Study of Etching Behavior of Gold Nanorods as Model for Glucose 6-phosphate Dehydrogenase (G6PD) Detection

Natthawoot Liwatthananukul

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การศึกษาพฤติกรรมการกร่อนของอนุภาคนาโนทองแท่งเพื่อเป็นต้นแบบสำหรับการตรวจเอนไซม์ Glucose 6-phosphate Dehydrogenase (G6PD)

ณัฐวุฒิ หลีวัฒนานุกูล

ปริญญานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรวิศวกรรมศาสตร์บัณฑิต

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สถาบันเทคโนโลยีพระจอมเหล้าเจ้าคุณทหารลาดกระบัง

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Title	**Study the Etching Behavior of Gold Nanorods as Model for Glucose 6-phosphate Dehydrogenase (G6PD) Detection**
By	**Natthawoot Liwatthananukul**
Field of Study	**Petrochemical Engineering**
Advisor	**Asst. Prof. Dr. Teeraporn Suteewong**

Accepted by the school of Engineering, King Monkut's Institute of Technology Ladkrabang in Partial Fulfillment of the Requirements for the Degree of Bachelor of Engineering (Petrochemical Engineering).

Thesis Committee

TEERAPORN

_____ **Chairman**

(Asst. Prof. Dr. Teeraporn Suteewong)

Duangkamol

_____ **Committee**

(Assoc. Prof. Dr. Duangkamol Na-Ranong)

T. Samanmulya

_____ _____ **Committee**

(Asst. Prof. Dr. Thachanan Samanmulya)

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Abstract

Glucose 6-Phosphate Dehydrogenase (G6PD) deficiency is x- linked chromosome disease. It is found approximately 12% male and 2% female suffer from G6PD deficiency disease in Thailand and has linked to the malaria-risked area. Thus, it is necessary to develop the simple and portable G6PD diagnostic kit for rural area. In this project gold nanorods (GNRs) were synthesized as a model for colorimetric detection of G6PD dur to their broad plasmon color. Their color depends on the aspect ratio. Thus they can be used to detect hydrogen peroxide (H_2O_2), an oxidizing agent found in G6PD patient, because oxidizing agent can etch GNRs. GNRs having aspect ratio from 2.4 to 2.6 were prepared through the reduction of Au(III) using cetyl trimethyl ammonium bromide as stabilizer/shape director and $NaBH_4$ as reducing agent. Then, etching of GNRs was performed. Using H_2O_2 as oxidizing agent. It was found that the presence of halide ions increased the etching rate. Among those, iodide ion has strongest effect on etching behavior within 1 min.

Keywords: G6PD deficiency, detection, gold nanorods, plasmonics, optical phenomenon

เรื่อง การศึกษาพฤติกรรมการกร่อนของอนุภาคนาโนทองแท่งเพื่อเป็นต้นแบบสำหรับการตรวจเอนไซม์ Glucose 6-phosphate Dehydrogenase

โดย ณัฐวุฒิ หลีวัฒนานุกูล

อาจารย์ที่ปรึกษา ผศ.ดร. ธีรพร สุธีวงศ์

สาขาวิชา วิศวกรรมปิโตรเคมี

สังกัด ภาควิชาวิศกรรมเคมี คณะวิศวกรรมศาสตร์

บทคัดย่อ

โรคพร่องเอนไซม์ Glucose 6-phosphate dehydrogenase (G6PD) เป็นโรคที่เกิดมาจากความผิดปกจิทางพันธุกรรมที่อยู่บนโครโมโซม x ในประเทศไทยมี 12% ในผู้ชายและ 2 % ในผู้หญิงที่เป็นโรคพร่องเอนไซม์ G6PD และมีภูมิประเทศที่ตั้งอยู่บนพื้นที่เสี่ยงต่อโรคไวรัสมาลาเรีย ดังนั้นการพัฒนาชุดเครื่องมือที่ใช้ง่ายและพกพาสะดวกจึงมีความจำเป็นเพื่อใช้ในพื้นที่ห่างไกล โดยโปรเจคนี้อนุภาคนาโนทองแท่งได้สังเคราะห์ขึ้นมาเพื่อเป็นต้นแบบสำหรับการตรวจ G6PD โดยใช้สีเนื่องจากมีช่วงสีของ plasmon ที่กว้าง โดยสีของ plasmon ที่ปรากฏออกมาจะขึ้นอยู่กับสัดส่วนระหว่างความยาวต่อความกว้างของอนุภาค ดังนั้นอนุภาคนาโนทองแท่งเหล่านี้สามาถใช้เพื่อตรวจสอบการมีอยู่ของ H_2O_2 ในผู้ป่วยที่เป็นโรคพร่องเอนไซม์ G6PD ที่ทำหน้าที่เป็น oxidizing agent เพื่อทำให้อนุภาคนาโนทองแท่งสั้นลงและส่งผลต่อสีที่เปลี่ยนไปในสารละลาย โดยอนุภาคนาโนทองแท่งที่มีค่า aspect ratio ระหว่าง 2.4-2.6 ได้ถูกสังเคาระห์โดยทำการรีดิวซ์ Au(III) ในอยู่ใน cetyl trimethyl ammonium bromide ที่ทำหน้าที่เป็นตัวกลางที่ทำให้สารละลายมีความเสถียรและเป็นโครงต้นแบบในการเกิดรูปร่างของอนุภาคนาโนทองอแท่ง การกร่อนของอนุภาคนาโนทองแท่งทำได้โดยใช้ H_2O_2 จากการศึกษาค้นพบว่า halide ion สามารถเพิ่มความเร็วการกร่อนของอนุภาคนาโนทองแท่งนี้ได้ โดยมี iodide ion ที่สามารถกร่อนอนุภาคนาโนทองแท่งภายใน 1 นาที

คำสำคัญ: G6PD deficiency, detection, gold nanorods, plasmonics, optical phenomenon

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Natthawoot Liwatthananukul

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CHAPTER I
INTRODUCTION

1.1. Background

Glucose-6-Phosephate dehydrogenase is inherited as an x-linked recessive that means male have more chance to be G6PD deficiency than female. G6PD affects approximately 500 million people worldwide, In Thailand there were found 12% of male and 2% of female. Moreover, there are more than 200 variances of G6PD deficiency genotype. The deficiency of G6PD leads to abnormal protein structure effects to the cell impatient with oxidative stress, Thus the symptom will not act up unless the oxidative stress in cell is activated by external stimuli such as naphthalene, fava bean, infection (fever, hepatitis virus or dengue virus) and antibiotics (sulfa aspirin, 8-aminoquinolin). These chemicals arise the formation of reactive oxygen and cause hemolysis of red blood cell, which may lead to renal failure. Furthermore, hemolysis of red blood cell cause anemia due to lack to blood's pigment, i.e., hemoglobin, that is necessary for oxygen carrier to the whole body. Thus, the effective G6PD deficiency diagnosis is important especially in Thailand where malaria is frequently found. Diagnosis of G6PD level before taking malaria remedy would lower the risk of acute hemolysis.

In the present, there are several G6PD deficiency diagnosis methods such as fluorescence spot test, analysis of enzyme activity, reduction of methhemoglobin, cytochemical assay, WST-8 Formazan, formazan DEAE Sephacel. Binaxnow and Carestart. None of them are suitable for the field work or point-of-care. Some are difficult and rely on the laboratory equipment. Some are not accuracy and can only be used in a limited range of temperature (18-25 ºC). The other main problem leading to false detection is the large mutation of G6PD deficiency that are specifically found in southeast Asia, especially in Thailand.

The tunable optoelectronic is one of the most interesting properties of noble metal based on size, size distribution, shape, charge, and surface chemistry of metal nanoparticle in the solution controlled by various method[1] affecting to the solution color occurring in the solution. similar color of gold nanoparticle solution depends on the particle shape, which cause unique phenomenon called surface plasmon resonance. The plasmonics based sensor is applied as analytical device for wide range of applications[2]. For example, the reversible aggregation and segregation of gold nanospherical in N,N´-dimethylaminopyridine (DMAP) / Polyvinulpyrrolidone (PVP) as stabilizer and tetrachloroaurate ion ($AuCl_4^-$) solution is used for colorimetric sensitive detection of G6PD deficiency[1].

The principle for G6PD deficiency diagnosis is the investigation of existence of Nicotinamide adenine dinucleotide phosphate (NADPH) which act as electron carrier and produced by enzyme G6PD in red blood cell. Detection using gold nanospherical is based on the aggregation and segregation of Gold nanospherical in DMAP/PVP stabilized with $AuCl_4^-$ as positive charged neutralize and suppress the steric stabilization of PVP molecule resulting in aggregation of Gold nanospherical. In the presence of NADPH, Au^{3+} from $AuCl_4^-$ is reduced to Au^+ in the solution resulting in positive charge regeneration inside the aggregated gold nanospherical, then the electrostatic force of positive push each other's cause segregation of gold nanospherical. Generating a shift in surface plasmon absorbance in which a visible color changing can be observed, a visible color change from bluish to red was demonstrated.

However, using gold nanospherical is able to investigate the existence of NADPH in solution but the final color was similar to human blood and auto aggregation of the particle may occur[3]. This could make it difficult to distinguish between blood color and gold color of nanospherical solution might lead to lack of accuracy when examine with human blood. Adaptation of gold nanoparticle with anisotropic property for investigating G6PD deficiency is an interesting topic. The gold nanorods (GNRs) have various color depending on aspect ratio (the ratio of length and width) of the particles and the aspect ratio of GNRs is able to adjust by oxidation-reduction reaction. Instead of using aggregation based detection of gold nanospherical, this work intend to use non-aggregated based detection by adjusting aspect ratio of monodisperse GNRs via oxidation-reduction, affecting to the change of particle morphology to anticipated aspect ratio (approximately 2.4-2.6). This can be detected by naked eye and by damping/shift plasmon band causing the evolution of size, shape and dielectric environment[3]. The synthesized GNRs particle with satisfied particle size and color will be used as model for color transformation induced by hydrogen peroxide (H_2O_2) which can be found in G6PD patient.

In this work, we focused on syntheses of GNRs with desired shape by approaching thermodynamics (e.g. surface capping, facets side and shape selectivity of GNRs crystalline) and kinetics (concentration, temperature and the supplementary chemical species)[4]. Investigation of G6PD deficiency was simulated using H_2O_2 by the existence of H_2O_2 in a sample as oxidizing agent to etch GNRs.

1.2. Objectives

1.2.1. To synthesize GNRs with different aspect ratios and stabilizer.

1.2.2. To study the etching behavior of GNRs from 1.2.1. using H_2O_2 as oxidizing agent.

1.2.3. To study the effect of halide ions on etching behavior of GNRs.

1.3. Scopes of Work

1.3.1. Syntheses of GNRs with different aspect ratios by varying concentration of gold precursor, silver nitrate, additive (sodium salicylate) and temperature.

1.3.2. Characterization of GNRs in prepare in 1.3.1

1.3.3. Study of the effect of H_2O_2 on etching behavior of GNRs.

1.3.4. Study effect of halide ions on etching behavior of GNRs.

1.4. Expected Outputs

1.4.1. The suitable size and aspect ratio of GNRs solution along with the expected color.

1.4.2. The solution of synthesized GNRs is able to response with H_2O_2 leading to change the color of solution.

CHAPTER II
LITERATURE REVIEW

2.1. Glucose-6-Phosphate Dehydrogenase deficiency

Glucose- 6- Phosphate Dehydrogenase Deficiency (G6PD Deficienc) is genetic disorders which the gene located on X-linked recessive chromosome; it is more commonly happen in male than female. In particular patient's symptom does not occur unless it is aroused by oxidative stress, the severity depends on the group (variances) of the disorder**.** It mainly affects older red blood cell[5]. The Figure 2.1 demonstrates the phenomenon that occur in red blood cell, the patient who is G6PD deficiency unable to produce NADPH and reduced glutathione as reducing agent for defending the cell from oxidative stress caused by reactive oxidant (i**.** e**.** H_2O_2). Therefore, NADPH production by G6PD is critically important for the proper functioning of the RBC antioxidant system[6].

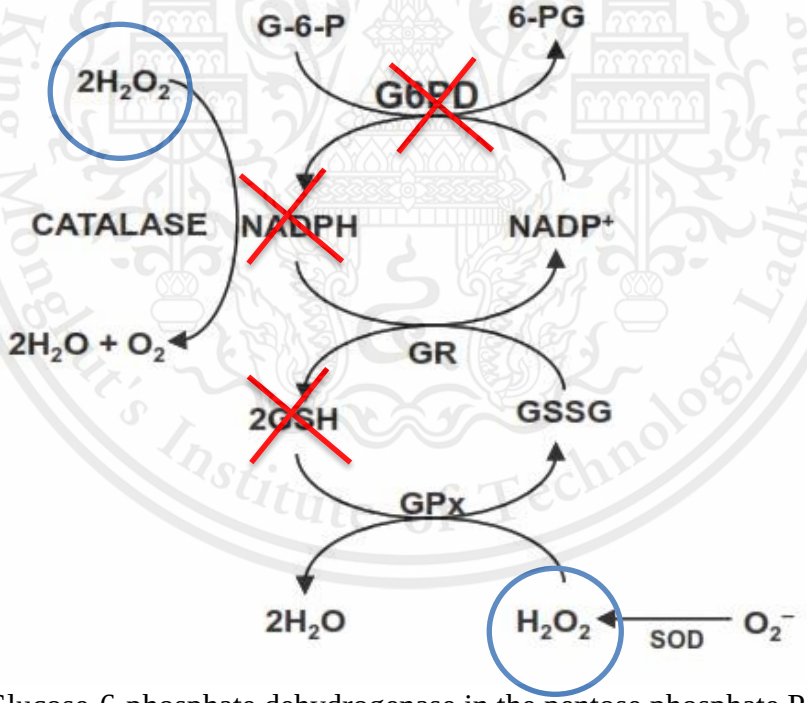

Figure 2.1: Glucose-6-phosphate dehydrogenase in the pentose phosphate Pathway[6].

2.2. G6PD Diagnostics Kit

In chapter 1 explained how severity of hemolysis of red blood cell when faces with oxidative stress occurred in G6PD deficiency patient**.** Defending those serious situations, the development of G6PD diagnostics kit has been developed for many years**.** The one of the challenging task is an evaluation of diagnostics method with radical treatment of *Plasmodium vivax* (Malaria disease) with radical generator medicine as primaquine (8-aminoquinoline compounds)[7]. Especially in countryside area where relatively high chance to face with malaria, accessing to G6PD testing might be expanded. There are a quite large number of the commercial rapid G6PD diagnostic kits have been released in the market as Carestart™ and Binax now®.

2.2.1. BinaxNOW® G6PD Test

Binaxnow® test is a qualitative enzyme chromatographic test (ECT) kit consist of 3 things shown in Figure 2.1. Test devices: A card board, book-shaped, and hinged test device containing the test strip, 2. Reagent A: Tris buffer containing detergent and red dye and 3. Sample preparation vials: Vials used to mix reagent A with whole blood samples prior to transfers to the test devices[8]. The BinaxNOWG6PD Test is a simple, rapid test for the detection of G6PD enzyme activity using whole blood collected by venous draw, test takes less than 10 minutes per sample to complete, does not require the use of special equipment.

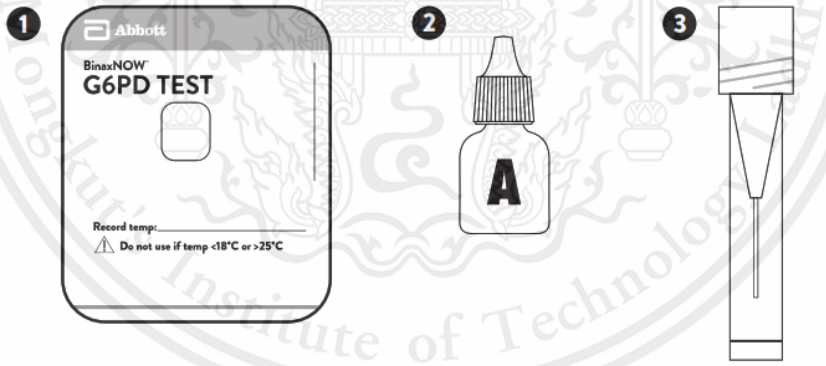

Figure 2.2: BinaxNOW® G6PD test composed of 1. Test devices, 2. Tris buffer containing detergent and red dye, and 3. sample preparation vials[7].

The BinaxNow® G6PD test must be operated at the temperature between 18-25 C is recommended**.** The procedure for G6PD deficiency test, the whole blood mixed with lysing reagent in a sample preparation vial after that prepared sample is drawn in to the test device sample pad**.** The lysed blood sample move up the test strip, drawn again reagents in the reaction pad and the device is closed**.** The results are able to read visually, If no change in color of the sample it is presumed to be G6PD deficient**.** If the color of the sample change

from red sample to brown/black color on the upper half of the reaction pad[8]**.** The example of the results is demonstrated in the Figure 2.3**.**

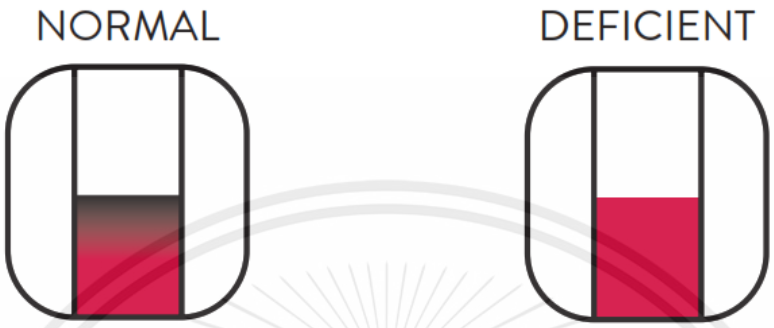

Figure 2.3: The BinaxNow® G6PD test sample results: Normal G6PD enzyme activity (Left) sample the black/brown in the top half of the pad. Deficient G6PD enzyme activity (Right) sample the color is unchanged[7].

The refrigerated blood must be allowed to be at the appropriate testing condition (18-25 ºC) and mix well prior to testing.

2.2.2. Carestart™ G6PD deficiency Test

Carestart G6PD deficiency test is a qualitative rapid G6PD deficiency diagnostic kit contains: strips in test device contained G6PD assay mixture **(** based on formazan method using tetrazolium compound**)**[9], assay buffer, and sample pipette**.** The procedure for using this kit is divided in to 2 main parts as follow:

2.2.2.1. Blood Collection

1**)** Clean the area to be lanced with alcohol swab.

2**)** Squeeze the end of fingertip with sterile lancet.

3**)** Wipe away the first drop of drop of blood with sterile gauze or cotton.

4**)** Take a sample pipette provided, while gently squeezing the tube, immerse the open end in the blood drop and then gently release the pressure to draw blood into the sample pipette up to black line as Figure 2.4.

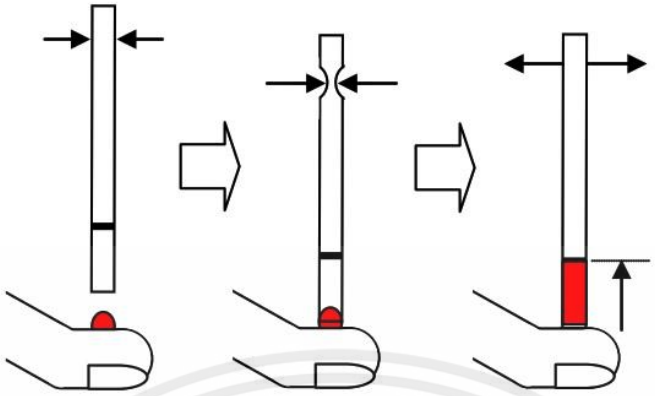

Figure 2.4: The schematic illustrate blood collection procedure from left to right[8].

2.2.2.2. G6PD Test Procedure

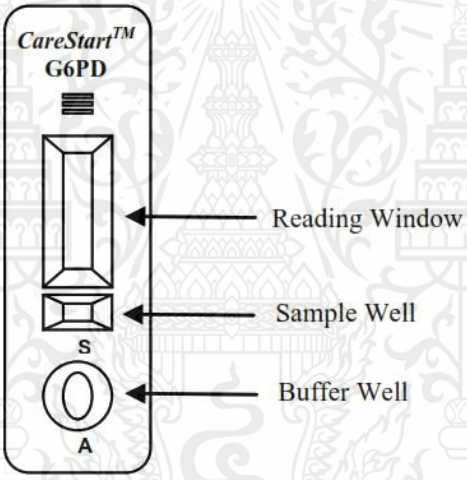

Figure 2.5: Carestart™ G6PD deficiency test device[8].

1) Add 2 µL of whole blood into sample well (small well) by squeezing sample pipette.
2) Immediately add 2 drops (60 µL) of assay buffer into Buffer Well**.**
3) Read the test result in 5-10 min (Caution: Do not read test results after 10 min)

2.2.2.3. Interpretation of the test

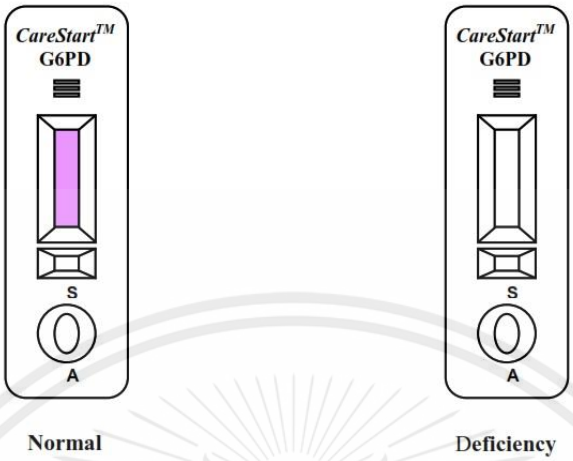

Figure 2.6: The interpretation of the G6PD test[9].

Normal: A distinct purple color appears in reading window within 10 min.

Deficiency: No purple color appears in reading window within 10 min.

2.3. Gold Nanorods

Gold nanoparticles have been researched for a century because of their wide range of application such as electronics[10], photodynamics therapy[11], theraeutic agent therapy[12], sensors[13], probe[14], diagnostics[15], catalysis[16]. Due to the tunable optical and electronics properties having its own specific properties for the specifics tasks by changing gold nanoparticle size and shape. In this report is focusing on GNRs synthesis used as a diagnostic prototype for G6PD deficiency by using its optical phenomenon known as surface plasmon resonance for indicating the existence of NADPH in human red blood cell.

Gold nanopairticles is noble metal-based localized surface plasmon resonance (LSPR) which the characteristics of LSPR strongly depend on size, shape and metallic properties[2]. Especially in gold nanorod have a unique property with a tunable longitudinal plasmon band by adjusting its aspect ratio.

2.3.1. The principle of Localized Surface Plasmon

Surface plasmon resonance is an optical phenomenon occurring at the surface of metal nanoparticles when the light strike on a surface the electron at the surface of the particle is conducted to oscillate in a resonance frequency of light. The influence of electric field of light facing with free electron on a surface of metal nanoparticle resulting in Coulomb repulsive force make the free electron move in the opposite direction**.** This phenomenon is call localized surface plasmon resonance. The different size and shape of surface plasmon lead to various demonstrations of LSPR as shown in Figure 2.7 and Figure 2.8.

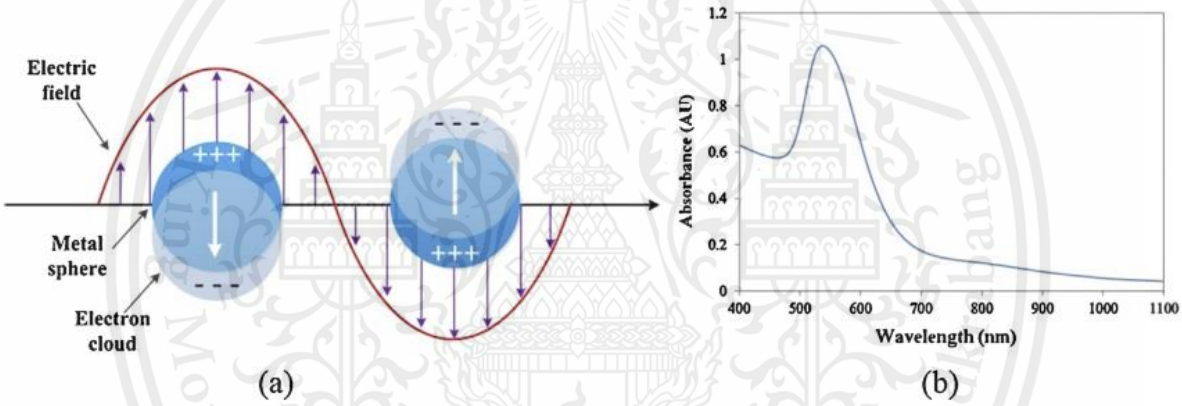

Figure 2.7: (a) Schematic illustration of LSPR excitation for GNSs. (b) A typical LSPR absorption band of GNSs[2].

The illustration of excitation of LSPR for gold nanosherical (GNSs) show only one band in absorbance spectrum compared with gold nanorods (GNRs) there are 2 absorption bands composed of transverse plasmon band (TPB) and longitudinal plasmon band (LPB) in absorbance spectrum shown in Figure 2.7. This is clearly observed the electron oscillation at the length side and width side of GNRs. It is emphasized that LSPR is sensitive to size, shape and dielectric properties of environment[2].

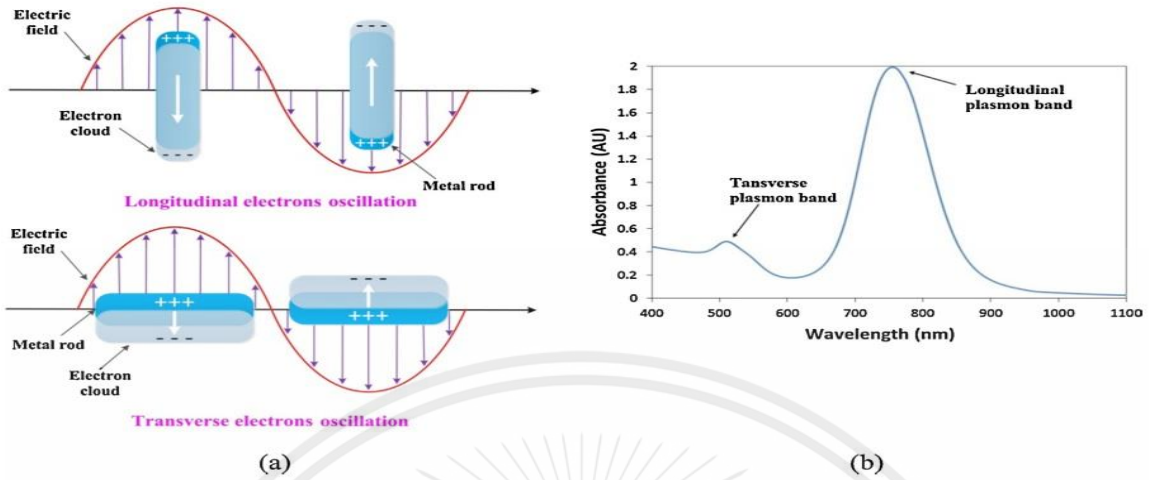

Figure 2.8: (a) Schematic illustration of LSPR excitation for GNRs and (b) LSPR absorption bands of GNRs[2].

longitudinal and transverse plasmon bands corresponding to the electron oscillation along the long axis (Figure 2.8(a) top) and the short axis (Figure 2.8(a) bottom) of GNR respectively.

Based on Gan's theory describe the behavior of GNRs surface plasmon would split into two distinct mode because of surface curvature and geometry of the metal nanoparticles[17]. Gan's theory is able to use for explaining the optical behavior of GNRs according to Gan's formula.

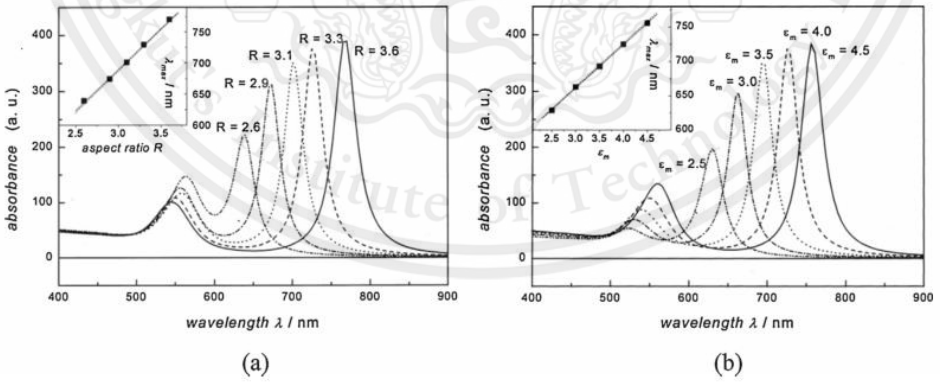

Figure 2.9: (a) Simulated LSPR absorption spectra of GNRs with various aspect ratios in the medium with a constant dielectric constant (b) simulated LSPR absorption spectra of GNRs with a constant aspect ratio in the medium with varying dielectric constant[17].

El-sayed et al. simulated the absorption spectra of gold nanorod in various of dielectric constant but the same aspect ratio. In Figure 2.9(a) It was observed that TPB is insensitive to size, shape and aspects ratio of GNRs, however there is only a little blue shift (the peak move to left hand side) occurred in contrast with LPB is very sensitive to the change of aspect ratio a large red shift is observed for LPB. The linear relationship between aspect ratio and peak wavelength of LPB is demonstrated in the upper left corner of Figure 2.9(a). For the Figure 2.9(b) demonstrate a behavior of wavelength peak and vary dielectric constant observed that not only size, shape and aspect ratio effect to LSPR but also dielectric constant of the surrounding medium. The difference behavior of wave length peak from varying is both of TPB and LPB have a red shift as higher dielectric constant.

2.3.2. Synthesis of Gold Nanorods

Gold nanosperical is the initial development of gold nanoparticle synthesis, the general method for creating gold nanospherical is citrate reduction by boiling the gold precursor and citrate. To regulate the size of the gold nanospherical by adjusting the ratio between citrate and gold precursor concentration. However, the unique characteristics of GNRs (e.g., able to produce various of optical color in the solution) becomes more interesting in research area. Thus, there are many of researchers intend to create the synthesis protocol of GNRs for the beneficial application in wide range area of work. In this report the sunthesis method is divide into 4 mains method are seed-mediated method, photochemical and electrohemical method.

2.3.2.1. Seed-mediated Method

The seed-mediated method is the most frequently used due to the ease of intrument preparation, simplicity of the protocol, flexibility in structural modification, able to follow what is the most significant parameters and etc. This method is divided into 2 steps preparation are seed solution and growth solution. In 2000s Nikookbath and El-sayed [18]create the method which is the prototype for developing GNRs synthesis. They used Cetyl trimethylammonium bromide (CTAB) as stabilizer and surfactant mixed with gold precursor ($HAuCl_4$) in both of seed solution and growth solution. Controlled the aspect ratio of GNRs by adjusting the amount of silver nitrate $AgNO_3$ or ascorbic acid in growth solution. The seed solution is prepared by reducing gold ion by $NaBH_4$ creating nucleation of gold. Finally added seed solution into growth solution for growing rods and left it for 16-20 hr.

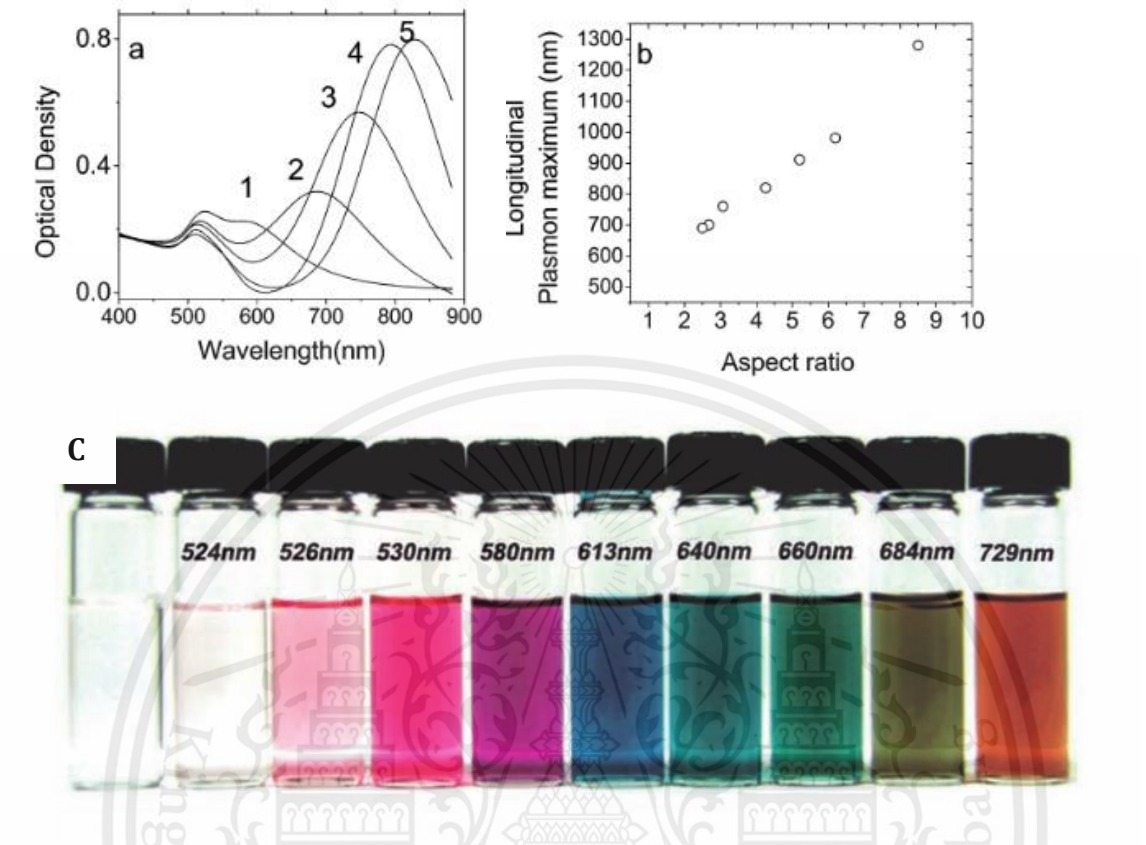

Figure 2.10: (a) Visible spectra of 5 identical growth solutions in which the silver content increases from sample no. 1 to 5. By controlling the Ag ion concentration, the length of the NRs can be adjusted. (b) The aspect ratios of NRs obtained from size measurements show an increase with longitudinal plasmon maximum[17]. (c) GNRs solution and their variuos highest absorbance peak result in color solution appearance[19].

Due to simplicity of GNRs preparation that directly adding sodium borohydride to growth solution instead of separately forming seed solution and growth solution[19], one-pot synthesis or generally called "seedless" method of GNRs synthesis is an attractive method that use lesser time to form GNRs compared with seed mediated method. Min Gu et al.[20] studied the quantity of silver nitrate and temperature effect to kinetics formation and morphology of GNRs they found that both of parameter effect the final morphology of synthesized GNRs. However the beneficial of ultrafast high-temperature is able present a better system for fast production of GNRs[20] but even quality of freshly prepared borohydride can be fluctuate and lack of visible seed during the procedure, reproducibility of seedless method could be fairly low[19].

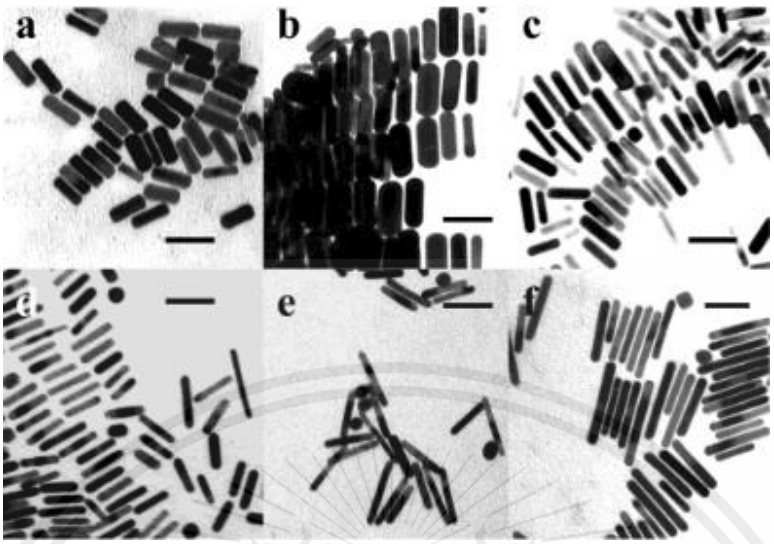

Figure 2.11: TEM images of GNRs with plasmon band energies at (a) 700, (b) 760, (c) 790, (d) 880, (e) 1130, and (f)1250 nm. The scale bar is 50 nm[18].

2.3.2.2. Other Methods

Photochemical method, Peidong Yang et al.[21] synthesized GNRs with regulate aspect ratio by photochemistry. A brief procedure, they used a mixed of CTAB, gold precursor, cyclohexane and silver nitrate ($AgNO_3$). Then the mixed solution is irradiated with a 254-nm UV light (420 micoW/cm^2) for 30 hr. The purified by centrifugation and redisperse with deionized water. In this protocol demonstrates the effects of UV-light, silver nitrate used to create GNRs and show an optical color of GNRs solution as a Figure 12. It is seemed less complicated than the seed-mediated methods but this method operate in very small bath and hard to scale up for more amount of the solution.

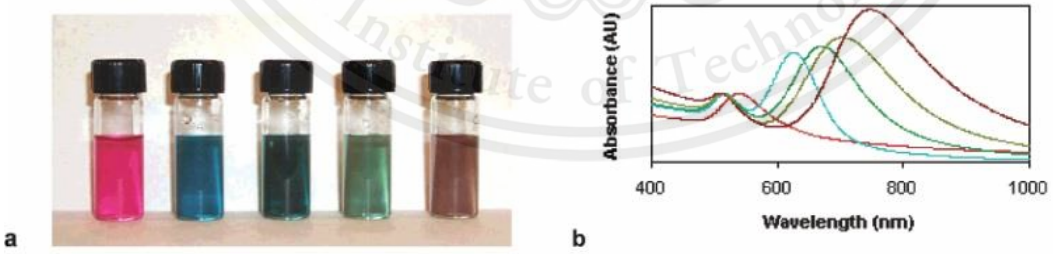

Figure 2.12: (a) Image of photochemically prepared GNRs solution,and (b) corresponding UV-vis spectrum. The leftmost solution was prepared with no silver ion addition. The other solutions were prepared with addition of 15.8, 31.5, 23.7, 31.5 µL of silver nitrate[21].

Electrochemical method, In 1990s, Wang and co- workers create a unique synthesis methods for preparing high yield of GNRs stabilized by cation surfactant[22], Using electrochemical technique to grow GNRs in the solution by inventing the simple two-electrode-type (Anode: gold and Cathode: platinum) electrochemical cell as Figure 2.13; both of electrode are immersed in to electrolytic aqueous solution (CTAB) which also act as surfactant and stabilizer. The solution is composed of acetone, cyclohexane, and silver plate. In the glass of electrolytic cell install ultrasonic cleaner for sonicating down while electrolysis[22]. They found that the amount of silver ions helps to speed up the releasing of GNRs as the more totally area of silver plate that immersed the longer GNRs obtained at the end. The forming of GNRs in electrolysis and the mechanism of silver assisted is still unknown.

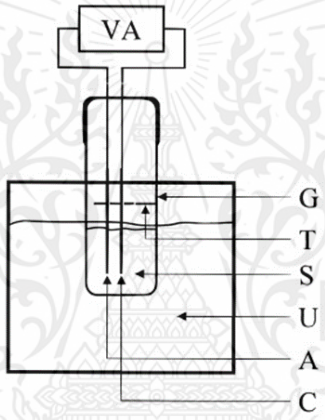

Figure 2.13: Schematic diagram of the setup for preparation of Au nanorods. The electrochemical system contains the following: VA, power supply; G, glassware electrochemical cell; T,Teflon spacer and the electrode holder; S, electrolytic solution;U, ultrasonic cleaner[22].

2.3.3. Practical Techniques for Gold Nanorods Synthesis

From the mentioned protocol above the possible way to synthesize GNRs in this project is seed-mediated method. The specification of GNRs for this work are tunable size of GNRs by oxidation-reduction reaction, monodisperse of GNRs particle, large scale synthesis of GNRs available, and reproducibility. To obtain the optimized GNRs, the synthesis requires thermodynamics and kinetics control which significantly increase the number of parameters that might be taken into account[4]. As describe in the Figure 2.14.

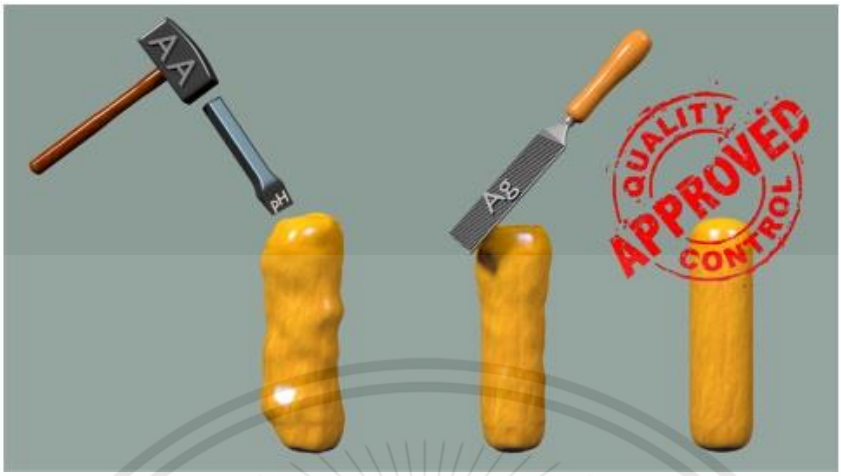

Figure 2.14: Optimization of synthesis methods achieved through careful tuning of various parameters such as reactant concentrations, which influence thermodynamic and kinetic aspects of seeded growth[4].

The successful synthesis of GNRs need the influence of others reagent for supporting the growth rate, the rod shape and the stability of the particle as explain schematically in the Figure 2.14. The fundamental things that need to realize when synthesize GNRs are: water quality: The presence of contaminant in water is able to make irreproducible events in GNRs[4]. Chemical supplier: If synthesis GNRs need to follow the reproducible protocol it need to check the chemical supplier for reducing difference result, Since Smith and Korgel get completely difference result when they changed CTAB supplier. This the reason why need the same suplier[4]**.** Store stocks solution: It need to keep record for every stock solution, especially the first time opening or use reagents must be recorded.

The main reagents used to synthesis GNRs is composed of CTAB, gold precursor, reductant, silver ion, and additives**.** in this section will be explained about how these reagents and other parameters influence to gold nanorod synthesis.

Surfactant, the required surfactant for GNRs synthesis must have important duty in aniotropic growth. So CTAB has bromide ion as counterion that helping anisotropics growth property. The fundamental requirement of the surfactant needs to have quaternary ammonium surfactant head group able to form Au**-** CTAB complex, bromide ion for encouraging single crystalline, tail length of surfactant should long enough and soluble at room temperature.

Gold precursor, commonly use Tetrachloroauric acid ($HAuCl_4$). When gold precursor and CTAB form complex there is the oxidation state of gold ion in the solution will be converted from Au(III) to Au(I) to Au(0)**.** The schematically demonstrate of ligand exchange of gold complex**.**

$$[AuCl_4]^- \longleftrightarrow [AuCl_3Br]^- \longleftrightarrow [AuCl_2Br_2]^- \longleftrightarrow [AuClBr_3]^- \longleftrightarrow [AuBr_4]^-$$

$[AuBr_4]^-$ ions will form an ion pair with the quaternary ammonium of CTAB monomers, the $[AuBr_4]^-$**-** CTA influence their redox potentials resulting in growth kinetics of the GNRs.

The reductant, this reagent the one of the most important for synthetizing gold nanorod the it will help to reduce of Au(III) aqueous solution to Au(0) solid state (e.g. using of sodium borohydride to create nucleation of gold nanoparticle in seed solution before forming rods). In growth solution, the reductant still plays an important role to reduce Au(III). For the example the use of ascorbic acid to reduce Au(III) to Au(I) and it need to be realized that reducing agent cannot completely reduce from Au(I) to Au(0) that will lead to secondary nucleation[4]. Thus there are 2 possible mechanisms which able to convert Au(I) to Au(0). First the seed act as a catalyst that produce itself as

$Au^{3+}+2Au^0 \longleftrightarrow 3Au^+$ and then Au^{3+} is reduced to Au^+ by remaining reductant in the system and due to the le'chatelier principle the more concentration of Au^+ lead the equilibrium shift to the left and produce Au^0 in a solid state. Second the in situ reduction of Au^+ by Au^0 withdraw electron from reductant and catalyzed the reduction of Au^+.

Role of silver ion on formation of GNRs has three main mechanism[4] which are (1) Under potential deposition, (2) Formation of a $Ag[BrCTA]_2$ act as a facets specific capping agent, and (3) Adaptation of CTAB micelle formation through silver bromide interaction. For the single crystalline in the presence of silver ion there is high selectivity reduction at the tips of the rod to achieve higher length of rod (kinetics influencing) and stabilization of the facets (thermodynamically stabilizing). The reason of kinetic selectivity of the tips and thermodynamics stabilization of the facets, Philippe Guyot**-** Sinonest et al. applied electrochemical study on single crystalline substrate shown the UPD shift related to the surfaces structure[23]. Figure 2.15 show the direction of facets of the GNRs single crystalline needed to know before understand the reason of silver assisted.

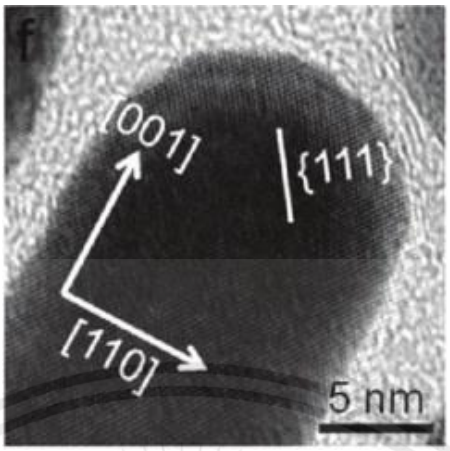

Figure 2.15: Demonstration of the facets direction of GNRs composed of [001], [110] and [111] [24].

From the UPD shift can be understood that at different facets have different work function, For Au and Ag the work function differences are 0.83, 0.85, and 0.57 eV for [001], [110] and [111] respectively[23]. It is shown that facets [111] have lower shift of Ag+ than others and the highest shift of Ag+ is [110]. This means at facets [110] the silver ion prefer to adhere to this facet more than others so it could have more possibility to block the gold particle growth in this facets (Thermodynamically favor). Thus at the tips (facets [111]) have less silver ion there which kinetically favor to grow the GNRs in [111] facets.

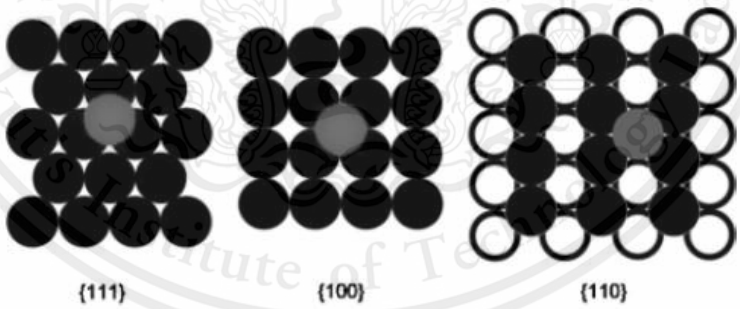

Figure 2.16: Underpotential deposited silver atom (gray circles) has more nearest neighbors on a more open facet[23].

For Au[100] facets, each silver atom has four nearest neighbors. For Au[110] facets, each silver atom has five nearest neighbors (one is in the second layer right beneath the silver atom). The gold atoms of the first layer are represented by black closed circles. For the Au[110] facets, the gold atoms of the second layer (open circles) are also partially exposed[23].

Seed, in the GNRs synthesis by seed mediated method is seed solution production. This seed solution affects the yield and aspect ratio of synthesis GNRs which are not only the concentration seed solution that use in growth step but also the size of seed crystalline also effect yield and aspect ratio in the final in of growth step. The more concentration gold precursors at first step of seed solution preparation get the larger seed particle in the seed solution leading to when synthesize GNRs from these of larger seed[25]. The larger diameter and shorter length of GNRs resulting lower aspect ratio of GNRs.

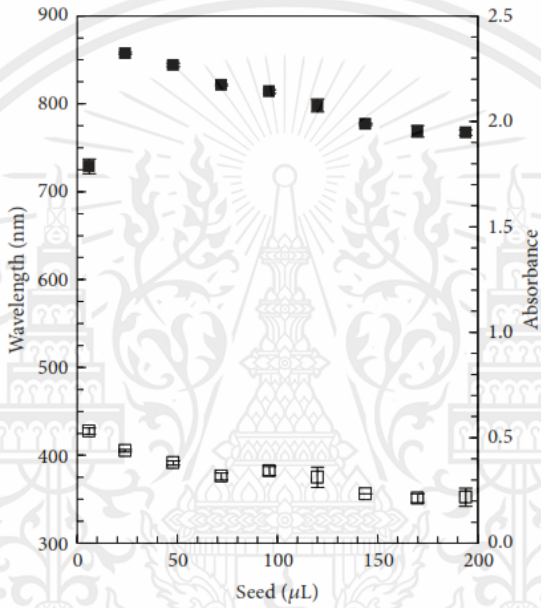

Figure 2.17: Peak absorbance of produced nanorods versus amount of seed added to growth solution. Solid symbols are wavelength at peak absorbance, open symbols are the absorbance of the growth solution at this wavelength[25].

Including to the concentration of seed solution in the growth step of GNRs synthesis, the more concentration of seed solution means that there are more seed particles in the system for growth[25]. Although there are several GNRs occur in the system but these of nanorods get lower aspect ratio because gold monomer in the growth solution separately reduce on each of seed particle, thus it is insufficient gold monomer to produce longer rods. As demonstrated in the Figure 2.17 the more amount of seed in growth solution cause longitudinal plasmon band significantly shift to shorter wavelength. Compare with less quantity of seed solution in the growth solution, Longitudinal surface plasmon shift to longer wavelength indicate that get higher aspect ratio of gold nanorod.

pH, In growth step of the solution pH controlled is very crucial parameter to regulate because pH value is directly effect to reducing agent (e.g. ascorbic acid). At low pH system of growth solution causing weaker reducing ability of ascorbic acid[26], thus the reduction from Au(III) to Au(I) by ascorbic acid is very slow that is the precondition to get higher aspect ratio of GNRs. Moreover, due to weak reducing potential in strong acidic system, ascorbic acid is not able to reduce silver ion to Ag(0) resulting that silver ion form AgBr in growth solution. At low pH system AgBr prefer to deposit on [110] facets than [001] and [111] facets because of high energy and low stability of facets[27]. Deposition of AgBr on [110] site can prevent the deposition of Au atom monomers. Thus, Au atom monomers prefer to deposit on [001] and [111] instead and lead to produce longer length of GNRs.

2.4. Etching of GNRs through Oxidation Reaction

The study of GNRs etching through oxidation reaction to obtain shorter GNRs, Chandrasekar et al[28] demonstrated the etching experiment of GNRs by separated into 2 types of GNRs solution for the experiments which are as– synthesized GNRs and centrifuged GNRs solution were etched by H_2O_2 solution. The final results show that longitudinal surface plasmon band of as-synthesized GNRs solution shift to the shorter wavelength that measured by UV-Vis spectrophotometer as show the extinction spectra in Figure 2.20.

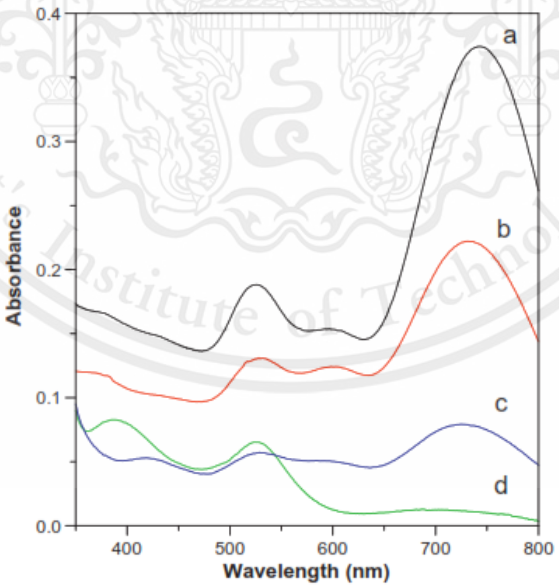

Figure 2.18: UV–Vis spectra of (a) as-synthesised GNRs, (b) centrifuged GNRs, (c) centrifuged GNRs after 2 h from H_2O_2 addition, and (d) as-synthesized GNRs after 2 h from H_2O_2 addition[28].

From as-synthesized GNRs solution in the Figure 2.18a to the Figure 2.18d that had been etching by H_2O_2 for 2 h The blue shift of longitudinal surface plasmon to shorter wavelength occur and finally complete disappearance compare with centrifuged GNRs solution in Figure 2.18b to Figure 2.18c. There is no blue shift observed meaning that it only decrease as their intensity of the extinction spectra of the centrifuged GNRs solution no change the color unlike as-synthesized GNRs solution the change of color was observed as show in the Figure 2.19.

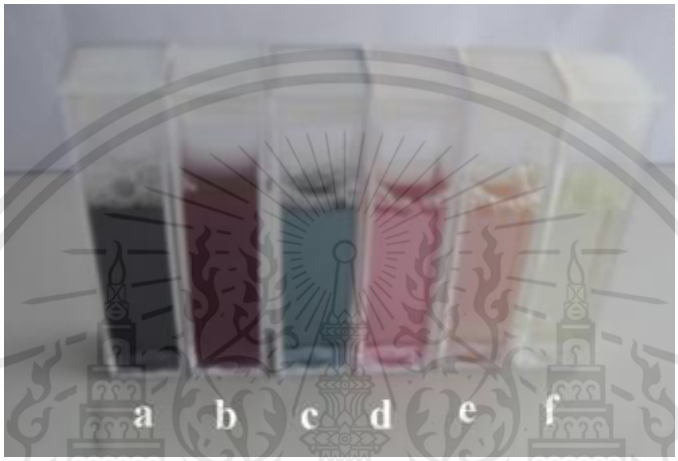

Figure 2.19: Picture of as-synthesized GNRs (a) before and after (b) 30 min, (c) 45 min, (d) 1 h 15 min, (e) 1 h 45 min, and (f) 3 h of oxidation with H_2O_2[28].

The blue shift of as-synthesized GNRs because of the excess of CTAB in the solution. The oxidative dissolution of GNRs particles have H_2O_2 acts as oxidizing agents of Au(0) and Br^- from excess CTAB as complexing agent of gold ion while oxidation reaction occurred in aqueous solution at ambient condition can de described by the redox reaction[28]:

$$AuBr_2^- + e^- \rightarrow Au + 2Br_2^- \quad (2.1)$$

$$AuBr_4^- + 3e^- \rightarrow Au + 4Br^- \quad (2.2)$$

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H2O \quad (2.3)$$

$$\text{Overall reaction: } 2Au + 3H_2O_2 + 6H^+ + Br^- \rightarrow 2[AuBr_4^-] + 6H_2O \quad (2.4)$$

Redox reaction preferentially takes place at the edge of the GNRs particle[28], which is the reaction between Au^0 and H_2O_2 oxidant to Au(III).

From the equations above show that bromide ion play an important role for advocating the oxidation of Au(0) to Au(I). Weihai et al[29] studied the role of bromide for CTAB capped gold nanorods oxidation reaction with hydrogen peroxide as a oxidizing agent in acidic system. In the system for operating the reaction, Hydrogen peroxide oxidizes bromide ion to elemental bromine in the presence of H^+. Then bromide ion and elemental bromine faces tribromide complex reaction get tribromide ion as a following equation[29]:

Slow: $H_2O_2 + 2H^+ + 2Br^- \rightarrow Br_2 + 2H_2O$ (2.5)

Fast: $Br_2 + Br^- \rightarrow Br_3^-$ (2.6)

Overall: $H_2O_2 + 2H^+ + 3Br^- \rightarrow Br_3^- + 2H_2O$ (2.7)

When adding reaction solution into GNRs solution, Br_3^- directly oxidizes Au(0) to Au(I) by complexing with $AuBr_2^-$ while Br_2 is reduced[30], resulting in shorter length and lower aspect ratio of GNRs.

Gold etching: $Br_3^- + 2Au + Br^- \rightarrow 2AuBr_2^-$ (2.8)

From the study of Weihai et al system indicated that hydrogen peroxide is not directly oxidize GNRs but they slowly oxidized bromide ion in acidic condition to get Br_3^- at the final and these of tribromide ion acts as both oxidizing agent and complex agent for dissolving Au into $AuBr_2^-$ [29]. The effect of bromide ion concentration and rate of GNRs etching was studied by varying the concentration of NaBr and the results was investigated by UV-Vis spectrophotometer as demonstrated in Figure 2.20.

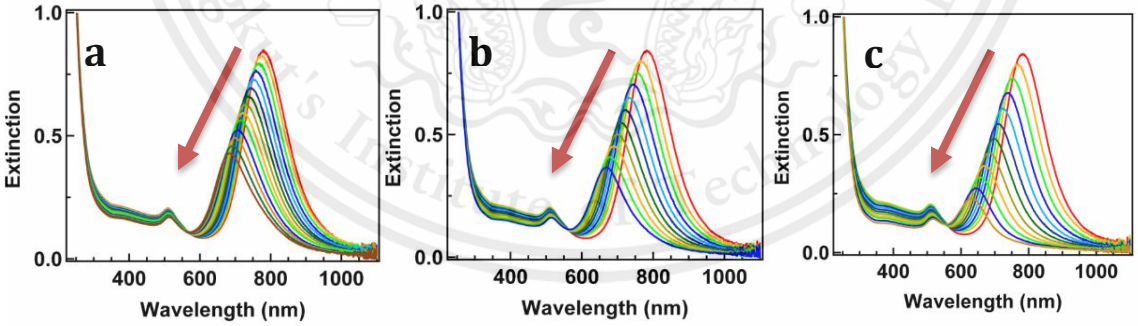

Figure 2.20: Time evolution of GNRs extinction spectra at various Br^- concentration: (a) 0.05, (b) 0.075, (c) 0.1 M respectively. The reaction solution contained 20 mM of HCl, 0.001 M CTA^+, and 30 mM H_2O_2 the spectra were recorded at 45 °C every 0.5 minute[29].

The extinction spectra of GNRs solution is significantly faster when using more concentration of bromide ion in the solution reaction as shown in the Figure 2.22 because the more concentration of Br^- in acidic system means that more of tribromide concentration

could produce in the solution for etching GNRs. In Figure2.26 describe concentration of CTA^+ and Br^- in oxidative reaction show that CTA^+ exhibits linear relation in two region separated by critical micelle concentration[29].

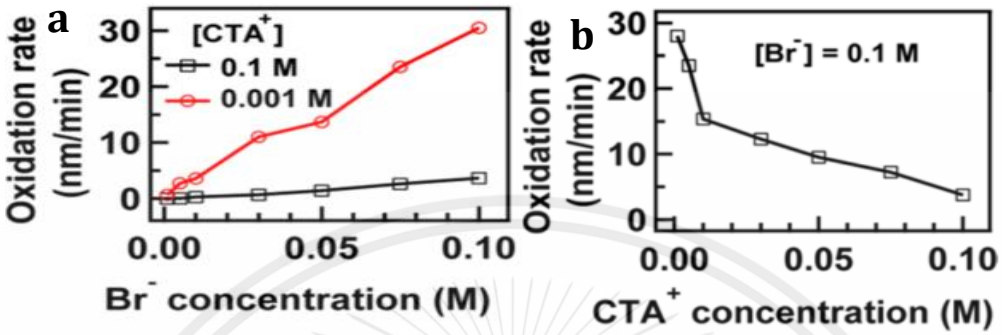

Figure 2.21: Effect of Br^- and CTA^+ concentration on the oxidation rate of GNRs. (a) Oxidation rate as a function of Br^- concentration at 0.001 and 0.1 M CTA^+. (b) Oxidation rate as a function of CTA^+ concentration at 0.1 M Br^-. H_2O_2 concentration was 30 mM, and the temperature was 45 ºC[29].

In Figure 2.21(a) the slope of the rate behavior varying CTA^+ concentration that equal to critical micelle concentration (cmc) (the red line) is steeper than above cmc (black line) indicate that at the cmc concentration, CTAB is incompletely cover on surface of GNRs particle. The oxidation rate of the GNRs is significantly faster as increasing bromide ion concentration in 0.001 M of CTA^+ system. The black line in Figure 2.21a together with Figure 2.21b investigated the effect of CTA^+ concentration and oxidation rate of gold nanorod show that the more concentration CTA^+ in the system the slower oxidation reaction of GNRs particles. In the oxidation system with CTA^+ above cmc, they form a protecting bilayer of micelle structure that cause tribromide ion barely to directly access to surfaces of GNRs.

The study of halide ion is able to trigger the oxidative etching of GNRs is done by Guojun Weng et al[31]. Comparation between Cl, Br, and I ion that can help to fasten the oxidation rate of GNRs in acidic condition with H_2O_2 and in order to study the etching behavior and particle morphology at the end of reaction. The study found that the gold nanrods etching had three stage[31]: short nanorods, big nanoshpere,and ion complex. The results of the etching behavior of three halide ion (Cl^-, Br^-, I^-) that I^- is able to trigger the etching of GNRs than others selected halide ion for the reason that the reduction potential of AuX^- (X= halide element), the oxidative trend as AuI^-> $AuBr,^-$> $AuCl^-$ [32]. The iodide ion had strong affinity to Au surfaces by chenisorption and ability to induce surface etching through oxidative reaction which oxidative bahavior analogous to equation (2.5), (2.6), (2.7),and (2.8).

CHAPTER III
RESEARCH METHODOLOGY

The objective of this work is to synthesize GNRs as a model for G6PD deficiency diagnostic by detection of H_2O_2 in the human red blood cell. GNRs are synthesized using seedless and seed mediated method. The synthesized GNRs are brought for etching investigation mechanism through oxidation reaction with H_2O_2 supported by halide ion. All of the solution reagents have to be freshly prepared for GNRs synthesis.

3.1. Chemicals

1) Cetyl trimethyl ammonium bromide (CTAB, ≥ 98%), Sigma Aldrich

2) Tetrachloroauric acid ($HAuCl_4$, ≥ 49%), Acros Organic

3) Silver nitrate ($AgNO_3$, ≥ 99%), Sigma Aldrich

4) L-Ascorbic acid, (99%), Sigma Aldrich

5) Sodiumborohydride ($NaBH_4$, 99%), Sigma Aldrich

6) Deionized water (DI-H_2O)

7) Sodium salicylate, (≥ 99%), Acros organic

8) Polyvinylpyrrolidone (PVP, Mw 10000), Sigma Aldrich

9) Sodium hydroxide (NaOH)

10) Hydrochloric acid (HCl, 37%)

11) Hydrogen peroxide (H_2O_2, 37%)

12) Sodium chloride (NaCl)

13) Sodium bromide (NaBr, ≥ 99%), Merck

14) Potassium iodide (KI)

3.2. Apparatus

1) Rounded-bottom flask

2) Oil bath

3) Temperature-controlled magnetic stirrer

4) Analytical balance

5) Aluminium foil

7) Iced water bath

8) Centrifuge conical tube

9) Volumetric flask

10) Analytical balance

11) Beaker

12) Spatula

13) Clamp

14) Magnetic bars

15) Centrifuge tube

15) Microcentrifuge tube

17) Centrifuge machine

18)UV-Vis Spectrophotometer (Evolution™ 201/220, Thermo Scientific)

19) Transmission Electron Microscope (JEM-2100, JEOL)

20) Shaking incubator

3.3. Synthesis of GNRs by Seedless Method

1) Prepare 5 mL aqeous solution containing 0.1 M CTAB and 0.5 mM $HAuCl_4$.
2) Add 30 µL of 0.12 mM $AgNO_3$ and 0.1 M ascorbic acid solution into the pre-formed $HAuCl_4$ solution under vigorous stirring (Adding in a sequence).
3) Quickly inject 2 µL of $NaBH_4$ 1.6 mM (Prepare $NaBH_4$ in iced cold bath).
4) Until the color of the solution occurs stop the reaction by centrifuge at 10000 rpm for 25 minutes at least.
5) Drain the supernatant, redisperse with DI-H_2O, and store in centrifuge canonical tube.
6) Adjusting aspect ratio of GNRs can be done by varying concentration of $AgNO_3$.
7) Varying temperatures; 65, 75, and 80 $^{\circ}$C.

3.4. Synthesis of GNRs by Seed Mediated Method using Aromatic Additive

3.4.1. Preparation of Seed Solution

1) Divide 1250 µL of gold solution in the first step of preparation of Growth solution and dilute with 1250 µL DI-H_2O in 10 mL rounded bottom flask.
2) Add 2500 µL of CTAB (0.2 M) into diluted gold solution.
3) Inject 500 µL of $NaBH_4$ (6 mM) under vigorously stir for 2 minutes and leave undisturbed at 30 ºC for 30 minutes before use.

3.4.2. Preparation of Growth Solution

1) Prepare 15 mL of 0.1 mM gold solution solution in beaker.
2) Prepare 15 mL solution of 0.54 g of CTAB containing 48 mg of sodium salicylate in beaker.
3) Add 240 µL of $AgNO_3$ (4 mM) into CTAB – sodium salicylate solution. Stir well and leave undisturbed at 30 ºC for 15 minutes.
4) Mix the 10 mL of Solution No. 1 and No. 2 together in the temperature-controlled jacket reactor, stir at 400 rpm for 15 minutes.
5) Inject 40 µL of ascorbic acid (0.064 M) into jacket reactor under vigorously stir for 30 seconds.
6) Inject 32 µL of seed solution into jacket under vigorously stiring for 30 seconds and left undisturbed at 30 ºC for 12 hours.

3.5. Characterization of Materials

3.5.1. Transmission Electron Microscope (TEM)

The collected images from TEM are used to investigate the morphology of GNRs prepared from seed method and seed mediated method. GNR solution was dropped on a 300-mesh copper-coated carbon grid and air dry.

3.5.2. Ultraviolet-Visible Spectroscopy (Uv-Vis Spectroscopy)

The absorption of GNR solution was analyzed using Uv-Vis spectroscopy with instrument setting:

Data mode:	Absorbance	**Scan speed:**	200 nm/min
Start wavelength:	1000.00 nm	**Data interval:**	1.00 nm
End wavelength:	300.00 nm		

3.6. Investigation of Etching Behavior

1) Prepare 3 vials that contain 500 µL of:
   -No.1: 0.1 M of NaCl, 40 mM of HCl, 30 mM of H_2O_2
   -No.2: 0.1 M of NaBr, 40 mM of HCl, 30 mM of H_2O_2
   -No.3: 0.1 M of KI, 40 mM of HCl, 30 mM of H_2O_2
2) Incubate all vials in an incubator at 55 ºC for 30 minutes.
3) Add 500 µL of GNRs solution into each vial. To investigate etching behavior, take a picture every 5-minute interval for 30 minutes.
4) Repeat the procedure from step 1) to 4) but vary the concentration of H_2O_2 to 20 mM, and 10 mM, respectively.

CHAPTER IV
RESULTS AND DISCUSSION

In this experiment GNRs

were synthesized using 2 protocols ,i.e., seedless and seed mediated method. The goal is to obtain GNRs with aspect ratio approximately 2.4-2.6 and color of the solution which is completely different from human blood.

4.1. Particle Morphology and Size

4.1.1. GNRs synthesized by seedless method

Figure 4.1((a)-(c)) shows TEM images of GNRs prepared by seedless method. It was found that, at [$AgNO_3$] = 0.05 mM, the aspect ratios of GNRs increased with decreasing temperature from 1.78 ± 0.359 at 80 ºC to 2.01 ± 0.364 at 65 ºC, which the latter condition yeilded desired aspect ratio. We further investigate the synthesis condition by increasing the concentration of $AgNO_3$ to 0.12 mM. Agreed with what has been reported, aspect ratio of GNR increased to 2.75 ± 0.620. Both [$AgNO_3$] and temperature affect the final aspect ratio of GNRs. Investigating [$AgNO_3$] generate the large number of silver ion (Ag+) to form complex with CTAB, $Ag[BrCTA]_2$, which selectively deposit on lateral side of GNRs[4] due to stronger affinity between silver complex and gold crystalline side [010] facet[23]. These capping species will slow down anisotropic growth of gold particle from the lateral side. Thus, the tip facets of GNRs composing of [111] and [001] facets, which $Ag[BrCTA]_2$ is less preferential to deposit on, interact with Au(III) and grow anisotropically to longer shape or higher aspect ratio. Temperature also effect final morphology of GNRs but on the reverse effect. Increasing temperature condition smaller aspect ratio. Injection of $NaBH_4$ (strong reducing agent) at elevated temperature promote the conversion from Au(III) to Au(0) as a nuclei[22]. Thus less number of Au(III) is available for anisotropic growth.

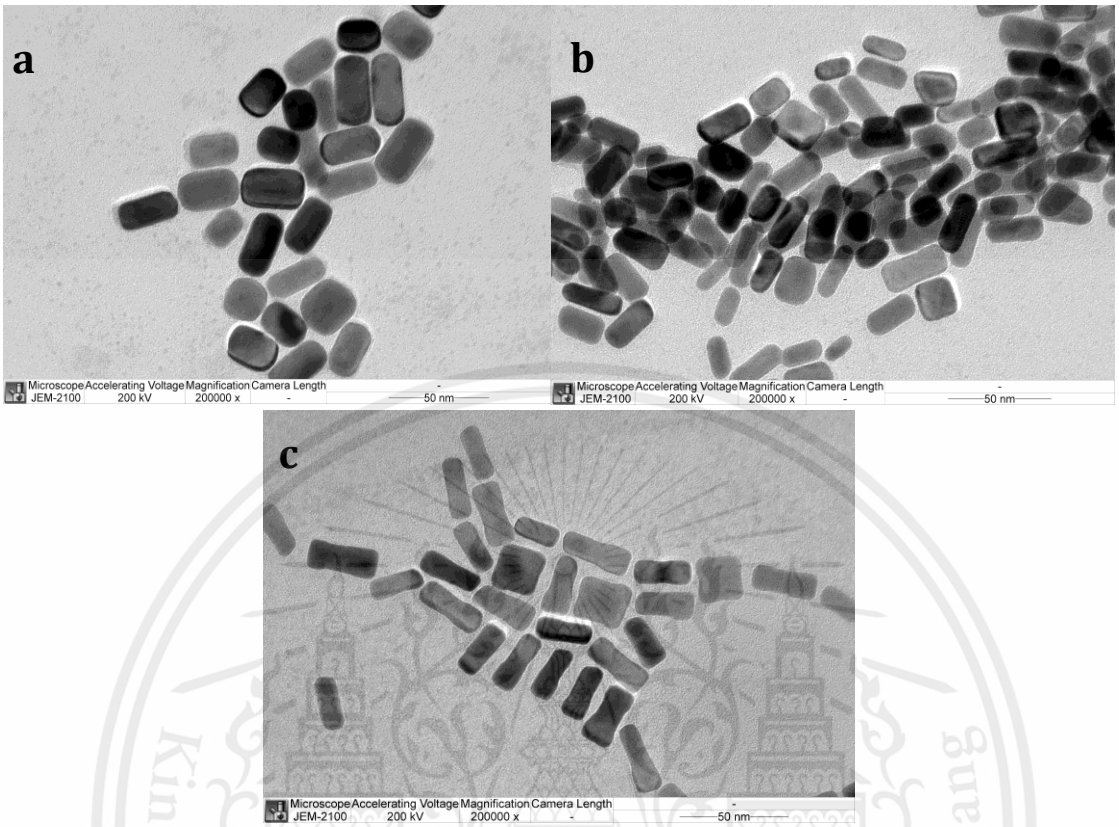

Figure 4.1: TEM images of GNRs synthesized by seedless method and at different [$AgNO_3$] and temperature: (a) [$AgNO_3$] = 0.05 mM at 80 ºC. (b) [$AgNO_3$] = 0.05 mM at 65 ºC. (c) [$AgNO_3$] = 0.12 mM at 65 ºC.

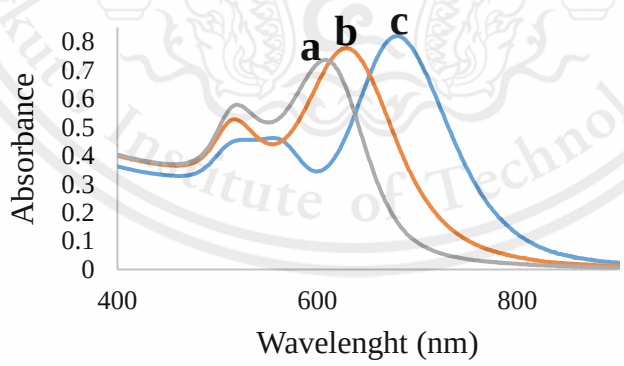

Figure 4.2: Absorption spectra of GNRs synthesized by seedless method at different [$AgNO_3$] and temperature: (a) [$AgNO_3$] = 0.05 mM at 80 ºC. (b) [$AgNO_3$] = 0.05 mM at 65 ºC. (c) [$AgNO_3$] = 0.12 mM at 65 ºC.

Figure 4.2((a)-(c)) show corresponding UV-Vis spectra of GNRs synthesized at different [$AgNO_3$] and temperature. Aspect ratio of GNRs relate with the absorption wavelength of longitudinal absorption peak of Uv-Vis spectra, The higher aspect ratio the higher wavelength of longitudinal absorption peak. Focusing [$AgNO_3$] at the same temperature, spectra (a), and (b) show red shift (longitudinal surface plasmon moves to longer wavelength) of absorption spectra from 679.30 nm to 608.35 nm because high silver complex species promote anisotropic growth of GNR. In contrast, raising temperature led to the shorter aspect ratio and blue shift (longitudinal surface plasmon moves to shorter wavelength) of absorption spectra, due to insufficient amount of Au(III) to direct the elongation of GNRs.

4.2. Effect of H_2O_2 on Etching of GNRs

Pentose Phosphate pathway indicates that G6PD activity is inverse with H_2O_2 concentration, It is possible to simulate the existence of H_2O_2 at high concentration in blood for substantiating the ability of enzyme G6PD to create NADPH by reducing reactive oxygen species (e.g. H_2O_2). The experiment was operated by mixing H_2O_2 and as-synthesized GNRs in various volume ratios.

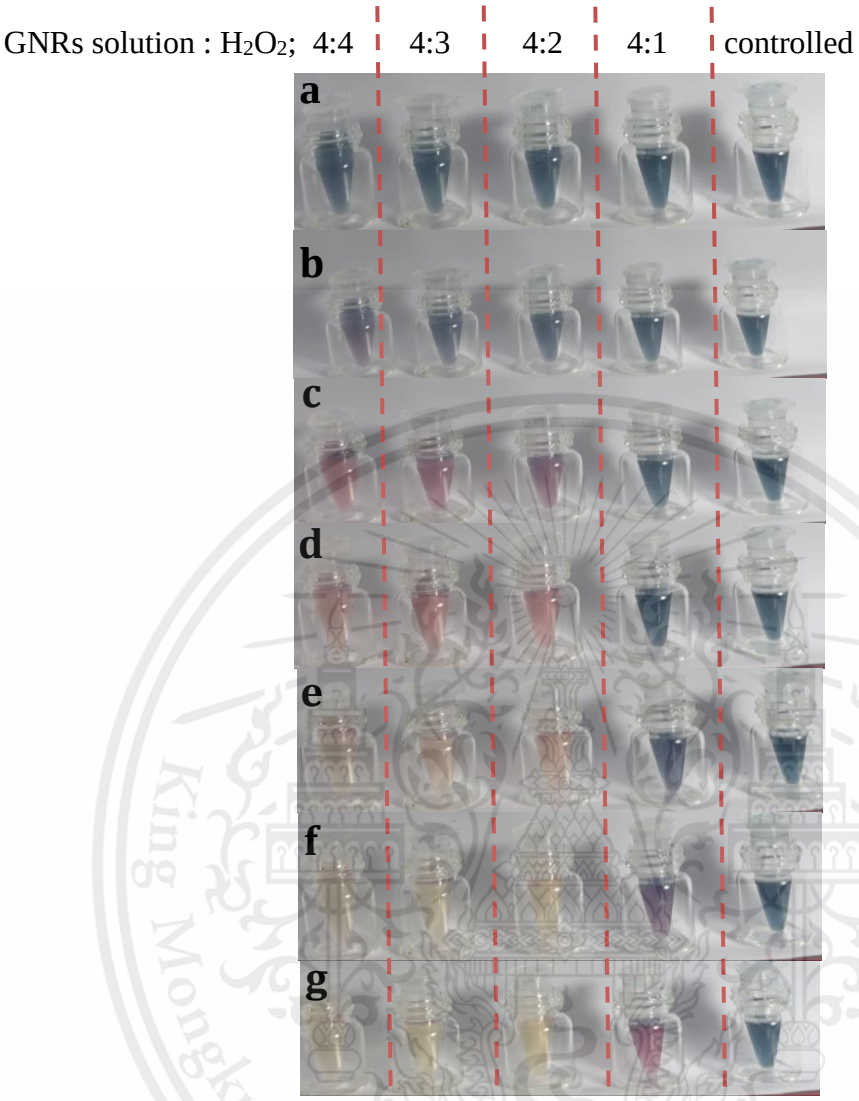

Figure 4.3: Photo of transition of etched as-synthesized GNRs: (a) before, and after adding H_2O_2 for (b) 15 min, (c) 20 min, (d) 25 min, (e) 30 min, (f) 35 min, and (g) 40 min.

Volume ratio between as-synthesized GNR solution and 37% w/w H_2O_2 solutions were varied from 4:4, 4:3, 4:2, 4:1, and controlled solution, respectively. The fastest color changing was observed at 4:4. This results indicate the fastest oxidation reaction because high concentration of H_2O_2 in the system can form large amount of radical to oxidize GNRs. The observed color during oxidation reaction directly reflected plasmon absorption band peak and aspect ratio of GNRs. Yellow solution in Figure 4.3(e) at 4:4 ratio and Figure 4.3(f)-(g) indicate the formation Au(III)-CTAB complex and no GNRs. The oxidation occurred by the reaction equation (2.1), (2.2), (2.3), and (2.4) respectively, which means that the bromide ion (Br^-) from excess CTAB of as-synthesized GNRs is the critical parameter for dissolution or etching of GNRs through oxidation reaction.

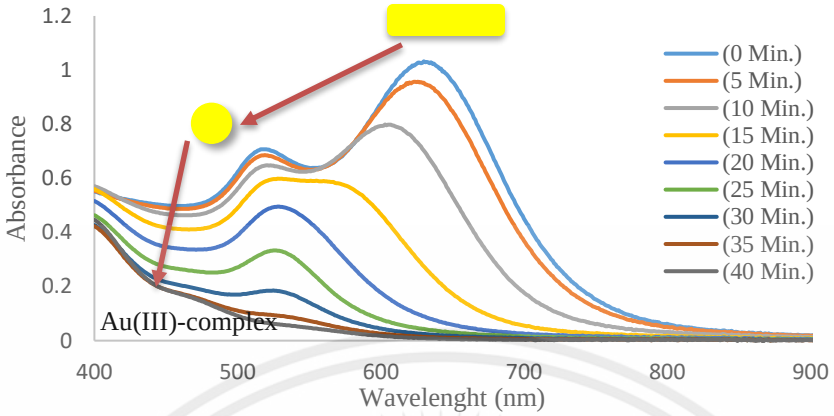

Figure 4.4: Time evolution of extinction spectra during etching of GNRs through oxidation reaction (using with 4:4 volume ratio of as-synthesized GNR solution and 37%w/w (12.78 M) H_2O_2 solution).

Time evolution of absorption spectra during oxidation reaction of GNRs with H_2O_2 at 4:4 volume ratio acquired every 5 minutes are shown in Figure 4.4. Blue shift of longitudinal surface plasmon (LSP) was observed, while blue shift of transverse surface plasmon (TSP) was insignificant. These result support the visual observation in Figure 4.3. It also confirm that the etching occurred at the tips of GNRs and their width were barely affected. The disappearance of LSP at t = 20 minutes means that the particle was transformed from rods to spherical shape. Then intensity of TSP gradually decrease, which implied the oxidation of gold nanospheres. Complete oxidation was at around 35 minutes as no plasmon peak was observed. Since bromide ion involves in oxidation process, next experiment is to investigate the role of Br^- on acceleration of oxidation reaction of GNRs, instead of increasing by higher concentration of H_2O_2. For G6PD deficiency diagnostics, concentration of H_2O_2 in blood of G6PD deficiency patient is much lower than H_2O_2 concentration used in this stimulate system.

4.3. Effect of Bromide Ion on GNRs Etching

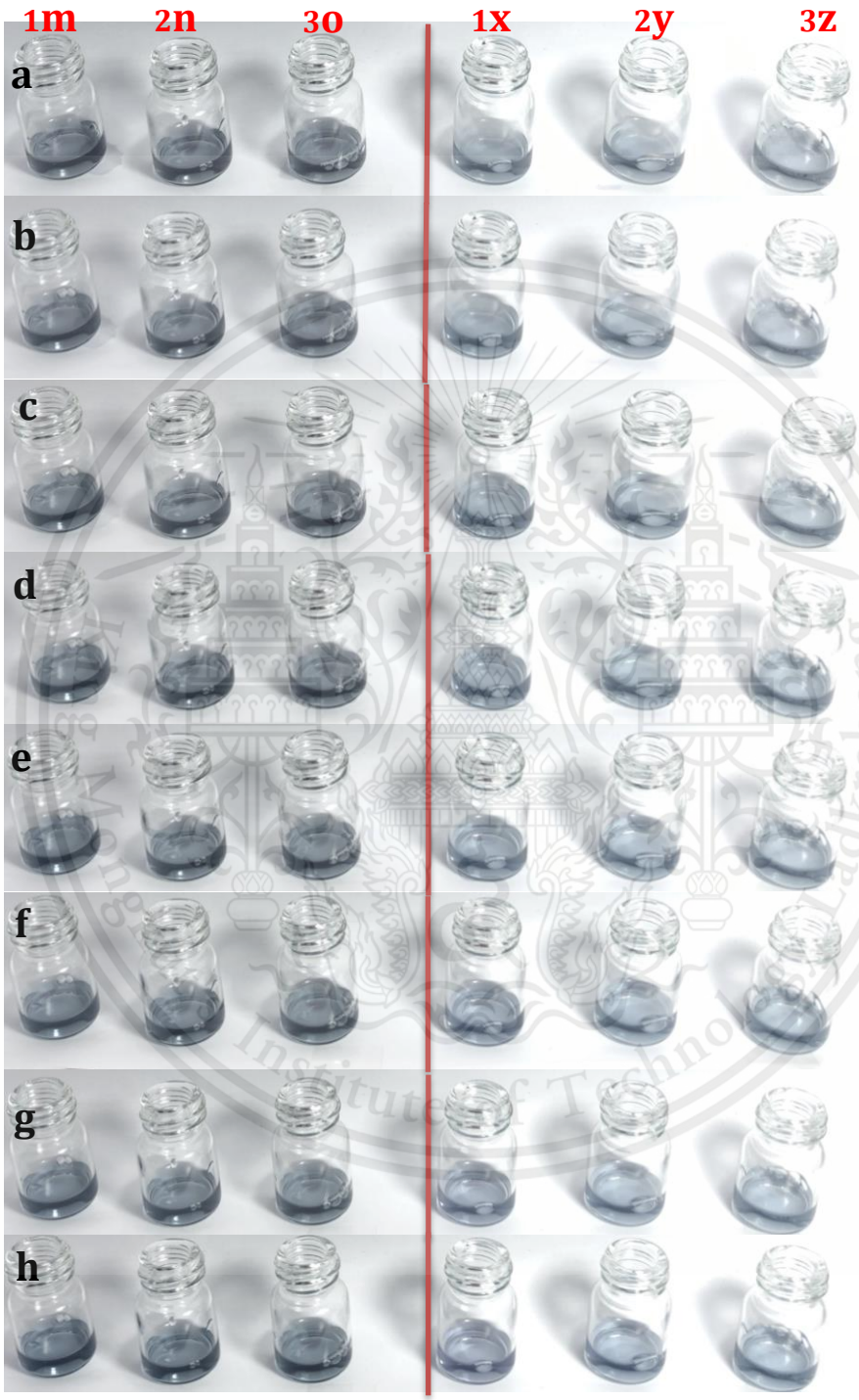

Figure 4.5: Photo of color transition of the mixture between GNR solution and 30 mM H_2O_2. As-synthesized GNRs were used in left columns (m, n, and o). Centrifuged GNRs

are used in the right column (x, y, and z).The photos were imaged at (a) Before, after adding H_2O_2 for (b) 5 min, (c) 10 min, (d) 15 min, (e) 20 min, (f) 25 min, (g) 30 min and (h) 35 min. 3 concentration of Br^- were studied, i.e., 0.1 M (m,x), 0.075 M (n,y), and 0.05 M (o,z). All vials also contained 20 mM HCl.

To evaluate role of excess Br^- on etching, 2 types of GNRs were used, i.e., as-synthesized GNRs and centrifuged GNRs, which excess CTAB was removed. There was no color transition was observed. It is implied that the affinity bromide ion and gold is not enough to suppress bilayer of CTAB to operate etching process. In comparison, barely color change was observed, concentration of H_2O_2 used in this session is much lower than that of in session 4.2. This experiment pointed out that Br^- is not efficiently involve in accelerating the etching process. Thus it would be interesting to explore role of other halide ion that can support H_2O_2 in etching GNRs.

4.4. Effect of Iodide Ion on GNRs Etching

There has been reported that the affinity between iodide ion [I^-] and Au(0) is higher than that of Br^-[31]. It is expected than iodide might have better ability to accerelate the etching process.

Figure 4.6: Photo representing the etching of GNRs after adding oxidizing solution containing H_2O_2: (a) 30 mM, (b) 20 mM, and (c) 10 mM. Each of vials have 0.1 M of iodide ion supported, and 20 mM of HCl. The photo was taken immediately after adding oxidizing solution.

Upon the addition of oxidizing solution the color of GNR solution immediately turn brown to turbid brown, as showed in Figure 4.6. The turbidity resulted from the precipitation of AuI[31]. So, it was difficult to investigate the color change during oxidation. This problem was solved by slowly adding oxidizing solution into GNR solution.

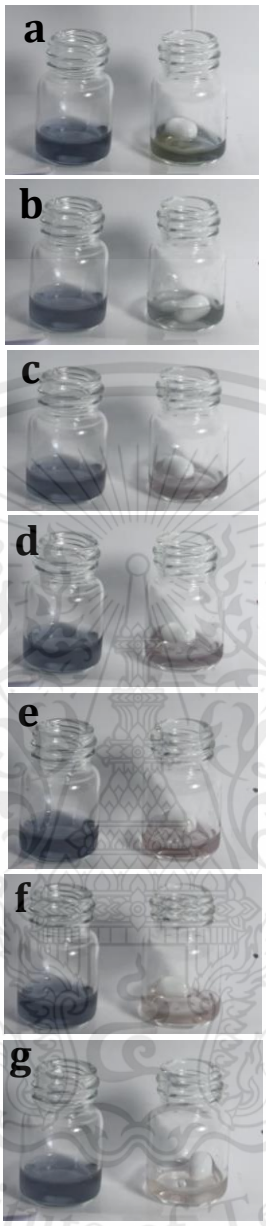

Figure 4.7: Photo of etched GNRs (a) before and after adding oxidizing solution for (b) 10 seconds, (c) 20 seconds, (d) 30 second, (e) 40 seconds, (f) 50 seconds and (g) 60 seconds. The oxidizing solution was 10 µL containing 5 mM H_2O_2, 0.1 mM of I^-, and 20 mM of HCl. Left vial was a control system.

Figure 4.7 shows that etching process was much faster than the Br^- added system. The color of GNR solution changed from blue to colorless with 60 second. In addition no precipitation was found because there is not enough of I^- to form insoluble AuI. All of I^- formed AuI_2^- at the final step of oxidation reaction instead. Comparing the overall concentration of halide ion that used in session 4.3 (used 500 µL of oxidizing solution

contained 0.1 mM bromide ion) and this session (used 10 µL of oxidizing solution contained 0.1 mM iodide ion) with 500 µL GNR solution ,approximately 50 times lesser mole quantity of iodide in used this session for observing etching behavior with iodide. This is a clear evidence that using iodide ion can support H_2O_2 – based etching for development of highly sensitive and rapid test model for G6PD deficiency diagnostic.

Because of difficult to reproduce GNRs synthesized by seedless method, see with Figure different UV-Vis spectra results and different solution color. The current protocol, seedless method, was difficult to reproduced as showed in Figure 4.8 because there was no seed to promote the uniform nucleation. Seed-mediated method was then used to prepared GNRs and employed for further investigation.

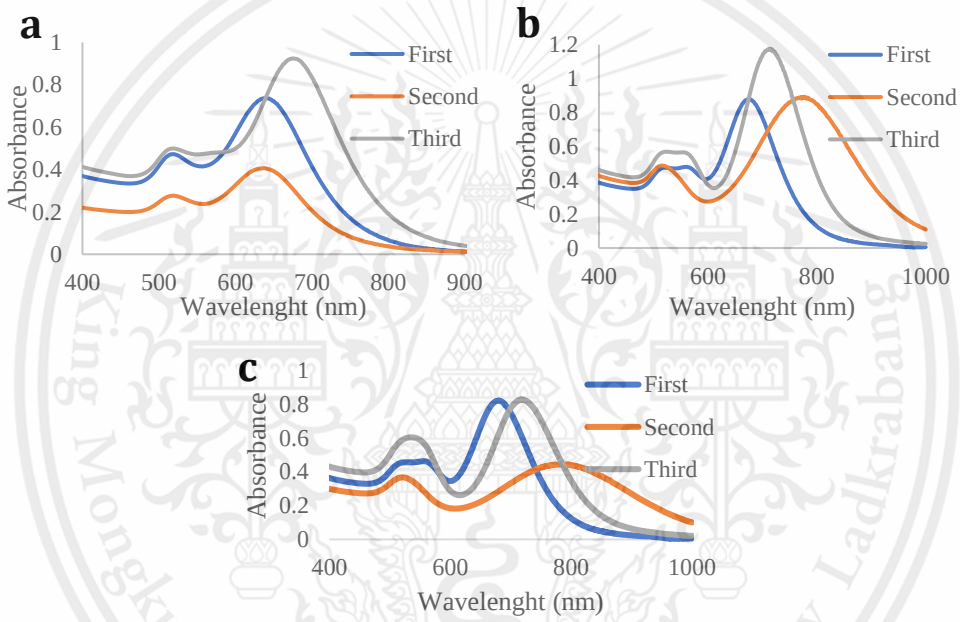

Figure 4.8: Absorption spectra of 3 times GNRs synthesized by seedless method at 65 ºC. and 3 concentrations of $AgNO_3$ varied: (a) 0.05 mM, (b) 0.09 mM, (c) 0.12 mM.

4.5. GNRs Synthesized by Seed Mediated Method using Sodium Salicylate as Aromatic Additive

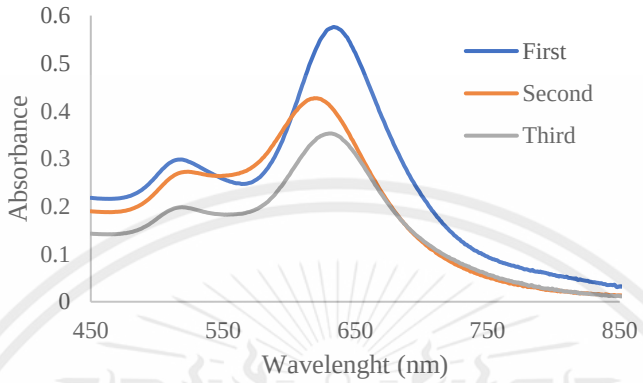

Figure 4.9: Absorption spectra of 3 batches GNRs synthesized by seed mediated method using sodium salicylate as aromatic additive.

Figure 4.9 shows UV-Vis spectra of 3 different batches of GNR synthesized by seed mediated method using sodium salicylate as aromatic additive. All LSP are at 633.88, 629.25, and 631.86 nm, which are relatively close. Also it is comparable to the desired GNRs, prepared from the seedless method. It is observed that the use of the small amount of sodium salicylate as an additive is able to reduce the use of CTAB compare with seedless method[20] and El-sayed et al method[18].

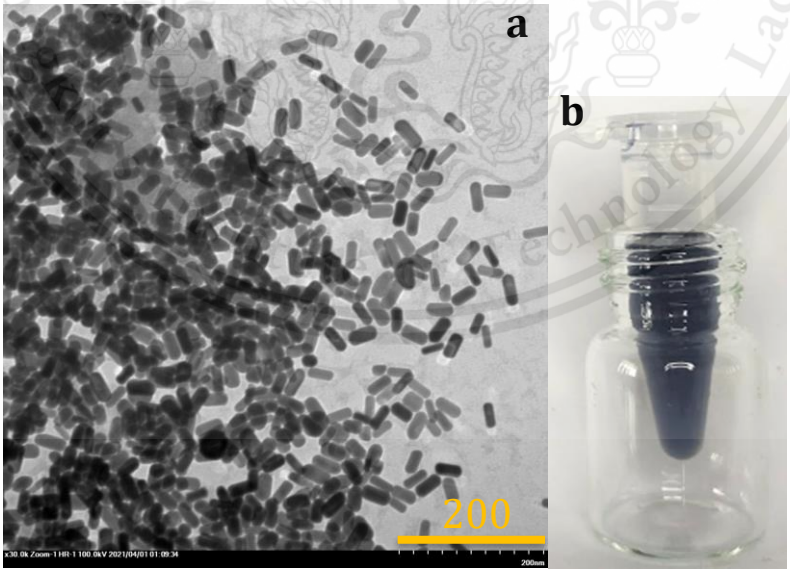

Figure 4.10: (a) TEM image of GNRs and (b) The image of GNRs solution nanorods synthesized by seed mediated method using sodium salicylate.

Benzene ring of sodium salicylate penetrates in to alkyl chain tail of CTAB. The COO^- of salicylate anion reduces the electrostatic repulsion between the quaternary ammonium group (cation head of CTAB miclle), inducing the rodlike micellar[24]. This phenomenon makes GNRs producible at lower concentration of CTAB (approximately 50% less than general recipes). This method not only reduces the use of CTAB bur also yields monodisperse GNRs and the most satisfying color as presented in Figure 4.10(a) and (b), respectively. the most satisfying color

4.6. Effect of Halide Ion on GNRs Etching

Influence of halide ion i.e., Cl^-, Br^-, I^- on etching of GNRs was investigated using H_2O_2 as oxidizing agent in acidic condition. The procedure for this operation was modified from Weihai et al[29]. Figure 4.5((a)-(g)) present solution color of GNRs during etching with H_2O_2 in the presence of different halide ion. Due to GNRs synthesized by seed mediated method is selected for further etching investigation, for more conprehensive behavior observation of halide ion on GNRs etching and the trend of GNRs etching with different halide ion. The Cl^-, Br^-, I^- is needed to observe simultaneouly.

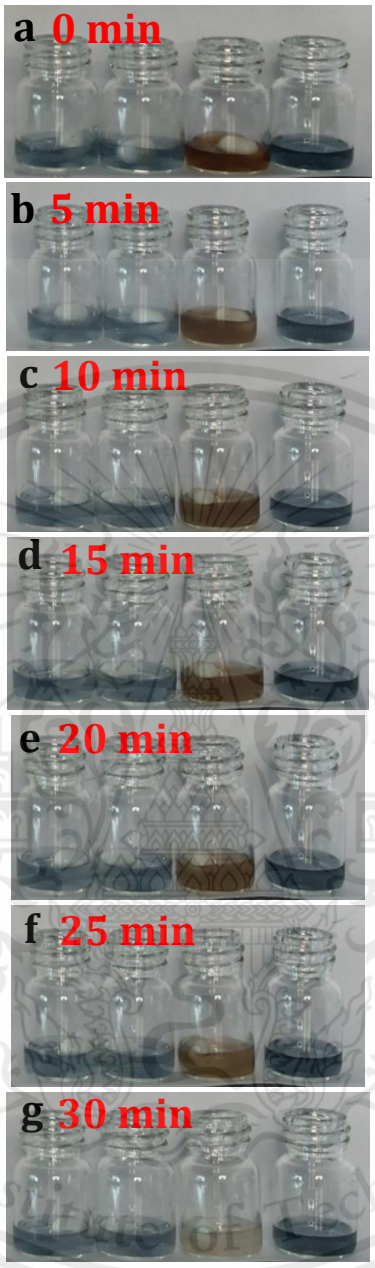

Figure 4.11: The oxidation of GNRs consisted of Cl^-, Br^-, I^-, and controlled solution from left to right respectively. Each of vials started reaction by adding 500 µL of the solution contained 0.1 M of Halide ion solution, 40 mM of HCl, and 30 mM of H_2O_2 to 500 µL of GNRs solution. The images were recorded every 5 minutes for (a)-(g) at room temperature.

It was clearly seen that solution color of system containing Cl^- and Br^- barely changed, even after 30 minutes. UV-Vis spectrophotometer could detect this invisible change but not the naked eye. Thus, these 2 ions are not suitable for plasmonic-based sensor. In case of I^- system, color of the solution begin to change at 20-25 minutes from brown-yellow color gradually faded out to the pale yellow at the end. This pale yellow indicate, that GNRs were completely etched. Based on the visually detectable change in color, the iodide-aided oxidation can be described as followed.

$$\text{Slow:} \quad H_2O_2 + 2H^+ + 2I^- \rightarrow I_2 + H_2O \tag{4.1}$$

$$\text{Fast:} \quad I_2 + I^- \rightarrow I_3^- \tag{4.2}$$

$$\text{Overall:} \quad H_2O_2 + 2H+ + 3I^- \rightarrow I_3^- + 2H_2O \tag{4.3}$$

$$\text{Etching GNRs:} \quad I_3^- + 2Au + I^- \rightarrow 2AuI_2^- \tag{4.4}$$

The occurrence of I_2 in the solution while the etching reaction was arising in the system, presenting the brownish yellow solution (Both Cl_2 and Br_2 solution are colorless) at beginning and during oxidation reaction. The overall reaction and the disappearance of yellow solution with no blue color of GNRs at the end of the reaction can imply that might be entirely etched by I_3^- through oxidation reaction before the reduction of I_2 was complete.

CHAPTER V
CONCLUSION

5.1. Conclusion

In this work GNRs were synthesized and characterized to achieve the appropriate size, shape, and aspect ratio, which yield suitable color for naked eye detection G6PD. Carefully adjusting chemicals and yield the expected aspect ratio of 2.5, which exhibit plasmon peak at approximately 633 nm, corresponding to visible blue color. It was found that etching rate depended not only concentration of H_2O_2 but also the presence of free halide ion. Among those halide ion induce and accelerate oxidation reaction even at low concentration of H_2O_2,i.e., 30 mM, color of GNRs change from blue to colorless within 1 min in the assistance of G6PD diagnostic kit. However, due to the intense brown color of I_2 solution, further experiment are need to optimize the detection process.

5.2. Suggestion

In this work show that iodine combine with hydrogen peroxide is able to cause gold dissolution and color of solution changing. Focusing on the range of hydrogen peroxide in G6PD deficiency patient blood, GNRs etching system need to regulate the sensitivity for effectively responding the suspect sample. Adjusting the quantity of GNRs solution, concentration of halide ion, quantity of suspect sample may help to find the most effective system for qualitatively test G6PD deficiency patient.

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