โพรไฟล์การสลายตัวของแผ่นระดับนาโนเมตรของเงินภายใต้ภาวะออกซิเดชันโดยใช้ไฮโดรเจนเปอร์ออกไซด์

นายกฤชพล นิตินัยวินิจ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

DECOMPOSITION PROFILES OF SILVER NANOPLATES UNDER OXIDATIVE CONDITIONS U

SING HYDROGEN PEROXIDE

Mr. Kritchapon Nitinaivinij

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2014

Thesis Title	DECOMPOSITION PROFILES OF SILVER NANOPLATES UNDER OXIDATIVE CONDITIONS USING HYDROGEN PEROXIDE
By	Mr. Kritchapon Nitinaivinij
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Kanet Wongravee, Ph.D.
Thesis Co-Advisor	Associate Professor Sanong Ekgasit, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯ Dean of the Faculty of Science

(Professor Supot Hannongbua, Ph.D.)

THESIS COMMITTEE

⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯ Chairman

(Associate Professor Vudhichai Parasuk, Ph.D.)

⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯ Thesis Advisor

(Assistant Professor Kanet Wongravee, Ph.D.)

⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯ Thesis Co-Advisor

(Associate Professor Sanong Ekgasit, Ph.D.)

⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯ Examiner

(Professor Thawatchai Tuntulani, Ph.D.)

⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯⋯ External Examiner

(Associate Professor Chinapong Kritayakornupong, Ph.D.)

กฤชพล นิตินัยวินิจ : โพรไฟล์การสลายตัวของแผ่นระดับนาโนเมตรของเงินภายใต้ภาวะออกซิเดชันโดยใช้ไฮโดรเจนเปอร์ออกไซด์ (DECOMPOSITION PROFILES OF SILVER NANOPLATES UNDER OXIDATIVE CONDITIONS USING HYDROGEN PEROXIDE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. คเณศ วงษ์ระวี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร.สนอง เอกสิทธิ์, 62 หน้า.

อนุภาคระดับนาโนเมตรของโลหะเงิน หรือซิลเวอร์นาโน (AgNPs) ได้ถูกนำมาประยุกต์ใช้อย่างแพร่หลายในปัจจุบัน ด้วยคุณสมบัติที่โดดเด่นในเชิงแสง และความหลากหลายในการประยุกต์ใช้ งานวิจัยนี้จึงได้ทำการศึกษาโพรไฟล์การสลายตัวของแผ่นระดับนาโนเมตรของเงินภายใต้สภาวะออกซิเดชัน โดยใช้ไฮโดรเจนเปอร์ออกไซด์ การสลายตัวแผ่นซิลเวอร์นาโนสามารถติดตามปฏิกิริยาได้จากเทคนิคยูวี วิสิเบิล แอบซอบชั่น สเปกโทรสโกปี (UV-Visible Absorption Spectroscopy) และกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (Transmission Electron Microscope) การเปลี่ยนแปลงรูปร่างของแผ่นซิลเวอร์นาโนสามารถวิเคราะห์ได้จากปรากฏการณ์โลคัลไลซ์เซอร์เฟสพลาสมอนเรโซแนนซ์ (Localized Surface Plasmon Resonance : LSPR) ผ่านเทคนิคยูวี วิสิเบิล และการเปลี่ยนแปลงสีของสารละลาย เมื่อทำการผสมไฮโดรเจนเปอร์ออกไซด์ที่ความเข้มข้นต่าง ๆ กับซิลเวอร์นาโน และติดตามปฏิกิริยาเป็นเวลา 60 นาที พบว่าสัญญาณ LSPR ที่ตำแหน่งอินเพลนไดโพล (in-plane dipole) ซึ่งสัมพันธ์กับขนาดของแผ่นซิลเวอร์นาโน มีความเข้มลดลง และเกิดบลูชิฟท์ (blue-shift) และสัญญาณที่ตำแหน่งเอาท์ออฟเพลนควอดรูโพล (out-of-plane quadrupole) ซึ่งสัมพันธ์กับอัตราส่วนระหว่างความกว้าง และความหนาของแผ่นซิลเวอร์นาโน มีความเข้มลดลงและเกิดเรดชิฟท์ (red-shift) แสดงถึงการเปลี่ยนแปลงรูปร่างของแผ่นซิลเวอร์นาโนจากแผ่นทรงกลมขนาดใหญ่ เป็นแผ่นทรงกลมขนาดเล็ก และบางลงทันทีหลังจากการเติมไฮโดรเจนเปอร์ออกไซด์ การเปลี่ยนแปลงรูปร่างของแผ่นซิลเวอร์นาโนส่งผลให้เกิดการเปลี่ยนสีของสารละลายจากสีแดงไปเป็นชมพู ส้ม เหลือง และใส ขึ้นกับความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์ นอกจากนี้การเปลี่ยนตำแหน่งของ LSPR ยังมีความสัมพันธ์แบบเส้นตรงกับความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์ แสดงให้เห็นว่าการเปลี่ยนแปลงรูปร่างของแผ่นซิลเวอร์นาโนสามารถประยุกต์ใช้เป็นเซ็นเซอร์ตรวจวัดปริมาณไฮโดรเจนเปอร์ออกไซด์ด้วยตาเปล่าได้ โดยไม่จำเป็นต้องมีการปรับปรุงสภาพพื้นผิวของแผ่นซิลเวอร์นาโน จากการผลการทดลองพบว่าโพรไฟล์การสลายตัวของแผ่นซิลเวอร์นาโนสามารถตรวจวัดปริมาณไฮโดรเจนเปอร์ออกไซด์ได้ต่ำสุดถึง 1.57 ไมโครโมลาร์ นอกจากนี้โพรไฟล์การสลายตัวของแผ่นซิลเวอร์นาโนด้วยไฮโดรเจนเปอร์ออกไซด์ยังสามารถประยุกต์ใช้เป็นเซ็นเซอร์ตรวจวัดปริมาณกลูโคสในระบบกลูโคสออกซิเดส (glucose-oxidase) ด้วยตาเปล่าได้อีกด้วย

ภาควิชา	เคมี	ลายมือชื่อนิสิต	....
สาขาวิชา	เคมี	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
ปีการศึกษา	2557	ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

KRITCHAPON NITINAIVINIJ: DECOMPOSITION PROFILES OF SILVER NANOPLATES UNDER OXIDATIVE CONDITIONS USING HYDROGEN PEROXIDE. ADVISOR: ASST. PROF. KANET WONGRAVEE, Ph.D., CO-ADVISOR: ASSOC. PROF. SANONG EKGASIT, Ph.D., 62 pp.

Silver nanoparticles (AgNPs) have been widely used nowadays due to their unique optical properties and potential applications. In this study, the decomposition profiles of silver nanoplates (AgNPls) under oxidative conditions using hydrogen peroxide (H_2O_2) were investigated. The AgNPls were morphologically decomposed by a low concentration of H_2O_2 revealed by UV-visible absorption spectroscopy and transmission electron microscopy (TEM). The morphological changes of AgNPls can be observed by the localized surface plasmon resonances (LSPR) in UV-visible spectrum. After incubating AgNPls with various concentrations of H_2O_2 and monitoring for 60 minutes, the in-plane dipole LSPR peak, which is related to the lateral size of AgNPls, was decreased and blue-shifted. The out-of-plane quadrupole LSPR, which is related to the aspect ratio (lateral size/thickness) of AgNPls, was decreased and slightly red-shifted. This observation indicates the AgNPls were immediately converted from regular disk into smaller and rounder disk after H_2O_2 were added. The morphology changes of AgNPls led to an appreciable color change in the AgNPl solution from red to pink, orange, yellow and finally transparency due to the H_2O_2 concentration. Furthermore, a good linear relationship between the wavelength shifts ($D\ell$) of AgNPls and the H_2O_2 concentration can be obtained. The solution phase detection of H_2O_2 by the direct morphological change can be accomplished without any surface modification of AgNPls. Therefore, a new and simple colorimetric strategy based on the chromaticity analysis of AgNPl solution was demonstrated. The hydrogen peroxide concentration at 1.57 micromolar can be recognized by naked-eye observation with good accuracy, stability and reproducibility. In addition, the proposed protocol can be applied to determine the glucose concentration through the glucose-oxidase system. The new colorimetric determination of hydrogen peroxide utilizing digital image analysis on color changes from AgNPl shape decomposition will open up an alternative method for simple, rapid and reliable detection of hydrogen peroxide and can realize its future applications in biochemical analysis or clinical diagnosis.

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LIST OF ABBREVIATIONS & SYMBOLS

MNPs	: Metal Nanoparticles
AgNPs	: Silver Nanoparticles
AgNSs	: Silver Nanospheres
AgNPls	: Silver Nanoplates
AuNPs	: Gold Nanoparticles
$AgNO_3$	: Silver Nitrate
$NaBH_4$	: Sodium Borohydride
H_2O_2	: Hydrogen Peroxide
LSPR	: Localized Surface Plasmon Resonance
UV	: Ultraviolet
GOx	: Glucose-oxidase Enzyme
TEM	: Transmission Electron Microscope
ATR FT-IR	: Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
w/v	: Weight by Volume
mL	: Milliliter
g	: Grams
mg	: Milligram
M	: Molar (mole/liter)
μM	: Micromolar
ppm	: Part Per Million
s	: Seconds

nm   : Nanometers

λ_{max} : wavelength at maximum

                                      absorption

$\Delta\lambda$: λ_{max} initial $-$ λ_{max} reaction

CHAPTER I

INTRODUCTION

1.1 Metal nanoparticles

Metal nanoparticles (MNPs) such as silver and gold nanoparticles have stimulated a great research interest due to their shape- and size- dependent optical, electronic and chemical properties and many potential applications. As for silver nanoparticles (AgNPs), the MNPs have the anti-bacterial properties which make them more interesting in the research field, not only in the academic studies but also in the industrial field. In nanoscale, MNPs has a unique optical property different form the bulk materials. The different in optical property is the result form the phenomena called "localized surface plasmon resonance" (LSPR) which is based on the coupling between an external electromagnetic field and the collective electron oscillations in the conduction band of the metal [1]. LSPR bands of MNPs can be observed in the UV-visible region. This unique property is strongly related to the color of the colloidal MNP solution which can be employed in various applications such as visual sensors.

1.2 Silver nanoplates

Silver nanolates (AgNPls) are an example of 2D structure of silver nanoparticles (AgNPs) with lateral size and thickness dimensions. The optical property of AgNPls is tunable due to the different shape and size is resulting in varieties of colloidal colors.

AgNPls have been used in many applications due to their easily tune optical property such as biological and chemical sensing, substrate in raman scattering technique and antibacterial.

Since the shape and size of AgNPls effect the color of colloidal solution, the changing of morphological after reacting with etching agent leading to the changing color of AgNPl solution make them capable of applying as rapid, accuracy and simple colorimetric sensor without any further surface modification.

1.3   Decomposition profiles of silver nanoplates.

Silver nanoparticles have been widely studied due to their unique optical properties and many potential applications. There are many researches studied on how to synthesize and how to apply these noble metal nanoparticles. However, there are not many researches studied about the decomposition mechanisms of silver nanoparticles. In this research, the decomposition profiles of silver nanoplates induced by hydrogen peroxide were studied and the application of this decomposition mechanism was also illustrated.

The studies of the decomposition mechanisms of silver nanoparticles were mostly based on the effect of halide ions, such as chloride or bromide ions [2-6]. The studies show that halide ions can act as both oxidative agent and stabilizer. Halide ions oxidized AgNPls at the edge and stabilized the decomposed silver atoms to form silver nanospheres (AgNSs). However, the decomposition mechanism induced by hydrogen peroxide was mostly based on AgNSs and concentrated on applying as spectroscopic hydrogen peroxide sensor. The studies show that hydrogen peroxide can oxidized AgNSs, leading to the changing in intensity of localized surface Plasmon resonance of AgNSs which corresponding to the concentration of added H_2O_2.

As for the applications, the decomposition profiles of AgNPls were not only resulting in the changing in LSPR shifting and intensity but also the changing in color of the AgNPl solution, thus, this phenomena suggest that the decomposition profiles of AgNPls induced by H_2O_2 may apply as colorimetric sensor for H_2O_2.

1.4   Hydrogen peroxide

Hydrogen peroxide (H_2O_2) has been broadly use in many industrial fields, such as, electronic circuit cleaning, water treatment, more over H_2O_2 also used in medical propose, for example, wound cleaning and dressing.

Despite of its useful properties, the excess H_2O_2 also induced cellular damaging, thus, the determination of excess H_2O_2 in environment or in waste water before releasing into environment is needed. Conventionally, the quantitative determination of H_2O_2 using many techniques, such as, chemiluminescence and voltammetry, but these techniques are bulky, costly and require many sample preparations [7-10], thus, the easier, rapid, accuracy and portable technique is required.

1.5   Colorimetric sensing

Colorimetric sensing was brought to interest recently due to its easy observation and many applications related to the digital camera and smart phone. Conventionally, the colorimetric sensing was based on the aggregation of metal nanoparticles, such as, AgNPs or gold nanoparticles (AuNPs). However, the aggregation of nanoparticles might not be appropriate for quantitative detection, thus, the colorimetric sensing using the particle transformation strategy was developed.

For the decomposition mechanism of silver nanoplstes induced by hydrogen peroxide, there was a studied on applying the relation between the color and LSPR of AgNPls as naked eye glucose sensor using the reaction between glucose and glucose oxidase enzyme which create hydrogen peroxide and further react with AgNPls leading to the changing in LSPR and color of the silver colloidal solution which corresponding to the concentration of added glucose.

This research is based on the decomposition mechanism of silver nanoplates induced by hydrogen peroxide and, moreover, studied the possibility in applying this understanding as naked eye sensor for hydrogen peroxide and glucose.

1.6   The objectives

The objectives of this research are to propose the decomposition mechanism of silver nanoplates under oxidative condition using hydrogen peroxide and to illustrate the possibility in applying this mechanism as a naked eye sensor for hydrogen peroxide and glucose.

1.7   Scopes of research

1. Synthesize silver nanoplates using soluble starch as stabilizer.
2. Study the decomposition profiles of silver nanoplates using various concentrations of hydrogen peroxide.
3. Study parameters
   3.1 Effect of hydrogen peroxide concentration to the decomposition rate of silver nanoplates
   3.2 Effect of size and shape of silver nanolates to the decomposition rate
   3.3 Effect of silver nanoplates concentration for applying as naked eye sensor

CHAPTER II

THEORETICAL BACKGROUND

2.1 Localizes surface plasmon resonance

Localized surface plasmon resonance (LSPR) is the phenomena based on the coupling between an external electromagnetic field and collective electron oscillations on the surface of metal nanoparticles (MNPs) and resulting as extinction spectra which can be observed via absorption spectra in UV-visible absorption technique [1, 11-16].
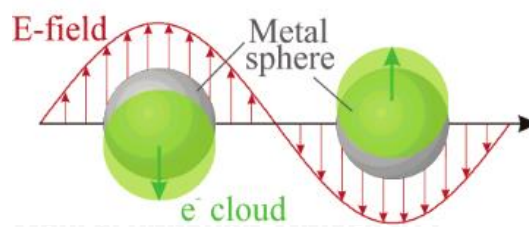

Figure 2.1   The coupling phenomena between electromagnetic field and collective electron oscillation on the surface of MNPs

The different shape and size of MNPs effect the LSPR due to the different electron oscillation patterns on the surface of MNPs which resulting in the different patterns of LSPR.

(A) In-plane dipole LSPR = 500 nm

(B) Out-of-plane dipole LSPR = 400 nm

(C) In-plane quadrupole LSPR = 470 nm

(D) Out-of-plane quadrupole LSPR = 340 nm
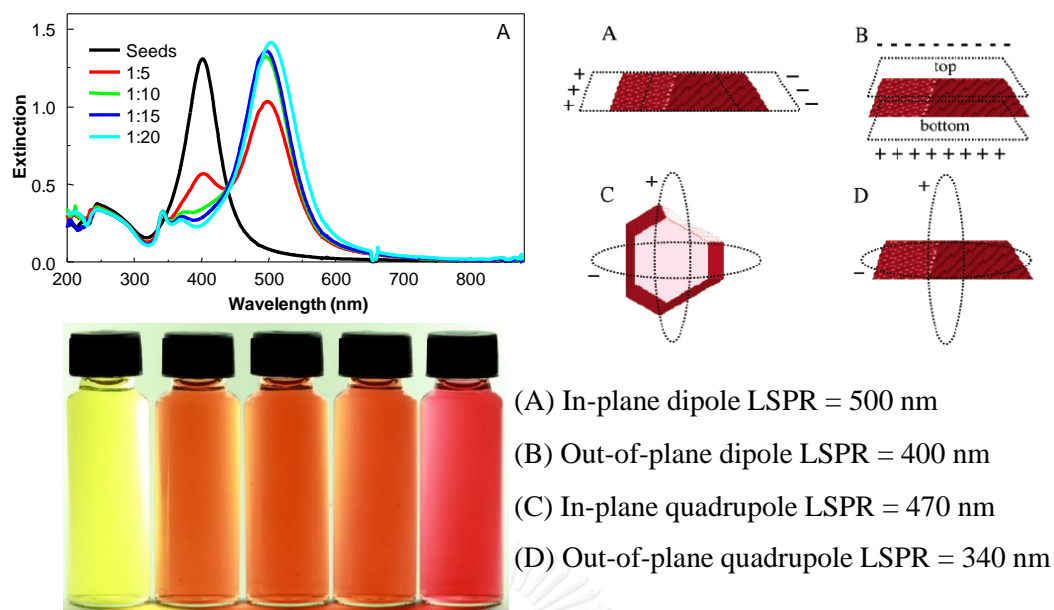
Figure 2.2 The relation of LSPR, size, shape and solution color of AgNPls

LSPR can be observed via UV-Visible absorption spectroscopy. The absorption band in UV-Visible spectra is called extinction spectra for LSPR. The different patterns in extinction spectra can predict the size and shape of MNPs. For silver nanoparticles (AgNPs), the sphere-like AgNPs (silver nanospheres: AgNSs) has characteristic LSPR band at 400 nm, but for plate-like AgNPs (silver nanoplates: AgNPls), there are 2 characteristic bands, at over 400 nm, the band refers to the lateral size of plate-like structure, called in-plane dipole LSPR band, and at 340 nm, the band refers to the ratio between lateral size over thickness of plate-like structure, called out-of-plane quadrupole LSPR band. The appearance of out-of-plane quadrupole LSPR band refers to the formation of plate-like silver nanostructure and the intensity of extinction spectra refers to the amount of nanoparticles in the solution.

Moreover, the changing in LSPR spectra of MNPs also affects the MNP solution color. The different size and shape of MNPs are resulting in different extinction spectra in visible region, thus, the appearing color of MNPs are also different.

2.2   Silver nanoparticles

Silver nanoparticles (AgNPs) have been widely studied nowadays due to their unique properties and many potential applications, such as catalysis, optical sensor, surface enhance raman substrate and antibacterial property which has been apply in many household products.

In nanoscale, the metal nanoparticles show unique different properties from bulk materials, especially the optical property. In case of silver metal, the silver nanoparticles show a significant different appearance form the solid silver. The AgNPs in colloidal solution have variety of color depending on the size and shape of the nanostructure. The different solution color of AgNPs is due to the phenomena called localized surface plasmon resonance (LSPR).

2.2.1 Silver nanospheres

There are many approaches to synthesis AgNPs such as photo reduction or chemical reduction [14, 17]. In this research, silver nanospheres (AgNSs) were firstly synthesized via chemical reduction of silver nitrate ($AgNO_3$) by sodium borohydride ($NaBH_4$) with soluble starch as stabilizer. The reaction follow

$$Ag^+ + BH4^- \rightarrow Ag^0 + \tfrac{1}{2}H_2 + \tfrac{1}{2}B_2H_6$$
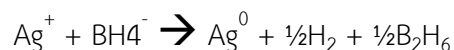
After the reduction, the solution of $AgNO_3$ turned dark brown indicated the formation of AgNSs. Soluble starch was used as stabilizer to prevent the aggregation and precipitation. The dark brown colloidal solution of AgNPs was characterized by UV-visible spectroscopy and shows an extinction band at 400 nm, called out-of-plane dipole, which is the character of AgNSs.

2.2.2 Silver nanoplates

Silver nanoplates (AgNPls) have been broadly used in many researches and industries due to their tunable optical property. By adjusting their morphology, the optical, physical and chemical properties of AgNPls change.

In industrial field, the antibacterial property of AgNPls was applied due to their stability comparing with AgNSs and silver ions. In research field, AgNPls were applied in many approaches such as substrate for raman spectroscopy or biological and chemical sensor.

Herein, AgNPls were fabricated using AgNSs as initial seeds and 30 % wt. H_2O_2 as transforming agent [18, 19]. By adjusting molar ratio between H_2O_2 and AgNSs, the morphology of AgNPls can be tuned in varieties and resulting in various optical characteristic and colloidal solution colors.
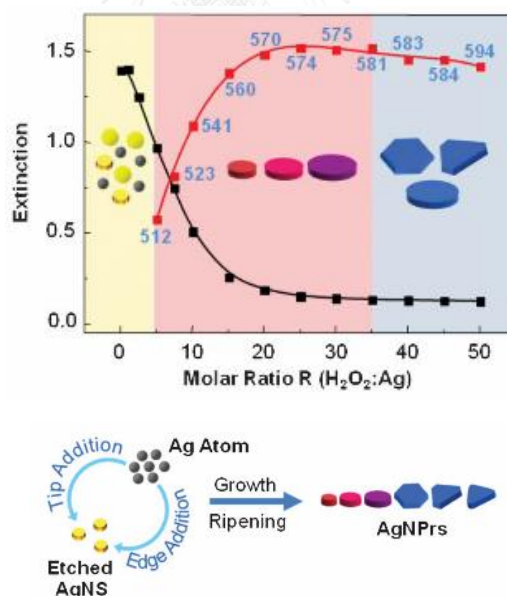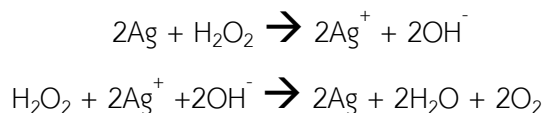

Figure 2.3 The shape conversion reaction of AgNSs and H_2O_2

The reaction follow

$$2Ag + H_2O_2 \rightarrow 2Ag^+ + 2OH^-$$

$$H_2O_2 + 2Ag^+ + 2OH^- \rightarrow 2Ag + 2H_2O + 2O_2$$

After H_2O_2 was added into AgNSs, H_2O_2 underwent the catalytic decomposition leading to the decomposition of AgNSs at unstable facet, then, silver ions were reduced back on the more stable facet leading to the formation of plate-like structure and further transformed AgNSs to AgNPls.

Due to LSPR phenomena, the changing in morphology of AgNPls leads to the changing in extinction spectra of AgNPls and resulting in the changing in color of the AgNPl colloidal solution.

2.3   Decomposition of silver nanoplates

Due to the different properties and potential applications of AgNPls, the morphology controlling approaches were studied and the decomposition reaction was brought to interest.

To control morphology of AgNPls, the decomposition reaction was chosen due to the fast and rapid reaction between AgNPs and etchants. Previously, the decomposition of AgNPls was studied using halide ions such as Cl^-, Br^- or I^-. The decomposition of AgNPls induced by halide ions can rapidly observe after etchants were added through the changing in color of AgNPl solution. Halide ions firstly selectively coordinate with Ag atoms on the active facet of AgNPls forming halide-coordinate Ag and dissociate from the main structure. After that, the halide-coordinate Ag forms clusters with halide ions as stabilizer and redeposit on the surface of deformed AgNPl structure and transform into AgNSs. This phenomenon can be observed through the changing in LSPR spectra, color changing and the

schematic of the decomposition mechanism can be carried out using transmission electron micrographs.
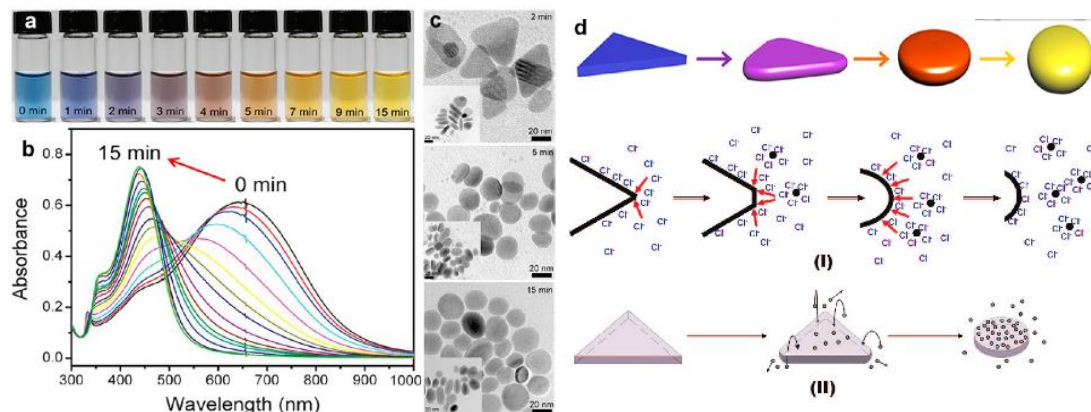
Figure 2.4 The decomposition mechanism of AgNPls induced by halide ions

In other hand, the decomposition profiles of AgNPls induced by H_2O_2 were yet to study. The studies about the effect of H_2O_2 to the decomposition of Ag nanostructures were mostly based on AgNSs. The objective of all the studies was to demonstrate the possibility in applying the changing in LSPR band of AgNSs as the spectroscopic sensor for determining the quantity of H_2O_2 [8, 10, 20, 21].

Figure 2.5 The decomposition of AgNSs induced by H_2O_2

For AgNPls, the decomposition of AgNPls was applied as colorimetric hydrogen peroxide sensor but none has ever applied as quantitative colorimetric hydrogen peroxide sensor. The morphology and color changing of AgNPls were studied and used as LSPR base sensor for H_2O_2, but none has ever clarify the capability in using the changing in color of AgNPls as quantitative sensing for H_2O_2. In this research, not only AgNPls were applied as quantitative LSPR base sensor for H_2O_2 but the changing in color of AgNPl solution was also coupled and applied as quantitative colorimetric sensor for hydrogen peroxide.

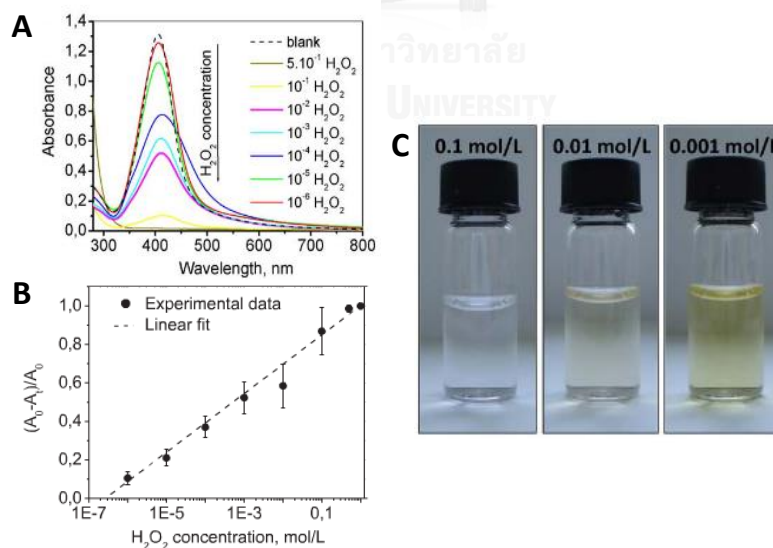## 2.4   Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is widely used in many industries as a strong oxidizing agent in water treatment, circuit cleaning and wound cleaning. In the presence of silver, H_2O_2 is possible to act as both reducing agent and oxidizing agent depending on the pH of the solution. The chemical reactions of oxidative and reductive reaction between H_2O_2 and silver are shown below [19]

H_2O_2 as a reducing agent

$$H_2O_2 + 2OH^- \rightarrow 2H_2O + O_2 + 2e^-$$
$$H_2O_2 + 2Ag^+ + 2OH^- \rightarrow 2Ag + 2H_2O + O_2$$

H_2O_2 as oxidizing agent

$$Ag^+ + e^- \rightarrow Ag$$
$$H_2O_2 + 2e^- \rightarrow 2OH^-$$
$$2Ag + H_2O_2 \rightarrow 2Ag^+ + 2OH^-$$
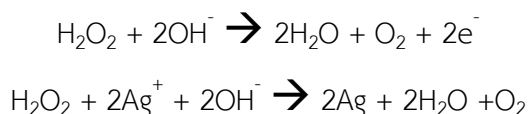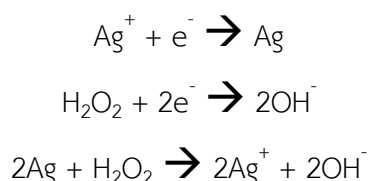
The reaction leads to the decomposition of silver metal into silver ions, thus, in case of AgNPls, the morphology changes, resulting in the changing in LSPR and solution color.

The fact that H_2O_2 can induce the cell damages made it important to detect the amount of H_2O_2 before releasing into environments. Conventionally, the determination of H_2O_2 based on the spectrophotometry or voltammetry [7-10, 20, 22] but these techniques are costly, bulky and complicated. Thus, the rapid, accuracy and portable technique such as colorimetric sensing is preferred.

In this research, when H_2O_2 was introduced to AgNPls, the AgNPls rapidly decomposed, leading to the changing in their morphology, resulting in changing of LSPR and solution color. Thus, the decomposition of AgNPls may apply as quantitative colorimetric sensing for hydrogen peroxide.

In addition, for biomolecules such as glucose or cholesterol, the reaction between these molecules and their oxidase enzyme produce H_2O_2 and in the presence of AgNPls, such reaction can induce the decomposition of AgNPls, thus, the decomposition profiles of AgNPls may also apply as potential quantitative colorimetric sensor for biomolecules.

2.5   Colorimetric sensing

Colorimetric method was brought to interest recently due to its simple and accurate result. The method uses the advantage of the aggregation of MNPs, by adding salt or using centrifugation, the MNPs aggregate, leading to the changing in optical property and significantly the solution color. However, the aggregation method required the modification on the surface of MNPs; thus, this method is not appropriate for quantitative sensing.

Previously, there are researches reported on utilizing AgNPs as colorimetric sensor for H_2O_2 but none of those can apply as quantitative colorimetric sensor. For example, Filippo E. et al. [10] reported the use of AgNSs as colorimetric hydrogen peroxide sensor. By determining the changing of LSPR band, the detection limit of AgNSs as hydrogen peroxide sensing can be as low as 10^{-6} M, but the changing in color cannot. The color of AgNSs after react with H_2O_2 fade away and hard to determine, thus, the use of AgNSs is not suitable for applying as quantitative colorimetric sensor for H_2O_2.
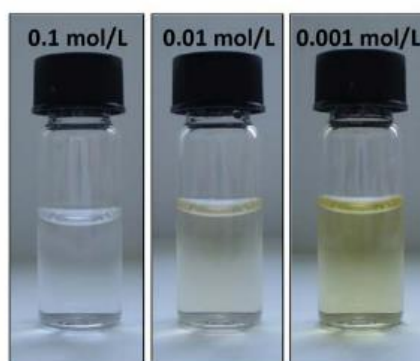
Figure 2.6 The color of AgNSs after react with various concentrations of H_2O_2

In this research, the decomposition of AgNPls induced by H_2O_2 result in a variety of solution colors corresponding to the H_2O_2 concentration added; thus, the decomposition profiles of AgNPls under oxidative condition using hydrogen peroxide may apply as colorimetric sensing for hydrogen peroxide.

Not only hydrogen peroxide sensing, the decomposition profiles of AgNPls can also apply as glucose sensing, due to the reaction between D-glucose and Glucose oxidase enzyme (GOx). The reaction between GOx and glucose produced H_2O_2, thus, in the present of AgNPls, the produced H_2O_2 leads to the decomposition of AgNPls resulting in the changing of AgNPl morphology and solution color. Xia Y. et al. [23] illustrated the colorimetric visualization of glucose using silver nanoprisms (AgNPrs). The study showed that the homogeneous solution of blue AgNPrs and GOx

can be used as colorimetric sensor for glucose with detection limit as low as 2.0×10^{-7} M.
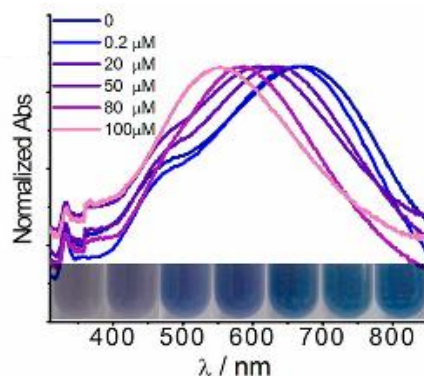
Figure 2.7   The changing in LSPR and color of AgNPr solution when using as colorimetric sensor for glucose

The changing in both LSPR and color of AgNPl solution depends on concentration of glucose, so, the decomposition profiles of AgNPls can be applied as colorimetric sensor for glucose and this colorimetric sensing required no surface modification.

Furthermore, in this research, the image processing method was used to help examining the changing in color. By using photograph of each experiment, the chromatic information of color code in red, green, blue (RGB) signal can be collected. The chromaticity level is related to the intensity of each RGB signal, thus, by applying this method to the decomposition of AgNPls, the changing in morphology of AgNPls can be detected and by this advantage, the quantitative analysis of colorimetric sensing can be carried out.

2.6   Characterization techniques

2.6.1 UV-Visible spectroscopy

UV-Visible spectroscopy is the common technique using to characterize nanoparticles. The technique measure the light that transmit through samples onto the detector. The extinction spectra of MNPs can be observed through the absorption band in the visible region of UV-visible absorption technique. Due to the relation between shape and size of nanoparticles with LSPR, the changing in shape and size of nanoparticles also affects the absorbance spectra in UV-visible technique. Since the changing in color of solution effects the changing of absorption spectra, thus, the changing in solution color of nanoparticles refers to the changing in size and shape of nanoparticles.

2.6.2 Transmission electron microscope

Transmission electron microscope (TEM) was a useful technique for studying the size, size distribution and morphology of MNPs. The technique using accelerated electron emitted in vacuum, deflected in a small angle by sample's atoms and transmitted through samples. These deflected electrons are magnified by magnetic lens and generate bright field on fluorescent screen.
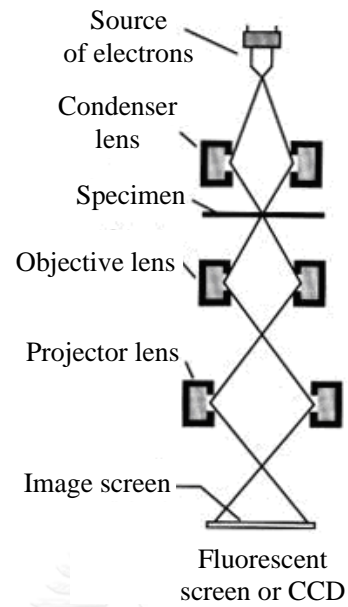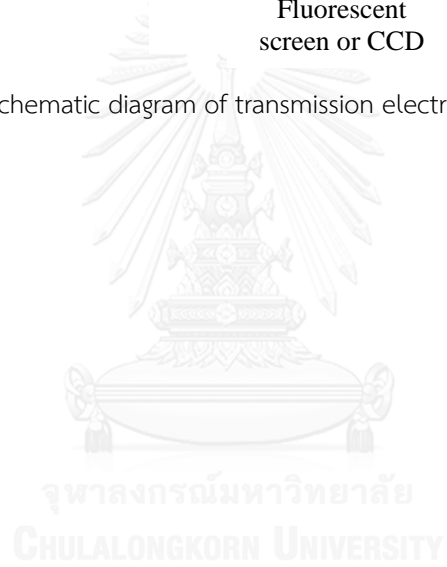
Source
of electrons

Condenser
lens

Specimen

Objective lens

Projector lens

Image screen

Fluorescent
screen or CCD

Figure 2.8 Schematic diagram of transmission electron microscope (TEM)

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CHAPTER III

EXPERIMENTS

3.1 Chemicals and materials

3.1.1 Silver nitrate (Carlo Erba.)

3.1.2 Sodium borohydride (MERCK)

3.1.3 Soluble starch (MERCK)

3.1.4 30 % wt. Hydrogen peroxide (MERCK)

3.1.5 D-glucose (Carlo Erba.)

3.1.6 Glucose Oxidase from Aspergillus niger (Sigma-Aldrich)

3.2 Silver nanoplate fabrication

3.2.1 Synthesis of starch-stabilized silver nanospheres (200 ppm)

Silver nanospheres (AgNSs) were synthesized with soluble starch as a stabilizer [19]. Firstly, a soluble starch solution of 2% (w/v) was prepared by dissolving 20 g of soluble starch in 1000 mL of boiling deionized water and heated for another 30 minutes. After the starch was completely dissolved, the solution was cooled down to room temperature. Next, the silver nitrate solution was prepared by dissolving 0.315 g $AgNO_3$ in 500 mL of the 2 % (w/v) soluble starch solution and the sodium borohydride solution was prepared with 0.109 g $NaBH_4$ dissolved in 500 mL of 2 % (w/v) soluble starch solution. To generate starch-stabilized silver nanospheres, the prepared $AgNO_3$ solution was slowly injected into the $NaBH_4$ solution under vigorous stirring using syringe at rate of 2 mL/s. Dark brown AgNSs colloidal solution

at 200 ppm was slowly developed. The excess $NaBH_4$ was eliminated by gently heating the colloidal AgNS solution for 2 hours. . The synthesized AgNSs were characterized by UV-visible absorption spectrophotometer.

3.2.2 Synthesis of starch-stabilized silver nanospheres (500 ppm)

Following the process described in 3.2.1, 500 ppm AgNSs can be synthesized by using $AgNO_3$ to 0.787 g and $NaBH_4$ to 0.2590 g.

3.2.3 Silver nanoplate fabrication

Silver nanoplates (AgNPls) were synthesized via shape transformation reaction of silver nanospheres (AgNSs). The shape transformation of AgNSs to AgNPls was induced by 30 % wt. hydrogen peroxide (H_2O_2) [18, 19].

Red silver nanoplates were synthesized by injecting 0.158 mL of 30 % wt. H_2O_2 into 50 mL of 200 ppm AgNSs under vigorous stirring using syringe pump (NE-1000 Programmable Single Syringe Pump, New Era Pump System, Inc.) at rate of 10.09 mL/min. The colour of the solution gradually changed from yellow to red wine at mole ratio of H_2O_2:AgNSs equal to 15:1. The generated AgNPl solution was incubated for 24 h. to ensure the reaction was achieved and excess H_2O_2 was completely decomposed. The synthesized AgNPls were characterized by UV-visible absorption spectrophotometer.

Follow the red AgNPls fabrication, 500 ppm AgNSs were converted to blue AgNPls by injecting 1.051 mL of 30 % wt. H_2O_2 into 50 mL of 500 ppm AgNSs under vigorous stirring using syringe pump (NE-1000 Programmable Single Syringe Pump, New Era Pump System, Inc.) at rate of 4.204 mL/min and incubated for 24 h. The mole ratio of H_2O_2:AgNSs is 50:1. The synthesized AgNPls were characterized by UV-visible absorption spectrophotometer.
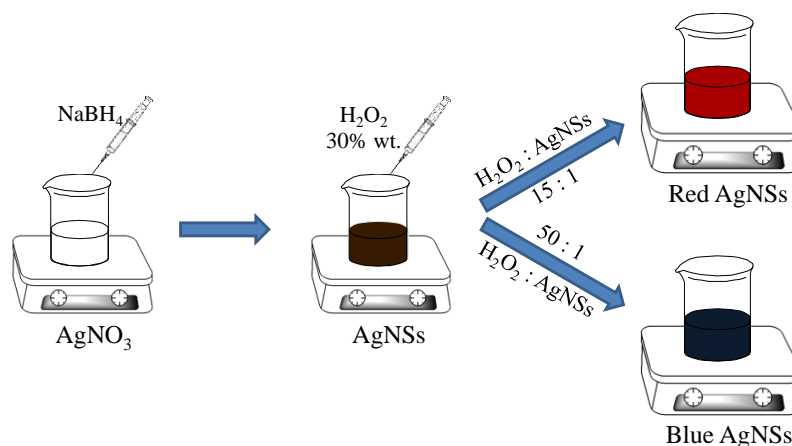
Figure 3.1 AgNPls fabrication procedure

3.3 Decomposition profiles of silver nanoplates induced by H_2O_2

AgNPls and 30 % wt. H_2O_2 were diluted to the desired concentrations using deionized water. 20 ppm of AgNPls was mixed with various concentrations of H_2O_2 at volume ratio 1:1 (v/v) and monitored the decomposition reaction with UV-visible spectrophotometer from incubating time 1–120 minutes. The relationship between the wavelength shifts, $\Delta\lambda_{max}$, of in-plane plasmon resonance band (λ_{max} of the original AgNPls – λ_{max} of decomposed AgNPls at each time) was plotted against the incubating time to monitor the decomposition reaction.

3.4 Protocol for naked eye H_2O_2 sensing

10 ppm of AgNPls was mixed with various concentrations of H_2O_2 at volume ratio of 1:1 (v/v) and monitored the changing in LSPR through time. The relation between $\Delta\lambda_{max}$ and concentrations of H_2O_2 was illustrated to find the linearity relation (regression model). In addition, the solution color was monitored and investigated in order to develop as a potential naked eye sensor for H_2O_2.
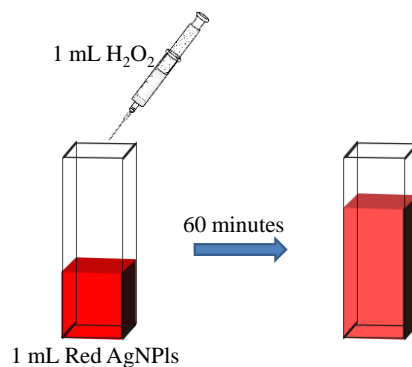
Figure 3.2 Schematic for H_2O_2 sensing using AgNPls

3.5   Influences of pH, temperature on the sensing capability

The effect of pH and temperature on AgNPls was tested to investigate the capability of AgNPls in using as colorimetric sensor. The temperature was varied from 28 $^{\circ}$C (room temperature) to 90 $^{\circ}$C using water bath. For pH, pH was varied from 3 – 10 using either 20 % acetic acid or 10 % ammonium solution.

3.6   Protocol for naked eye glucose sensing

D-glucose stock solution (5.55 x 10^{-3} M) was prepared by dissolving 0.01 g of D-glucose in 10 mL of deionized water and diluted to the desire concentrations, 20 – 100 μM. 10 mg/mL glucose oxidase enzyme was prepared by dissolving 20 mg of glucose oxidase enzyme in 2 mL of deionized water. After that 1 mL of 20 ppm silver nanoplates was mixed with 20 μL of glucose oxidase enzyme and incubated for 5 minutes, then 1 mL of desire glucose concentration was added into the mixture and incubated for 1 h. The changing in LSPR was monitored through UV-visible absorption spectrophotometer. In addition, the solution color was monitored and investigated in order to develop as a potential naked eye sensor for glucose.
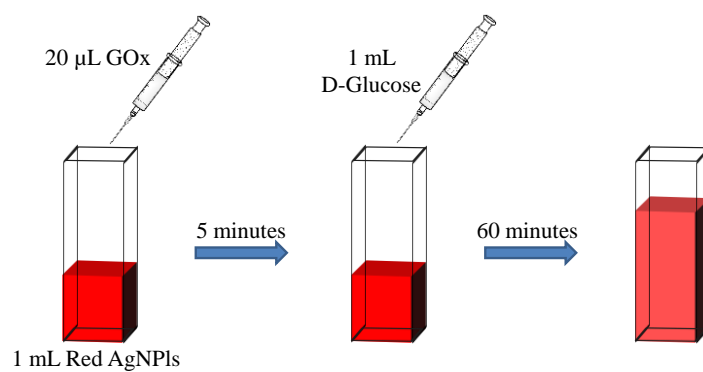
Figure 3.3 Schematic for glucose sensing using AgNPls

CHAPTER IV

RESULTS AND DISCUSSION

4.1   Silver nanoplate fabrication

Silver nanospheres were firstly synthesized via the reduction reaction of silver nitrate ($AgNO_3$) with sodium borohydride ($NaBH_4$) using soluble starch as stabilizer.

$$Ag^+ + BH_4^- \rightarrow Ag^0 + \tfrac{1}{2}H_2 + \tfrac{1}{2}B_2H_6$$

The dark brown solution was suddenly observed after slowly mixing $AgNO_3$ into $NaBH_4$ in soluble starch solution. This indicates that the silver nanospheres were generated. The dark brown colloidal solution shows the characteristic dipole LSPR of silver nanospheres, monitored by UV-visible absorption spectroscopy (λ_{max} = 400 nm) as shown in figure 4.1. The pristine AgNSs have a single LSPR at 400 nm with a narrow full width at half maximum (FWHM) of 55 nm indicating spherical AgNSs generated with narrow size distribution (high uniformity). In this study, AgNSs were prepared with concentration at 200 and 500 ppm as a stock solution to be used in further experiments.
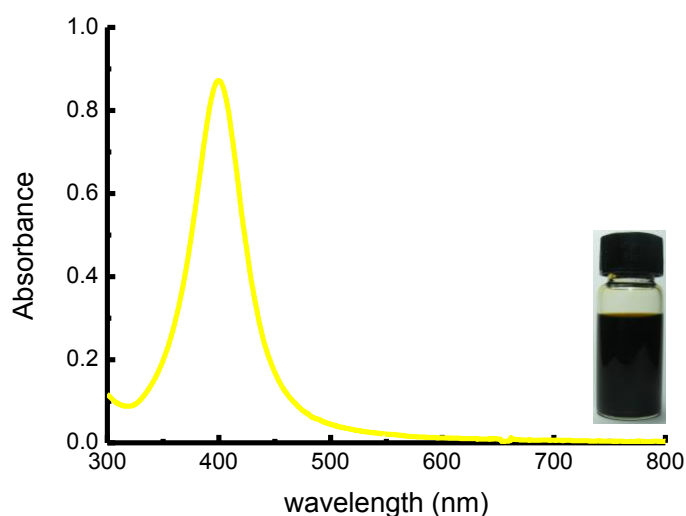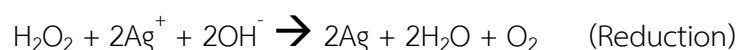
Figure 4.1 UV-visible absorption spectrum and inset photograph of AgNSs

To fabricate silver nanoplates (AgNPls), H_2O_2 was used, in order to transform AgNS morphological structure. By mixing hydrogen peroxide with AgNS colloidal solution, AgNSs can immediately convert into AgNPls. Shape and size of the developed AgNPls can be controlled by adjusting a different mole ratio of H_2O_2:AgNSs.   It is theoretically possible that hydrogen peroxide can function as both a facet selective etchant toward metallic Ag and a reducing agent for ionic Ag species. The total reaction of H_2O_2 which act as etching agent and reducing agent is shown below [19].

$$2Ag + H_2O_2 \rightarrow 2Ag^+ + 2OH^- \qquad \text{(Etching mechanism)}$$

$$H_2O_2 + 2Ag^+ + 2OH^- \rightarrow 2Ag + 2H_2O + O_2 \qquad \text{(Reduction)}$$

H_2O_2 is known as a strong oxidizing agent but in this study, it was used to convert the morphology of AgNPs from AgNSs to AgNPls via Oswald-ripening reaction. For evolution of AgNSs by H_2O_2, hydrogen peroxide was firstly utilized as the powerful oxidative etching agent which singles out the seeds with highest stability.

Secondly, the etched ionic Ag (Ag^+) is reduced to form Ag^0 by the reducing species from H_2O_2 on the specific facets. H_2O_2 can act as reducing agent due to the pH of the solution. In acidic environment, H_2O_2 act as strong oxidizing agent but in alkaline environment, H_2O_2 can play a role as mild reducing agent. In the study, the shape conversion reaction was performed in a slightly alkaline condition; therefore, hydrogen peroxide can reduce Ag^+ to the stable seed of AgNPls. This process promotes the formation of AgNPls as shown in scheme 4.1 [19].
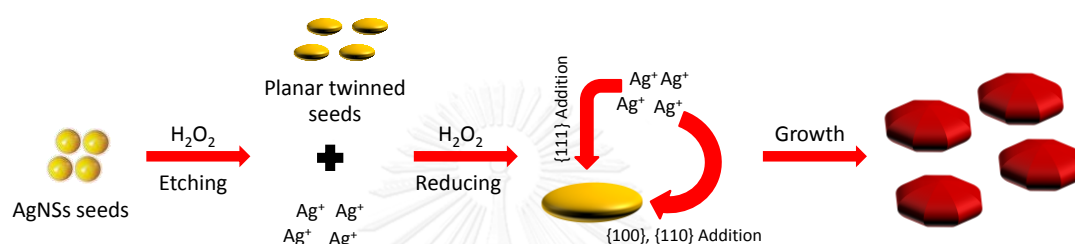
Scheme 4.1 The formation of AgNPls using H_2O_2

For red-AgNPls, 30% w/v H_2O_2 was injected into 50 mL of 200 ppm AgNSs at mole ratio of H_2O_2:AgNSs equal 15:1 to convert yellow AgNSs into red AgNPls. After adding H_2O_2 into AgNS colloidal solution under vigorous stirring, the color of the colloid immediately changed from yellow to red. This indicates that a sphere-to-plate conversion is achieved. The red colloidal solution shows two new distinct LSPR bands as shown in figure 4.2.
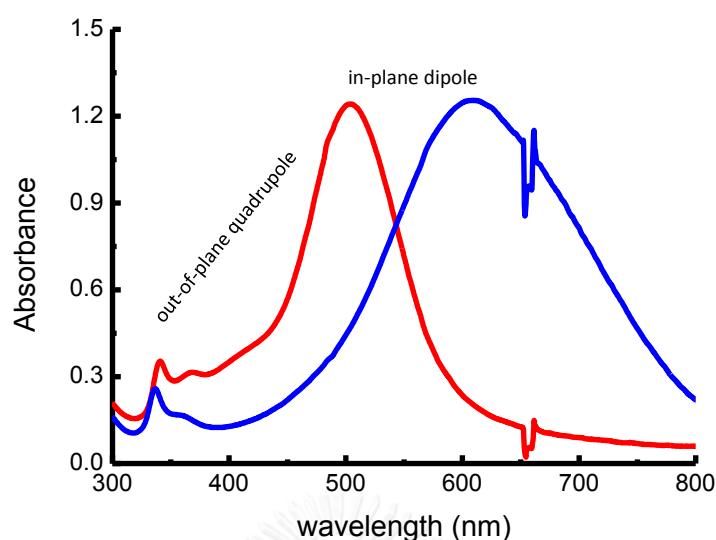
Figure 4.2 UV-visible spectrum shows characteristic LSPR bands of red- and blue- AgNPls

A LSPR band at 504 nm associates with an in-plane dipole oscillation, which directly relates to lateral size of AgNPls, and another one at 340 nm represents an out-of-plane quadrupole oscillation corresponding to aspect ratio (thickness/lateral size) of AgNPls. This observation suggests that AgNSs were converted to plate-like AgNPs. An absence of LSPR band around 400 nm suggests that there are no AgNSs or residual of small-diameter nanoparticles in the final colloid. It indicates that the conversion of AgNSs to AgNPls is completely achieved.

For blue-AgNPls, 50 mL of 500 ppm AgNSs was used. The mole ratio of H_2O_2:AgNSs used in the shape conversion of AgNSs to AgNPls was adjusted to 50:1. After mixing H_2O_2 into AgNSs, the dark yellow colloidal solution turned to dark blue colloidal solution indicating the morphological changes of AgNSs. With an increase in hydrogen peroxide, the in-plane dipole oscillation is red-shifted up to ~600 nm. The red-shift implies a particle growth with an increase in lateral size or plate diameter.

The red-AgNPls were smaller in lateral size compared with blue-AgNPls due to a lower wavelength of in-plane dipole LSPR band. Lower maximum wavelength of

the LSPR band indicates higher energy of the oscillating electron on the surface of AgNPls following the equation; $E=hf$, when $f = c/\lambda$ (where E = energy of oscillated electrons, h = planck's constant, f = oscillated electron frequency, c = speed of light and λ = wavelength (nm)) [11, 12, 15-17]. In the case, particle with the high oscillating energy (high oscillating frequency) represents small lateral size of AgNPls. On the other hand, particle with low oscillating energy is obtained with a large lateral size. To sum up, a lower wavelength of in-plane dipole LSPR band indicate a smaller plate size of AgNPls. For out-of-plane quadrupole LSPR band, the band relates to the aspect ratio, the ratio of lateral size over thickness, of the plate-like structure. A similar maximum wavelength at ~340 nm is obtained in both red and blue AgNPls suggesting that the aspect ratio of red- and blue- AgPls are similar.

4.2   Decomposition profiles of AgNPls induced by H_2O_2

The decomposition profiles of AgNPls were studied using 20 ppm of AgNPls mixing with H_2O_2 at volume ratio of 1:1 and monitored using UV-visible absorption technique. Real time monitoring experiment was performed in order to gain the insight information of the decomposition mechanism of AgNPls induced by H_2O_2. The changing in in-plane dipole LSPR band of AgNPls after H_2O_2 was introduced can be used to demonstrate the changing in lateral size of AgNPls. In addition, the changing of out-of-plane quadrupole LSPR band can be used to reveal the changes of aspect ratio of AgNPls. In this study, AgNPls with 20 ppm were prepared by diluting the stock solution with customized deionized water which is slightly acid. Therefore, hydrogen peroxide could acts as oxidizing agent due to the acidic environment of AgNPls in this study.

For both red- and blue-AgNPls, 20 ppm of AgNPls was mixed with 100 µM of H_2O_2 and monitored for 15 minutes using UV-visible absorption spectrophotometer.

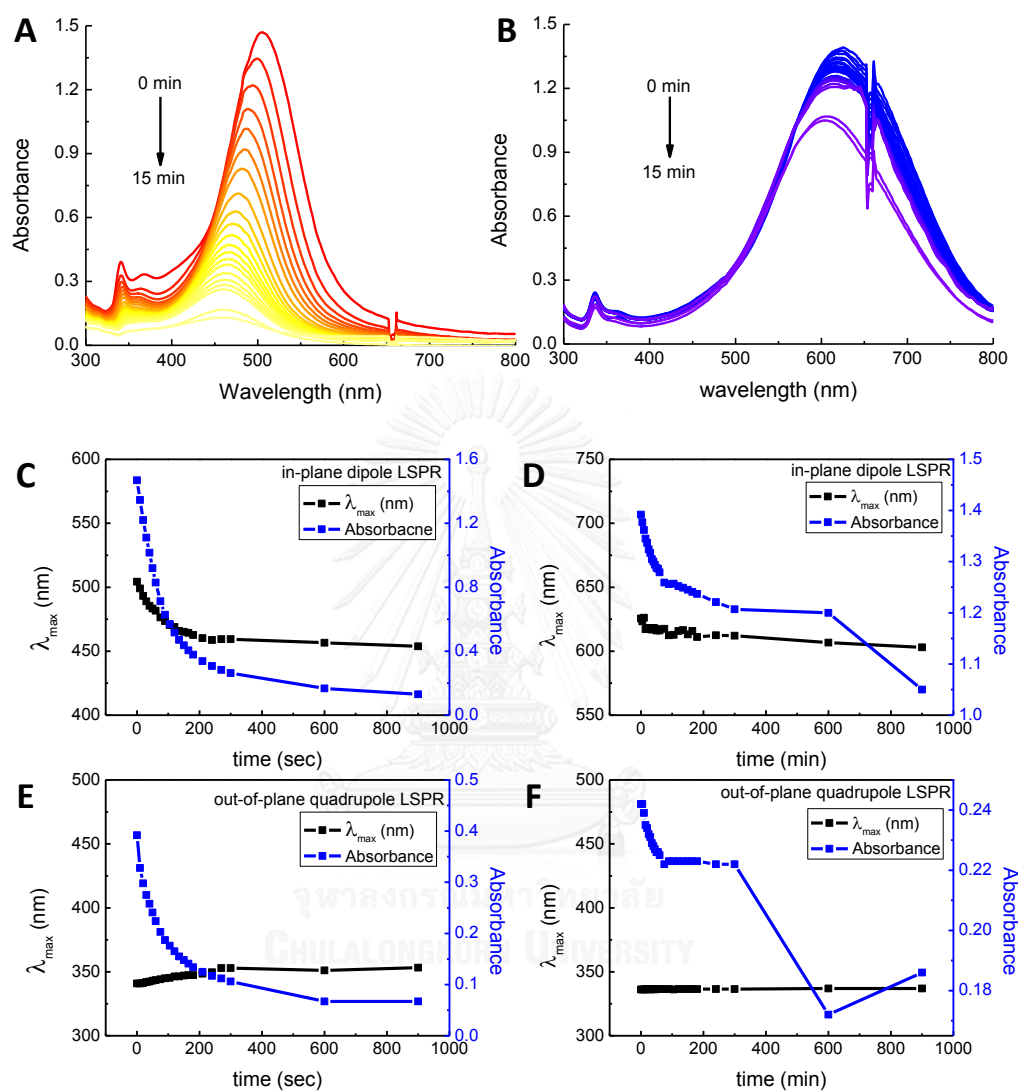The extinction spectra show that the decomposition immediately occurred after the addition of H_2O_2.

Figure 4.3   The real time decomposition spectra and plots between the changing of in-plane dipole and out-of-plane quadrupole LSPR band of (A), (C), (E) red- and (B), (D), (F) blue-AgNPls after the addition of H_2O_2 100 μM for 15 minutes

From figure 4.3, the spectra of red-AgNPls changed immediately after H_2O_2 was added into AgNPl solution. For in-plane dipole LSPR band, the intensity drastically decreased with approximately 50 nm of blue-shifts, shifted to lower wavelength. This phenomenon indicates that a lateral size of AgNPls immediately decreases after H_2O_2

was added. In case of out-of-plane quadrupole LSPR band, the intensity decreased and slight red-shift, shift to higher wavelength, was observed. This result suggests that the aspect ratio of AgNPls slightly changed after H_2O_2 was added which implied that the thickness of AgNPls also changed or decreased along with the decreasing in lateral size. From the decomposition profiles, it can be seen that H_2O_2 act as unselectively etching agent because both facet {110}, {100} (thickness) and facet {111} lateral size of the metallic silver are concurrently etched. The decomposition profile of blue-AgNPls also gives a very similar trend. However, the shifting of in-plane dipole LSPR was smaller than that of red-AgNPls and no shifting in out-of-plane quadrupole LSPR was observed. These results demonstrate that blue-AgNPls also decreased in both lateral size and thickness but less than that of red-AgNPls. From AgNPl fabrication procedure [19], it shows that red-AgNPls are thicker than blue-AgNPls. Thus, red-AgNPls tend to survive longer after the decomposition and able to show the shifting in in-plane dipole LSPR while blue-AgNPls tend to decompose to thinner plate-like structure and turn to silver ions before the changing in lateral size can affect the LSPR.

The decreasing in intensity LSPR can refer to the decreases of the AgNPl population. This etching reaction of hydrogen peroxide induces the degradation of silver atoms on AgNPls to silver ions as follow:

$$H_2O_2 + 2Ag \rightarrow 2Ag^+ + 2OH^-$$

To verify the proposed etching mechanism, an experiment was performed using 1,000 µM of H_2O_2, added into 20 ppm of red AgNPls at volume ratio 1:1 (v/v) and incubated for 2 hours. The solution after incubation turned from red to transparency solution. After that, a drop of freshly prepare $NaBH_4$ (strong reducing agent) was added into transparency solution and incubated for another 1 hour. The

spectra and color of all solutions were shown in figure 4.4. After adding H_2O_2, AgNPls were decomposed, therefore, the solution turned from red to transparency. In-plane dipole LSPR band of red-AgNPls was vanished without any new developed band. This suggests that no nanoparticles presents in the system. After adding $NaBH_4$ into the transparency solution, the solution turned yellow with a generated LSPR band at ~400 nm. This LSPR band reveals the characteristic band of the generated AgNSs. From the test, it indicates that AgNPls were decomposed to silver ions (Ag^+) and the released Ag^+ ions were reduced by $NaBH_4$ back to AgNSs.
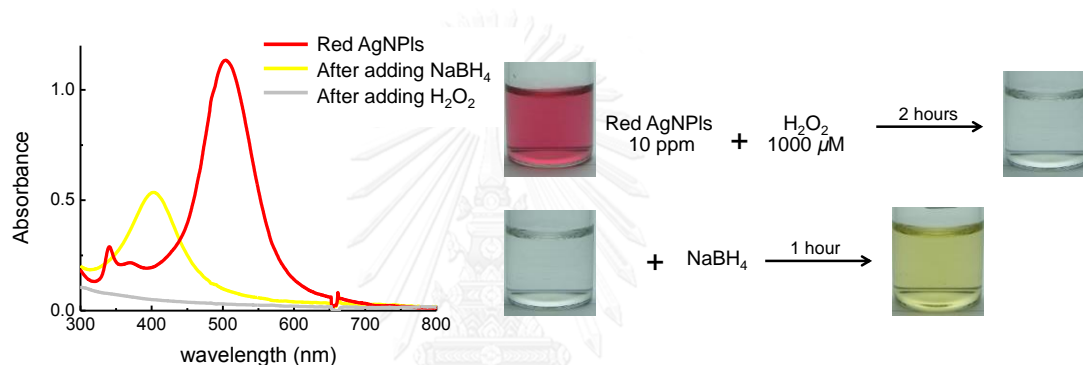

Figure 4.4   Digital images and UV-visible spectra with LSPR bands of red-AgNPls decomposed red-AgNPls induced by H_2O_2, and decomposed red-AgNPl solution reduced by $NaBH_4$

From the observations, the in-plane LSPR peak of AgNPls blue-shifted and the intensity decreased in the presence of H_2O_2. This suggests that the population of the AgNPls might be etched or destroyed by H_2O_2. To gain an insight into the morphological transformation of AgNPls, TEM analysis was employed. Figure 4.5 shows the TEM micrographs, including the colloidal solution of the initial AgNPls and the AgNPls with orange and yellow color obtained after the etching process by H_2O_2 at 50 and 100 μM, respectively.
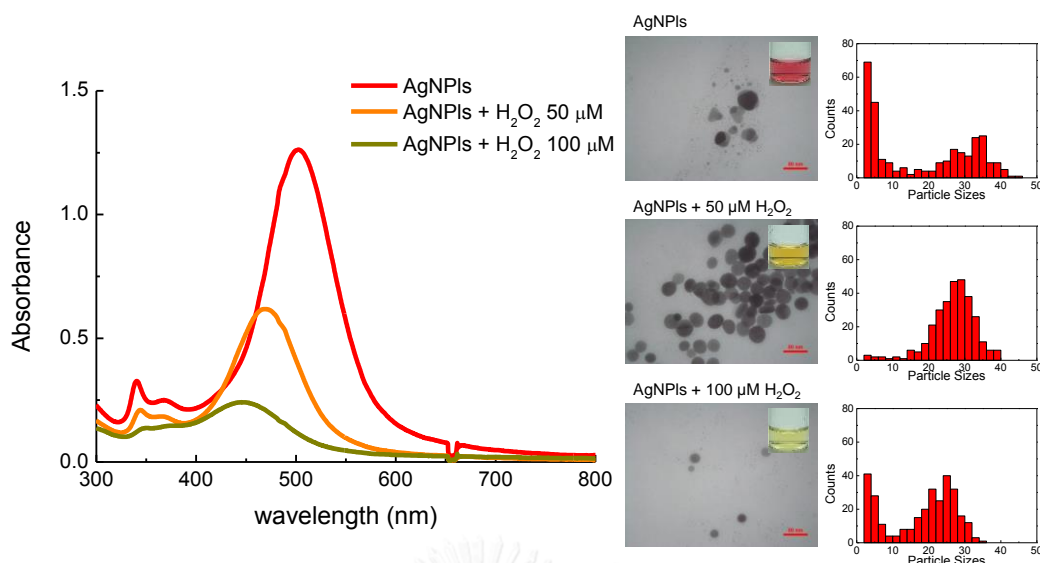
Figure 4.5   Digital images, LSPR spectra, TEM images and size distribution of initial red-AgNPls and red-AgNPls after introduced H_2O_2

The lateral size distribution plots of AgNPls were measured from 300 representative particles using ImageJ program [24]. The initial red-AgNPls before interacting with H_2O_2 exhibited regular nanodisks with various sizes, with two distributions of edge lengths at average size of ~5.5 and 32 nm. When the initial AgNPls were incubated with H_2O_2, most of the AgNPls were rounded and transformed into uniform nanodisks with a unimodal lateral length distribution centered at average size of ~27 nm. The small initial AgNPls at ~5 nm disappear suggesting that the small AgNPls were completely decomposed into Ag^+. When AgNPls were exposed to H_2O_2 at a concentration of 100 μM, the AgNPls were still in a disk shape. However, the two distributions of edge lengths with an average size of ~5.5 and 24 nm were observed again. This phenomenon suggests that the AgNPls randomly disintegrated in every dimension from a larger disk to a smaller disk. The lateral length contraction of AgNPls could result in a drastic change in the in-plane dipole plasmon resonance position, correlating with a large shift of the colloidal solution color from red wine to orange and yellow. The extent of the AgNPl decomposition

directly correlated with the H_2O_2 concentration. The morphological decomposition of AgNPls by H_2O_2 etching represents the pathway by which AgNPls degenerated to smaller nanodisks accompanying silver ion solubilization. This observed phenomenon is different from the previous reports on halide ion etching of AgNPls. Halide ions, i.e. Cl^-, Br^-, and I^-, can selectively modify the AgNPls by rounding the tip or edge which finally yields nanodisks or nanospheres while H_2O_2 totally disintegrates AgNPls to Ag^+ without preference of the final shape. A schematic drawing of the possible morphological degeneration mechanism is shown in scheme 4.2.
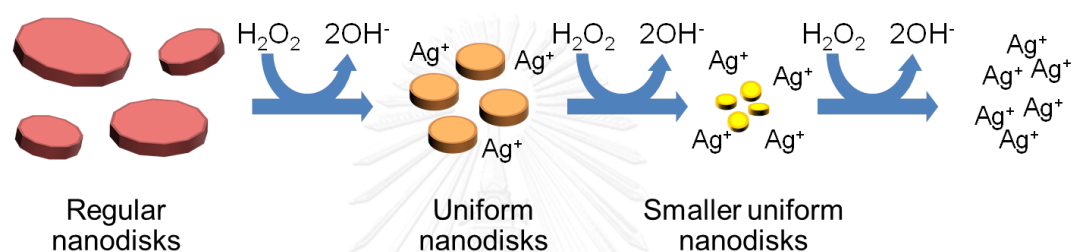
Scheme 4.2 The decomposition mechanism of AgNPls induced by H_2O_2

4.3 Protocol for naked eye H_2O_2 sensor

After studied the decomposition mechanism of AgNPls, red-AgNPls was chosen to apply as a naked eye sensor due to its variety of color when react with H_2O_2 while blue-AgNPls was observed fading but no significant changing in color.
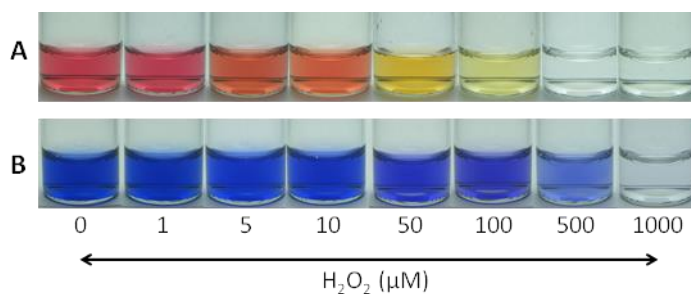

Figure 4.6   Color comparisons between (A) red- and (B) blue-AgNPls after incubating with various concentration of H_2O_2 for 60 minutes

Various concentrations of H_2O_2, 1 – 1,000 µM, were introduced to the prepared 20 ppm red-AgNPls to find the suitable range of H_2O_2 concentration for applying as colorimetric sensor. The change in its optical characteristics with incubation time (0, 5, 15, 30, 60 and 120 min) was monitored through the in-plane dipole LSPR band (at ~504 nm) in the extinction spectra. The changing of $\Delta\lambda$ ($\Delta\lambda$ = λ_{max} of initial AgNPls – λ_{max} of AgNPls after incubated with H_2O_2) represents the changing of the optical characteristics of the LSPR-based hydrogen peroxide sensor with time is shown in figure 4.7A.
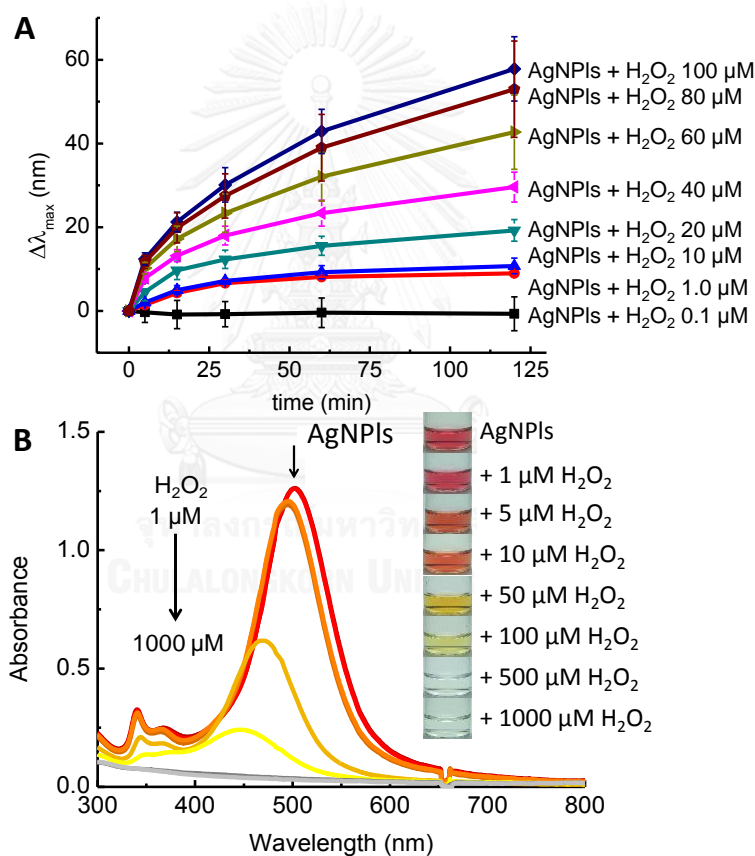

Figure 4.7   (A) $\Delta\lambda_{max}$ of AgNPls after incubated with various concentrations of H_2O_2 at different times and (B) Digital images and LSPR spectra of red AgNPls after incubated with 1 – 1000 µM H_2O_2, for 60 minutes

A drastic change in $\Delta\lambda$ could be observed after 60 minutes of incubation time. This suggests that the optimal incubation time to investigate the change of the optical characteristic of AgNPl solution interacting with the added H_2O_2 was 60 minutes. Figure 4.7B shows the extinction spectra and the corresponding solution color of AgNPls after reacting with H_2O_2 at various concentrations for 60 minutes. The color of AgNPl suspension changed noticeably from red to orange, yellow and transparent, respectively, corresponding with the amount of H_2O_2 added. In addition, a blue-shift of the in-plane dipole LSPR band of AgNPls and a concomitant decrease of the peak intensity were observed. On the other hand, the out-of plane quadrupole LSPR band at 340 nm was unchanged. These observed phenomena indicate that the lateral size of AgNPls decreased, but the particle aspect ratio remained the same. Therefore, the thickness of AgNPls concomitantly decreased along with the lateral size. The spectral change of AgNPls in the solution is a manifestation of the morphological transformation that occurred with the AgNPls. From figure 4.7B, the sensitivity of the corresponding color change of AgNPls is promising in the range of H_2O_2 concentrations 10-100 μM with the incubating time at 60 minutes.

The suitable concentration of AgNPl solution was considered due to the effect of that to color of the solution. Various concentrations of red AgNPls (5 – 50 ppm) were validated with H_2O_2 concentration in the range of 1 – 100 μM.
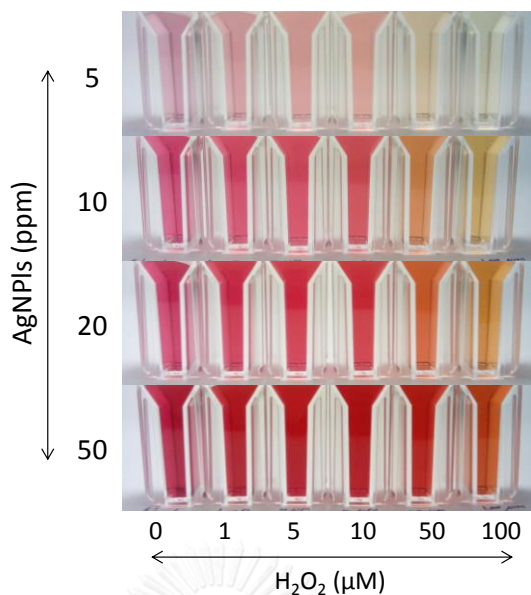
Figure 4.8 The colors of AgNPl solution after incubation with various concentrations of H_2O_2

After incubated AgNPls with H_2O_2 for 60 minutes, the color of AgNPl solution changed depending on H_2O_2 concentration. A distinct color change of the AgNPls (Red $\rightarrow$ dark orange $\rightarrow$ light orange $\rightarrow$ yellow $\rightarrow$ pale yellow) can be clearly observed as the concentration of H_2O_2 increases. Thus, this colorimetric method for detection of H_2O_2 is promising and more importantly the detection range and the accuracy of the sensor can be tuned by adjusting the concentration of AgNPls used for the detection. The larger concentration gradient of AgNPls might provide a wider detection range but lower accuracy in colorimetric measurement (color is too vivid and difficult to differentiate). Therefore, the 10-20 ppm of AgNPls were chosen to use for hydrogen peroxide colorimetric sensor.

After optimized the conditions e.g. incubating time, concentration of AgNPls, concentration range of H_2O_2, the sensitivity and other performance indices of the proposed H_2O_2 naked-eye sensor were further investigated. As discussed above, the AgNPls were completely disintegrated to silver ions and the colloidal solution became clear and transparent, when AgNPls were exposed to H_2O_2 at a

concentration higher than 100 μM. Therefore, H_2O_2 concentration at 100 μM was set as an upper limit for naked eye detection. A series of H_2O_2 solutions with various concentrations ranging from 10-100 μM were added to the homogenous system of the AgNPl solution and incubated for 60 minutes. A relationship between the LSPR band shifts ($\Delta\lambda$) calculated from the difference of the in-plane LSPR peak from the original AgNPls and the transformed AgNPls, along with the corresponding solution color, are illustrated in figure 4.9.
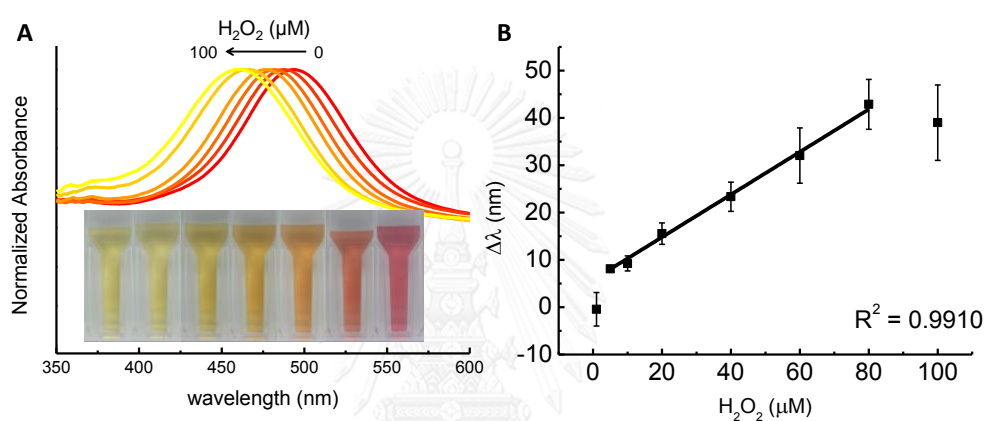

Figure 4.9   (A) Normalized absorbance spectra with observing color of red-AgNPls after incubating with various concentrations of H_2O_2 and (B) the relation between LSPR band shifts and H_2O_2 concentrations

As shown in figure 4.9, a gradual blue shift of the in-plane LSPR band and a distinct color change of the AgNPl solution (red $\rightarrow$ orange $\rightarrow$ yellow) can clearly be observed as the H_2O_2 concentration increases. In addition, a good linear relationship between the LSPR peak shifts ($\Delta\lambda$) and the concentrations of H_2O_2 ranging from 10-80 μM with R^2 = 0.9910 is observed. The limit of detection, LOD (calculated from 3.3 × standard deviation / slope of the regression line) for H_2O_2 was 1.57 μM.

The contrastable color change of starch-stabilized AgNPls responding to the interaction with H_2O_2 offers the possibility for developing the AgNPls as a quantitative naked-eye sensor for H_2O_2. Since the extent of color change of starch-stabilized

AgNPls exposed to H_2O_2 is a systematic response to the H_2O_2 concentration, a colorimetric approach utilizing RGB image capturing is proposed for the quantitative determination of hydrogen peroxide. The digital photograph of each experiment was taken and converted into RGB chromatic level. A schematic drawing of the RGB imaging set up is shown in figure 4.10.
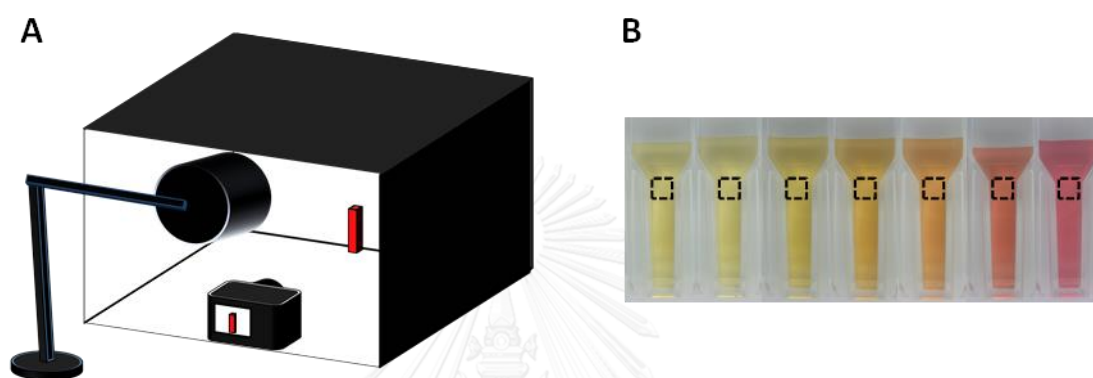

Figure 4.10   (A) Photo taking process and (B) The cropped area of each digital image for detecting the possibility in applying the decomposition profiles of AgNPls as colorimetric sensor

The photo taking process consists of a customized box (21.0 x 29.7 x 21.0 cm in dimensions), the FUJIFILM Finepix S5700 digital camera and 20 W SYLVANIA Minilyx Economy light source. The camera was adjusted follow: JPEG (3072 x 2304 pixels) and ISO 100. The distance between samples and the camera was fixed at 7.0 cm. Each sample was prepared by mixing 10 ppm of red AgNPls with various concentrations of H_2O_2 at range of 10 – 100 μM in transparent disposable cuvette. After image taking, the bottle neck of cuvette from each image was cropped into 30 x 30 pixels area and converted to RGB format using MATLAB version R2012a (7.14.0.739).   From RGB format, the red channel was selected to investigate the reaction due to the strongest intensity and highest selectivity compared to others.

The red chromaticity level (r) was calculated following (r) = R/(R+G+B) [25]. The relation between red chromaticity and H_2O_2 concentrations was plotted.
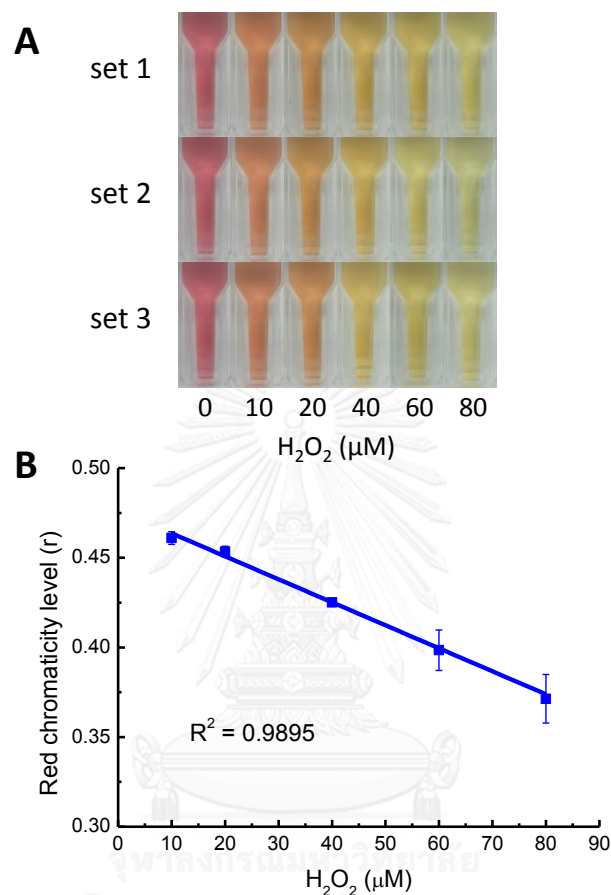

Figure 4.11 (A) Digital images of each experiment for detecting the changing in color of red AgNPls after incubated with H_2O_2 and (B) The relation between the changing in red chromaticity level and concentration of H_2O_2

From figure 4.11B, the plot between red chromaticity level and H_2O_2 concentrations of three experiments shows a good linear relation with R^2 = 0.9895. The linear relation suggests that the red chromaticity level decreased corresponding to the H_2O_2 concentrations. The results show that the different concentrations of H_2O_2 induced the different changing in color of AgNPl solution which corresponding to the changing in morphology of AgNPls. From the observations, the decomposition

profiles of AgNPls induced by H_2O_2 can be applied as potential H_2O_2 quantitative colorimetric sensor. The LOD using $\Delta\lambda$ and red chromaticity level using AgNPls as chemical sensor is 1.57 µM and 6.19 µM respectively. Table 4.1 summarizes the detection limit (LOD) of our optimized procedure and of other comparable procedures based on utilizing silver nanoparticles combined with either spectroscopic technique or colorimetric approach. Our protocol is comparable with the other works utilizing silver nanoparticles and UV-visible spectroscopy for H_2O_2 detection. In fact, the sensitivity of our sensors can be improved by decreasing the concentration of AgNPls because the greater concentration of AgNPls, the higher H_2O_2 concentration is required. However, a concentration (20 ppm) of AgNPls was employed in order to prevent the color distortion of AgNPl solution under the light illumination.

Table 4.1 Limit of H_2O_2 detection from previous reports comparing with this work

Year	References	LOD	Technique	Detection mechanism
2011	P. Vasileva, et al., Colloids Surf. A., 2011, 382, 203. [8]	0.9 µM	UV-Visible Spectroscopy	Decomposition of starch-stabilized silver nanoparticles
2012	V.K. Shukla, et al., J. Hazardous Material, 2012, 213, 161. [26]	1 µM	Potentiometry	Amperometric response of silver nanoparticle modified on GC electrode
2012	G.L. Wang, et al., Biosens. Bioelectron., 2012, 31, 337. [20]	10 nM	UV-Visible Spectroscopy	Aggregation of functional silver nanoparticles
2013	C.K. Tagad, et al., Sens. Actuators, B, 2013, 183, 144. [27]	10 µM	UV-Visible Spectroscopy	Decomposition of LBG polysaccharide stabilized silver nanoparticles

2014	S. Mohan, et al., Carbohydr. Polym., 2014, 106, 469. [21]	1 µM	UV-Visible Spectroscopy	Decomposition of spherical silver nanoparticles
2014	This work	1.57 µM	UV-Visible Spectroscopy	Decomposition of silver nanoplates
		6.19 µM	Colorimetric	

4.4 Stability of AgNPl as chemical sensor

In our system, AgNPls were stabilized by soluble starch. Starch molecules can be degraded and fragmented to smaller organic acids (e.g. formic acid, acetic acid, etc.) under specific circumstances, such as alkaline treatment and oxidative environments. In order to investigate whether soluble starch was chemically interfered with H_2O_2, i.e. whether hydrogen peroxide acted as a strong oxidizing agent, or not. The molecular characteristics of starch on AgNPl surfaces under various experimental conditions were further investigated by ATR FT-IR spectroscopy (as shown in figure 4.12).
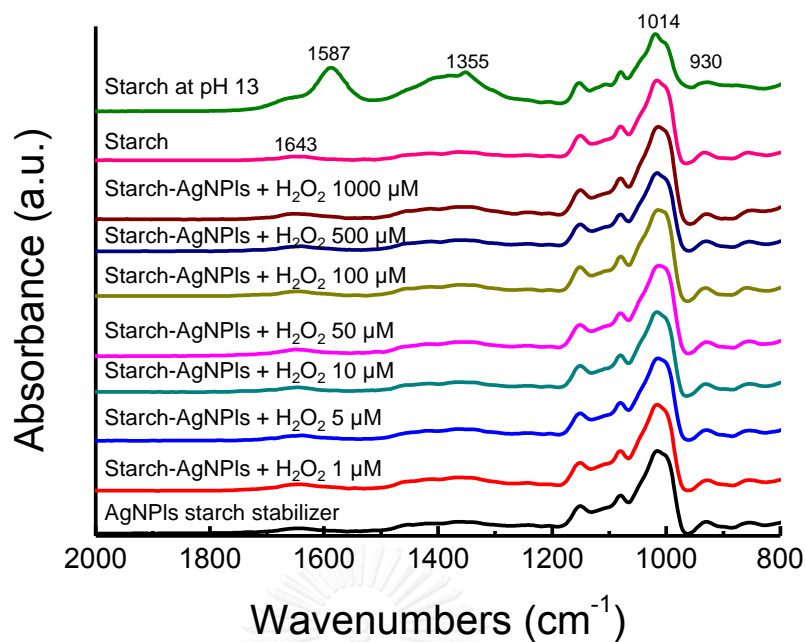
Figure 4.12 ATR-FTIR spectra of starch-stabilized AgNPls after incubated with various concentration of H_2O_2, soluble starch and decomposed soluble starch at pH13

Table 4.2 Band assignment of soluble starch

Infrared band (cm^{-1})	Infrared band assignment
860	CH$_2$ deformation
930	Skeleton mode vibration of α-1,4 glycosidic linkage (C-O-C)
1200-900	Bridge β C^1-O-C^4
1500-1300	Vibration band related to the carbon and hydrogen atoms
1610-1500/1420-1300	COO$^-$ stretching vibration (carboxylic acid salt)
1642	Water adsorbed in the amorphous region of starch
1765	C=O stretching vibration of carboxylic acid

Starch has a few common ATR FT-IR bands at 1200-900 cm^{-1} and at 930 cm^{-1} representing the bridge (β C_1–O–C_4) stretching and the skeleton mode vibration of the α-1,4 glycosidic linkage (C-O-C), respectively. From the ATR FT-IR spectra of pure starch, starch-stabilized AgNPls and starch-stabilized AgNPls after the addition of H_2O_2, no significant change in these common bands was observed at any concentration of H_2O_2. Starch can be oxidatively degraded by H_2O_2 only when divalent metal ions, notably Fe^{2+} or Cu^{2+}, were presented [28, 29]. However, after the decomposition reaction, there were only monovalent metal ions (Ag^+) present in our system. Therefore, there was no appropriate catalyst for starch degradation and soluble starch did not interfere with our analysis. These results suggested that H_2O_2 directly interacted only with AgNPls.

The influences of pH and temperature might affect the sensitivity and the stability of our chemical sensors (AgNPls). The influence of incubation time (analyzing time) is previously discussed in Figure 4.7. The effect of pH and temperature on the synthesized of AgNPls was monitored by using UV-visible spectroscopy. A pH of AgNPl solution was adjusted to pH 3-10 using either 20% v/v of acetic acid or 10% (v/v) of NH$_4$OH. A temperature of AgNPl solution was adjusted in the range of 28°C (room temperature) – 90°C using water bath. The Δλ of AgNPls and corresponding solution color at various pH and temperature are depicted in figure 4.13. The results reveal that no significant changes in LSPR of AgNPls (Δλ = 0) in the range of pH 3-8. In addition, no considerable change in LSPR of AgNPls is observed in the temperature range of 28-45 $^\circ$C. The Δλ increases and the color of the AgNPl solution become pale orange only when pH > 9 or temperature > 45 ºC. From the results, we believe that our sensor based on the decomposition of AgNPls works well in a normal temperature and pH range.
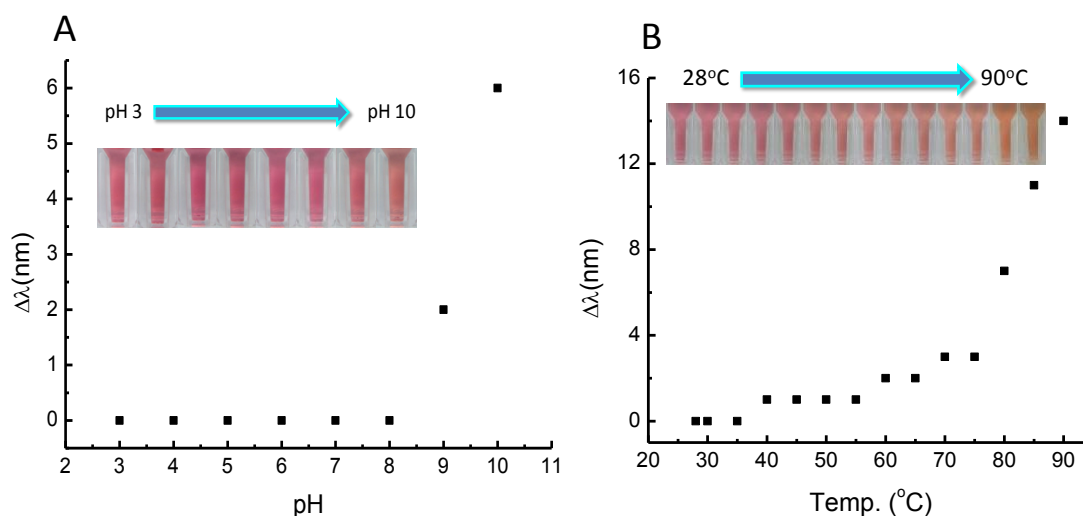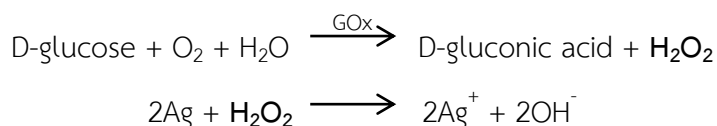
Figure 4.13 The effect of pH and temperature on color and LSPR of AgNPls

4.5 Protocol for glucose sensing

We further investigated the sensitivity and performance of the developed chromatic analysis protocol for the quantitative determination of glucose utilizing AgNPls. The shape transformation and corresponding solution color of AgNPls were caused by the etching reaction of H_2O_2 generated from the enzymatic oxidation of glucose [23].

$$D\text{-glucose} + O_2 + H_2O \xrightarrow{GOx} D\text{-gluconic acid} + \mathbf{H_2O_2}$$

$$2Ag + \mathbf{H_2O_2} \longrightarrow 2Ag^+ + 2OH^-$$

A series of glucose solutions with concentrations ranging from 20 to 100 μM were added to the homogenous AgNPl solution mixed with glucose oxidase enzyme (GOx) and incubated for 60 minutes. As shown in figure 4.14, the color of AgNPls changed from red →orange→yellow with respect to the concentrations of D-glucose. The changing in color of AgNPls was due to the H_2O_2 which created from the reaction between GOx and D-glucose. The created H_2O_2 induced AgNPls to be

decomposed resulting in the changing in color and LSPR band. The higher D-glucose concentration generates large amount of H_2O_2 which induces the shifts in color and LSPR band. The shifting in LSPR band of AgNPls after mixing with GOx and D-glucose was linearly related to the concentrations of added D-glucose with R^2 = 0.9945. The results suggest that the decomposition mechanism of AgNPls induced by H_2O_2 has potential to apply as chemical sensor to quantify glucose.
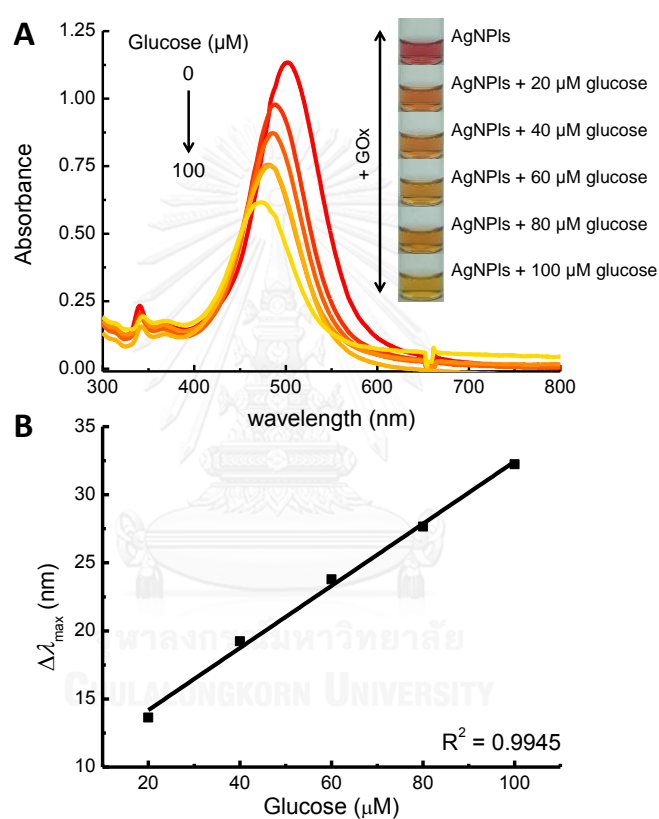

Figure 4.14 (A) LSPR spectra and digital images of AgNPls and (B) The changing of in-plane dipole LSPR band after incubated with various concentrations of D-glucose

To demonstrate the potentiality in applying the AgNPl as naked eye sensor to quantify glucose, the set up experiment with 10 ppm of red AgNPls, 10 mg/mL of GOx and various concentrations of D-glucose (20 – 100 μM) was performed. A distinct color change of the AgNPl solution and a red chromaticity level shift can be

observed as the concentration of glucose increased. A good linearity between the red chromaticity level and the glucose concentration ranging from 20–60 µM was observed with R^2 = 0.9801. Therefore, glucose could also be determined at a micro-molar level using the chromatic analysis approach with an accuracy and precision comparable to reported nanoparticle-based techniques.
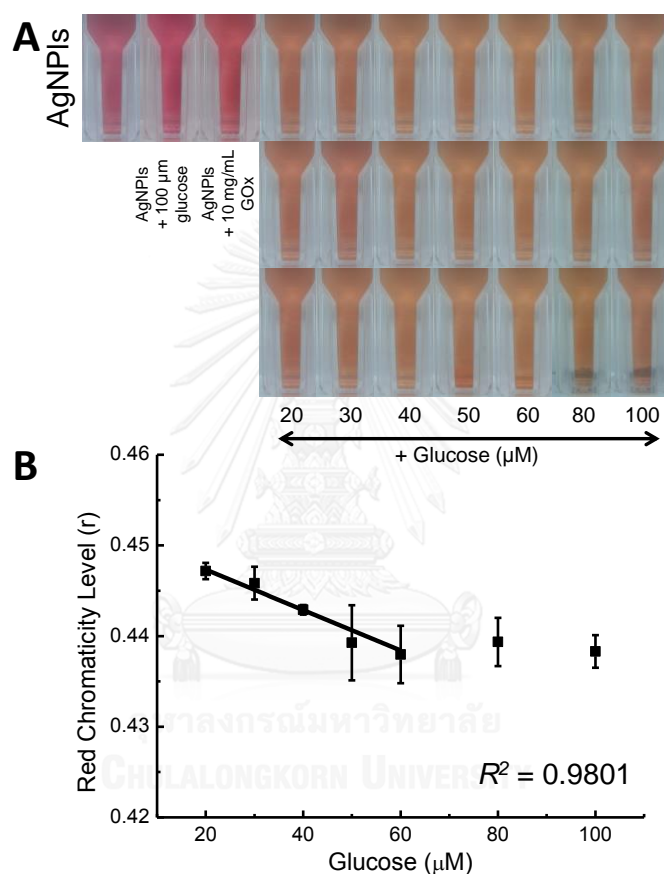

Figure 4.15   (A) Digital images and (B) The changing of red chromaticity level of red-AgNPls after incubated with various concentrations of D-glucose for 60 minutes

Furthermore, we also investigated the effects of glucose analogues species e.g. fructose, lactose, maltose and sucrose to red chromaticity level of the AgNPls. These species were separately added to the AgNPls with glucose oxidase enzyme and the red chromaticity level of AgNPls was measured under identical conditions (Figure 4.16). It can be seen that the red chromaticity level dramatically dropped only in the

glucose system, while the red level remains almost unchanged in the other systems.

This suggests that this protocol gives very high selectivity for glucose determination.
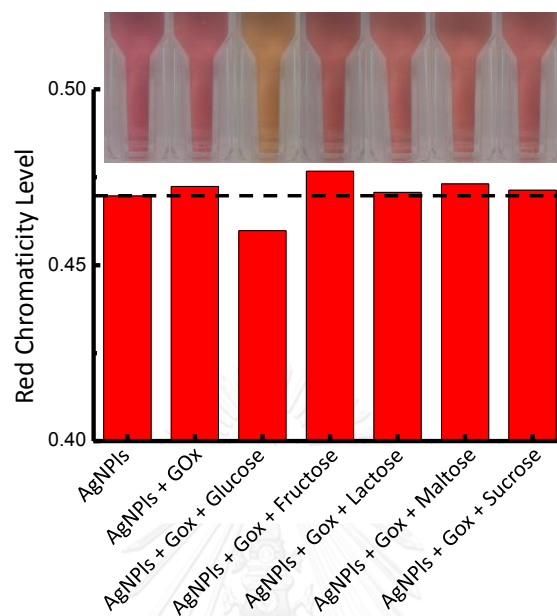

Figure 4.16   The changing in Red Chromaticity Level of AgNPls after 60 minutes incubation with

different analytes

CHAPTER V

CONCLUSIONS

Silver nanoplates (AgNPls) were fabricated through the green synthesis via the shape transformation of starch-stabilized silver nanospheres (AgNSs) to nanoplates induced by hydrogen peroxide. The shape conversion was carried out by injecting hydrogen peroxide into a starch-stabilized AgNSs under ambient conditions. The in-plane dipole LSPR band of AgNPls can be controlled by the mole ratio of H_2O_2:AgNSs. By the fabrication protocol, the in-plane dipole LSPR can be selectively tuned across visible and near infrared regions. In this study, the red-AgNPls and blue-AgNPls were fabricated by controlling the mole ratio of H_2O_2:AgNSs equal to 15:1 and 50:1, respectively. The decomposition profiles of AgNPls under oxidative conditions inducing by H_2O_2 were investigated by UV-visible spectroscope and transmission electron microscope (TEM). The decomposition mechanism is spontaneous and rapid. It started immediately after the addition of H_2O_2. The decomposition progress can be monitored in real time with the shifts of in-plane LSPR band of AgNPls and with the naked eye via the color change of the colloidal solution. TEM images reveal the decomposition phenomenon which initiates with the oxidative etching of the regular silver nanodisks into smaller and rounder particles. The morphological decomposition of AgNPls by H_2O_2 etching represents the pathway by which AgNPrs degenerated to smaller nanodisks accompanying silver ion solubilization.

From the observations, the color change of AgNPl solution is directly correlated with the morphological decomposition of AgNPls when interacted with H_2O_2. Systematic color changes of red-AgNPls when exposed to H_2O_2 at

submicromolar concentrations can be clearly observed with naked eyes. In this study, the application of using AgNPls as colorimetric H_2O_2 sensor was demonstrated. Red-AgNPls was chosen as colorimetric sensor due to the strong color changing during the decomposition reaction induced by H_2O_2. A good linear relation between LSPR shift and H_2O_2 concentrations in the range of 10 – 80 µM was obtained with R^2 = 0.9910. The limit of H_2O_2 detection using LSPR shift was 1.57 µM. Furthermore, the simple and rapid setup of image analysis utilizing a hand-held digital camera in combination with the red chromaticity level extracted from the captured digital images of AgNPl solutions was proposed in order to simplify the quantitative analysis protocols of hydrogen peroxide. A very low hydrogen peroxide concentration at 6.19 µM can be recognized by using the set-up strategy with good accuracy, stability and reproducibility. The strategy can be further applied for the quantitative analysis of glucose by determining the hydrogen peroxide generated from the glucose oxidase enzyme reaction.

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APPENDIX

Analytical Methods

PAPER

Colorimetric determination of hydrogen peroxide by morphological decomposition of silver nanoprisms coupled with chromaticity analysis†

Kritchapon Nitinaivinij, Tewarak Parnklang, Chuchaat Thammacharoen, Sanong Ekgasit and Kanet Wongravee*

A novel colorimetric method with image colour analysis for highly sensitive and accurate detection of hydrogen peroxide using starch-stabilized silver nanoprisms (AgNPrs) is proposed. AgNPrs were morphologically decomposed by a low concentration of hydrogen peroxide revealed by UV-visible absorption spectroscopy and transmission electron microscopy (TEM). The morphological changes of AgNPrs led to an appreciable colour change in the AgNPr solution from red to orange, and finally yellow. A good linear relationship between the wavelength shift of AgNPrs and the H_2O_2 concentration can be obtained. The solution phase detection of H_2O_2 by the direct morphological change can be accomplished without any surface modification of AgNPrs. In addition to the conventional determination of the H_2O_2 concentration utilizing spectroscopic data, a new and simple colorimetric strategy based on the chromaticity analysis of AgNPr solution was demonstrated. The strategy can be employed not only for visual detection of H_2O_2 by the naked eye but also for reliable and convenient methods for quantification of H_2O_2. The hydrogen peroxide concentration at 1.57 μM can be recognized by naked-eye observation with good accuracy, stability and reproducibility. Furthermore, the proposed protocol can be applied to determine the glucose concentration through the glucose-oxidase system. A good linearity between the red chromaticity of the solution colour and the glucose concentrations was observed. The new colorimetric determination of hydrogen peroxide utilizing digital image analysis on colour changes from AgNPr shape decomposition will open up an alternative method for simple, rapid and reliable detection of hydrogen peroxide and can realize its future applications in biochemical analysis or clinical diagnosis.

Sensor Research Unit (SRU), Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330. E-mail: kanet.w@chula.ac.th; Fax: +66 2218 7585; Tel: +66 2218 7589

Introduction

Metal nanoparticles (MNPs) have stimulated a great deal of research interest due to their shape- and size-dependent optical, electrical and chemical properties.[1-3] The well-known optical property of MNPs, called "localized surface plasmon resonance (LSPR)", is based on the coupling between an external electromagnetic field and the collective electron oscillations in the conduction band of the metal. The shape and size of MNPs present in a dielectric environment can induce different collective oscillations of electrons on MNP surfaces. In the case of silver and gold nanoparticles, this unique nano-associated property is strongly related to the colour of the colloidal MNP solution observed in the UV-visible region which can be employed in various applications such as visual sensors.[4-8]

Colorimetric approaches have extensively grown in chemical sensing applications due to their simplicity in detection by the naked eye. With the recent advancement of nanotechnology, designing colorimetric sensors by MNPs is a promising field which shows increasing interest, especially for gold and silver nanoparticles, which contain a strong plasmonic signal. This

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