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DETECTION OF GLUCOSE BY SILVER NANOPLATES PAPER-BASED SENSOR

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ใช้อนุภาคเงินระดับนาโนเมตรแบบแผ่นเพื่อตรวจวัดไฮโดรเจนเปอร์ออกไซด์และกลูโคสที่ความเข้มข้นต่ำ ๆ โดยอาศัยการสลายตัวของอนุภาคเงินระดับนาโนเมตรแบบแผ่น โดยเตรียมกระดาษที่ถูกดัดแปรด้วยอนุภาคเงินระดับนาโนเมตรแบบแผ่นแบบใหม่ขึ้นโดยการตรึงอนุภาคเงินระดับนาโนเมตรแบบแผ่นบนกระดาษกรองที่เคลือบด้วย 3-เมอแคปโตโพรพิล ไตรเมทอกซีไซเลน สำหรับการตรวจวัดไฮโดรเจนเปอร์ออกไซด์ สีของกระดาษที่ถูกดัดแปรด้วยอนุภาคเงินระดับนาโนเมตรแบบแผ่นเกิดการฟอกจางเนื่องจากเกิดออกซิเดชันของอนุภาคเงินระดับนาโนเมตรแบบแผ่นด้วยไฮโดรเจนเปอร์ออกไซด์เมื่อจุ่มกระดาษทดสอบลงในสารละลายไฮโดรเจนเปอร์ออกไซด์ สามารถใช้กระดาษทดสอบในการตรวจวัดไฮโดรเจนเปอร์ออกไซด์ในช่วงความเข้มข้น 0.7 ถึง 3.0 มิลลิโมลาร์ได้ด้วยตาเปล่า และสามารถได้ข้อมูลในเชิงปริมาณโดยใช้โปรแกรมอิมเมจเจ สำหรับการตรวจวัดกลูโคสทำการเติมกลูโคสออกซิเดสลงในตัวอย่างปัสสาวะเพื่อทำปฏิกิริยากับกลูโคสและทำให้เกิดไฮโดรเจนเปอร์ออกไซด์ ในการวิเคราะห์นี้ ได้ใช้กระดาษแผ่นยาวที่เคลือบด้วยอนุภาคเงินระดับนาโนเมตรแบบแผ่น โดยใช้วิธีเติมสารมาตรฐานและการเจือจางสารตัวอย่างเพื่อลดผลกระทบจากองค์ประกอบของปัสสาวะ และเติมซิลเวอร์ไนเตรตลงในสารละลายตัวอย่างปัสสาวะเพื่อตกตะกอนคลอไรด์ ซัลเฟต และฟอสเฟตไอออน ในการตรวจวัดของกลูโคสในตัวอย่างปัสสาวะ มีช่วงความเป็นเส้นตรงของการตรวจวัด คือ 0 ถึง 1.4 มิลลิโมลาร์ ค่าร้อยละการได้กลับคืนของกลูโคสในการวิเคราะห์ของตัวอย่างปัสสาวะที่เติมกลูโคสอยู่ในช่วง 94–105% และมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ของผลการวิเคราะห์ในช่วง 5.9–21.4% สามารถใช้วิธีตรวจวัดโดยใช้กระดาษทดสอบนี้สำหรับการตรวจวัดกลูโคสในปัสสาวะ โดยให้ผลวิเคราะห์ที่มีความแม่นและความเที่ยงที่ยอมรับได้

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KRITCHAPORN KHOONRUGSA: DETECTION OF GLUCOSE BY SILVER NANOPLATES PAPER-BASED SENSOR. ADVISOR: ASST. PROF. FUANGFA UNOB, Ph.D., CO-ADVISOR: PROMPONG PIENPINIJTHAM, Ph.D., 57 pp.

Silver nanoplates (AgNPls) were employed to detect a low level of hydrogen peroxide (H_2O_2) and glucose based on the decomposition of AgNPls. A new AgNPls-modified paper was fabricated by depositing AgNPls on a filter paper coated with (3-mercaptopropyl) trimethoxysilane (MPTMS). For H_2O_2 detection, the color of AgNPls-modified paper was bleached due to the oxidation of AgNPls by H_2O_2, when immersed in H_2O_2 solution. It could be used to detect H_2O_2 in the concentration range from 0.7 to 3.0 mM by naked eyes detection and the quantitative data was measured *via* ImageJ software. For glucose detection, glucose oxidase was added into urine samples to react with glucose and generate H_2O_2. AgNPls-paper strip was prepared and used in this method. The standard addition method and sample dilution were performed to overcome the matrix effect from human urine. Chloride, sulfate and phosphate ions in urine samples were eliminated by adding $AgNO_3$ into the urine sample solutions to form precipitates. In the determination of glucose in urine samples, the dynamic linear range was in the range from 0 to 1.4 mM. The recovery of glucose added to urine samples in the range of 94 to 105% was obtained with the relative standard deviation of the results in the range of 5.9–21.4%. The paper-based method could be used for glucose detection in human urine with acceptable accuracy and precision.

Department:	Chemistry	Student's Signature	.....
Field of Study:	Chemistry	Advisor's Signature	.....
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LIST OF ABBREVIATIONS

M	Molar
mM	Millimolar
mg	Milligram
mg/L	Milligram per liter
µL	Microliter
µM	Micromolar
mL	Milliliter
cm	Centimeter
mm	Millimeter
°C	Degree Celsius
AgNPls	Silver nanoplates
AgNPs	Silver nanoparticles
AgNSs	Silver nanospheres
MPTMS	3-mercaptopropyltrimethoxysilane
LSPR	Localized surface plasmon resonance
H_2O_2	Hydrogen peroxide
GOx	Glucose oxidase

CHAPTER I
INTRODUCTION

1.1 Statement of background and problem

Glucose is one of 6-carbon monosaccharides. It can be found in many sources such as rice, fruits and vegetables. When glucose is taken into human body, it is stored in blood for nourishing cell and small amounts are released in urine.

Diabetes mellitus is a worldwide crucial health problem. According to the World Health Organization (WHO), there are globally 422 million people with diabetes and a number will probably be more than double in the next 20 years. In 2012, the direct cause of 1.5 million deaths was diabetes. Moreover, 80% of diabetes deaths are in low- and middle-income countries [1]. The glucose level in blood is very important because it relates to diabetes mellitus. A person with diabetes has insufficient insulin hormone production, which causes high level of glucose in blood. Two primary techniques convenient for determining diabetes include self-monitoring of blood glucose and self-monitoring of urine glucose. Generally, in clinical diagnosis detects the level of glucose in blood because of accurate values. However, glucose in blood detection is not appropriate with a person who is trypanophobia or cannot be injured. It is a drawback of this method. Another choice is the detection of glucose in urine. Normal person releases a little bit glucose concentration in urine. If glucose level in urine is higher than 25 mg/dL (1.4 mM), it indicates that the person may have something abnormal such as a high level of plasma glucose or a deteriorated renal glucose absorptive capacity [2-4].

Nowadays, the method for medical screening should be performed rapidly and easily using simple instruments. We should be able to bring the analysis on-site such as laboratory in a hospital and undeveloped country. Furthermore, the target analytes at low concentrations in sample should be detected with this method.

Paper-based colorimetric sensor, which is simple, portable, inexpensive and disposable, has been used in different applications including the detection of various

analytes such as metals, organic compounds, bacteria in foods, proteins and the analysis of lung cancer for rapid screening and diagnosis [5-10].

Currently, nano-size silver is widely used. Its properties such as optical property and catalytic activity depend on its shape and size. For example, spherical silver nanoparticles (AgNPs) show yellow color while silver nanoplates (AgNPls) with mixed geometries *e.g.,* hexagonal, truncated triangular, rounded-tip triangular prisms, and circular disks show red, purple, or blue colors. Because of localized surface plasmon resonance (LSPR), AgNPls show characteristic colors depending on their shape, size, and environment [11]. In this work, AgNPls are chosen due to a change in their distinct color with a change in size of AgNPls.

AgNPls are applied to determine a small amount of hydrogen peroxide (H_2O_2), which is a strong oxidizer. The decomposition (oxidation) of AgNPls by H_2O_2 causes an alteration in shape and size of AgNPls, which result in a change in the color of AgNPls. By following the change in color, the degree of AgNPls decomposition related to the amount of H_2O_2 could be determined [12].

In this research, AgNPls are used to detect H_2O_2 and glucose by following the decomposition of H_2O_2. From previous works, AgNPls were employed in solution system [12-17]. Each system is not portable and inconvenient for utilizing in daily life. Therefore, a new paper-based method was developed in this work to determine H_2O_2 and glucose. The (3-mercaptopropyl)trimethoxysilane (MPTMS) was used to coat filter paper on which AgNPls were further deposited. Glucose oxidase (GOx), a specific enzyme for glucose, is used to generate H_2O_2 from a glucose sample while silver nitrate ($AgNO_3$) is used to precipitate chloride, sulfate and phosphate ions present in human urine before detection. When the AgNPls on surface of modified filter paper reacted with generated H_2O_2, the color of AgNPls was bleached due to the oxidation of AgNPls by H_2O_2 to silver ion ($Ag^0 + 2H_2O_2 \longrightarrow Ag^+ + 2O^{2\bullet-} + 2H_2O$) [13]. Standard addition method was also applied to decrease the matrix effect in a real sample. The color change was observed by naked eyes and measured by ImageJ software to construct a calibration curve and obtain quantitative data.

1.2 Research objectives

1.2.1 To develop the paper-based sensor for determining H_2O_2 and glucose using AgNPls

1.2.2 To develop method for H_2O_2 and glucose detection by naked eyes and ImageJ software

1.2.3 To apply this method for glucose detection in real samples

1.3 Scope of the research

For the paper-based H_2O_2 detection, the concentration of H_2O_2 which is determined is in the range of 0–3 mM. The parameters affecting on glucose detection (*i.e.*, size of AgNPls, MPTMS concentration, AgNPls concentration, detection time, volume of sample solution) were optimized.

For the detection in human urine, real samples were obtained from Environmental Analysis Research Unit (EARU) members. The effect of AgNPls concentration, urine matrix, $AgNO_3$ volume and the presence of co-existing species such as urea, creatinine, ascorbic acid, chloride ions, sulfate ions and phosphate ions were examined.

The color intensity was observed by naked eyes and measured the taken photo *via* ImageJ software to construct a calibration curve and obtain quantitative data.

Glucose detection method was compared to standard method such as hexokinase method and Combur[7] and URS-2P dipsticks.

1.4 The benefit of this research

To obtain a new paper-based method for naked-eye detection of H_2O_2 and glucose using AgNPls.

CHAPTER II
THEORY AND LITERATURE REVIEW

2.1 Relationship between glucose and diabetes

Glucose is one of 6-carbon monosaccharides that can be found in many sources such as rice, fruits, beverages and vegetables. When glucose is taken into human body, it is stored in blood for nourishing cell and small amounts of it are released in urine.

Diabetes mellitus is a worldwide crucial health problem. According to the World Health Organization (WHO), there are globally 422 million people with diabetes and the number will probably be more than double in the next 20 years. In 2012, it was reported that the direct cause of 1.5 million deaths was diabetes. Moreover, 80% of diabetes deaths occurred in low- and middle-income countries [1]. Diabetes can be classified into 4 general categories: type 1 diabetes (due to ß-cell destruction, usually leading to complete insulin deficiency), type 2 diabetes (due to a progressive insulin secretory defecting on the background of insulin resistance), gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy) and specific types of diabetes due to other causes [18]. Patients with diabetes are at greater risk of having cardiovascular disease (CVD) and most of patients have type 2 diabetes. Glucose measurements are important for the diagnosis and treatment of abnormal of carbohydrate metabolism such as diabetes mellitus. A person with diabetes has insufficient insulin hormone production, which causes high level of glucose in blood. Two primary techniques convenient for detecting diabetes include self-monitoring of blood glucose and urinary glucose. Generally, the analysis of glucose in blood is usually performed in clinical diagnosis as it gives the accurate values. However, this kind of detection is not appropriate with a person who has trypanophobia or cannot be injured. The detection of urinary glucose is an alternative for the preliminary diagnosis or screening.

Small amount of glucose are normally excreted by the kidney [19]. The result from glomerular filtration is glucosuria, glucose in urine. It happens in all normal individuals in the level of glucose up to 25 mg/dL (1.4 mM). An abnormally increasing glucosuria level (> 25 mg/dL in fresh urine) can be the result from either a high level of plasma glucose or a deteriorated renal glucose absorptive capacity or both. Many semi-quantitative glucosuria tests are commercially available. However, they can detect only high glucose level in urine (50–250 mg/dL or 2.8–14 mM) [3]. Moreover, human urine contains different species such as urea, creatinine and salts [20-22] that may have interfering effect on glucose detection. Hence the detection of low concentration of glucose in urine is difficult.

2.2 Silver nanoparticles (AgNPs)

Nano-size silver is used in many applications including using as antibacterial agent, biosensing due to their unique properties [23]. The important properties such as optical property and catalytic activity strongly depend on its shape and size. For example, spherical silver nanoparticles (AgNPSs) show yellow color while silver nanoplates (AgNPls), 2D structure of AgNPs, with mixed geometries *e.g.,* hexagonal, truncated triangular, rounded-tip triangular prisms, and circular disks show red, purple or blue color. When the electromagnetic wave couple to the collective oscillations of the electrons on nanoparticles surface, it leads to strong absorption and scattering as shown in Figure 2.1. This phenomenon is called localized surface plasmon resonance (LSPR). Consequently, AgNPls show characteristic colors in the visible region of the spectrum depending on their shape, size and environment. Therefore, a designed chemical interaction between the analyte and AgNPls surroundings can lead to a change of color for visual determination of the target analyte. Thus, nanoparticle LSPR-shift based determination provides a high sensitivity for biosensing [11, 24-26].

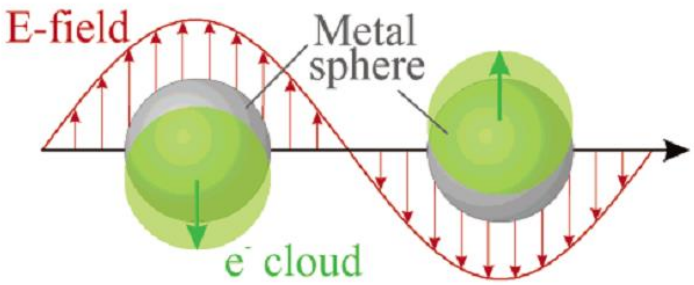


Figure 2.1 The coupling between electromagnetic field and the collective oscillations of the electrons on metal nanoparticles surface [27].

Metal nanoparticles having the different shapes and sizes affect the LSPR because of different patterns of electron oscillation on the metal nanoparticles surface resulting in the different patterns of LSPR as displayed in Figure 2.2.

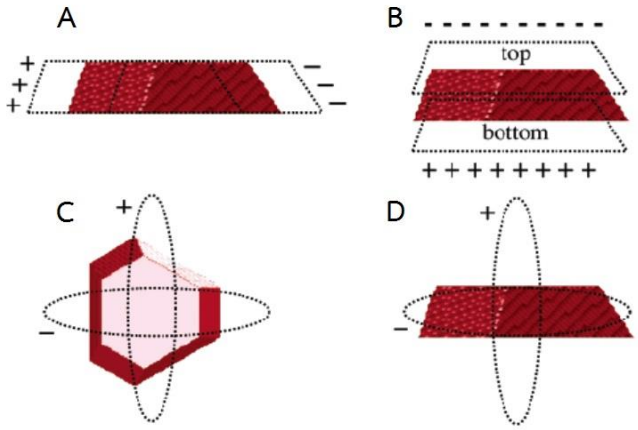


Figure 2.2 The pattern of LSPR of silver nanoprisms of AgNPls (A) In-plane dipole LSPR (500 nm) (B) Out-of-plane dipole LSPR (400 nm) (C) In-plane quadrupole LSPR (470 nm) (D) Out-of-plane quadrupole LSPR (340 nm) [28].

AgNPls has been fabricated by using various methods such as photoinduced method [24, 29], and chemical reaction methods [25, 30, 31]. A new method based on the chemical reaction with H_2O_2 is used to prepare AgNPls in this research. Parnklang et al. [11] synthesized AgNPls by shape transformation of silver

nanospheres (AgNSs) by H_2O_2. H_2O_2 can be both an oxidizing agent (etching AgNSs to Ag^+) and a mind reducing agent (reducing Ag^+ in solution to Ag^0 that added on the edge of etched AgNSs). The growth process of AgNPls in the presence of H_2O_2 by AgNSs etching and regeneration of Ag atom is shown in Figure 2.3.

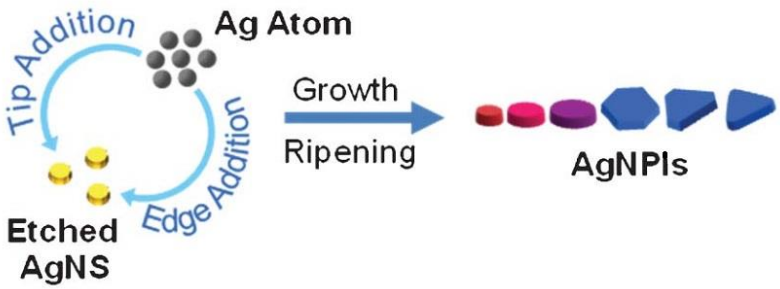


Figure 2.3 AgNPls formation in the presence of H_2O_2 by etching of AgNSs and regeneration of Ag atom.

AgNPls have been used to detect many analytes such as halide ions [16, 32-34], Hg^{2+} [15, 35, 36], Cu^{2+} ions [9, 37] and H_2O_2 [12, 14, 17, 38]. In this work, AgNPls are chosen to detect H_2O_2 and glucose by observing a distinct change in their color with a change in AgNPls size. Starch is utilized as eco-friendly and nontoxic biopolymer stabilizer of AgNPls [38].

2.3 Method for glucose detection

The level of glucose can be measured by spectrophotometric [17], potentiometric [39], chemiluminesence [40, 41] and colorimetric methods [2, 42, 43]. The detail of these methods is described as follows.

2.3.1 Spectrophotometric method

In spectrometric method, the measurement is based on the interaction between an interest compound and an incident monochromatic radiation. If the compound can absorb the radiation, the absorption of the radiation is directly

proportional to the quantity of compound. The advantages of using a UV-Vis spectrophotometer are its quick analysis ability and ease of use. On the other hand, the major disadvantage of this method is its low selectivity. The examples of glucose detection by spectrophotometric method are given below.

Hexokinase method is a standard method for glucose detection used in a general clinical laboratory. Hexokinase and adenosine triphosphate (ATP) are used to convert glucose to glucose-6-phosphate (G-6-P). In a second step, under the influence of glucose-6-phosphatedehydrogenase (G-6-PDH) and nicotine-amide-adenine-dinucleotide-phosphate (NADP), 6-phosphogluconate and reduced NADP ($NADPH_2$) are formed in proportion to the glucose content in the sample. The content of $NADPH_2$ can be determined spectrophotometrically at 366 nm [18].

Bo *et al.* [42] proposed a colorimetric assay for detecting glucose in urine using G-quadruplex-based DNAzymes and 10-acetyl-3,7-dihydroxy phenoxazine (ADHP). Hemin-G quadruplex DNAzyme was used to transduce the oxidation of glucose into the color change of ADHP. A stable G-quadruplex structure was form by oligonucleotide. H_2O_2 that was produced in the reaction of glucose and oxygen catalyzed by glucose oxidase, oxidized colorless ADHP to red resorufin with the presence of horseradish peroxidase as catalyst. The absorbance of resorufin observed at 570 nm and the dynamic range of glucose detection was 3.0–100 mM.

Su *et al.* [43] used $ZnFe_2O_4$ magnetic nanoparticles (MNPs) in the detection of glucose in urine by colorimetric detection based on their peroxidase activity. GOx was utilized to generate H_2O_2 from glucose. $ZnFe_2O_4$ MNPs could adsorb H_2O_2 and hydroxyl radicals (·OH) were generated through the reaction of H_2O_2 with the bound Fe^{3+} or *via* partial electron exchange interaction. TMB was oxidized by ·OH to form a blue color product. The blue signal came from the charge-transfer complex, consisting of a cation radical and TMB. The linear range for glucose detection was 1.25–18.75 μM with the detection limit of 0.3 μM.

2.3.2 Potentiometric method

Potentiometry is an electrochemical method based on the voltage measurement of an electrochemical cell when no current flows using two electrodes; working (indicating electrode) such as ion selective electrode (ISE) and reference electrodes. As an example of applying this technique in glucose detection, Ngeontae *et al.* [39] used AgNPs as a potentiometric redox marker. H_2O_2 generated from reaction of glucose and oxygen catalyzed by GOx was able to oxidize AgNPs to free Ag^+ ions. The amount of Ag^+ ions related to glucose concentration could be directly measured using a specifically prepared Ag-ion selective electrode (Ag-ISE). The limit of glucose detection was 10 μM.

2.3.3 Chemiluminesence method

Chemiluminescence (CL) is observed when light is emitted as a result of a chemical reaction. For instance, peroxyoxalate chemiluminescence (PO-CL) is a powerful technique for the sensitive and selective determination of a large variety of analytes including glucose. However, this assay requires a specific instrumentation.

Zargoosh *et al.* [40, 41] utilized carbon nanotubes and gold nanoparticles (AuNPs) as catalyst of the reaction of H_2O_2 (generated in enzymatic reaction of GOx and glucose) for sensitive determination of glucose by peroxyoxalate chemiluminescence (PO-CL) detection. Generally, PO-CL concerns the oxidation of an aryl oxalate ester by H_2O_2 in the presence of a fluorophore as activator. The reaction may occur through a chemically initiated electron exchange luminescence (CIEEL) mechanism including a high energy intermediate formation such as 1,2-dioxetanedione. These metastable intermediates form complexes with the fluorophore so that one electron can be transferred back to the fluorophore promoting it to an excited state and eventually releasing “light” during its relaxation. The emitted light intensity is related to H_2O_2 concentration.

The linear range of glucose determination was 2.25–175 μM and the detection limit was 1 μM.

2.3.4 Colorimetric method

Colorimetric method is based on the detection of color changes resulted from specific reactions of analytes and reagents. Since color changes can be detected by the naked eyes, this method does not require expensive or sophisticated instrumentation and can be applied to field analysis and point-of-care diagnosis.

For example, Radhakumary *et al.* [2] presented the application of gold nanoparticles (AuNPs) functionalized with thiol groups for the detection of glucose in urine sample by naked eyes. The thiol capped AuNPs was functionalized with glucose oxidase using carbodiimide chemistry. The interaction of GOD-functionalized AuNPs with glucose caused visible color change from red to blue due to aggregation of AuNPs when the glucose concentration exceeded 100 μg/mL.

The naked eyes detection is an interesting and challenged approach in the determination of glucose in human urine and it can also be applied in the paper-based system.

2.4 Paper based detection of glucose

Nowadays, the method for medical screening should be rapid and easy to perform with simple instruments. It will be beneficial if it can be applied for the analysis on-site or in a laboratory of a hospital. Furthermore, for diagnosis, it should be sensitive enough to detect the target analytes at low concentrations in samples.

Paper-based colorimetric detection, which is simple, portable, inexpensive and disposable, has been used in different applications including the detection of metals, organic compounds, bacteria in foods, proteins and the analysis of lung cancer for

rapid screening and diagnosis [2, 5-10, 35, 44-46]. The paper-based glucose detection is one of interesting and challenging approaches and could be beneficial for self-monitoring of urine glucose.

In this research, two commercially available paper dipsticks for glucose detection in human urine are described as follows.

1. Combur^{7} test (dipstick)

   For this dipstick, the glucose determination is based on the specific glucose-oxidase/peroxidase reaction (GOD/POD method). Glucose reacts with glucose oxidase to generate gluconic acid and hydrogen peroxide. Peroxidase further catalyzes the reaction of hydrogen peroxide with 3,3',5,5'-tetramethylbenzidine (TMB) chromogen. The oxidized form of TMB has green color and the color of the dipstick changes from pale yellow to dark green depending on the level of glucose. The detection range is from 0–55 mM with the detection limit of 2.8 mM glucose.

2. Urine reagent strip for urinalysis (URS-2P)

   This test is based on two sequential enzyme reactions. Glucose oxidase catalyzes the oxidation of glucose resulting in a formation of gluconic acid and hydrogen peroxide. Another enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with potassium iodide chromogen. The chromogen color changes from blue-green to greenish-brown, brown and dark brown, respectively, depending on the level of glucose. The detection range is from 0–110 mM with the detection limit of 5 mM glucose.

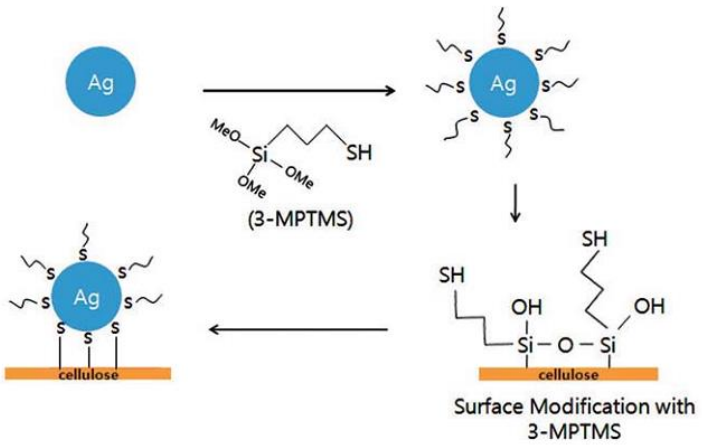
In this research, a new AgNPls paper-based method were prepared and used to detect hydrogen peroxide (H_2O_2) and glucose by following the decomposition of AgNPls. The (3-mercaptopropyl)trimethoxysilane (MPTMS) was used to coat filter paper on which AgNPls were further deposited.

2.4.1 (3-mercaptopropyl)trimethoxysilane (MPTMS) coated paper surface

MPTMS contains a thiol group (–SH) and siloxane groups (–O–Si). It can be used to coat paper and react with AgNPs by chemisorption.

In most cases, silver(I) ions will form complexes with organic thiols. Silver(I) ion forms the thermodynamically favorable complex of silver(I) thiolate (AgSR). In the case of AgNPs and thiols, it has been shown that with organothiols, Ag-SR remained on the surface, forming a shell, as evidenced by scanning electron microscopy [23].

MPTMS was used as a binding bridge between AgNPs and cotton surface [47] which contained cellulose as a main composition. It is assumed that chemisorption between AgNPs and thiol groups occurred. In addition, hydrolyzed siloxane might react with hydroxyl groups in cellulose. The proposed reaction mechanism is demonstrated in Scheme 2.1 [47].



Scheme 2.1 Reaction scheme between silver nanoparticles, MPTMS and cotton.

Not only thiol group is used to coat paper surface and attach AgNPs but it also stabilized AgNPs. The thiol molecule is grafted to the AgNPs surface through Ag–S chemical bonds [48]. Kelly et al [33] investigated the stabilization of triangular AgNPls treated with thiols to protect them from the degradation by chloride ions.

2.5 Literature review

AgNPs are applied to determine a small amount of H_2O_2, which is a strong oxidizer and the resulting product from the reaction between glucose and oxygen with glucose oxidase as catalyst. The decomposition (oxidation) of AgNPs by H_2O_2 causes transformation of shape and size of AgNPs, which result in a change in the color of AgNPs. By following the change in color, the degree of AgNPs decomposition related to the amount of H_2O_2 could be determined [12]. For this reason, researchers have reported the H_2O_2 detection with AgNPs by spectrophotometric and colorimetric method.

Pangdam *et al*. [12] proposed the method of H_2O_2 detection based on the oxidation reaction of H_2O_2 and AgNPs aggregation. The change in LSPR was measured by UV-visible spectrophotometer at a wavelength of 395 nm. The color of AgNPs changed from light blue to pink, when increased the concentration of H_2O_2. The linear range of the detection was 0.1–0.7 ppm H_2O_2.

Filippo *et al*. [14] synthesized directly AgNPs in Poly(vinyl alcohol) (PVA) matrix by thermal treatment of PVA containing $AgNO_3$ salt without reducing agent and used as H_2O_2 sensor. The degradation of AgNPs was induced by the reaction with H_2O_2. It caused a change in LSPR absorbance and the yellow color AgNPs-polymer solution turned to the transparent and colorless solution. Moreover, the yellow color solution could be regenerated by thermal treatment. The detection limit of this method was 1 μM H_2O_2.

Zhang *et al.* [17] studied the AgNPs formation induced by H_2O_2 and UV irradiation. This method was developed for H_2O_2 detection by spectrophotometric assay. H_2O_2 acted as a reducing agent in the AgNPs formation in which Ag^+ was reduced to Ag^0 by O_2^- generated *via* the H_2O_2 decomposition in alkaline media. In contrast, photoreduction of Ag^+ to Ag^0 under UV irradiations also led to the nanoparticles formation. The linear range of H_2O_2 detection was 0.5–60 μM and the detection limit was 0.2 μM.

Nitinaivinij *et al*. [38] presented a H_2O_2 colorimetric detection using starch-stabilized AgNPls based on AgNPls decomposition as a result of a reaction with H_2O_2. AgNPls was changed to silver ion. The mixture color changed from red to colorless. The linear range of H_2O_2 detection was 10–80 μM with R^2 = 0.9910 and the detection limit was 1.57 μM.

Chen *et al*. [13] created in situ growth of AgNPs on the graphene quantum dots (GQDs) surface for colorimetric detection of H_2O_2 and glucose. GQDs could be used as both a stabilizer and a reducing agent of AgNPs. The reduction of H_2O_2 was catalyzed with GQDs/AgNPs hybrid. The oxidation of glucose by adding glucose oxidase enzyme generated H_2O_2 for glucose detection. The detection limit of H_2O_2 and glucose detection was 33 nM and 170 nM, respectively.

Moreover, researchers have reported the glucose detection by paper-based methods.

Zhou *et al*. [49] developed a paper-based colorimetric method for H_2O_2 and glucose detection using cross-linked siloxane 3-aminopropyltriethoxysilane (APTMS) as probe. When APTMS was cross-linked with glutaraldehyde (GA), brick-red color of APTMS-GA complex occurred. This complex was oxidized by H_2O_2 leading to a change of color from brick-red to colorless. The color intensity was measured via ImageJ software. Glucose oxidase was immobilized onto modified-paper to react with glucose and generate H_2O_2 for glucose detection. The range of H_2O_2 and glucose detection was 2.5–500 mM and 0.5–30 mM, respectively.

Noiphung *et al.* [50] fabricated paper-based microfluidic devices for glucose electrochemical detection from whole blood. The H_2O_2 generated from the reaction between glucose and the GOx enzyme was detected by a Prussian blue modified screen printed electrode. The linear range for glucose assay was 0–33.1 mM (R^2 = 0.987).

CHAPTER III

EXPERIMENTAL

3.1 Chemicals

All of chemicals were of analytical reagent (AR) grade. The list of chemicals is shown in Table 3.1. MilliQ water was thoroughly used as a solvent in this study.

Table 3.1 List of chemicals

Chemicals	Supplier/ Grade
Hydrogen peroxide 30%	Merck/ AR grade
D-(+)-Glucose	Sigma-Aldrich/ ACS reagent
Glucose oxidase from Aspergillus niger	Sigma
Silver nanoparticles	Sensor Research Unit
3-mercaptopropyltrimethoxysilane 95%	Aldrich/ reagent grade
Silver nitrate	AENCORE/ AR grade
Sodium hydroxide	Merck/ for analysis
Creatinine	Aldrich/ reagent grade
Ethanol	Merck/ for analysis
Urea	Merck/ for analysis
Sodium chloride	CARLO ERBA/ for analysis
di-Sodium hydrogen orthophosphate	BDH/ AR grade
Potassium dihydrogen phosphate	Merck/ for analysis
L-ascorbic acid	UNILAB/ reagent grade
Sodium sulfate anhydrous	Fisher Scientific/ AR grade
Tri-sodium citrate	Fisher Scientific/ AR grade

3.2 Preparation of glucose oxidase (GOx) solution

A 0.1 M phosphate buffer solution (PBS) was prepared by mixing 61.2 mL of 0.1 M Na_2HPO_4 solution with 38.8 mL of 0.1 M KH_2PO_4 solution. Then, a 2.5 mg/mL (500 U/mL) glucose oxidase solution was prepared by dissolving 12.5 mg of GOx powder in 5 mL of 0.1 M PBS. This solution was used to generate H_2O_2 in glucose detection.

3.3 Synthesis of silver nanospheres (AgNSs)

AgNSs were prepared by the chemical reduction of $AgNO_3$ (1.86×10^{-2} M, 500 mL) with $NaBH_4$ (2.88×10^{-2} M, 500 mL) utilizing 2 %w/v soluble starch as a stabilizer. $AgNO_3$ solution was slowly added into $NaBH_4$ solution under vigorous stirring. The obtained 1000 ppm colloidal AgNSs solution appeared as a dark yellow-brown solution. This colloidal AgNSs was used in the preparation of AgNPls paper.

3.4 Synthesis of silver nanoplates (AgNPls)

AgNPls were prepared by shape transformation reaction of AgNSs. A solution of 30 %wt H_2O_2 was added into the colloidal AgNSs solution under vigorous stirring. At a mole ratio of H_2O_2 : AgNSs equal to 15 : 1, the color of the colloid gradually changed from yellow to red wine, which the 200 ppm red colloid of AgNPls was finally obtained. At a mole ratio of H_2O_2 : AgNSs equal to 30 : 1, the color gradually changed from yellow to purple, which the 300 ppm purple colloid of AgNPls was finally obtained. At a mole ratio of H_2O_2 : AgNSs equal to 50 : 1, the color gradually changed from yellow to blue, which the 500 ppm blue colloid of AgNPls was finally obtained. These three colloidal AgNPls were used in the preparation of AgNPls paper.

3.5 Characterization method

The absorbance spectra of AgNPls, AgNSs and their mixtures were measured by a UV-visible spectrophotometer in the wavelength range from 300 to 750 nm with 1 cm path length cell. The light source is deuterium and tungsten lamp. AgNPs

colloidal solutions were diluted to 5 ppm for measuring absorbance spectra. So as to confirm that AgNPls were still in their original size and concentration (no dissolution or aggregation), LSPR spectra of AgNPls colloidal solution was observed by UV-visible spectrophotometer before preparing the testing papers.

3.6 Paper-based H_2O_2 detection

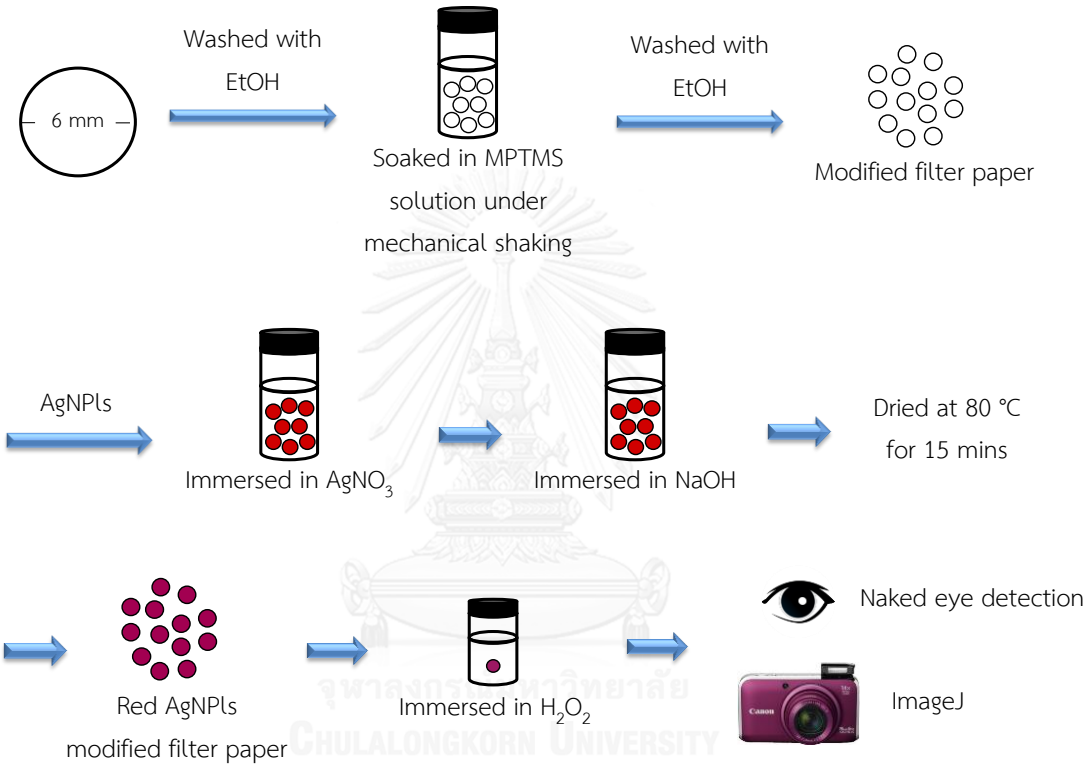
3.6.1 Preparation of AgNPls paper for H_2O_2 detection

Disk-shaped filter paper was cut into a small disk with a hole puncher, having a diameter of 6 mm. These paper disks were washed with ethanol to clean and prepare the surface for MPTMS coating. Then, it was soaked in 0.5 %v/v MPTMS in ethanol under mechanical shaking for 5 minutes. MPTMS was used to attach the AgNPls likely through complexation and stabilize AgNPls. The MPTMS-modified paper was rinsed with ethanol to remove unattached MPTMS. A 5 µL of 180 ppm colloidal AgNPls was dropped onto MPTMS-modified papers. The obtained paper was further immersed in a 1 mM $AgNO_3$ solution and 1 mM NaOH solution, subsequently and incubated at 80°C in an oven for 15 minutes to form thin layer of Ag_2O on the surface. The layer of Ag_2O on the AgNPls was thought to catalyse the H_2O_2 decomposition with AgNPls. Thus, it can enhance the detection. The color of the paper surface changed from white to the color of AgNPls. The prepared paper was kept for H_2O_2 detection.

3.6.2 H_2O_2 detection

For H_2O_2 detection, the AgNPls-modified filter paper was immersed in 5 mL of solutions containing different concentrations of H_2O_2 for 10 minutes. When AgNPls on paper surface were reacted with H_2O_2 solution, the color of AgNPls was bleached due to the oxidation of AgNPls to silver ion catalyzed with thin layer of Ag_2O by H_2O_2. The change of paper color was observed by naked

eyes. To construct a calibration curve and produce a color calibration chart for quantitative analysis, the intensity of papers color was measured by analyzing the photo of filter papers using ImageJ software. The intensity was observed in gray scale mode. The preparation of AgNPls paper and H_2O_2 detection method are shown in Scheme 3.1.



Scheme 3.1 The preparation of AgNPls paper and H_2O_2 detection method.

3.6.3 Optimization of paper-based H_2O_2 determination method

To gain the optimal condition for H_2O_2 detection, the effects of some parameters as shown in the topic of 3.6.1 and 3.6.2 (*i.e.*, AgNPs size, MPTMS concentration, AgNPls concentration, detection time and sample solution volume) were investigated. The value ranges of each parameter are presented in Table 3.2.

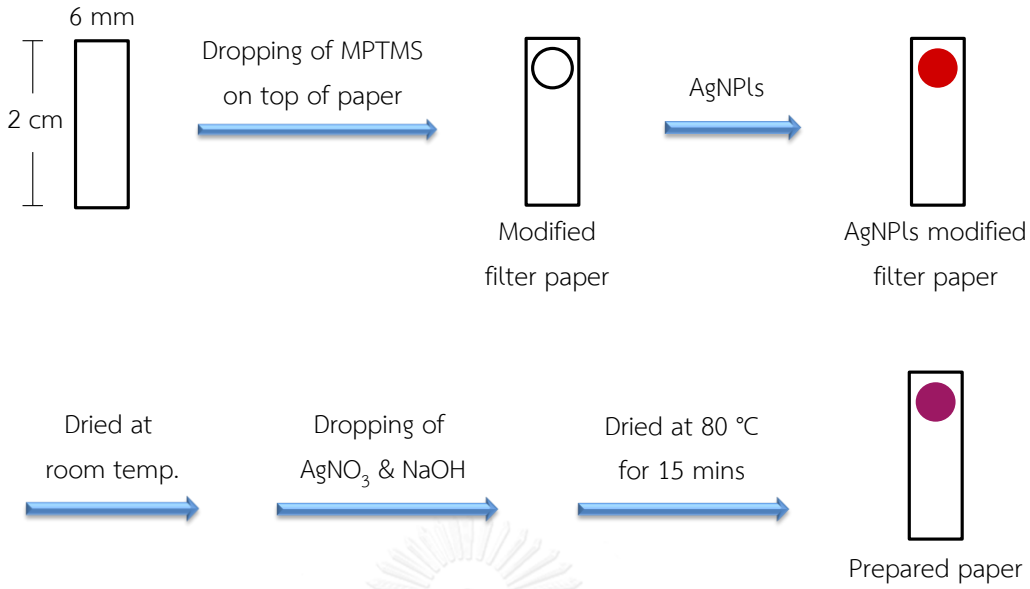
Table 3.2 The value ranges of each parameter for H_2O_2 determination method

Parameters	Range of values
AgNPs size	AgNSs, AgNPls and their mixtures
MPTMS concentration	0.1–5 %v/v
AgNPls concentration	125–200 ppm
detection time	5–30 minutes
sample solution volume	20 µL and 2–10 mL

3.7 Paper-based glucose detection in human urine samples

3.7.1 Preparation of AgNPls-paper strip for glucose detection

The filter paper was cut into rectangle shape with dimension of 6 mm × 2 cm. A 1 µL of 0.5% v/v MPTMS in ethanol solution was dropped on one end of the filter paper. A 1 µL of 100 ppm colloidal red color of AgNPls was further dropped onto MPTMS-modified area of the paper and dried at room temperature for tight attachment of AgNPls and MPTMS on paper surface. A 1 µL of 2.5 mM $AgNO_3$ solution and 1 µL of 2.5 mM NaOH solution were subsequently dropped onto the same modified area of the filter paper. The paper was incubated in an oven at 80°C for 15 minutes to form thin layer of Ag_2O on the modified surface. The color of the modified area on filter paper was the same as the AgNPls color. The prepared AgNPls-paper strips were kept for glucose detection. The preparation method is shown in Scheme 3.2.

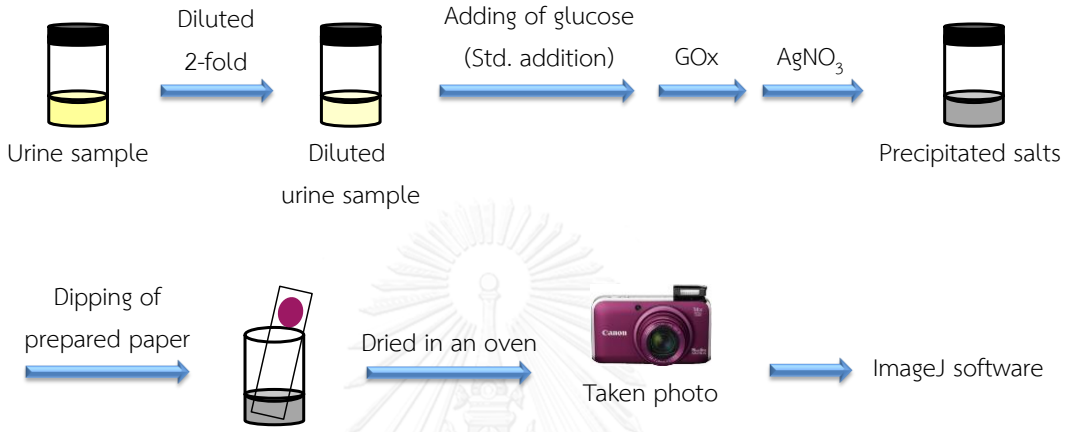


Scheme 3.2 The preparation method of AgNPls-paper strip for glucose detection.

3.7.2 Glucose detection in human urine samples

For glucose detection, since human urine contains different interfering species, the external calibration curve was not suitable for the detection. Thus, the standard addition method was used to overcome the matrix effect from human urine. Moreover, the real urine sample was diluted 2-fold with MilliQ water to reduce the content of interference. The different concentrations of glucose standard were added into a set of 1 mL of diluted samples to construct a calibration curve. A 20 µL of 2.5 mg/mL GOx was added into each solution to react with glucose and generate H_2O_2 for 30 minutes. Furthermore, chloride, sulfate and phosphate ions in urine sample were eliminated by adding 50 µL of 5 M $AgNO_3$ in the solution to form precipitates and the excess silver ions could also catalyse the oxidation of AgNPls. Then, a AgNPls-paper strip was dipped in the sample solution by the unmodified end while the AgNPls-modified area was on top. The paper strip was left in solution for a few minutes in order that H_2O_2 in the solution diffused toward AgNPls-modifed area. Then, the paper strip was dried in an oven at 80°C for 5 minutes to reduce the detection time. The photo of the paper was taken and then the color intensity of AgNPls-modified area was

determined by ImageJ software in gray scale mode to get data for calibration curve construction. The quantitative data was obtained after calibration curve extrapolation toward x-axis. The sample preparation and glucose detection method are shown in Scheme 3.3.



Scheme 3.3 The sample preparation and glucose detection method of AgNPls-paper strip.

3.7.3 Optimization of paper-based glucose determination method

To get the optimal condition for glucose determination in human urine, the effect of some parameters as shown in the topic of 3.7.1 and 3.7.2 (*i.e.*, AgNPls concentration, $AgNO_3$ solution volume and the presence of co-existing species) were investigated. The value ranges of each parameter are presented in Table 3.3.

Table 3.3 The value ranges of each parameter for glucose determination method

Parameters	Ranges of value
AgNPls concentration	50–180 ppm
urine matrix and $AgNO_3$ volume	25 and 50 µL
co-existing species	urea, creatinine, ascorbic acid, chloride ion, sulfate ion and phosphate ion

3.7.4 Method validation

The method of paper-based glucose detection in human urine samples was validated. The accuracy and precision of the presented method were judged under a chosen condition.

The accuracy and the precision of the obtained analytical results were compared with the results from the analyses using a standard hexokinase method and using Combur[7] and URS-2P dipsticks. The method of spiked sample was done under appropriate condition. A glucose standard solution was added into human urine samples to obtain the concentration in the samples of 0.7 and 1.5 mM. The level of glucose in these samples was determined according to the proposed protocol. The photo of the paper was taken and then the color intensity of AgNPls-modified area was determined by ImageJ software in gray scale mode to get data for calibration curve construction. The data were presented in terms of %recovery and %RSD.

CHAPTER IV
RESULTS AND DISCUSSION

This chapter is divided into three main parts. The first part is the characterization of silver nanoparticles (AgNPs) including AgNPls, AgNSs and their mixtures. The second part is focused on the paper-based H_2O_2 detection. The last part covers a paper-based method for glucose detection in human urine samples.

4.1 Characterization of AgNPs and mixed AgNPs

AgNPs of different sizes (red, purple, blue AgNPls and yellow AgNSs) were used in this research. They were used as single colloidal AgNPs and also as mixtures in different concentration ratios, as shown in Table 4.1, for H_2O_2 detection. To estimate the size of these AgNPs, the extinction spectra of these AgNPs were recorded by using a UV-visible spectrophotometer and the maximum wavelength was observed. The extinction spectra and colors of these AgNPs are presented in Figure 4.1. Due to the localized surface plasmon resonance (LSPR), AgNPls exhibit characteristic colors depending on their shape, size, and environment. The spectra of the red, purple and blue AgNPls show extinction wavelength maxima at 504, 551 and 623 nm, respectively, which are attributed to in-plane dipole plasmon resonance of these AgNPls. It indicates that the blue AgNPls (100 nm) have the biggest size, followed by the purple AgNPls (47 nm) and the red AgNPls (33 nm), respectively [11, 38]. On the other hand, the extinction spectrum of AgNSs (in yellow color) shows an extinction maximum at 405 nm, revealing that the size of AgNSs (6 nm) [11, 38] is the smallest compared to the others. The spectra of the mixtures of AgNPls and AgNSs also displayed mixed characteristic spectra of each AgNPs.

Table 4.1 List of colloidal AgNPs and their mixtures

Colloidal AgNPs	Mixing ratios
Red AgNPls 200 ppm	–
Yellow AgNSs 200 ppm	–
Purple AgNPls 200 ppm	–
Blue AgNPls 200 ppm	–
Red AgNPls 133 ppm : Blue AgNPls 67 ppm (R 2 : B 1 AgNPs)	2 : 1
Red AgNPls 100 ppm : Yellow AgNSs 100 ppm (R 1 : Y 1 AgNPs)	1 : 1
Blue AgNPls 67 ppm : Yellow AgNSs 133 ppm (B 1 : Y 2 AgNPs)	1 : 2

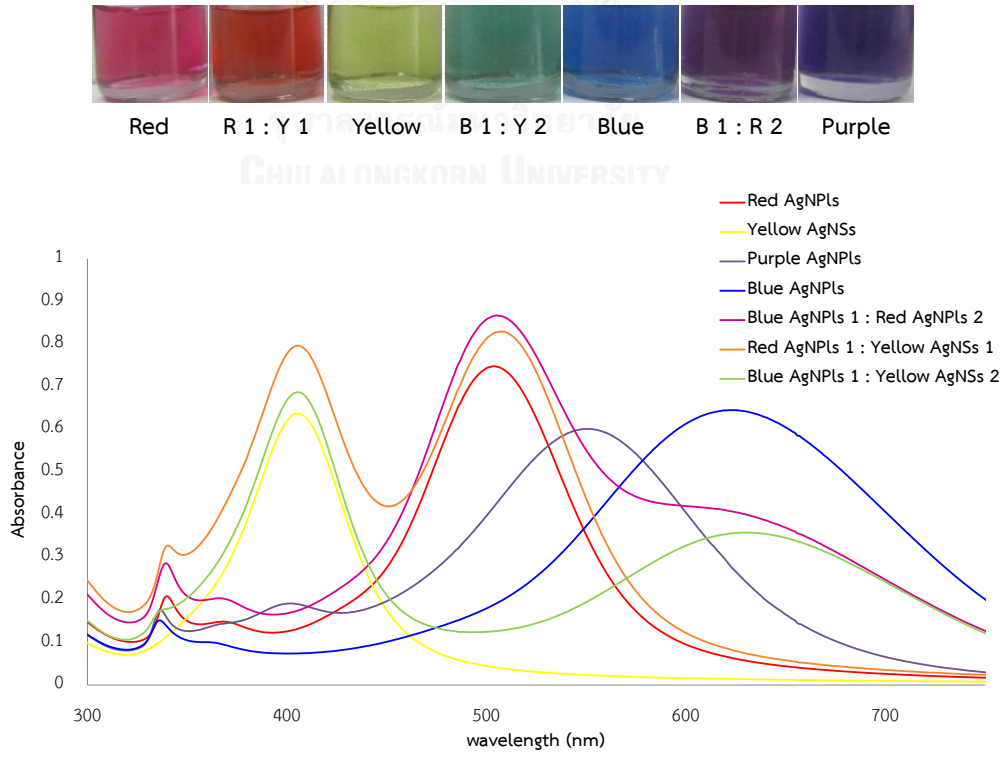


Figure 4.1 The colors and extinction spectra of AgNPls, AgNSs and their mixtures.

4.2 Paper-based H_2O_2 detection

4.2.1 Effect of AgNPs size on the detection of H_2O_2

The objective of this work is to develop a colorimetric method for H_2O_2 detection at low concentration level; therefore, colloidal AgNPls of different colors *i.e.*, red, purple, and blue AgNPls and colloidal solution of AgNSs (yellow color) were used to prepare the test papers. Moreover, these colloidal AgNPs were mixed in different concentration ratios to create colloids with mixed color tones from mixed-size AgNPs (Table 4.1). The effect of AgNPs size was investigated with a total AgNPs concentration of 200 ppm. Figure 4.2 displays the modified papers after being coated by single and mixed colloidal AgNPs.

Figure 4.3 shows colors of the AgNPs modified papers after being immersed in 5 mL of solutions containing different H_2O_2 concentrations (0–1.5 mM) for 10 minutes. The color of the papers was faded due to the bleaching of AgNPs by H_2O_2 [11, 38]. The color of the dried AgNPs modified papers was paler than their colors observed immediately after the reaction with H_2O_2, indicating that the surface bleaching or the reaction of H_2O_2 left on the paper with AgNPs still occurred. The red AgNPls-modified paper containing small AgNPls showed a noticeable change in color at low H_2O_2 concentrations, compared to purple and blue AgNPls-modified papers. They exhibited distinct tone of colors when used to detect H_2O_2 of different concentrations and it changed from magenta to orange and yellow, respectively, with an increase in a H_2O_2 concentration. Moreover, preparing the testing papers with single color AgNPls is easy and convenient. Thus, red AgNPls were chosen for the detection of H_2O_2 at low concentration levels in this study.

Figure 4.2 The papers modified with single and mixed 200 ppm colloidal AgNPs.

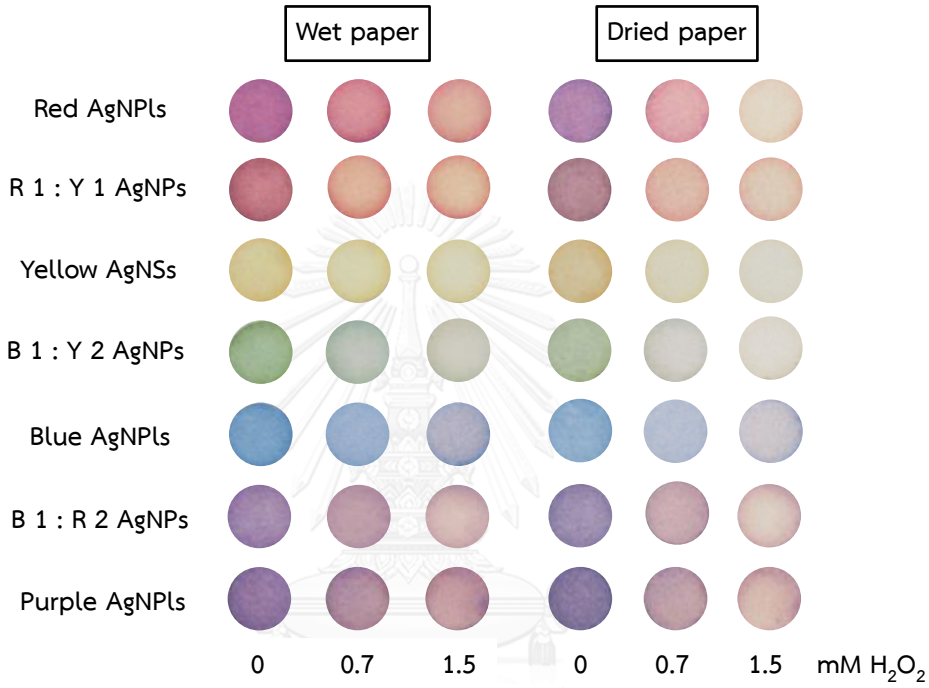


Figure 4.3 Color of papers prepared by using single and mixed 200 ppm AgNPs after the reaction with H_2O_2 (sample volume 5 mL, contact time 10 minutes).

4.2.2 Effect of MPTMS concentration

MPTMS is used to attach AgNPls onto the paper surface and stabilize AgNPls. The results of preliminary studies revealed that without the MPTMS coating, AgNPls decomposed or degraded on the papers surface. Furthermore, the amount of AgNPls on the paper surface would be related to MPTMS concentration. Therefore, when coated the paper with MPTMS of various concentrations, the different amounts of AgNPls would be attached onto the

paper resulting in different intensity of paper color. A MPTMS solution with concentration of 0.1, 0.5 or 5.0 %v/v was separately coated on filter papers prior to the modification with 200 ppm red AgNPls (Figure 4.4 and Figure 4.5). The color of a paper coated with 0.1 %v/v MPTMS was not homogeneous because of an insufficient amount MPTMS on the surface, and thus the paper surface was not well covered by AgNPls as shown in Figure 4.4. Figure 4.5 displays the papers prepared by coating of 0.5 and 5.0 %v/v MPTMS on their surface. The papers surface could be fully coated with homogeneous surface and intense color. When these modified filter papers were immersed in H_2O_2 solutions (0–1.2 mM), the papers coated with 5.0 %v/v MPTMS were slightly bleached by the oxidation reaction of H_2O_2 in the studied concentration range because of a high content of AgNPls deposited on the surface. On the other hand, the bleaching of the AgNPls on the papers coated with 0.5 %v/v MPTMS could be clearly observed by naked eyes. It could be used to detect H_2O_2 at a concentration as low as 0.3 mM by naked eyes. Thus, 0.5 %v/v MPTMS was chosen to prepare the testing papers in this research.

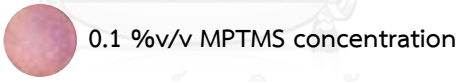


Figure 4.4 The color of AgNPls paper coated with 0.1 %v/v MPTMS and 200 ppm AgNPls.

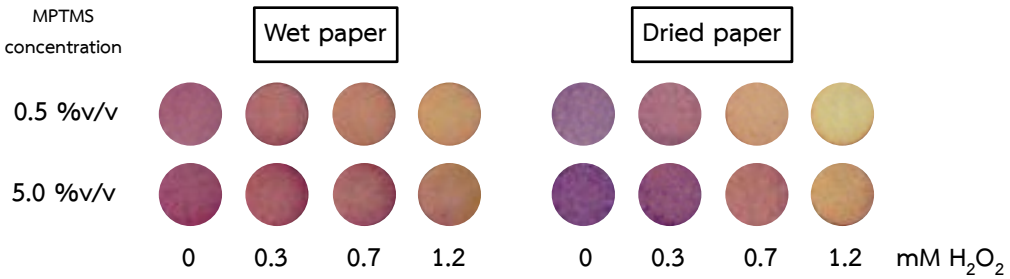


Figure 4.5 Comparison of AgNPls papers prepared by using different MPTMS concentrations (AgNPls concentration 200 ppm, sample volume 5 mL, contact time 10 minutes).

4.2.3 Effect of AgNPls concentration

In order to clearly observe the change in color of testing paper and to reduce the cost of production, the concentration of AgNPls was varied as 125, 150, 160, 170, 180 and 200 ppm. Figure 4.6 shows AgNPls modified papers prepared by using different AgNPls concentrations after being immersed in 5 mL of solutions containing different H_2O_2 concentrations (0–3 mM) for 10 minutes. By using high AgNPls concentrations, the papers color was intense due to a high content of AgNPls on the paper. When used to detect H_2O_2, these testing papers exhibited distinct color change that could be observed by naked eyes.

Furthermore, in order to obtain a more reliable detection than the naked eye detection, the intensity of paper color was observed by converting the photo of paper into gray scale mode and the mean gray values of the photo were determined. If the paper color is in white tone, the mean gray values will be high. In contrast, a photo color in darker or black tone gives low mean gray values. The calibration curves were constructed between mean gray values against H_2O_2 concentration as shown in Figure 4.7 and Figure 4.8. The results of using 180 and 200 ppm AgNPls in the detection of H_2O_2 show good linearity and high slope in both cases of using the photos of wet and dried papers. However, the resulting curves obtained by observing dried testing paper exhibited a better linearity and higher sensitivity regarding to a higher slope. The testing papers prepared by using 180 ppm AgNPls also showed good results with respect to the linearity and sensitivity. Therefore, a concentration of 180 ppm was used in this study to reduce cost.

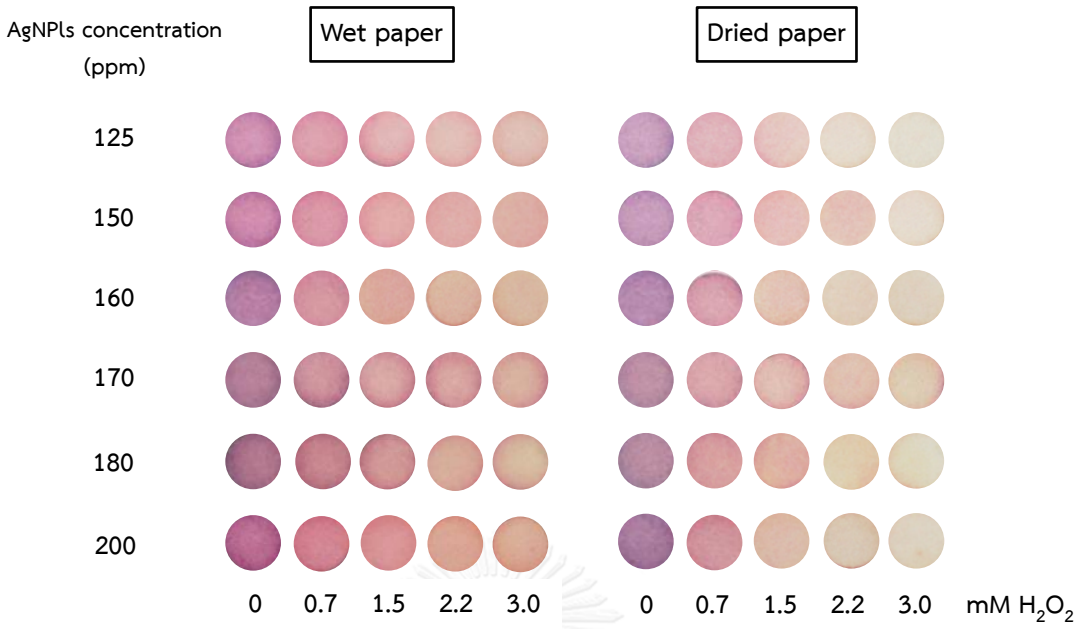


Figure 4.6 Comparison of AgNPls papers prepared by using different AgNPls concentrations after the reaction with H_2O_2 (sample volume 5 mL, contact time 10 minutes).

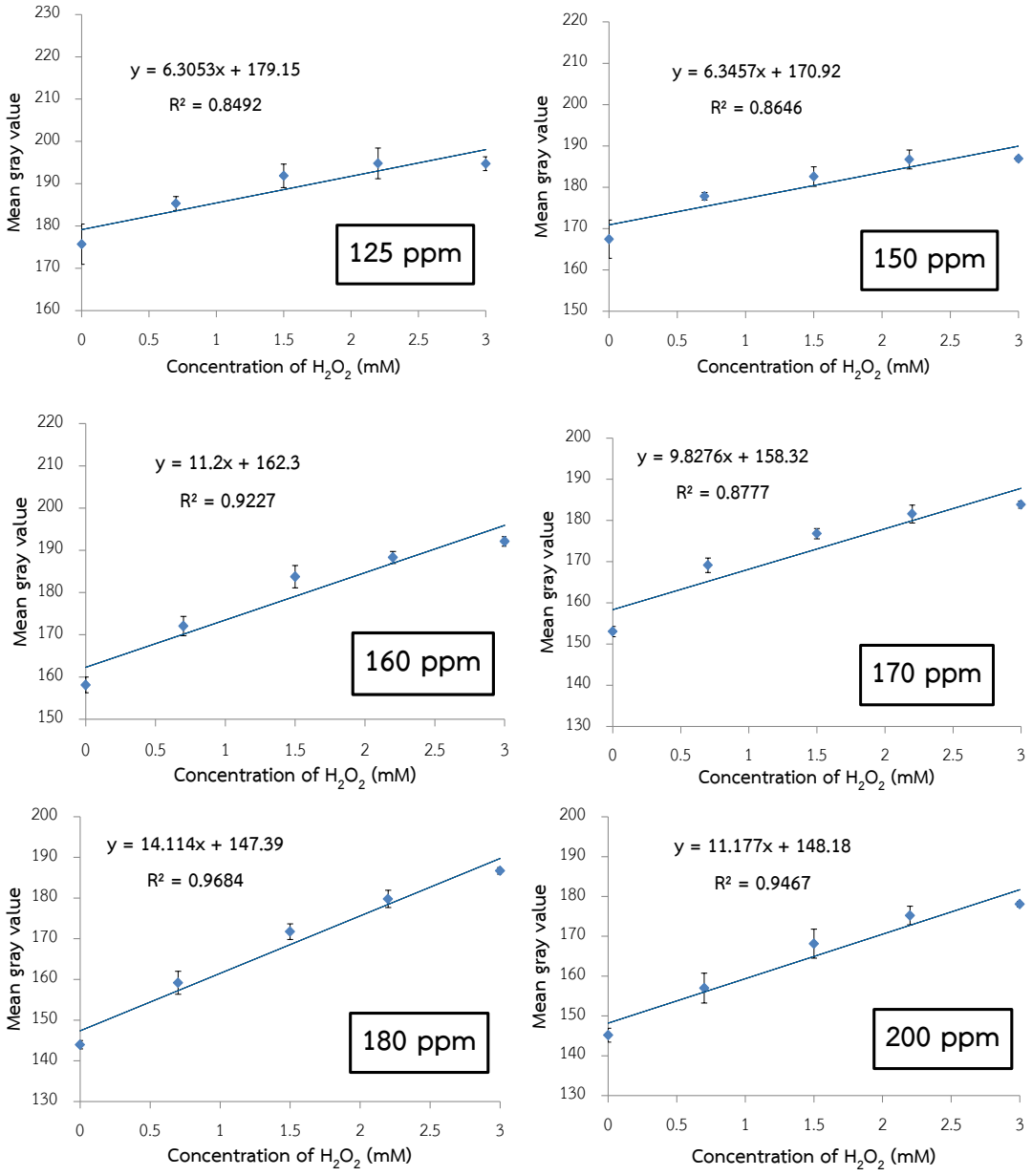


Figure 4.7 Calibration curves for the determination of H_2O_2 obtained by using testing papers modified by different AgNPls concentrations (wet testing papers) (sample volume 5 mL, contact time 10 minutes).

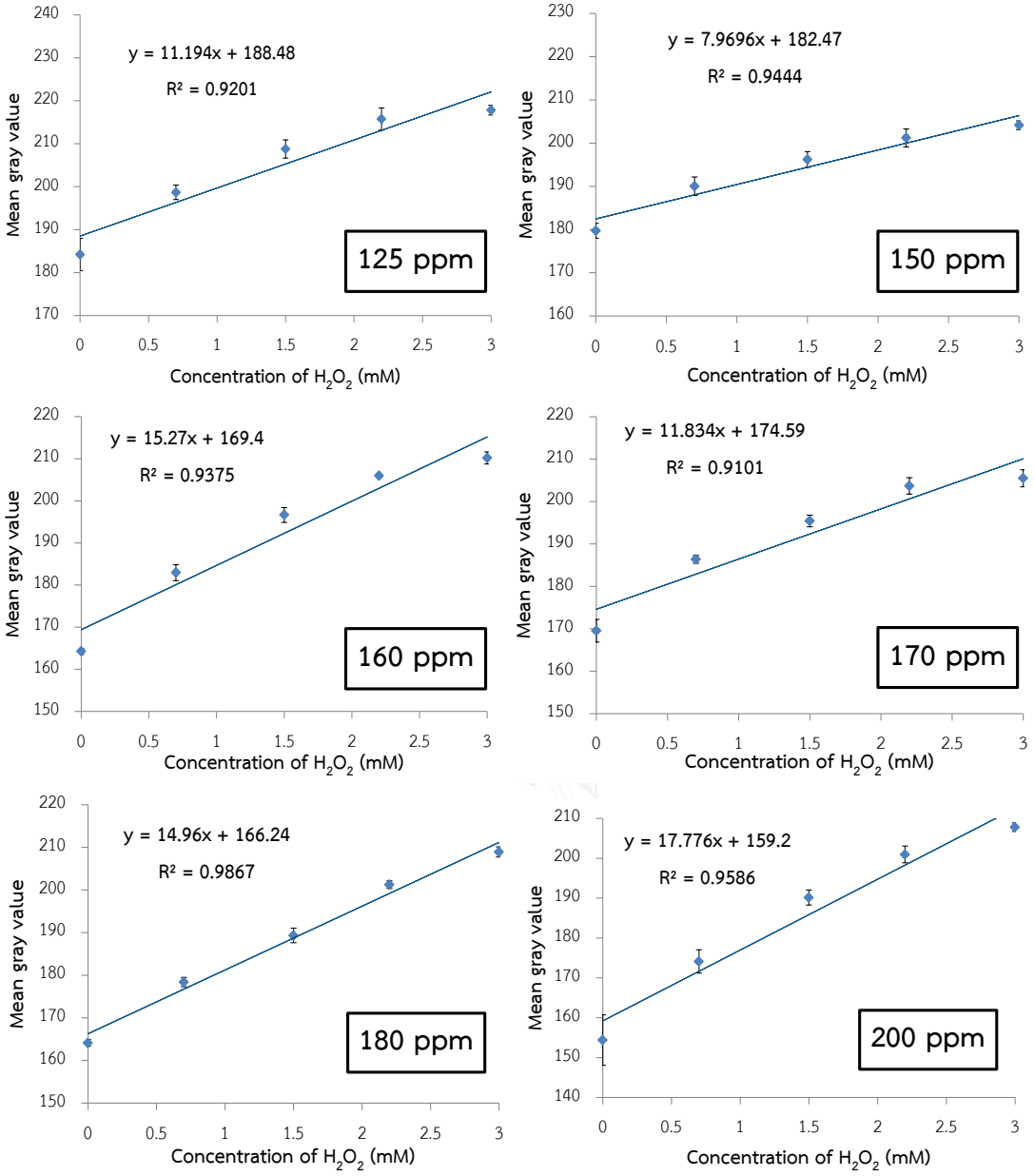


Figure 4.8 Calibration curves for the determination of H_2O_2 obtained by using testing papers modified by different AgNPls concentrations (dried testing papers) (sample volume 5 mL, contact time 10 minutes).

4.2.4 Effect of detection time

The effect of contact time was examined in order to obtain a suitable time for color observation as the degree of AgNPls bleaching would depend on the reaction time between the AgNPls on the modified paper and H_2O_2. Figure

4.9 displays the AgNPls papers after being immersed in 5 mL of solutions containing different H_2O_2 concentrations (0–3 mM) at various contact times (*i.e.* 5, 10, 15 and 30 minutes). At contact time of 5 minutes, the change of the paper color was not clearly observed by naked eyes. Using a contact time of 10 minutes, the AgNPls bleaching related to H_2O_2 concentration resulted in a more noticeable color change when compared to other contact times. When used a contact time of 15 or 30 minutes, the testing papers did not exhibit different colors at high concentrations of H_2O_2 (*i.e.* 2.2 and 3 mM). Due to a too long reaction time and a limited content of AgNPls, all AgNPls on the papers would be totally decomposed at high H_2O_2 concentrations, resulting in total bleaching of the papers color. Thus, the results of the analysis of 3 mM glucose were not used to construct the calibration curve. The calibration curves constructed from results of using different contact times are shown in Figure 4.10 and Figure 4.11. The graphs obtained from using the detection time of 10, 15 and 30 minutes show linear relationship in both case of using wet and dried papers. The sensitivity and the linearity observed in case of dried testing papers were better than the case of wet testing papers. To reduce the analysis time, the detection time of 10 minutes was chosen for this analysis.

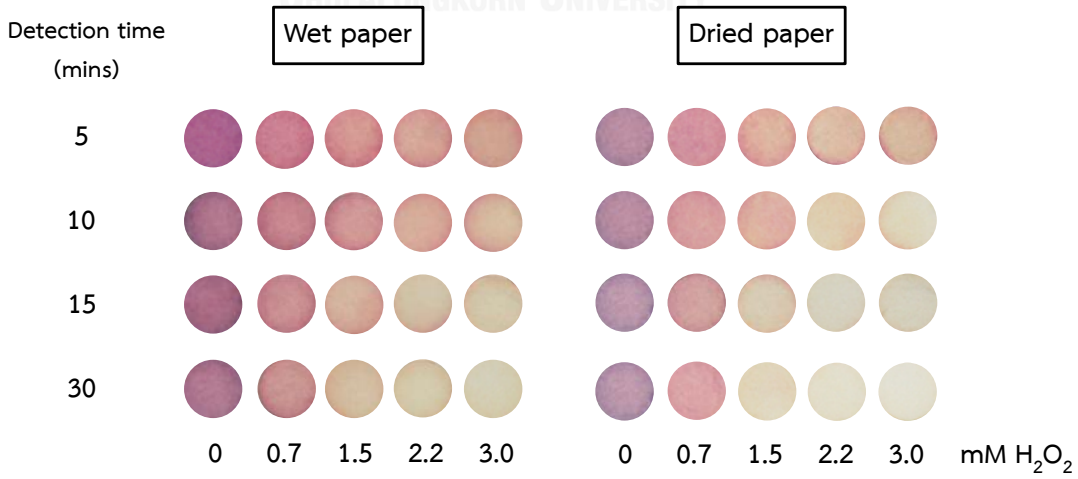


Figure 4.9 Comparison of AgNPls papers after the reaction with H_2O_2 at different contact times (sample volume 5 mL).

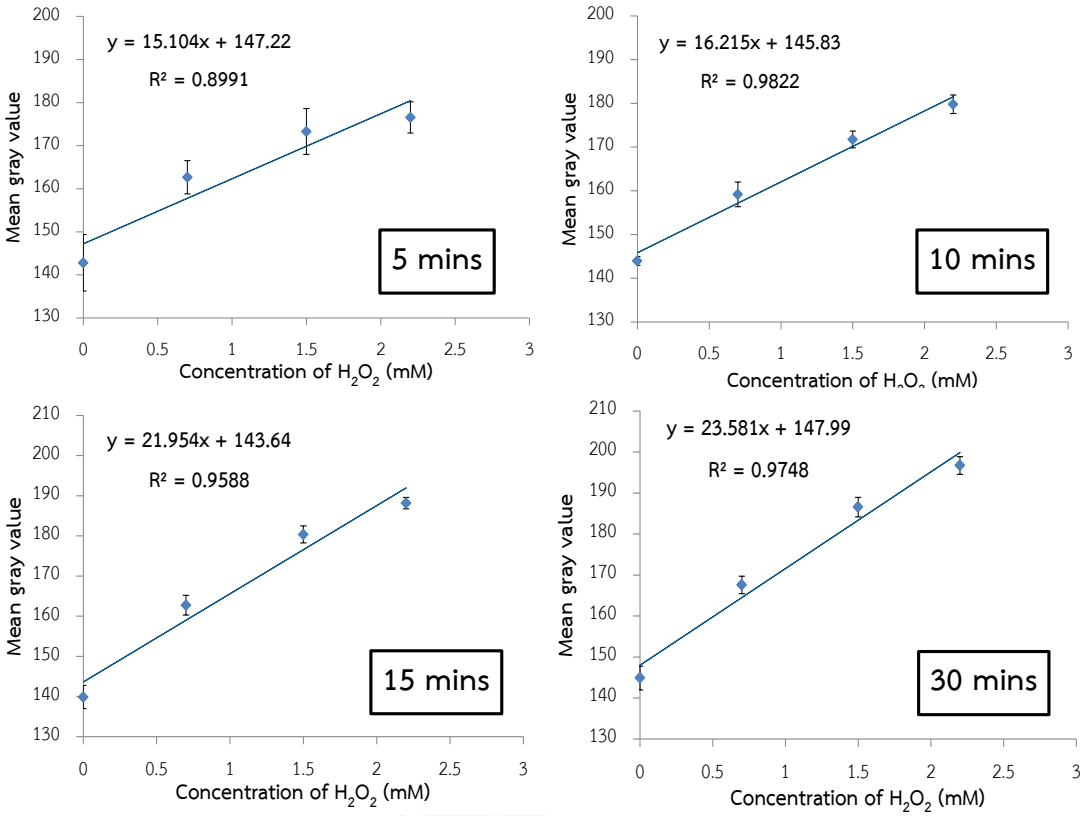


Figure 4.10 Calibration curves for the determination of H_2O_2 using AgNPls papers after the reaction with H_2O_2 at different contact times (wet testing papers) (sample volume 5 mL).

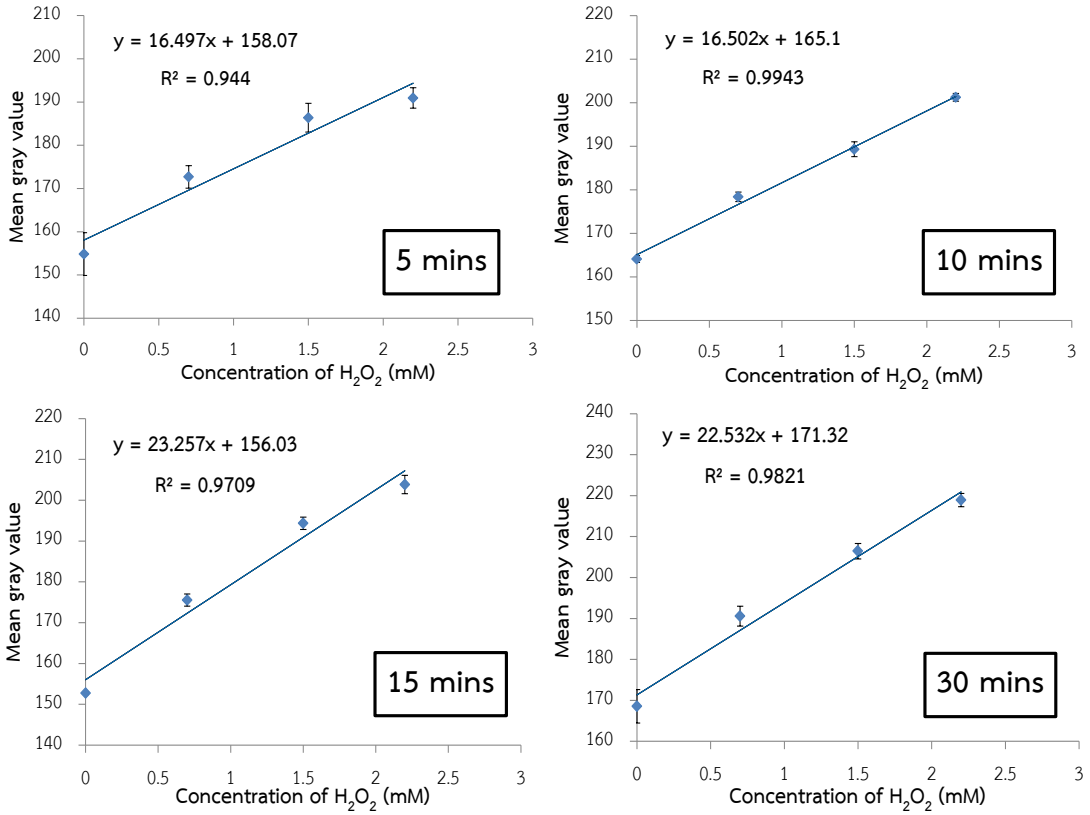


Figure 4.11 Calibration curves for the determination of H_2O_2 using AgNPls papers after the reaction with H_2O_2 at different contact times (dried testing papers) (sample volume 5 mL).

4.2.5 Effect of sample solution volume

In order to find the sample solution volume suitable for the detection, the sample volume was varied by 20 µL to 10 mL. When the sample volume of a H_2O_2 solution was increased, the number of H_2O_2 moles also increased. Figure 4.12 displays the modified filter papers reacted with H_2O_2 solutions of different volumes for 10 minutes. It could be seen that dropping 20 µL of H_2O_2 solution onto the papers did not show obvious color change due to a low amount of H_2O_2, compared to the content of AgNPls on the papers. On the other hand, the papers immersed in 2, 5 and 10 mL of H_2O_2 solution resulted in a noticeable color change and a larger volume of solution gave clearer color change due to a

higher amount of H_2O_2. The calibration curves obtained by applying different sample volumes are displayed in Figure 4.13 and Figure 4.14. However, the color intensity observed when used the papers to detect 3.0 mM H_2O_2 was not different from that of 2.2 mM H_2O_2. Therefore, the limit of linearity was 2.2 mM H_2O_2. When a sample volume of 5 mL was used, the obtained results show a better linear relationship between the color intensity and H_2O_2 concentration and a higher slope than using 2 mL or 10 mL of sample. Thus, a sample volume of 5 mL was chosen in this study.

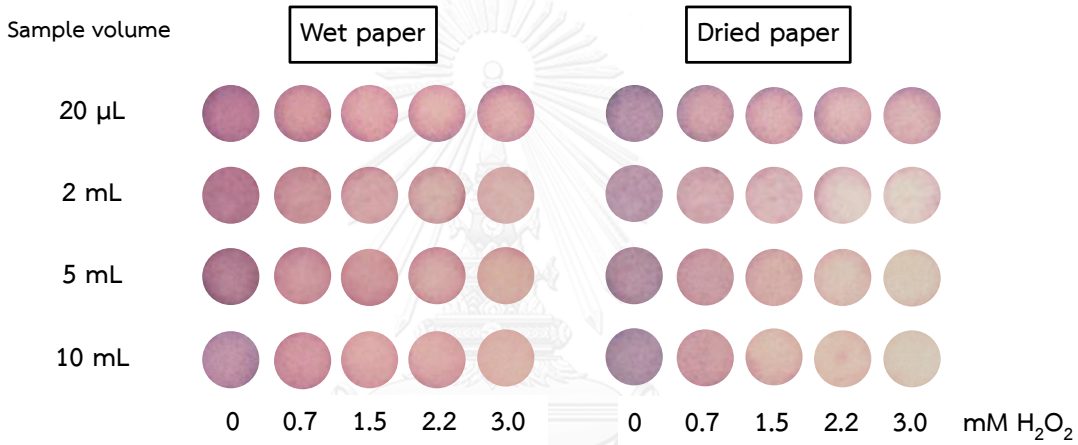


Figure 4.12 Comparison of AgNPls papers after being immersed in different volumes of H_2O_2 solutions (contact time 10 minutes).

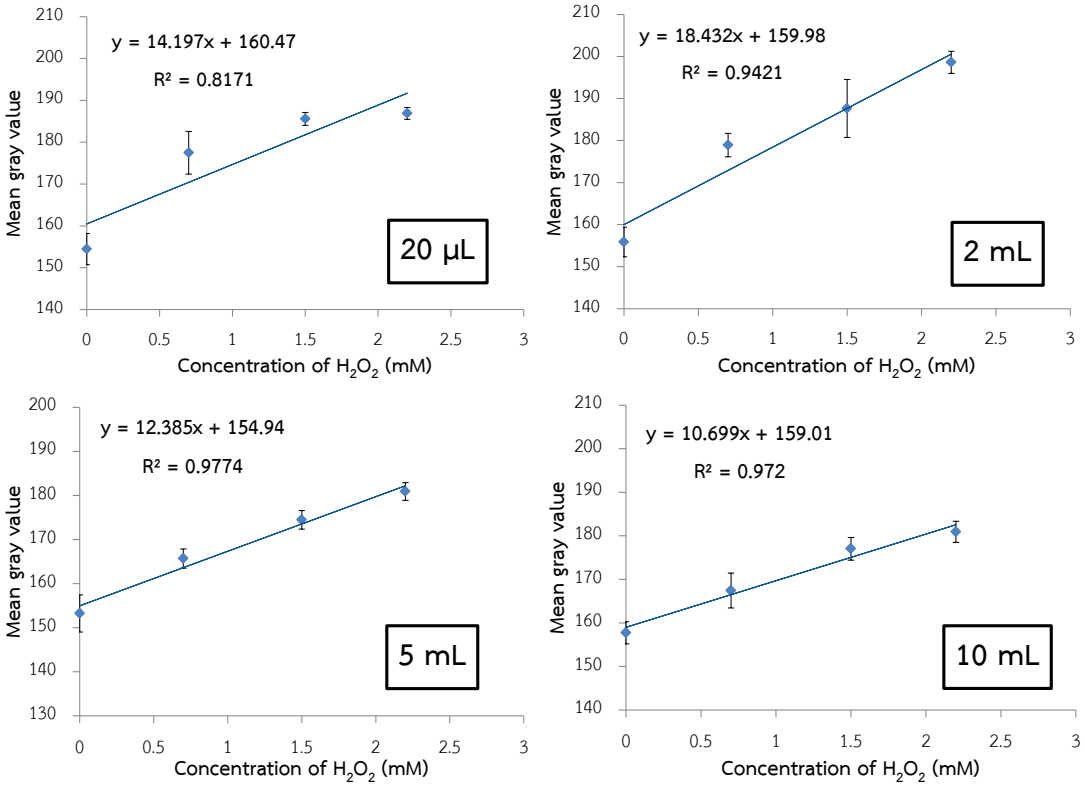


Figure 4.13 Comparison of calibration curve of AgNPls papers after the reaction with H_2O_2 solutions of different volumes (wet testing papers) (contact time 10 minutes).

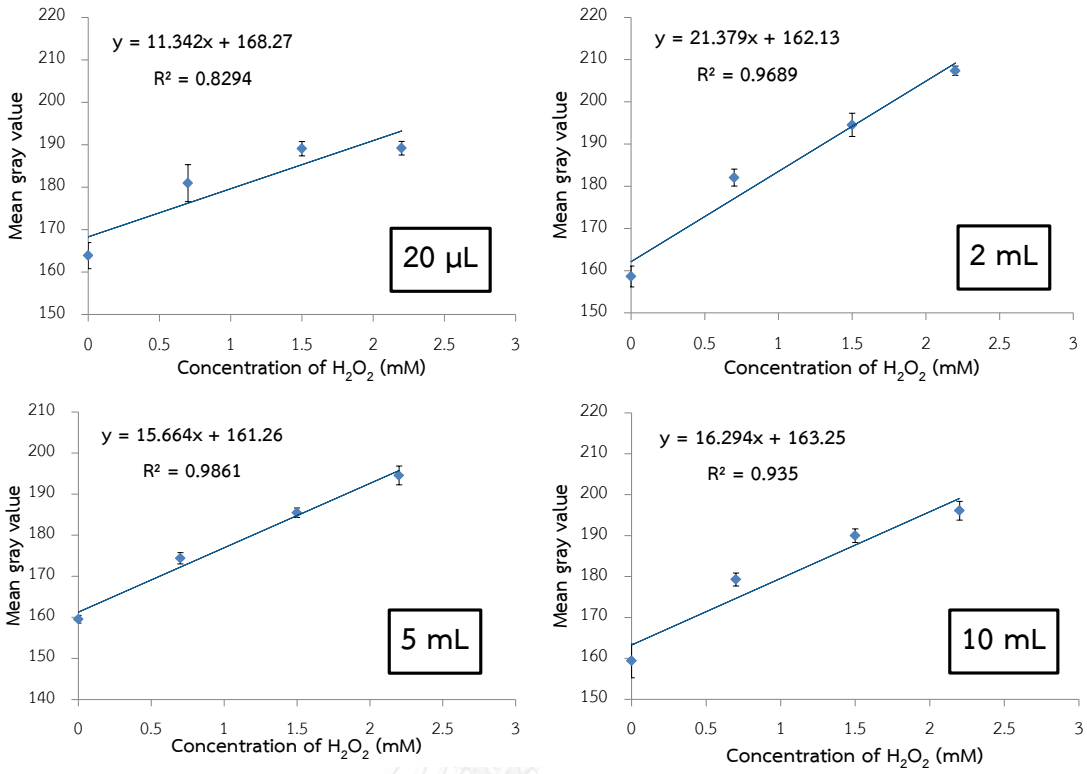


Figure 4.14 Comparison of calibration curve of AgNPls papers after the reaction with H_2O_2 solutions of different volumes (dried testing papers) (contact time 10 minutes).

For the paper based H_2O_2 detection, the testing papers observed when dried after the reaction with H_2O_2 showed distinct color changes and a better linear relationship between mean gray values and concentrations of H_2O_2 than using results from wet testing papers. However, a major drawback was a long detection time. In the next part, an oven was used to dry the testing papers after the reaction to reduce analysis time.

4.3 Paper-based glucose detection in human urine samples

In the detection of glucose in urine sample, due to the interfering effect of urine matrix including chloride, sulfate and phosphate ions, these ions were eliminated by adding $AgNO_3$ solution to form precipitates. An excess amount of

$AgNO_3$ was required to assure the precipitation of these ions. To perform the analysis with the testing papers, the paper was not immersed in the sample as the residual silver ions in sample would attach onto the paper surface. As a result, a thick layer of Ag_2O occurred on the paper surface when dried. Consequently, the paper color tone changed to darker tone and the AgNPls bleaching could not be properly observed. Thus, the shape of the filter papers and the detection procedure was modified. A rectangle shape and a flow system were chosen instead of a disk shape and immersing system, respectively. The sample volume was also reduced to minimize the matrix effect and the usage of $AgNO_3$ solution. Glucose in standard solutions and urine samples reacted with glucose oxidase to produce H_2O_2 that would further be detected by AgNPls paper strips.

4.3.1 Effect of AgNPls concentration

In this system, the paper strip was dipped into a sample solution to allow a small volume of sample to flow to the testing area. Hence, only small but suitable amount of AgNPls was required in the testing area. In this study, the paper strips were prepared with solutions of AgNPls at concentration of 50, 75, 100 and 180 ppm and glucose standard solutions were tested instead of a human urine sample. Thus, a $AgNO_3$ solution for chloride, sulfate and phosphate ions precipitation was not used in this experiment. Figure 4.15 presents the color of AgNPls test area on the paper strips after being dipped in solutions containing different glucose concentrations (0–3 mM). By using 75 and 50 ppm AgNPls concentration, the original color of the test area was pale and a change in color after the reaction with H_2O_2 was not clear because of a low content of AgNPls. However, when increase the AgNPls concentration to 180 ppm, the color was intense and it was difficult to differentiate the colors due to high content of AgNPls. The suitable AgNPls concentration to prepare the testing strips was 100 ppm. It displayed clear color change from pink to white in increasing H_2O_2 concentration. To compare the results of AgNPls bleaching by H_2O_2, mean gray values of these testing areas were measured and used to construct calibration

curves as shown in Figure 4.16. The results also confirmed that using the strip with 100 ppm AgNPls gave highest slope (good contrast for different colors) and good linear relationship. However, it should be noticed that the color intensity observed in the detection of 3.0 mM glucose was close to that of 2.0 mM, probably due to a limited amount of AgNPls on the surface. Thus, an upper limit of detection was limited to 2.0 mM.

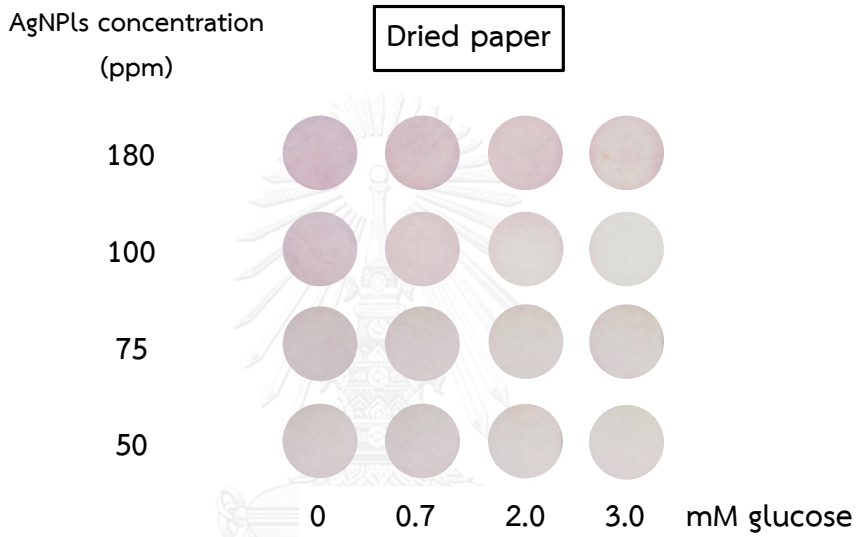


Figure 4.15 Comparison of AgNPls papers prepared by using different AgNPls concentrations in the detection of glucose.

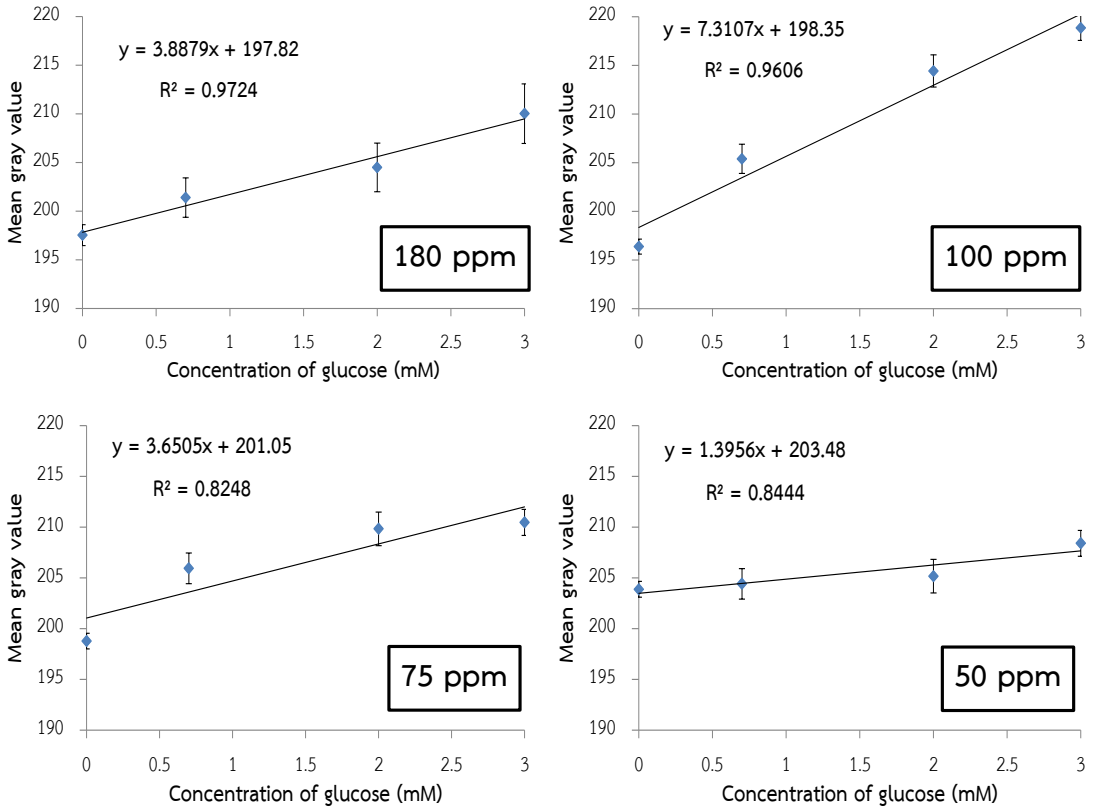


Figure 4.16 Calibration curves for the determination of glucose obtained by using testing papers modified by different AgNPls concentrations.

4.3.2 Effect of urine matrix and $AgNO_3$ volume

In general, a human urine sample contains a certain matrix including chloride, sulfate and phosphate salts that may affect the analysis using AgNPls. AgNPls would release a small amount of silver ions after the reaction with H_2O_2 that form precipitates with these matrix ions. It might form a thin layer of precipitates that covers AgNPls surface and obscures the actual color of AgNPls. The change of color regarding AgNPls bleaching was observed by naked eyes only when $AgNO_3$ was added to urine samples. Therefore, all these chloride, sulfate and phosphate ions must be eliminated before the analysis and the addition of $AgNO_3$ into urine samples was applied for this purpose. Furthermore, standard addition method was adopted to overcome the matrix effect because urine samples from different individuals contain different matrices. The glucose

concentration added into urine samples was in the range of 0–1.6 mM by considering the linear range of the detection and the actual level of glucose in human urines. because of adding more glucose with standard addition method.

In this study, a human urine sample was 2-fold diluted to reduce the content of interference. A volume of 25 or 50 µL of 5 M $AgNO_3$ solution were added into 1 mL of human urine solution prior to the addition of glucose oxidase and the analysis with testing papers. Figure 4.17 shows the color of AgNPls testing area when used in the analysis of a urine sample spiked with standard glucose to have different glucose concentrations (0–1.6 mM). The calibration curve in this part was plotted by using Δ mean gray values which were obtained from the difference of the values of the starting AgNPls-strip color intensity and those of AgNPls after the reaction with H_2O_2 generated from glucose. Δ mean gray values would represent accurately the degree of AgNPls bleaching by H_2O_2 (analyte). The standard addition calibration curve was used for calculating the actual glucose level in a urine sample. The urine sample used in this experiment had glucose level in range of 0.1–0.3 mM measured by standard hexokinase method. When used 50 µL of $AgNO_3$ solution, the glucose level obtained from standard addition method (0.2 mM) was close to values from hexokinase method, showing better accuracy than the use of 25 µL $AgNO_3$ (1.1 mM glucose). The results are shown in Figure 4.17 and Figure 4.18. Apart from precipitation of chloride, sulfate and phosphate ions in the matrix, residual silver ions in solution may catalyze the oxidation of AgNPls [51, 52]. Thus, the volume of 50 µL $AgNO_3$ was chosen in this study.

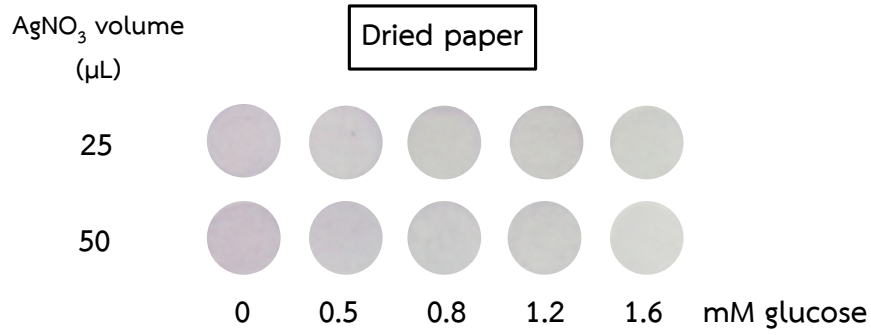


Figure 4.17 Comparison of 100 ppm AgNPls papers with different adding volumes of $AgNO_3$ in 2-fold diluted urine sample.

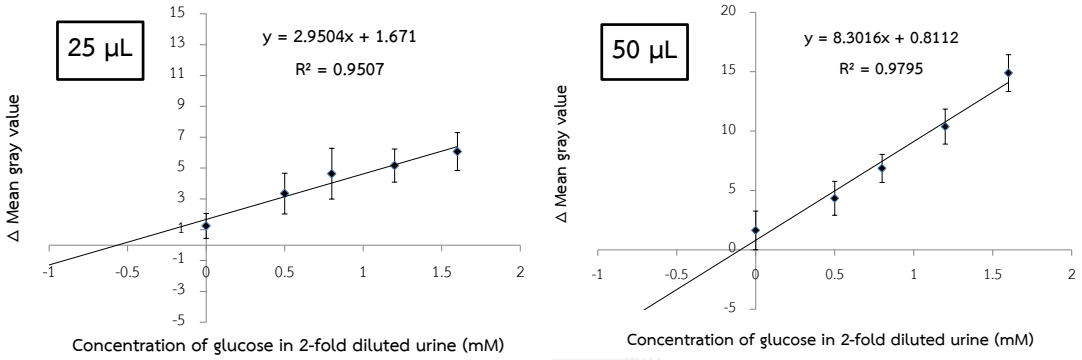


Figure 4.18 Comparison of standard addition calibration curves obtained in the analysis of a urine sample with different adding volumes of $AgNO_3$.

4.3.3 Effect of co-existing species

The effects of co-existing species were examined by adding potential interfering substances into human urine samples. The interfering effect of these species was investigated under an extreme condition. The spiked urine sample contained both the urine matrix and an additional species which was 0.25 M urea, 0.2 M NaCl, 0.02 M creatinine, 0.02 M Na_2SO_4 or 2.5 mM tri-sodium citrate. These concentration levels were the maximum concentration of these species found in human urine. Moreover, the tolerance limit of the potential interfering species including ascorbic acid and KH_2PO_4 was also determined. Urea and creatinine are the major species in human urine. Urea might interact with metal

nanoparticles owing to its $-NH_2$ functional group [53] and they might affect the analysis. Furthermore, tri-sodium citrate and ascorbic acid are reducing agents that could react with H_2O_2. The presence of these reducing substances might result in a decrease in degree of AgNPls bleaching because of the reduction of H_2O_2 concentration. Moreover, citrate ions may also act as stabilizers protecting AgNPls surface from the reaction with H_2O_2.

It was found that phosphate ions and ascorbic acid were only the two substances that affected the analysis if present in high concentrations. Phosphate ions readily formed Ag_3PO_4 precipitate with silver ions from $AgNO_3$ solution (K_{sp} = 8.9 x 10^{-17}). Therefore, less amount of silver ions were left in solution after matrix precipitation to catalyze the oxidation of AgNPls, compared to urine without extremely high concentration phosphate. The tolerance limit of phosphate ion and ascorbic acid were 2 and 0.4 mM, respectively.

Figure 4.19 shows standard addition calibration curve of the analysis of urine samples containing each co-existing species compared to that of non-spiked urine. The quantitative data was obtained after calibration curve extrapolation toward x-axis, as shown in Table 4.2. The glucose levels found in non-spiked urine and spiked urine were not significantly different. The slope values of these graphs were similar to each other. The results indicated that the presence of these species did not affect the analysis and the effect of interfering chloride, sulfate and phosphate ions in the matrix was minimized with the addition of $AgNO_3$ solution.

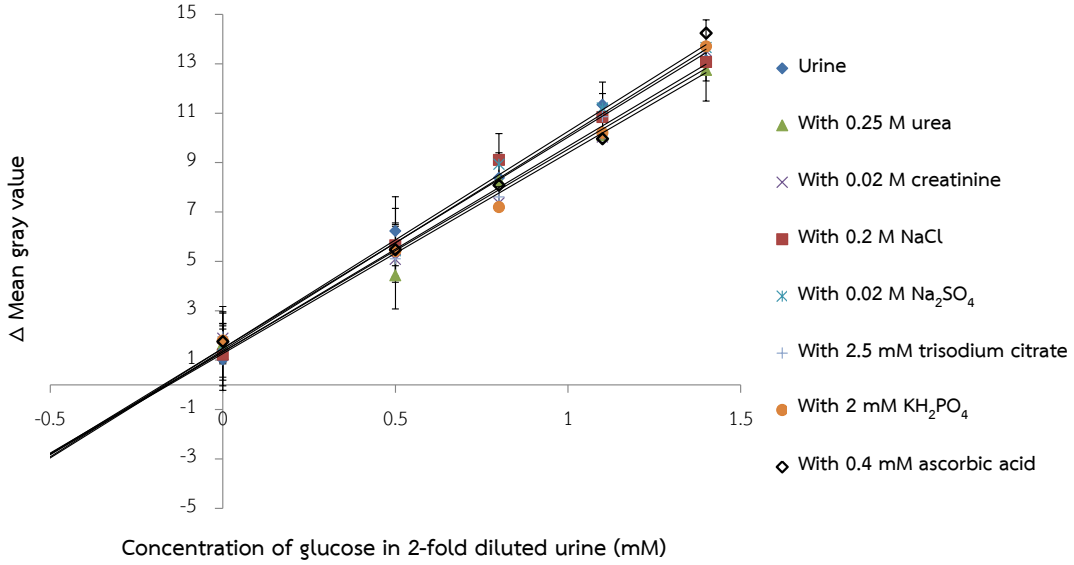


Figure 4.19 Standard addition calibration curves of the determination of spiked and non-spiked urine samples.

Table 4.2 The effect of certain substance on the determination of glucose in spiked and non-spiked urines by standard addition method

Spiked substance	Equations*	R^2*	glucose values*
None	y = 8.7091x + 1.3986	0.9922	0.16±0.06
urea 0.25 mM	y = 8.1240x + 1.2652	0.9841	0.16±0.11
creatinine 0.02 M	y = 8.2005x + 1.3380	0.9836	0.17±0.05
NaCl 0.2 M	y = 8.5489x + 1.4802	0.9902	0.18±0.09
Na_2SO_4 0.02 M	y = 8.7851x + 1.4776	0.9956	0.17±0.06
trisodium citrate 2.5 mM	y = 8.5354x + 1.3618	0.9863	0.16±0.09
KH_2PO_4 2 mM**	y = 8.3236x + 1.3345	0.9825	0.16±0.06
ascorbic acid 0.4 mM**	y = 8.5938x + 1.3647	0.9814	0.16±0.09

* Results of 2-fold diluted urine samples ** Tolerance limit of this method

4.3.4 Method validation

The proposed paper-based method was validated and the accuracy and precision were evaluated under the chosen condition. The obtained analytical results were compared to the results from the analysis using a standard hexokinase method and Combur[7] and URS-2P dipsticks for glucose detection in urine.

Fresh urine samples were collected from Environmental Analysis Research Unit (EARU) members. The standard addition calibration curve plotted between Δ mean gray values and concentrations of glucose (0–1.4 mM or 0–25 mg/dL) was constructed as presented in Figure 4.20 and all results are shown in Table 4.3. The results of all methods (paper-based method, hexokinase method, Combur[7] and URS-2P dipstick) were compared in Table 4.4. The results from URS-2P dipstick were all 'negative' (not shown in the table) due to a high limit of detection (5 mM) of this dipstick. The Combur[7] dipsticks indicated only vaguely the level of glucose in samples. On the other hand, the results of the paper-based method and the standard hexokinase method were not significantly different at 95% confidence level. The recovery of glucose in spiked sample detected by paper-based method was in the range of 94 to 105% and 100 to 105% for hexokinase method. The relative standard deviation of the results obtained from paper-based method and hexokinase method was 5.9–21.4% and 0–3.9%, respectively. These results indicated that the accuracy of the paper-based method was acceptable [54] and comparable to the standard hexokinase method. However, the precision of the results of the detection of extremely low level of glucose in some samples was low and not in the acceptable range. All of results confirmed that AgNPls paper-based glucose detection can be an alternative for urinary glucose detection.

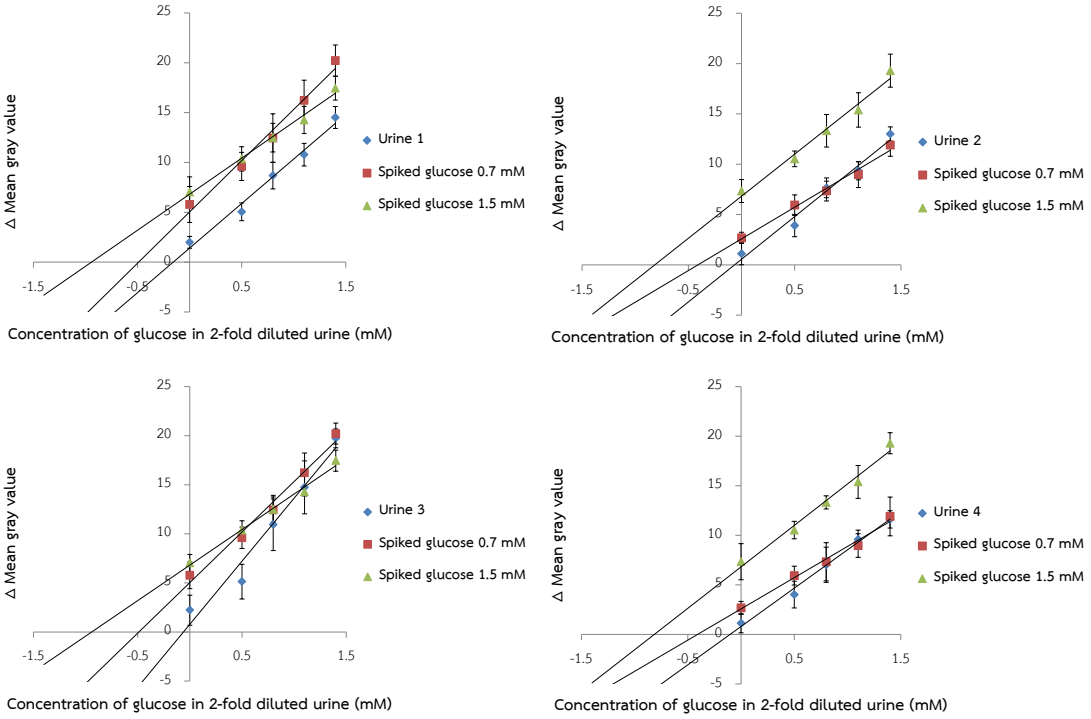
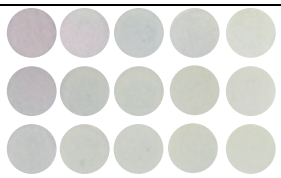


Figure 4.20 The standard addition calibration curves for the determination of glucose in non-spiked and glucose spiked urine samples.

Table 4.3 The paper colors, equations and R^2 of the standard addition calibration curves for the analysis of urine samples

Samples*	Amount added (mM)	Paper colors (0, 0.5, 0.8, 1.1, 1.4 mM)	Equations	R^2
Urine 1	0		y = 8.9420 + 1.4083	0.9833
	0.35		y = 10.292 + 5.0300	0.9828
	0.75		y = 7.2386 + 6.8172	0.9905
Urine 2	0		y = 8.4853 + 0.5568	0.9791
	0.35		y = 6.2979 + 2.5806	0.9855
	0.75		y = 8.3623 + 6.8225	0.9819
Urine 3	0		y = 12.843 + 0.7887	0.9633
	0.35		y = 12.372 + 4.8382	0.9681
	0.75		y = 6.7202 + 5.5546	0.9616
Urine 4	0		y = 7.9496 + 0.5677	0.9944
	0.35		y = 10.713 + 4.5295	0.9687
	0.75		y = 8.6909 + 7.2200	0.9848

* Samples were 2-fold diluted.

Table 4.4 Determination of glucose in urine samples by the paper-based method, standard hexokinase method and a commercial dipstick

Samples	Amount added (mM)	Paper-based method			Hexokinase method			Combur[7]
		Found* (mM)	Recovery (%)	RSD (%)	Found* (mM)	Recovery (%)	RSD (%)	Found (mM)
Urine 1	0	0.31±0.03	–	9.7	0.33	–	0	0
	0.7	0.98±0.09	95	9.2	1.06	103	0	0
	1.5	1.88±0.11	105	5.9	1.91±0.03	105	1.7	0-2.8
Urine 2	0	0.13±0.02	–	15.4	0.11	–	0	0
	0.7	0.81±0.07	98	8.6	0.83	103	0	0
	1.5	1.63±0.13	100	8.0	1.67	104	0	0-2.8
Urine 3	0	0.12±0.02	–	16.7	0.11	–	0	0
	0.7	0.78±0.07	94	9.0	0.81±0.03	101	3.9	0
	1.5	1.65±0.13	102	7.9	1.63±0.03	101	2.0	0-2.8
Urine 4	0	0.14±0.03	–	21.4	0.11	–	0	0
	0.7	0.85±0.06	100	7.1	0.81±0.03	101	3.9	0
	1.5	0.85±0.06	101	6.6	1.61±0.06	100	3.4	0-2.8

* mean±SD (n=3)

CHAPTER V
CONCLUSION

5.1 Conclusion

In this research, small-sized red AgNPls were used to detect H_2O_2 and glucose based on the redox reaction between Ag(0) and H_2O_2 with paper-based system.

For H_2O_2 detection, the paper previously coated with 0.5 %v/v MPTMS was modified with a 180 ppm red AgNPls. The testing papers successfully detected H_2O_2 at a low concentration as 0.7 mM up to 3.0 mM (12.6–54 mg/dL) by immersing the AgNPls modified paper in 5 mL of H_2O_2 sample solutions for 10 minutes. When H_2O_2 concentration increased, the change of color from magenta to orange and yellow, respectively, could be clearly observed by naked eyes and the quantitative data was obtained *via* ImageJ software. The AgNPls modified papers showed a potential in the detection of H_2O_2 produced in reactions of certain biomolecules including glucose.

In glucose detection, AgNPls-paper strip was fabricated. To overcome the effect of human urine matrix, the standard addition method was used. Moreover, the 2 fold-dilution of urine samples was performed to reduce the content of interferences. Glucose oxidase solution was added into a set of urine samples to react with glucose and generate H_2O_2 for 30 minutes. Furthermore, chloride, sulfate and phosphate ions in urine samples were eliminated by adding 5 M $AgNO_3$ solution to form precipitates. The test strips were dipped in urine samples to let H_2O_2 flow toward detection area modified by a solution of 100 ppm AgNPls. The photos of the papers were taken to measure the color intensity by using ImageJ software in gray scale mode to get data for calibration curve construction. The linear range for the determination of glucose was in the range from 0 to 1.4 mM (0–25 mg/dL). The species commonly found in human urine (*i.e.*, urea, creatinine, chloride ion, sulfate ion and trisodium citrate) do not have effect on the detection of glucose excepting phosphate ion and ascorbic acid. The tolerance limit of phosphate ion and ascorbic

acid were 2 and 0.4 mM, respectively. The paper-based method could detect glucose in urine samples with an acceptable accuracy and precision.

5.2 Suggestion for future work

The paper-based H_2O_2 detection system should be applied to detect H_2O_2 in other samples or to detect H_2O_2 generated from reactions of other biomolecules of interest.

REFERENCES

[1] World Health Organization. World Health Day 2016: Beat diabetes [Online]. 2016. Available from: http://www.who.int/campaigns/world-health-day/2016/en/ [May 1, 2016]

[2] Radhakumary, C. and Sreenivasan, K. Naked eye detection of glucose in urine using glucose oxidase immobilized gold nanoparticles. Anal. Chem. 83(7) (2011): 2829-2833.

[3] Cowart, S.L. and Stachura, M.E. Glucosuria. in SYSTEM, X.E. (ed.)LABORATORY, pp. 653-657.

[4] Lu, J., et al. Comparable efficacy of self-monitoring of quantitative urine glucose with self-monitoring of blood glucose on glycaemic control in non-insulin-treated type 2 diabetes. Diabetes Res Clin Pract 93(2) (2011): 179-186.

[5] Alkasir, R.S., Ornatska, M., and Andreescu, S. Colorimetric paper bioassay for the detection of phenolic compounds. Anal. Chem. 84(22) (2012): 9729-9737.

[6] Hossain, S.M. and Brennan, J.D. beta-Galactosidase-based colorimetric paper sensor for determination of heavy metals. Anal. Chem. 83(22) (2011): 8772-8778.

[7] Jokerst, J.C., Adkins, J.A., Bisha, B., Mentele, M.M., Goodridge, L.D., and Henry, C.S. Development of a paper-based analytical device for colorimetric detection of select foodborne pathogens. Anal. Chem. 84(6) (2012): 2900-2907.

[8] Pozuelo, M., Blondeau, P., Novell, M., Andrade, F.J., Xavier, R.F., and Riu, J. Paper-based chemiresistor for detection of ultralow concentrations of protein. Biosens. Bioelectron. 49 (2013): 462-465.

[9] Ratnarathorn, N., Chailapakul, O., Henry, C.S., and Dungchai, W. Simple silver nanoparticle colorimetric sensing for copper by paper-based devices. Talanta 99 (2012): 552-557.

[10] Yildiz, U.H., Alagappan, P., and Liedberg, B. Naked eye detection of lung cancer associated miRNA by paper based biosensing platform. Anal. Chem. 85(2) (2013): 820-824.

[11] Parnklang, T., Lertvachirapaiboon, C., Pienpinijtham, P., Wongravee, K., Thammacharoen, C., and Ekgasit, S. H_2O_2-triggered shape transformation of silver nanospheres to nanoprisms with controllable longitudinal LSPR wavelengths. RSC Adv. 3(31) (2013): 12886-12894.

[12] Pangdam, A., Thammacharoen, C., and Ekgasit, S. Hydrogen peroxide determination via colorimetric sensor by silver nanoparticles aggregration. in 36th Congress on Science and Technology of Thailand. Bangkok, Thailand, 2010.

[13] Chen, S., Hai, X., Chen, X.W., and Wang, J.H. In situ growth of silver nanoparticles on graphene quantum dots for ultrasensitive colorimetric detection of H_2O_2 and glucose. Anal. Chem. 86(13) (2014): 6689-6694.

[14] Filippo, E., Serra, A., and Manno, D. Poly(vinyl alcohol) capped silver nanoparticles as localized surface plasmon resonance-based hydrogen peroxide sensor. Sens. Actuators, B 138(2) (2009): 625-630.

[15] Wang, G.L., Zhu, X.Y., Jiao, H.J., Dong, Y.M., and Li, Z.J. Ultrasensitive and dual functional colorimetric sensors for mercury (II) ions and hydrogen peroxide based on catalytic reduction property of silver nanoparticles. Biosens. Bioelectron. 31(1) (2012): 337-342.

[16] Yang, X.-H., Ling, J., Peng, J., Cao, Q.-E., Ding, Z.-T., and Bian, L.-C. A colorimetric method for highly sensitive and accurate detection of iodide by finding the critical color in a color change process using silver triangular nanoplates. Anal. Chim. Acta. 798 (2013): 74-81.

[17] Zhang, Y., et al. A quantitative colorimetric assay of H_2O_2 and glucose using silver nanoparticles induced by H_2O_2 and UV. Chin. Chem. Lett. 24(12) (2013): 1053-1058.

[18] American Diabetes Association. Standards of Medical Care in Diabetes-2016 Abridged for Primary Care Providers. Clin Diabetes 34(1) (2016): 3-21.

[19] Schersten, B. and Fritz, H. Subnormal Levels of Glucose in Urine. JAMA 201 (1967): 129-132.

[20] Tilley, E., Atwater, J., and Mavinic, D. Effects of storage on phosphorus recovery from urine. Environ. Technol. 29(7) (2008): 807-816.

[21] Sittiwong, J. Determination of creatinine in urine samples using gold nanoparticles and paper-based Jaffe reaction. Doctor of Philosophy Program, Chemistry Chulalongkorn, 2014.

[22] Boontham, S. Determination of creatinine by spectrophometry after extraction by functionalized MCM-41-coated magnatic particles. Master of Science Program, Chemistry Chulalongkorn, 2012.

[23] Shuang, T.H., Christopher, B.-M., Kristina, T., and G, C.R. Chemical interactions between silver nanoparticles and thiols: a comparison of mercaptohexanol against cysteine. Sci. China Chem. 57 (2014): 1199-1210.

[24] Lopez, I.A. and Gomez, I. Photoinduced shape transformation from nanospheres to silver triangular nanoprisms and nanodisks: citrate ion concentration and stirring effects. Rev. Mex. Fis. 58 (2012): 289-292.

[25] Wu, C., Zhou, X., and Wei, J. Localized Surface Plasmon Resonance of Silver Nanotriangles Synthesized by a Versatile Solution Reaction. Nanoscale Res Lett 10(1) (2015): 1058.

[26] Vilela, D., Gonzalez, M.C., and Escarpa, A. Sensing colorimetric approaches based on gold and silver nanoparticles aggregation: chemical creativity behind the assay. A review. Anal Chim Acta 751 (2012): 24-43.

[27] Kelly, K.L., Coronado, E., Zhao, L.L., and Schatz, G.C. The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment. The Journal of Physical Chemistry B 107 (2003): 668-677.

[28] Nitinaivinij, K. Decomposition profiles of silver nanoplates under oxidative conditions using hydrogen peroxide. Master of Science Program, Chemistry Chulalongkorn, 2014.

[29] Jin, R., Cao, Y., Mirkin, C.A., Kelly, K.L., Schatz, G.C., and Zheng, J.G. Photoinduced conversion of silver nanospheres to nanoprisms. Science 294(5548) (2001): 1901-1903.

[30] Tanimoto, H., Hashiguchi, K., and Ohmura, S. Growth Inhibition of Hexagonal Silver Nanoplates by Localized Surface Plasmon Resonance. The Journal of Physical Chemistry C 119(33) (2015): 19318-19325.

[31] Chen, S. and Carroll, D.L. Silver Nanoplates: Size Control in Two Dimensions and Formation Mechanisms. J. Phys. Chem. B 108 (2004): 5500-5506.

[32] Cathcart, N., Frank, A.J., and Kitaev, V. Silver nanoparticles with planar twinned defects: effect of halides for precise tuning of plasmon resonance maxima from 400 to >900 nm. Chem Commun (Camb) (46) (2009): 7170-7172.

[33] Kelly, J.M., Keegan, G., and Brennan-Fournet, M.E. Triangular Silver Nanoparticles: Their Preparation, Functionalisation and Properties. Acta Phys. Pol., A 122 (2012): 337-345.

[34] Tang, B., Xu, S., An, J., Zhao, B., Xu, W., and Lombardi, J.R. Kinetic effects of halide ions on the morphological evolution of silver nanoplates. Phys Chem Chem Phys 11(44) (2009): 10286-10292.

[35] Apilux, A., Siangproh, W., Praphairaksit, N., and Chailapakul, O. Simple and rapid colorimetric detection of Hg(II) by a paper-based device using silver nanoplates. Talanta 97 (2012): 388-394.

[36] Chen, L., Fu, X., Lu, W., and Chen, L. Highly sensitive and selective colorimetric sensing of Hg^{2+} based on the morphology transition of silver nanoprisms. ACS Appl Mater Interfaces 5(2) (2013): 284-290.

[37] Chaiyo, S., Siangproh, W., Apilux, A., and Chailapakul, O. Highly selective and sensitive paper-based colorimetric sensor using thiosulfate catalytic etching of silver nanoplates for trace determination of copper ions. Anal. Chim. Acta 866 (2015): 75-83.

[38] Nitinaivinij, K., Parnklang, T., Thammacharoen, C., Ekgasit, S., and Wongravee, K. Colorimetric determination of hydrogen peroxide by morphological decomposition of silver nanoprisms coupled with chromaticity analysis. Anal. Methods 6(24) (2014): 9816-9824.

[39] Ngeontae, W., Janrungroatsakul, W., Maneewattanapinyo, P., Ekgasit, S., Aeungmaitrepirom, W., and Tuntulani, T. Novel potentiometric approach in glucose biosensor using silver nanoparticles as redox marker. Sens. Actuators, B 137(1) (2009): 320-326.

[40] Zargoosh, K., Chaichi, M.J., Shamsipur, M., Hossienkhani, S., Asghari, S., and Qandalee, M. Highly sensitive glucose biosensor based on the effective

immobilization of glucose oxidase/carbon-nanotube and gold nanoparticle in nafion film and peroxyoxalate chemiluminescence reaction of a new fluorophore. Talanta 93 (2012): 37-43.

[41] Zargoosh, K., Shamsipur, M., Qandalee, M., Piltan, M., and Moradi, L. Sensitive and selective determination of glucose in human serum and urine based on the peroxyoxalate chemiluminescence reaction of a new fluorophore. Spectrochim. Acta A Mol. Biomol. Spectrosc. 81(1) (2011): 679-683.

[42] Bo, H., Wang, C., Gao, Q., Qi, H., and Zhang, C. Selective, colorimetric assay of glucose in urine using G-quadruplex-based DNAzymes and 10-acetyl-3,7-dihydroxy phenoxazine. Talanta 108 (2013): 131-135.

[43] Su, L., Feng, J., Zhou, X., Ren, C., Li, H., and Chen, X. Colorimetric detection of urine glucose based $ZnFe_2O_4$ magnetic nanoparticles. Anal. Chem. 84(13) (2012): 5753-5758.

[44] Xu, M., Bunes, B.R., and Zang, L. Paper-based vapor detection of hydrogen peroxide: colorimetric sensing with tunable interface. ACS Appl. Mater. Interfaces. 3(3) (2011): 642-647.

[45] Martinez, A.W., Phillips, S.T., Carrilho, E., Thomas, S.W., Sindi, H., and Whitesides, G.M. Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis. Anal. Chem. 80 (2008): 3699–3707.

[46] Ornatska, M., Sharpe, E., Andreescu, D., and Andreescu, S. Paper bioassay based on ceria nanoparticles as colorimetric probes. Anal. Chem. 83(11) (2011): 4273-4280.

[47] Kim, S.S., Park, J.E., and Lee, J. Properties and antimicrobial efficacy of cellulose fiber coated with silver nanoparticles and 3-mercaptopropyltrimethoxysilane (3-MPTMS). J. Appl. Polym. Sci. 119(4) (2011): 2261-2267.

[48] Battocchio, C., et al. Silver Nanoparticles Stabilized with Thiols: A Close Look at the Local Chemistry and Chemical Structure. J. Phys. Chem. C. 116 (2012): 19571–19578.

[49] Zhou, M., Yang, M., and Zhou, F. Paper based colorimetric biosensing platform utilizing cross-linked siloxane as probe. Biosens. Bioelectron. 55 (2014): 39-43.

[50] Noiphung, J., Songjaroen, T., Dungchai, W., Henry, C.S., Chailapakul, O., and Laiwattanapaisal, W. Electrochemical detection of glucose from whole blood using paper-based microfluidic devices. Anal. Chim. Acta 788 (2013): 39-45.

[51] Jana, N.R., Sau, T.K., and Pal, T. Growing Small Silver Particle as Redox Catalyst. J. Phys. Chem. B 103 (1999): 115-121.

[52] Shiraishi, Y. and Toshima, N. Colloidal silver catalysts for oxidation of ethylene. J. Mol. Catal. A: Chem. 141 (1999): 187-192.

[53] Campos, C., et al. Adhesion of gold and silver nanoparticles onto urea–alkylamine inclusion compounds. J. Inclusion Phenom. Macrocyclic Chem. 75(1-2) (2012): 165-173.

[54] Gustavo González, A. and Ángeles Herrador, M. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. TrAC, Trends Anal. Chem. 26(3) (2007): 227-238.

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