionalized on the AuNP surface to produce a surface plasmon resonance that is useful for signal transduction systems. The immobilization process of gold nanoparticles with the MB probe must be carried out in an appropriate environment as several things such as pH, temperature, and incubation time, as well as the strength of the chemical bonds between bonds, were critical things that would affect the success of the immobilization process (14). Prior to being used, reparation of tools as a medium for immobilization must first be soaked (soak) with alkaline solvent (NaOH). Soaking the vial with NaOH was intended to remove contamination attached to the vial and prevent nanoparticles from sticking to the vial surface, especially after adding NaCl. Nanoparticles attached to the vial would affect the concentration to interfere with the results of the immobilization carried out. (15). The immobilization process started by mixing the MB probe with DTT and acetate buffer. Dithiothreitol (DTT) is a reducing agent in solution samples often needed to maintain activity and stability. In this study, DTT was used to reduce the SH group in the MB probe and then immobilized with gold nanoparticles. It is said that the temperature that can be used in the immobilization process is between room temperature (25 ºC) to boiling water temperature (100 ºC), pH in the range 7-9, and was incubated for 30 minutes until all night. (16).

NaCl's presence in the reaction provides an ionic bonding effect that could increase and accelerate immobilization between the MB probe and gold nanoparticles (14). Another source explained that the immobilization process would run well under appropriate pH conditions, salt, and buffer concentrations. The test results using the Uv-Vis spectrophotometer showed a decrease in the absorbance value between pre and post immobilization, respectively, namely, 0.226 and 0.167 (Table 2). According to Firmansyah (2012) (17), immobilization was successful when there was a decrease in the absorbance value between pre and post immobilization. In measurements using SEM, the average size of the nanoparticles before immobilization was 21.7 nm. Furthermore, after immobilization, the size increased to 27.3 nm (Figure 2). It indicated that gold nanoparticles successfully bounded the MB probe.

Table 2 Comparison of test results between Uv-Vis spectrophotometer and intermediate SEM at pre and post immobilization

Method of Analysis	AuNP	AuNP-MB
Spectrophotometer Uv-Vis	Wavelength: 524 nm Absorbance: 0.226	Wavelength: 524.5 nm Absorbance: 0.167
SEM	Average of size: 21.7 nm Distribution: Clustered	Average of size: 27.3 nm Distribution: Clustered

4. CONCLUSION

The optimum conditions for the synthesis of nanoparticles used a temperature of 150° C and a speed of 270 rpm, which produced stable colloids within 3.5 months. Besides that, it gave good SEM results, with an average size of 21.7 nm. A decrease in the absorbance value between pre and post immobilization from 0.226 to 0.167 indicated that the immobilization process was successful.

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