A Smartphone-based Colorimetric Probe from (3-Aminopropyl)tri-ethoxysiloxane-glutaraldehyde Complex for Hydrogen Peroxide Determination in Antiseptic Commercial Product

Petcharawut Ramsiri

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Forensic Science

Prince of Songkla University

2023

A Smartphone-based Colorimetric Probe from (3-Aminopropyl)tri-ethoxysiloxane-glutaraldehyde Complex for Hydrogen Peroxide Determination in Antiseptic Commercial Product

Petcharawut Ramsiri

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Forensic Science
Prince of Songkla University
2023

Thesis Title	A Smartphone-based Colorimetric Probe from (3-Aminopropyl)-triethoxysiloxane-glutaraldehyde Complex for Hydrogen Peroxide Determination in Antiseptic Commercial Product
Author	Mr. Petcharawut Ramsiri
Major program	Forensic Science

Major Advisor	**Examining committee:**
……	……..Chairperson
(Asst. Prof. Dr. Apichai Phonchai)	(Dr. Nichanun Sirasunthorn)
	…….……..Committee
Co-advisor:	(Asst. Prof. Dr. Wongkot Phuphumirat)
……	…….. Committee
(Assoc. Prof. Dr. Aree Choodum)	(Asst. Prof. Dr. Apichai Phonchai)
	…….. Committee
	(Assoc. Prof. Dr. Aree Choodum)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Forensic science.

…….

(Asst. Prof. Dr. Thakerng Wongsirichot)

Acting Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due to acknowledgement has been made of any assistance received.

…….. Signature
(Asst. Prof. Dr. Apichai Phonchai)
Major advisor

…….. Signature
(Assoc. Prof. Dr. Aree Choodum)
Co-advisor

…….. Signature
(Mr. Petcharawut Ramsiri)
Candidate

I hereby certify that this work has not been accepted in substance for any degree and is not being currently submitted in candidature for any degree.

…….. Signature

(Mr. Petcharawut Ramsiri)

Thesis Title A Smartphone-based Colorimetric Probe from (3-Aminopropyl)-triethoxysiloxane-glutaraldehyde Complex for Hydrogen Peroxide Determination in Antiseptic Commercial Product

Author Mr. Petcharawut Ramsiri

Major program Forensic Science

Academic year 2023

ABSTRACT

This thesis aims to develop a smartphone-based colorimetric probe for hydrogen peroxide determination in an antiseptic solution. The colorimetric probe employed (3-aminopropyl)triethoxysiloxane-glutaraldehyde complex (APTES-GA) as a chemical reagent that could react to hydrogen peroxide and give off a bleached colorimetric product. The change of the brick-red color to a pale yellow can be observed through the naked eye and quantified using a smartphone-based digital image analysis method with the assistance of a custom-built photographic box. The parameters of APTES-GA preparation as a colorimetric probe were optimized. The method development for hydrogen peroxide detection was investigated including the effects of the color detection mode, the volume ratio of APTES-GA and sample solution, reaction time, and water content on colorimetric analysis. Under the optimum conditions, a linear calibration curve for the hydrogen peroxide was established in the range of 0.17 %w/v to 1.36 %w/v using green intensity (R^2 = 0.9909) as an analytical signal. The limit of detections (LOD) and limit of quantifications (LOQ) were calculated from the standard error of y-intercept and slope of the calibration curve and obtained as 0.10 %w/v and 0.32 %w/v, respectively. The intra-day and inter-day precisions were 0.074 - 0.363 %RSD and 0.268 - 0.910 %RSD, respectively. The intra-day and inter-day accuracies were 82.124 - 109.017% and 85.292 - 104.131%, respectively. The APTES-GA probe displayed an excellent shelf-life of evaluation and can be stored at 4 ºC and -18 ºC for up to 6 weeks without a decrease in performance. The proposed method was applied to detect hydrogen peroxide in antiseptic commercial products. The results agreed with those from $TiOSO_4$ spectrophotometry which was used as a reference

method. Overall, the device is simple, rapid, cost-effective, portable, disposable, and suitable for onsite screening.

Keywords: Hydrogen peroxide, Colorimetric probe, APTES-GA complex

ชื่อวิทยานิพนธ์ การพัฒนาอุปกรณ์ตรวจวัดแบบตรวจวัดสี จากสารเชิงซ้อนของ (3-อะมิโนโพรพิล)ไตรเอทอกซีไซลอกเซนและกลูตารัลดีไฮด์ สำหรับการวิเคราะห์ไฮโดรเจนเปอร์ออกไซด์ในผลิตภัณฑ์น้ำยาฆ่าเชื้อ

ผู้เขียน เพชราวุธ รามศิริ

สาขาวิชา นิติวิทยาศาสตร์

ปีการศึกษา 2566

บทคัดย่อ

วิทยานิพนธ์ฉบับนี้ ถูกจัดทำขึ้นโดยมีจุดประสงค์เพื่อพัฒนาอุปกรณ์ตรวจวัดแบบตรวจวัดสีด้วยโทรศัพท์มือถือ สำหรับตรวจวัดสารไฮโดรเจนเปอร์ออกไซด์ในผลิตภัณฑ์น้ำยาฆ่าเชื้อ โดยใช้สารเชิงซ้อนของ (3-อะมิโนโพรพิล)ไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ (APTES-GA) ที่มีสีแดงอิฐ เป็นสารตรวจวัด เมื่อสารเชิงซ้อน APTES-GA ทำปฏิกิริยากับไฮโดรเจนเปอร์ออกไซด์จะเกิดการเปลี่ยนแปลงสีจากสีแดงอิฐเป็นสีเหลืองซีด ซึ่งสามารถสังเกตเห็นได้ด้วยตาเปล่า และสามารถตรวจวัดปริมาณสารไฮโดรเจนเปอร์ออกไซด์ด้วยวิธีวิเคราะห์ภาพดิจิทัลโดยใช้โทรศัพท์มือถือเป็นเครื่องมือถ่ายภาพร่วมกับกล่องถ่ายภาพ วิทยานิพนธ์นี้ได้ศึกษาสภาวะที่เหมาะสมของการเตรียมสารเชิงซ้อน APTES-GA รวมไปถึงการศึกษาสภาวะที่เหมาะสมของการตรวจวัดสารไฮโดรเจนเปอร์ออกไซด์ ได้แก่ อัตราส่วนโดยปริมาตรของ APTES-GA ต่อสารละลายที่วิเคราะห์ ระยะเวลาการทดสอบ และผลของปริมาณน้ำในสารละลายที่วิเคราะห์ต่อการวิเคราะห์แบบตรวจวัดสี ภายใต้สภาวะที่เหมาะสม ช่วงความเป็นเส้นตรงของวิธีวิเคราะห์อยู่ในช่วงความเข้มข้น 0.17 %w/v ถึง 1.36% w/v (R^2 = 0.9909) เมื่อวิเคราะห์ด้วยความเข้มสีเขียว ค่าขีดจำกัดการตรวจวัดและค่าขีดจำกัดการตรวจวัดเชิงปริมาณ เท่ากับ 0.10 %w/v และ 0.32 %w/v ตามลำดับ โดยคำนวณจากส่วนเบี่ยงเบนมาตรฐานของจุดตัดแกน y และความชันของกราฟมาตรฐาน ความเที่ยงของการวิเคราะห์ภายในวันเดียวกันและระหว่างวัน มีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ในช่วง 0.074 - 0.363% และ 0.268 - 0.910% ตามลำดับ ความแม่นยำของการวิเคราะห์ภายในวันเดียวกันและระหว่างวัน อยู่ในช่วง 82.124 - 109.017% และ 85.292 - 104.131% ตามลำดับ อุปกรณ์ตรวจวัดที่เตรียมจากสารเชิงซ้อน APTES-GA มีอายุการเก็บรักษานาน 6 สัปดาห์ เมื่อเก็บไว้ที่อุณหภูมิ 4 ºC และ -18 ºC อุปกรณ์ตรวจวัดที่พัฒนาขึ้นสามารถตรวจวัดไฮโดรเจนเปอร์ออกไซด์ในผลิตภัณฑ์น้ำยาฆ่าเชื้อได้ และให้ผลการวิเคราะห์สอดคล้องกับวิธีมาตรฐานยูวีวิสิเบิลสเปกโตรโฟโตเมทรีที่

ใช้น้ำยาเคมีไทเทเนียมออกซีซัลเฟต ($TiOSO_4$) เป็นสารทดสอบ โดยสรุป อุปกรณ์ตรวจวัดที่พัฒนาขึ้นสามารถใช้งานได้ง่ายและรวดเร็ว มีราคาถูก พกพาสะดวก และเหมาะสมกับการใช้งานเพื่อวิเคราะห์เบื้องต้น

คำสำคัญ: ไฮโดรเจนเปอร์ออกไซด์, อุปกรณ์ตรวจวัดแบบตรวจวัดสี, สารเชิงซ้อน APTES-GA

ACKNOWLEDEGMENT

I owed this work to my major advisor, Asst. Prof. Dr. Apichai Phonchai, who supervised me with intensive care. Additionally, my sincere thanks to my colleagues for their all assistances and supports during this study that allowed this study to be completed.

I thank myself for going through a lot. This work took me months to complete due to unexpected issues that arose during the process. Honestly, the outcome of this work totally differed from initial expectation. However, the difficulties and trials I encountered during this research allowed me to learn and improve myself in another way to become the researcher that I would like to be. I am very grateful to everyone who lent me a hand whenever I needed, especially for the complements and encouragement I have received so far. Those kind words indeed carried me through till the end.

At last, I would like to thank the Development and Promotion of Science and Technology Talents Project (DPST) which provided financial support for this work. I also would like to thank the Forensic Chemistry research group, Faculty of Science, Prince of Songkhla University that provided the equipment, laboratory, and facilities that allowed me to accomplish the entire research.

Petcharawut Ramsiri

CONTENTS

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
AOAC	Association of Analytical Communities
AK	Actinic keratosis
APTES	(3-aminopropyl)triethoxysiloxane
APTMS	(3-aminopropyl)trimethoxysiloxane
B	Blue intensity
G	Green intensity
GA	Glutaraldehyde
GSH	Glutathione
HRP	Horseradish peroxidase
HSV	Herpes simplex virus method
ICH	International Conference on Harmonization
LCV	Leuco crystal violet
LOD	Limit of detection
LOQ	Limit of quantification
NATA	National Association of Testing Authorities
NPs	Nanoparticles
R	Red intensity
RE	Relative error
ROS	Reactive oxygen species
RSD	Relative standard deviation
SD	Standard deviation
SDE	Standard error
SK	Seborrheic keratosis
TMB	3,3’,5,5’-tetramethylbenzidine
TNT	Trinitrotoluene

CHAPTER 1

INTRODUCTION

1.1 Background and rationale

Hydrogen peroxide is a common substance that provides a wide range of utilities. Its usage covers several areas such as medicine, detergent, cosmetic, and precursor for other chemicals. The medicinal products of hydrogen peroxide are sold as a water solution with various concentrations depending on its purpose. Generally, a low-concentration hydrogen peroxide product is used as an antiseptic agent and hair-color bleaching agent, whereas a higher one is for industries and laboratories.

The degradation of hydrogen peroxide in a commercial product is inevitable because hydrogen peroxide can decompose readily when exposes to visible light. Hence, the container is mostly made of brown glasses or light-impermeable material to minimize decomposition. Nevertheless, the change of concentration of hydrogen peroxide occurs slowly over a period of time. Although the difference is negligible and can be disregarded, sometimes, it might affect the performance of the product greatly and the hydrogen peroxide medicine solution is one such case.

The common antiseptic product has a concentration of hydrogen peroxide ranging from 3% to 6%. It is a mild antiseptic that is used for cleaning shallow wounds or removing dead skin. In addition, it can be used to rinse the mouth. Of note, the hydrogen peroxide must be diluted according to the instruction prior to actual use. It is because a too-high concentration of hydrogen peroxide will cause irritation on applying area. Hereby, an observation of hydrogen peroxide concentration in medicine products is quite necessary to predict the performance of medicine. The development of analytical methods for the quality control of hydrogen peroxide content in antiseptic commercial products is important to ensure that products are properly labeled and to assure consumer safety. In addition, hydrogen peroxide has been reported as an oxidizing agent in the illicit production of cocaine and has occasionally been identified in clandestine cocaine laboratories. Therefore, detection of hydrogen peroxide is also

necessary for forensic investigations in order to detecting and identifying clandestine drug laboratories.

Many hydrogen peroxide determination methods were reported such as chemical titration (Kiassen et al., 1994), spectrophotometry (Cui et al., 2017; Wang et al., 2018; Yang et al., 2016; Zhang & Wong, 1994; Zong et al., 2018), fluorometry (Qu et al., 2020; Yuan et al., 2015; Zhou et al., 2020), and electrochemistry (Periasamy et al., 2011; Zor et al., 2014). Those mentioned methods cover a variety of samples and provided an efficient way for hydrogen peroxide determination, but it is unlikely an appropriate method for household measurement due to a complication and expensive equipment. Hydrogen peroxide sensors were developed into a cost-effective and reliable method for in situ measurements. Majority of reported sensors utilized colorimetry based on the oxidation of chromogen coupled with horseradish peroxidase (HRP) or peroxidase-mimic substances to promote the reactive oxygen species (Cui et al., 2017; Wang et al., 2018; Yang et al., 2016; Zhang & Wong, 1994; Zong et al., 2018). Despite a good and reliable performance, those mentioned sensors still required a complicated sample preparation and resulted in a long total analysis time. The paper-based siloxane complex sensor, however, showed a good potential for the determination of hydrogen peroxide in hydrogen peroxide medicine products with fewer preparation steps (Zhou et al., 2014). A cross-linked siloxane as probe was used for determination of hydrogen peroxide. The detection is based on the bleaching of the brick-red color of APTMS-GA, resulting a colorless product. The color change can be observed by naked eyes and quantified by software. The method was applied to three different kinds of analytes, hydrogen peroxide, glucose and protein biomarker and showed the ability for quantitative assessment. However, the directly coated reagent on the paper-based analytical device suffers from instability of the reagent.

Henceforth, this work developed a siloxane-glutaraldehyde complex as a colorimetric probe with smartphone-based digital image analysis for determination of hydrogen peroxide. The hydrogen peroxide analysis was based on the oxidation reaction of colorimetric probe by hydrogen peroxide. The camera of a smartphone was used to image the colorimetric products for qualitative and quantitative analysis. The developed method is simple and rapid, cost-effective, portable, disposable, and suitable

for onsite screening use which has the potential to be used for the quality control of antiseptic commercial product and forensic application.

1.2 Literature reviews

1.2.1 General information of hydrogen peroxide

Hydrogen peroxide was produced successfully in 1818 by Louis Jacques Thenard, and the production for industrial scale had been first corrected at the end of the 19th century. Hydrogen peroxide is the simplest molecule in organic peroxides and also reactive oxygen species (ROS). Its molecule comprises two hydrogen atoms and two oxygen atoms (the formula is H_2O_2) which are two oxygen atoms connected by a single covalent bond (O-O). The molar mass of hydrogen peroxide is 34.0147 g mol^{-1}. It appears light blue in its pure form but colorless in solution form, tastes bitter, and has a slightly sharp odor depending on concentration. Hydrogen peroxide is highly soluble in water and many organic solvents especially the solvent that can perform as either hydrogen bond donors or hydrogen bond acceptors. The O-O bond is slightly sensitive and that makes hydrogen peroxide very reactive. It readily undergoes decomposition even under sunlight exposure. Hydrogen peroxide is a strong oxidizing agent which readily oxidizes various oxidizable substances upon contact. The application of this chemical covers many areas such as medicine, detergents, cosmetics, and the chemical industry. (Abdollahi & Hosseini, 2014; National Center for Biotechnology Information, 2023)

1.2.2 Medical application of hydrogen peroxide

Hydrogen peroxide which is reactive oxygen species can damage the cellular biochemical molecules such as nucleic acids, proteins, and lipids via oxidation reaction. With how small hydrogen peroxide is, it can bypass the cell membrane and penetrate inside. The cellular enzymatic reaction of hydrogen peroxide takes place at this point and produces reactive species (e.g., hydroxyl radical (·OH), peroxide anion (O_2^-)) which affect organic molecules in cells, leading to cell membrane breakdown and cell destruction eventually (**Figure 1**). (Murphy & Friedman, 2019)

Several formulas of hydrogen peroxide products are present in a wide range of 1% to 45% of hydrogen peroxide. At low concentrations, 1% to 6% hydrogen

peroxide formulas are for the disinfection of open wounds and for removing dead skin. The efficiency of the antiseptic agent depends on the concentration level and contact time. The 1% formula is less effective compared to the 6% formula because the concentration of hydrogen peroxide is lower, so we need to prolong the contact time of the 1% formula treatment to achieve a similar result to the 6% formula. A diluted hydrogen peroxide solution is recommended for dentistry that can be used for mouth rinsing to remove mucus and recover from oral surgery. Apart from this, modified formulas of hydrogen peroxide at a low concentration that combine with necessary components, are able to treat venous insufficiency ulcers and acne. (Urban et al., 2019).

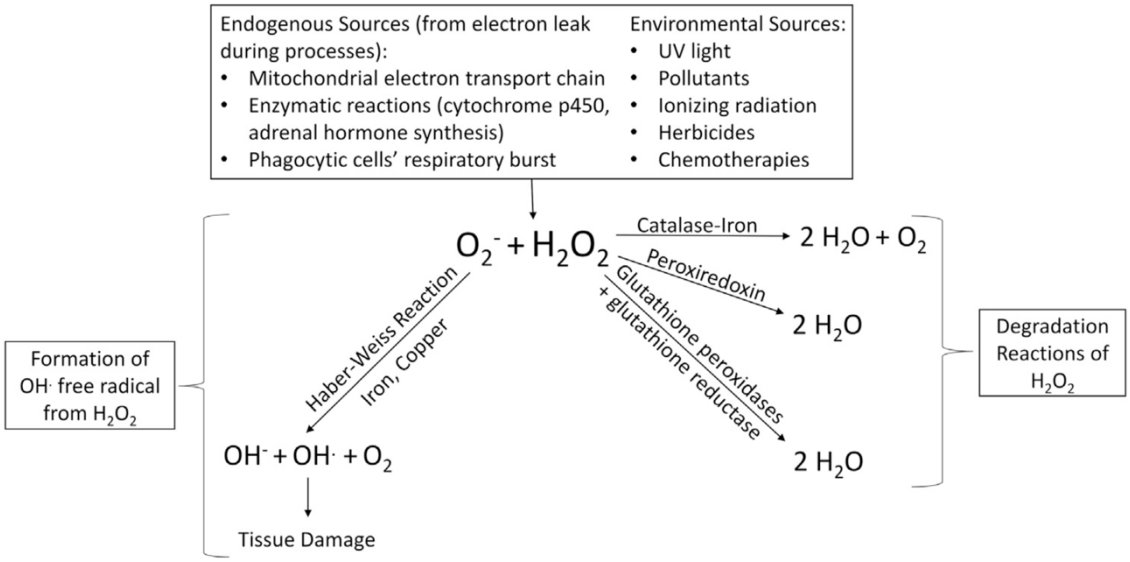


Figure 1 The production of hydrogen peroxide from diverse sources and its degradation in cells that produces reactive oxygen species. (Murphy & Friedman, 2019)

For 10% to 40% formula, the direct contact treatment must be carried out cautiously owing to the risk of severe skin damage. The medical step involving the high concentration of hydrogen peroxide is under investigation and clinical trials need more cases to observe. The research focus is on three skin problems: actinic keratosis (AK), seborrheic keratosis (SK), and common wart. The mechanism of the treatment for these three diseases likely relates to the Warburg effect. The excess hydrogen peroxide introduces oxidative stress into the irregular keratinocytes, causes cell transformation, hereby the change of cellular activities within affected keratinocytes

(**Figure 2**). This also suggested that the cell elimination occurs in transformed cells more than in regular ones because irregular keratinocytes are more sensitive to oxidative stress. The danger of medical treatment using hydrogen peroxide is still a concern because it affects surrounding cells in the exposure area regardless of whether they are desired targets or not. There is a possibility of irritation in the exposure area as well. A low concentration of hydrogen peroxide is obviously much safer to use than a higher one. Nevertheless, we can reduce the side effect to a smaller level if it is utilized carefully, by proper control of an applied amount, contact time, and frequency of treatment. (Liberti & Locasale, 2016; Murphy & Friedman, 2019; Urban et al., 2019).

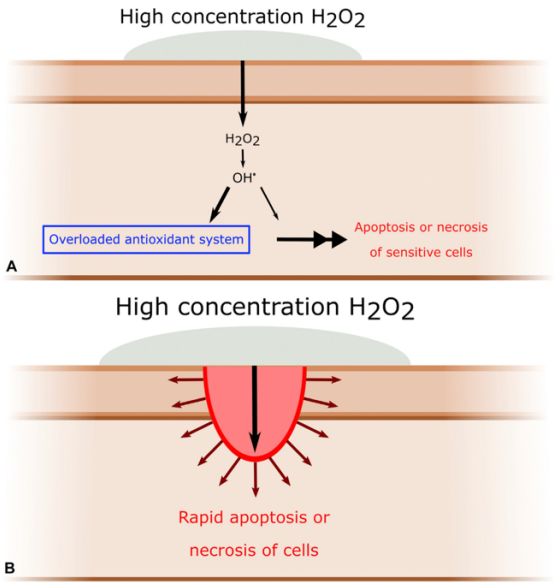


Figure 2 The possible effect of concentrated hydrogen peroxide in the different exposure skin: (A) the regular skin and (B) abnormal keratinocytes with stratum corneum as a barrier. (Murphy & Friedman, 2019)

In addition to skin treatment and mouth rinse, the other use of hydrogen peroxide includes disinfectant (on the object) and cerumenolytic. The disinfection of objects with hydrogen peroxide is similar to wound cleansing but with a higher concentration. Hydrogen peroxide can soften the ear wax resulting in removing the ear wax being easier with a low concentration of hydrogen peroxide (West, 2021).

1.2.3 Analytical method of hydrogen peroxide

The most common method for hydrogen peroxide determination is titration. Potassium permanganate ($KMnO_4$) titration methods are well-known to chemists because this method has been taught for years as a learning practice. In 1994, by the report of Kiassen et al., the potassium permanganate solution is slowly added into stirring acidified hydrogen peroxide solution. Permanganate anion (MnO_4^-) which is a stronger oxidant, will oxidize hydrogen peroxide under an acidic environment and produce manganese(II) ion (Mn^{2+}) and oxygen gases.

$$2MnO_4^-(aq) + 5H_2O_2(aq) + 6H^+(aq) \rightarrow 2Mn^{2+}(aq) + 8H_2O(l) + 5O_2(g) \quad \text{Equation 1.1}$$

The redox reaction causes the deep violet color of the permanganate solution changes to colorless if there is hydrogen peroxide remaining in the analyte solution. The excess permanganate makes the solution appears pink in color, resulting in the endpoint of the titration, which is obvious to bare eyes and could also be observed through absorption spectrophotometry at 525 nm. The permanganate titration method is simple but also complicated as it required several steps and an extensive procedure (Kiassen et al., 1994).

Colorimetric detections based on adsorption spectrophotometry are under focus in recent years. Several chromogenic substances and organic dyes are oxidizable and produce a product(s) whose color differs from the precursor. So, the change of color can serve as a response signal.

In 1994, Zhang and Wong reported the determination of hydrogen peroxide in marine water by leuco crystal violet and horseradish peroxidase system (LCV-HRP). The presence of horseradish peroxidase induced the production of reactive oxygen species from the hydrogen peroxide which is more reactive than hydrogen peroxide. Consequently, the leuco crystal violet that was originally colorless was oxidized by producing reactive oxygen, and the purple form, that is crystal violet was produced, resulting in the bleaching of the chemical reagent. The absorbance of the resulting solution was measured at 592 nm, at pH 4 by spectrophotometer. The LCV-HRP system displayed a good linear relationship between absorbance at 592 nm and hydrogen peroxide concentration at a range of 1 - 20 μmol L^{-1} with a detection limit

of 0.02 μmol L^{-1}. The LCV-HRP system provided good precision for hydrogen peroxide detection in marine water. water (Zhang & Wong, 1994).

Apart from crystal violet and its derivatives, 3,3,5,5-tetramethylbenzidine (TMB) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) are very popular for colorimetric reagent in hydrogen peroxide determination. Lots of research utilized its advantages to develop a colorimetric detection method based on the oxidation strategy. In case of TMB, the colorless TMB undergoes oxidation in the presence of oxidizing agent and produces radical cations that readily form a blue complex, resulting in a blue solution. Additional oxidation of radical cation gives diamine as a colorimetric product, so the color changes from blue to yellow. Both oxidation steps involve a one-electron transfer mechanism between the oxidizing agent and TMB (Harpaz et al., 2020).

The presence of the enzyme horseradish peroxidase is necessary for the hydrogen peroxide detection method to enhance the production of reactive oxygen species that will accelerate the oxidation reaction by far. However, the detection method becomes complicated since the enzyme is very sensible and required careful handling. In this case, many works utilized peroxidase mimics to imitate the activity of horseradish peroxidase, instead. Several substances exhibit a peroxidase-like activity including metal composites and metal nanoparticles that are very popular in this regard (Yang et al., 2016). Those peroxidase mimics can promote hydrogen peroxide to reactive species like horseradish peroxidase but be easier to handle and display many advantages over horseradish peroxidase.

In 2016, Yang et al. reported a detection method for hydrogen peroxide using CoS nanostructure as a peroxidase mimic, and TMB as a peroxidase substrate. The principal of this method is the color change from colorless to blue which is a result of the oxidation of TMB. The absorption of hydrogen peroxide solution was analyzed with a spectrophotometer at 652 nm. The absorbance of the analyte solution was subtracted by the absorbance of the blank and reported as ΔA. The method displayed linearity ranged from 0.05 - 0.8 mmol L^{-1} with a detection limit of 0.02 mmol L^{-1} (Yang et al., 2016).

In 2017, Cui et al. reported a detection method for hydrogen peroxide and xanthine utilizing iridium nanoparticles. Iridium nanoparticle prepared by Ir(III)

compound and tannin acid as a stabilizing agent. TMB was selected as a peroxidase substrate. It was mentioned that iridium nanoparticles perform a superior peroxidase-like activity that is better than horseradish peroxidase in certain situations. With the IrNPs-tannin-TMB system, the method achieves good linearity ranging from 1 - 100 μmol L^{-1} with a detection limit of 0.53 μmol L^{-1} (Cui et al., 2017).

In 2018, Wang et al. reported a colorimetric method for hydrogen peroxide determination with α-$AgVO_3$ microrods as peroxidase mimics. The chromogen used as reagent was TMB. The response of the method was observed through the greenish-blue product that exerts s strong adsorption at 652 nm. The linear calibration of hydrogen peroxide detection for the method ranged from 60 - 200 μmol L^{-1} with a detection limit of 2 μmol L^{-1}. The commercial antiseptic liquid sample was tested, and the result was in good agreement with the reference method (Wang et al., 2018).

In 2018, Zong et al. reported a visual colorimetric measurement of hydrogen peroxide and glucose with a citrated cap silver nanoparticle. The principal of the method lies in the edging of silver nanoparticles. Silver nanoparticles were prepared in round shape and according to their surface plasmon resonance, the dispersion of round shape silver nanoparticles gives a brown solution. The originally round shape silver nanoparticle will lose the matter on the surface causing the change of its shape and thus resulting in the shift of maximum absorption wavelength, so the color of the solution is changed and can be observed through a spectrophotometer. The reported method showed a linear relationship between ranged from 0.2 - 32 μmol L^{-1} with a detection limit of 90 nmol L^{-1} (Zong et al., 2018).

Another approach for hydrogen peroxide detection is fluorometry or fluorescence sensor. This method allows a particular molecule emits strong light upon being excited under irradiation by an electromagnetic wave at a certain wavelength. The emittance of fluorescence light is normally measured by a fluorometer.

In 2015, Yuan et al. reported a MnO_2-nanosheet nanosystem for fluorescence sensors for the detection of hydrogen peroxide and indirect detection of glucose in serum and blood. The upconversion nanoparticles which could be excited by near-infrared electromagnetic waves, and gave a visible light emission, were modified by MnO_2 nanosheet and resulted in MnO_2 nanosheet coated on the surface of

upconversion nanoparticles. The MnO_2 nanosheet induced fluorescence quenching at 450 nm of upconversion nanoparticles. The addition of hydrogen peroxide in the system, will etch the MnO_2 nanosheet layer and released Mn(II) ion (Mn^{2+}), hereby the restoration of fluorescence emission at 450 nm. The MnO_2 nanosheet-modified nano system provided a linear correlation between fluorescence emission at 450 nm and hydrogen peroxide concentration at a range of 0 - 150 µmol L^{-1} and 180 - 350 µmol L^{-1} with a detection limit of 0.9 µmol L^{-1} (Yuan et al., 2015).

In 2020., Qu et al. reported a fluorescence sensor using glutathione-capped copper nanocluster along with lead(II) and zirconium(IV) in one system (GSH-CuNCs-Pb^{2+}-Zr^{4+}) for hydrogen peroxide detection and applied to the determination of glucose and cholesterol in serum sample via hydrogen peroxide detection. The presence of Pb^{2+} ion and Zr^{4+} ion triggered aggregation of glutathione-capped copper nanocluster resulting in the fluorescence emission at 585 nm. The Cu^{2+} cation was produced after the introduction of hydrogen peroxide hence the quenching of fluorescence of glutathione-capped copper nanocluster. The reduction of fluorescence was reported as a fluorescence ratio of $(F_1-F_2)/F_1$. GSH-CuNCs-Pb^{2+}-Zr^{4+} system exhibited linearity ranging from 1 - 60 µmol L^{-1} with a detection limit of 0.6 µmol L^{-1} (Qu et al., 2020). In the same year, Zhou et al. reported a fluorescence sensor using a hybrid system of CdSe@ZnS quantum dots and Ag nanocluster (CdSe@ZnS QDs/AgNCs) for hydrogen peroxide detection in the milk sample. The CdSe@ZnS QDs emitted fluorescence at 510 nm which will be quenched by silver ion (Ag^{+}) released from the oxidation of AgNCs by hydrogen peroxide, through cation exchange between Ag^{+} and Zn^{2+}. The calibration model of this method was constructed using the fluorescence intensity at 510 nm that provided a linear relationship at a range of 0.5 - 60 µmol L^{-1} with a detection limit of 0.3 µmol L^{-1}. The detection of hydrogen peroxide in milk samples by CdSe@ZnS QDs/AgNCs showed recovery of 95.8 - 112.0 % for three fortified concentrations (Zhou et al., 2020).

In addition to spectrophotometry, the electrochemical sensor is an another approach for the detection of hydrogen peroxide by using modified electrodes. In 2011, Periasamy et al. reported an electrochemical sensor using a modified glassy carbon electrode that was functionalized by a multi-walled carbon nanotube and carminic acid. It was shown that carminic acid caused multiwalled carbon nanotubes to

disperse well in an aqueous solution and carminic acid was readily absorbed into the wall of carbon nanotubes. Under optimized conditions, the method provided linearity of hydrogen peroxide determination at a range of 10 - 32 mmol L^{-1} with a good selectivity and storage ability (Periasamy et al., 2011).

In 2014, Zor et al. reported an electrochemical biosensor prepared by reduced graphene oxide/nanopyrrole composite for hydrogen peroxide determination. The biosensor was developed on a glassy carbon electrode and investigated through cyclic voltammetry, differential pulse voltammetry, and chronoamperometry techniques to obtain the optimized condition for hydrogen peroxide detection. The reduced graphene oxide/nanopyrrole-modified electrode displays a linear relationship between analytical response and hydrogen peroxide concentration ranging from 0.1 - 4.0 μmol L^{-1} with a detection limit of 32 nmol L^{-1} (Zor et al., 2014).

Table 1 The analytical method for hydrogen peroxide determination.

Literatures	Techniques	Linear range	Limit of detection
Zang and Wong	spectrophotometry	1 - 20 μM	0.02 μM
Yang et al.	Spectrophotometry	0.05 - 0.8 mM	0.02 mM
Cui et al.	Spectrophotometry	1 - 100 μM	0.53 μM
Wang et al.	Spectrophotometry	60 - 200 μM	2 μM
Zong et al.	Spectrophotometry	0.2 - 32 μM	90 nM
Yuan et al.	Fluorometry	0 - 150 μM 180 - 350 μM	0.9 μM
Qu et al.	Fluorometry	1 - 60 μM	0.6 μM
Zhou et al.	Fluorometry	1 - 60 μM	0.6 μM
Periasamy et al.	Electrochemistry	10 - 30 mM	10 mM
Zor et al.	Electrochemistry	0.1 - 4 μM	32 nM

1.2.4 Smartphone-assisted digital image analysis

Digital colorimetric determination using a smartphone has recently become widely used in analytical applications, such as in environmental analysis, in

food analysis, in pharmaceutical analysis and in forensics. Smartphone based colorimetric detection is based on digital color images of the colorimetric products from a specific reagent reaction. The digital images contain image layers of primary colors (red, green, and blue; RGB) in the digital photo of the colorimetric product and can give precise and accurate results. The RGB digital images is captured with a smartphone. RGB data have been used in the quantification of target analytes, by converting RGB intensities to absorbance estimates with an external calibration technique (Lantam et al., 2020). The main attractions of smartphone based colorimetric detection in analytical applications include easy-to-operate, portable and rapid devices. Using a smartphone as a processor and detector have the potential to enable the execution of lengthy and complicated analytical chemistry protocols in the field, without the need for expensive equipment or high levels of expertise.

In 2013, Choodum et al. reported a method using a smartphone as a device for Trinitrotoluene (TNT) detection in soil sample. The TNT detection is based on a charge-transfer mechanism in which the dicyclohexylamine (DCHA) as the electron donating reagent and TNT acts as an electron acceptor, resulting red–violet products. The colored products were quantified by an application of smartphone. The calibration curve was plotted between RGB absorbance and TNT concentrations in the range of 1 - 500 mg L^{-1}. The inter-day precision of the method was in the range of 2.09 - 7.43 %RSD. The author found that smartphone gave the potential to be employed as a rapid on-site method for semi-quantification of TNT (Choodum et al., 2013).

In 2014, Zhou et al. proposed a paper-based biosensor for hydrogen peroxide and glucose using (3-aminopropyl)trimethoxysiloxane-glutaraldehyde complex (APTMS-GA). APTMS-glutaraldehyde was grafted on filter paper which resulted to the brick-red of APTMS-GA on filter paper. APTMS-GA could be oxidized by hydrogen peroxide that led to the color of reagent was bleached. The digital image of resulting probe was captured by camara and analyzed by ImageJ software to obtain mean grey value. The linear range of hydrogen peroxide determination by this paper-based probe was obtained at 2.5 - 500 mmol L^{-1} with detection limit of 2.5 mmol L^{-1} (Zhou et al., 2014).

In 2019, Hosu et al. developed enzymatic biosensor for hydrogen peroxide and glucose detection which utilized horseradish peroxidase, glucose oxidase,

horseradish peroxidase-glucose oxidase and tyrosinase enzymes on poly(aniline-co-anthranilic acid) composite film. aniline-co-anthranilic acid polymer will spontaneously undergo redox reaction upon contact with hydrogen peroxide which also was amplified by enzyme immobilized on the polymer. The color was change from green to blue. The response can be observed through digital images captured by smartphone and analyzed by ColorLab android application. The linear relationship of hue signal difference (ΔHue) and hydrogen peroxide concentration was obtained at 25 to 200 μmol L^{-1} with a detection limit of 51.2 μmol L^{-1} (Hosu et al., 2019).

In 2020, Khachornsakkul and Dungchai reported a colorimetric sensor for hydrogen peroxide detection using TMB as substate coupled to silver nanoparticles. The developed method utilized oxidation etching of silver nanoparticle that was enhanced by ultrasound. The oxidized TMB was produced by oxidation etching of silver nanoparticles without peroxidase which will be observed through Herpes Simplex Virus (HSV) method via application on smartphone. This method showed a linear range of hydrogen peroxide detection at a range of 25 to 1000 nmol L^{-1} with a detection limit of 2 nmol L^{-1} (Khachornsakkul & Dungchai, 2020).

1.3 Objectives

The aim of this research is to develop a colorimetric probe for hydrogen peroxide determination using (3-aminopropyl)triethoxysiloxane-glutaraldehyde complex with smartphone-based digital image analysis. The detail are as follows.

(1) To study the parameters for (3-aminopropyl)triethoxysiloxane-glutaraldehyde complex preparation as a colorimetric probe.
(2) To investigate the method development of hydrogen peroxide determination using (3-aminopropyl)triethoxysiloxane-glutaraldehyde complex and smartphone-based digital image analysis.
(3) To perform the method validation of (3-aminopropyl)triethoxysiloxane-glutaraldehyde complex with smartphone-based digital image method for hydrogen peroxide detection.

(4) To utilize the prepared colorimetric probe for determination of hydrogen peroxide in commercial antiseptic solution and compare to spectrophotometry as a reference method.

1.4 Expected benefits

The APTES-GA colorimetric probe is successfully prepared with simple and affordable procedure. The APTES-GA colorimetric probe can efficiently determine hydrogen peroxide in antiseptic solution leading to monitor the concentration of hydrogen peroxide in antiseptic product. The APTES-GA probe can be use indirectly determine other substances relating to hydrogen peroxide such as triacetone triperoxide.

CHAPTER 2

METHODOLOGY

2.1 Chemicals

(3-aminopropyl)triethoxysiloxane (APTES) was purchased from Tokyo Chemistry Industry Co. Ltd., Japan. Glutaraldehyde solution (25% in water), Hydrogen peroxide solution (30 % in water), ammonium sulfate ($(NH_4)_2SO_4$), ammonium nitrate (NH_4NO_3), magnesium nitrate ($Mg(NO_3)_2$), sodium nitrate ($NaNO_3$), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), sodium bicarbonate (Na_2CO_3), and potassium carbonate (K_2CO_3) were purchased from Sigma-aldrich., Germany. Titanium(IV) oxysulfate solution (1.9 - 2.1 %) for determination of hydrogen peroxide (H 15, DIN 38 409) and absolute ethanol were purchased from Sulpelco., Germany. Acetonitrile was purchased from Macron Fine Chemicals., United states. Ultrapure water was obtained from Simplicity UV system, 18.2 MΩ.cm, Millipore.

2.2 Hydrogen peroxide standard solution preparation

For hydrogen peroxide standard solution, 30 % hydrogen peroxide solution was pipetted, and then diluted with acetonitrile to obtain a 1000 mmol L^{-1} (3.40 %w/v) stock solution. The standard solution was prepared freshly for each experiment to minimize the influence of hydrogen peroxide degradation.

2.3 Digital image processing system and digital image analysis

Black color acrylic box with the size of 20 × 20 × 20 cm (width × length × height) was built according to the design (**Figure 3**) to assist in digital images processing. A LED lightbulb (3 Watts, Philips, Hong Kong) was planted inside on a wall of the box as a light source. The inner layer of the wall was covered with a white foam sheet to eliminate the dark background. The front side of the box was punctured to create a hole for image capturing by smartphone (Vivo Y50). The top acrylic plate was also punctured to create holes for positioning of 200-µL micro-PCR tubes 10 cm away from the front-side wall which is a suitable distance according to the focus

distance of the smartphone. The captured digital image was analyzed by Image J software (online version) to collect color intensity values with the R-G-B color system.

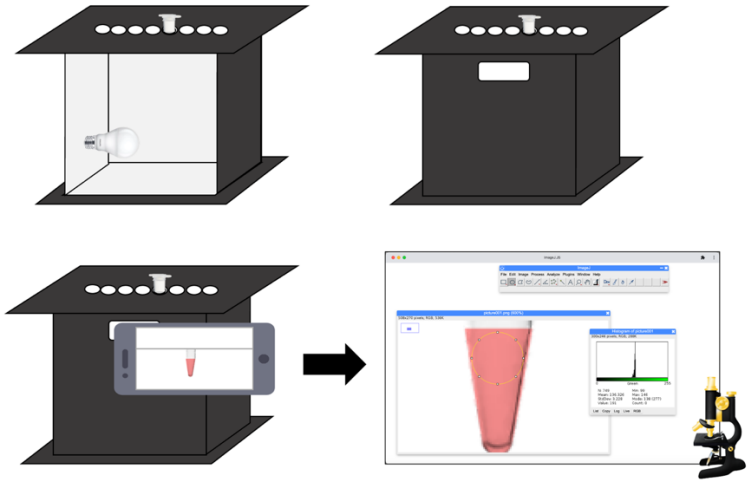


Figure 3 The picture illustrated the custom-build photographic box and digital image processing with a smartphone as an image capturing device.

2.4 UV-Visible absorption spectrophotometry

A spectrophotometer (Genesys^{TM} 10S, Thermo Fisher Scientific, Madison, USA) with 1.0 cm path length polystyrene cuvette was used to collect the UV-Vis absorption spectra. Absorbance spectra were generated by scanning between 380 nm to 780 nm with a spectral bandwidth of 1.8 nm and interval of 1 nm.

2.5 Fourier-transform infrared spectroscopic apparatus

Fourier transform infrared spectrometer, VERTEX 70, Bruker, Germany coupled with KBr pellet method was utilized in this work. A 1.8 mg of samples was glided and mixed together with 98.2 mg of KBr powder. The mixture powder was compressed to a pellet. The measurement was taken in a solid phase and the scanning wavenumber range started from 400 cm^{-1} to 4000 cm^{-1}.

2.6 Optimal conditions of APTES-GA reagent preparation

The optimal condition of (3-aminopropyl)triethoxysiloxane-glutaraldehyde complex (APTES-GA) preparation as a colorimetric prob was studied.

UV-Visible absorption spectrophotometry and smartphone-based digital image analysis were employed to collect absorbance value and color intensity, respectively. Two parameters of APTES-GA preparation were investigated; the volume ratio of APTES : 25% GA and incubation time.

2.6.1. The volume ratio of (3-aminopropyl)triethoxysiloxane to 25% glutaraldehyde solution

The volume ratio of (3-aminopropyl)triethoxysiloxane to 25% glutaraldehyde solution (APTES : 25% GA) was investigated from 0.5:1 to 5:1 v/v. A volume of 80 µL of 25% GA solution was set constant, while the volume of APTES was varied from 40 µL to 400 µL. The solution of APTES in absolute ethanol was first prepared, followed by the addition of 25% GA solution. The mixture solution was then allowed to stand at room temperature for 1 day before taken to measurement. For digital image analysis, three replicates of 200 µL of the resulting solution were transferred into a 200-µL PCR tube and followed by the digital image processing with the designed system. For UV-visible absorption spectrophotometry, three replicates of 200 µL of the resulting solution were diluted with 800 µL of absolute ethanol (five-fold dilution) prior to UV-visible absorption measurement in a range of 380 - 780 nm. The reagent blank was prepared in the same manner using ethanol instead of hydrogen peroxide solution.

2.6.2 The incubation time

The incubation time was investigated from 3 hours to 7 days. An optimal volume of APTES and 25% GA solution was mixed evenly in ethanol. For digital image analysis, three replicates of 200 µL of the resulting solution were transferred into 200-µL PCR tubes and allowed it to stand at room temperature. The resulting PCR tube was taken to the digital image processing with the designed system at designed interval time started from 3 hours to 7 days. For UV-visible absorption spectrophotometry, three replicates of 200 µL of the resulting solution were diluted with 800 µL of absolute ethanol (five-fold dilution) prior to UV-visible absorption measurement, at designed interval time started from 3 hours to 7 days.

2.7 Colorimetric determination of hydrogen peroxide

Colorimetric determination was based on the reaction of hydrogen peroxide with APTES-GA reagent, which forms brick-red color changed into colorless. The feasibility of hydrogen peroxide detection by APTES-GA colorimetric probe was observed through UV-Visible absorption spectrophotometry and Fourier-transform infrared spectrometry. APTES-GA solution was prepared with an optimized condition before treatment with hydrogen peroxide working solution.

2.7.1 UV-Visible absorption spectrophotometry measurement

The positive control was prepared by adding 700 µL of 300 mmol L^{-1} (1.02 %w/v) hydrogen peroxide solution (in acetonitrile) to 300 µL of APTES-GA solution. The mixture solution was transferred to a polystyrene cuvette and allowed to stand at room temperature for 5 minutes before measurement. Similarly, the negative control was prepared by adding 300 µL of APTES-GA solution to 700 µL of acetonitrile.

2.7.2 Fourier-transform infrared spectrometry measurement

1 mL of 1000 mmol L^{-1} (3.40 %w/v) hydrogen peroxide solution was added to 1 mL of APTES-GA solution. The mixture solution was allowed to stand at room temperature for 30 minutes. The resulting mixture and APTES-GA solution were heated separately in a ventilated oven at 40 ºC for 2 hours to assure that the solvent was entirely evaporated. Consequently, solid samples of colorimetric product and APTES-GA were obtained. The solidified APTES-GA was then taken to FT-IR spectroscopy measurement.

2.8 Method development for hydrogen peroxide detection

The colorimetric determination of hydrogen peroxide with the APTES-GA probe using a smartphone-based digital image analysis was developed for the detection of the target analyte in antiseptic commercial product. The effects of the color detection mode, the volume ratio of APTES-GA and analyte solution, reaction time and effect of water content on the color intensity signal were studied to achieve the maximum intensity which was chosen as the optimum condition.

2.8.1 The effect of the color detection mode

The effect of the color detection mode was evaluated in order to maximize sensitivity of the APTES-GA for hydrogen peroxide detection. Three solutions of hydrogen peroxide were prepared in concentrations of 250 mmol L^{-1} (0.85 %w/v), 750 mmol L^{-1} (2.55 %w/v), and 1250 mmol L^{-1} (4.25 %w/v). A 100 µL of APTES-GA solution and 100 µL of the analyte solution was mixed evenly in a 200-µL PCR tube. Three replicates for each analyte solution were investigated. The reaction was allowed to complete before image processing and digital image analysis. Digital images were then captured with built-in camera of the smartphone and an in-house light control box. The JPEG format image was transferred to Image J software for measurement of the RGB color intensity. The color intensity of each picture was measured as the red, green, and blue intensity.

2.8.2 The volume ratio of APTES-GA and analyte solution

The volume ratio of APTES-GA and analyte solution was investigated in a range of 3:7 to 7:3 v/v. APTES-GA solution and 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution was mixed evenly in a 200-µL PCR tube to obtain a final volume of 200 µL. Three replicates for each volume ratio were investigated. The reaction was allowed to complete before image processing and digital image analysis. Digital images were then captured with built-in camera of the smartphone and an in-house light control box. The JPEG format image was transferred to Image J software for measurement of the RGB color intensity. The color intensity of each picture was measured as the green intensity.

2.8.3 The effect of water content on colorimetric analysis

The water content in analyte solution was investigated in term of volume percentages of water. 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution was prepared in mixture of acetonitrile and ultrapure water to obtain the working solution with different water content, starting from 0% increase to 50%. APTES-GA solution and 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution was mixed evenly in optimal volume ratio in a 200-µL PCR tube to obtain a final volume of 200 µL. Three replicates for each water content were investigated. The reaction was allowed to

complete before image processing and digital image analysis. Digital images were then captured with built-in camera of the smartphone and an in-house light control box. The JPEG format image was transferred to Image J software for measurement of the RGB color intensity. The color intensity of each picture was measured as the green intensity.

2.8.4 Reaction time

The reaction time were observed from 1 minute to 25 minutes. APTES-GA solution and 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution was mixed evenly in optimal volume ratio in a 200-µL PCR tube to obtain a final volume of 200 µL. Three replicates for each time interval were investigated. The reaction was allowed to complete before image processing and digital image analysis. Digital images were then captured with built-in camera of the smartphone and an in-house light control box. The JPEG format image was transferred to Image J software for measurement of the RGB color intensity. The color intensity of each picture was measured as the green intensity.

2.9 Method validation

The validation of APTES-GA probe with smartphone-based digital image method for hydrogen peroxide detection in commercial antiseptic solution, was conducted under the optimized conditions selected previously. The linearity and range, limit of detection (LOD), limit of quantification (LOQ), intra-day and inter-day precision and accuracy, interference study, and shelf-life of evaluation were evaluated following the International Conference on Harmonization (ICH) tripartite guideline validation of analytical procedures: text and methodology Q2(R1) (Tietje & Brouder, 2010, Technical Note 17 - Guidelines for the validation and verification of quantitative and qualitative test methods (NATA, 2012), and the AOAC official methods of analysis, guidelines for standard method performance requirements, appendix F (AOAC international, 2016).

2.9.1 Linearity and range

A linearity of calibration of this work can be obtained from a relationship of concentration of an analyte and corresponding response of the color

green intensity of the digital image. A range of 1 - 1,500 mmol L^{-1} (0.003% w/v - 5.10 %w/v) of hydrogen peroxide solution was investigated. APTES-GA solution and hydrogen peroxide solution was mixed evenly in optimal volume ratio in a 200-µL PCR tube to obtain a final volume of 200 µL. The reaction was allowed to complete before image processing and digital image analysis. Seven replicates for each concentration were investigated. The color intensity of the analyte (y axis) were plotted against their concentration (x axis). The linear relationship was observed using regression analysis by Microsoft Excel.

2.9.2 Limit of detection (LOD)

A detection limit of this work is investigated to obtain the lowest concentration of hydrogen peroxide that can be detected by the developed method. The limit of detection was calculated using three different equations and compared.

$$\text{LOD} = \frac{3 \times \text{Standard deviation of y-intercept value}}{\text{Slope of calibration}} \qquad \text{Equation 2.1}$$

$$\text{LOD} = \frac{3 \times \text{Standard deviation of regression line}}{\text{Slope of calibration}} \qquad \text{Equation 2.2}$$

2.9.3 Limit of quantification (LOQ)

The limit of quantification is the lowest concentration of the analyte in the sample that can be determined with acceptable performance. The limit of quantification was calculated using three different equations and compared.

$$\text{LOQ} = \frac{10 \times \text{Standard deviation of y-intercept value}}{\text{Slope of calibration}} \qquad \text{Equation 2.3}$$

$$\text{LOQ} = \frac{10 \times \text{Standard deviation of regression line}}{\text{Slope of calibration}} \qquad \text{Equation 2.4}$$

2.9.4 Precision

Precision can be defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample. The precision of this work is investigated via relative standard deviation (%RSD) from several series of measurement. The intra-day precision can be achieved with three repeats a day and inter-day precision can be achieved with three repeats in three different days to obtain the most representative value. An aliquot of 50 µL of 1000 mmol L^{-1} (3.40 %w/v) hydrogen peroxide standard solution was spiked in sample solution to obtain three different concentration levels (low, medium and high levels) of fortified sample. APTES-GA solution and sample solution was mixed evenly in optimal volume ratio in a 200-µL PCR tube to obtain a final volume of 200 µL. The reaction was allowed to complete before image processing and digital image analysis. Three replicates for each sample solution were investigated. The acceptable relative standard deviation for precision is within ± 15%.

$$\%\text{RSD} = \frac{\text{Standard Deviation}}{\text{Mean Value}} \times 100 \qquad \text{Equation 2.5}$$

2.9.5 Accuracy

Accuracy is the difference between the expectation of the test results and an accepted reference value. The acceptable accuracy data is within ± 15% of actual value. Unless the accuracy is accepted, recovery is needed to show performance of the detection method. The recovery can be calculated as the percentage of the analyte response after sample workup compared to that of a solution containing the analyte at a concentration corresponding to recovery of 100%. The acceptable percentage of recovery is within 80 -115%. The intra-day accuracy can be achieved with three repeats a day and inter-day accuracy can be achieved with three repeats in three different days to obtain the most representative value. An aliquot of 50 µL of 1000 mmol L^{-1} (3.40 %w/v) hydrogen peroxide standard solution was spiked in sample solution to obtain three different concentration levels (low, medium and high levels) of fortified sample. APTES-GA solution and sample solution was mixed evenly in optimal volume ratio in a 200-µL PCR tube to obtain a final volume of 200 µL. The reaction was allowed to

complete before image processing and digital image analysis. Three replicates for each sample solution were investigated.

$$\text{Recovery (\%)} = \frac{(C_f - C_u)}{C_a} \times 100 \qquad \text{Equation 2.6}$$

where: C_f is the concentration of analyte measured in spiked sample

C_u is the concentration of analyte in real sample

C_a id the concentration of analyte (standard) added into the sample

2.9.6 Interference study

To assess specificity of the proposed method towards hydrogen peroxide, various ingredients that are potentially found in commercial antiseptic solution, including sodium chloride (NaCl), potassium chloride (KCl) and calcium chloride ($CaCl_2$) were used to investigate potential interferences. In addition, other substances which represented common ion were also investigated including ammonium sulfate ($(NH_4)_2SO_4$), ammonium nitrate (NH_4NO_3), magnesium nitrate ($Mg(NO_3)_2$), sodium nitrate ($NaNO_3$), bicarbonate (Na_2CO_3), and potassium carbonate (K_2CO_3). The tolerance limit for these potentially interfering chemical species was verified by spiking various concentrations of these species into a 300 mmol L^{-1} (1.02 %w/v) solution of hydrogen peroxide solution as a sample solution. APTES-GA solution and sample solution was mixed evenly in optimal volume ratio in a 200-µL PCR tube to obtain a final volume of 200 µL. The reaction was allowed to complete before image processing and digital image analysis. Three replicates for each sample solution were investigated. The green intensity obtained from each experiment would be compared to the green intensity of colorimetric product without interferences by the term of relative error. Tolerance limit refers to the highest concentration that did not alter, by a variation of more than ± 5%, the analytical signal of hydrogen peroxide at a concentration of 300 mmol L^{-1} (1.02 %w/v).

$$\text{Relative error (RE)} = \frac{G_{exp} - G_{ref}}{G_{ref}} \times 100 \qquad \text{Equation 2.7}$$

where: G_{exp} referred to green intensity of colorimetric product with interferences.

G_{ref} referred to green intensity of colorimetric product without interferences.

2.9.7 Shelf-life evaluation of colorimetric probe

Shelf-life of evaluation is studied to assure reliability of colorimetric sensing when keep in freeze storage for a long period. Therefore, it is defined as the chemical stability of an analyte in a given matrix under specific conditions for given time intervals. A 100 µL of APTES-GA solution was transferred into 200-µL PCR tubes. Three batches of APTES-GA probes were stored at three different conditions: room temperature (25 ºC), in refrigerator (4 ºC), and freeze refrigerator (-18 ºC). Three replicates of stored APTES-GA probes were treated with 100 µL of 300 mmol L^{-1} (1.02 %w/v) hydrogen peroxide standard solution at time intervals started from 1 day to 6 weeks. The reaction was allowed to complete before image processing and digital image analysis. Digital images were then captured with built-in camera of the smartphone and an in-house light control box. The JPEG format image was transferred to Image J software for measurement of the RGB color intensity. The color intensity of each picture was measured as the green intensity. The green intensity obtained from each experiment would be compared to the green intensity of colorimetric product obtained at the first day of measurement, by the term of relative error.

$$\text{Relative error (\%RE)} = \frac{G_{exp} - G_{ref}}{G_{ref}} \times 100 \qquad \text{Equation 2.8}$$

where: G_{exp} referred to green intensity of colorimetric product.

G_{ref} referred to green intensity of colorimetric product obtained at the first day of measurement.

2.10 Determination of hydrogen peroxide in real samples

Three commercial brands of hydrogen peroxide medicine were purchased from pharmacy stores in the Songkhla province of Southern Thailand. The

samples were labeled as Brand A, Brand B and Brand C which contained hydrogen peroxide at concentration of 3 %w/v , 6 %w/v, and 3 %w/v, respectively. Each sample was diluted with acetonitrile at a dilution factor of 1/5 and 1/10 to obtain a final volume of 1 mL and mixed thoroughly using a vortex mixer before analysis. APTES-GA solution and sample solution was mixed evenly in optimal volume ratio in a 200-µL PCR tube to obtain a final volume of 200 µL. The reaction was allowed to complete before image processing and digital image analysis.

The accuracy of the proposed method for sample analysis was evaluated by determining concentration of hydrogen peroxide in the three antiseptic commercial product spiked with hydrogen peroxide standards at 50 mmol L^{-1} (0.17 %w/v), 100 mmol L^{-1} (0.34 %w/v), and 200 mmol L^{-1} (0.68 %w/v) (low, medium and high levels). The fortified sample solutions were analyzed using the developed method.

2.11 Validation of the proposed method using $TiOSO_4$ method

The conventional method for hydrogen peroxide detection which was selected to be reference method of this work was $TiOSO_4$ method. This method was based on complex ion formation of Titanium complex and peroxo ligand which exhibits a strong absorption at 408 nm. The instrument used for UV-visible absorption spectrophotometry was Thermo-Scientific, GENESYSTM 10S model.

For sample analysis by $TiOSO_4$ method, medicine samples were diluted to obtain an estimated concentration in calibration range. Accorded to Üzer et al. (2017), the linear calibration of hydrogen peroxide detection with $TiOSO_4$ method was established in range of 0.1 mmol L^{-1} to 2 mmol L^{-1}. Therefore, Brand A and Brand C samples were diluted 1000-fold while Brand B sample was diluted 2000-fold to obtain the initial concentration in range of calibration. The hydrogen peroxide working solution was prepared in ultrapure water. A 0.4 mL of $TiOSO_4$ solution was added to 1 mL of hydrogen peroxide working solution, resulting volume ratio of $TiOSO_4$ solution to hydrogen peroxide working solution as 2 : 5 v/v. The yellow complex formed immediately after and resulted to yellow solution. The absorbance at 408 nm was used for analytical quantitation. The amounts of hydrogen peroxide in antiseptic samples, as obtained from smartphone-based colorimetric probe and $TiOSO_4$ method, were compared by percentage of relative error (RE). The accuracy of the $TiOSO_4$ method for

sample analysis was evaluated by determining concentration of hydrogen peroxide in the three antiseptic commercial product spiked with hydrogen peroxide standards at 0.196 mmol L^{-1}, 0.490 mmol L^{-1}, and 0.980 mmol L^{-1} (low, medium and high levels). The fortified sample solutions were analyzed using the reference method.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Optimal conditions of APTES-GA reagent preparation

3.1.1 Volume ratio of (3-aminopropyl)triethoxysiloxane to 25% glutaraldehyde solution

Our idea in this work suggested that the APTES-GA solution and analyte solution were mixed into a homogenous solution. The color would be distributed evenly and suitable for digital image analysis. To our knowledge, the high concentration of glutaraldehyde would risk the resulting mixture underwent gelation and lost the solution phase property. It was difficult to make APTES-GA gel and analyte solution mixed evenly due to two different phases. Therefore, the amount of glutaraldehyde in mixture solution at 0.5% v/v was fixed and the amount of APTES was varied from 1% to 10% v/v. We decided to report as APTES : 25% GA volume ratio which corresponded to the volume ratio of APTES and 25% glutaraldehyde solution used for synthesis. So, a range of 1%:0.5% v/v to 10%:0.5% v/v was converted to a range of volume ratio of 0.5:1 to 5.0:1 v/v.

The reaction of (3-aminopropyl)triethoxysiloxane and glutaraldehyde in ethanol provided brick-red APTES-GA complex (**Figure 4**). The color intensity of APTES-GA complex was significantly influenced by the amount of (3-aminopropyl)triethoxysiloxane and glutaraldehyde used in preparation. At the incubation time of 24 hours, the volume ratio of 0.5:1 v/v and 1.0:1 v/v gave dark yellow color and orange color, respectively (**Figure 5**). The volume ratio of 1.5:1 v/v provided the most intense red color and turned paler with the increase of APTES, from the volume ratio of 2.0:1 to 5.0:1 v/v.

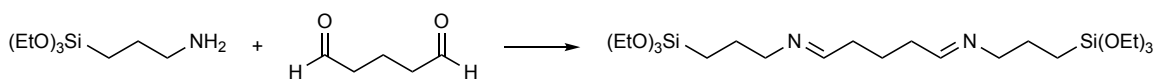


Figure 4 Proposed mechanism of APTES-GA complex formation.

Figure 5 Digital images of APTES-GA probes that were prepared from APTES and 25% GA solution at volume ratio ranged from 0.5:1 to 5.0:1 v/v. The image was captured after the incubation time of 24 hours.

The plot of color intensities (R-G-B) against the volume ratio of APTES : 25% GA showed that red intensity of all ten volume ratios was the same and almost constant (**Figure 6**). The blue intensity was the lowest at 0.5:1 v/v and much lower than its respective green intensity so the probe appeared as dark yellow color. The blue intensity increased as the fraction of APTES increased so the yellow color was less intense as the fraction of APTES increased. The green intensity, in another hand, decreased from 0.5:1 volume ratio till 1.5:1 volume ratio, and increased afterward. Hence, the green intensity obtained from 1.5:1 volume ratio was the lowest. In addition, 1.5:1 v/v provided similar values of green intensity and blue intensity, and both were far lower than red intensity. This contributed to the intense red color of the probe that prepared by using 1.5:1 volume ratio.

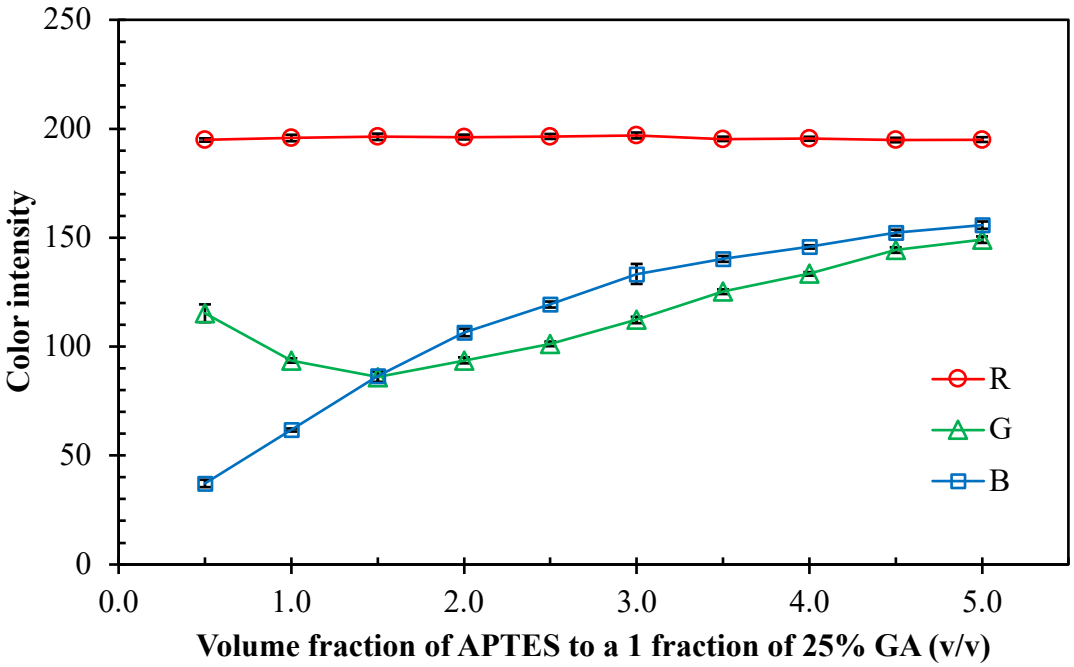


Figure 6 The plot of color intensity against the volume fraction of APTES per 1 fraction of 25% GA solution. The APTES-GA solution was incubated for 24 hours before measurement. (n=3)

The data from absorption spectrophotometry corresponded to the data obtained from digital image analysis. The APTES-GA from 0.5:1 v/v exhibited strong absorption and weak absorption at 440 nm and 540 nm, respectively (**Figure 7**, bright blue line). As a result, the intense yellow color was observed because the absorbance at 440 nm overwhelmed the absorbance at 540 nm. The absorbance at 440 nm is the highest at 0.5:1 v/v and decreased till it reached the lowest at 5.0:1 v/v so the yellow color became lighter as the fraction of APTES increase. The absorbance at 540 nm was further plotted against the volume ratio and the highest value was spotted on the 1.5:1 v/v (**Figure 8**). The most suitable volume ratio of APTES : 25% GA was, therefore, 1.5:1 v/v as it provided the deepest red color.

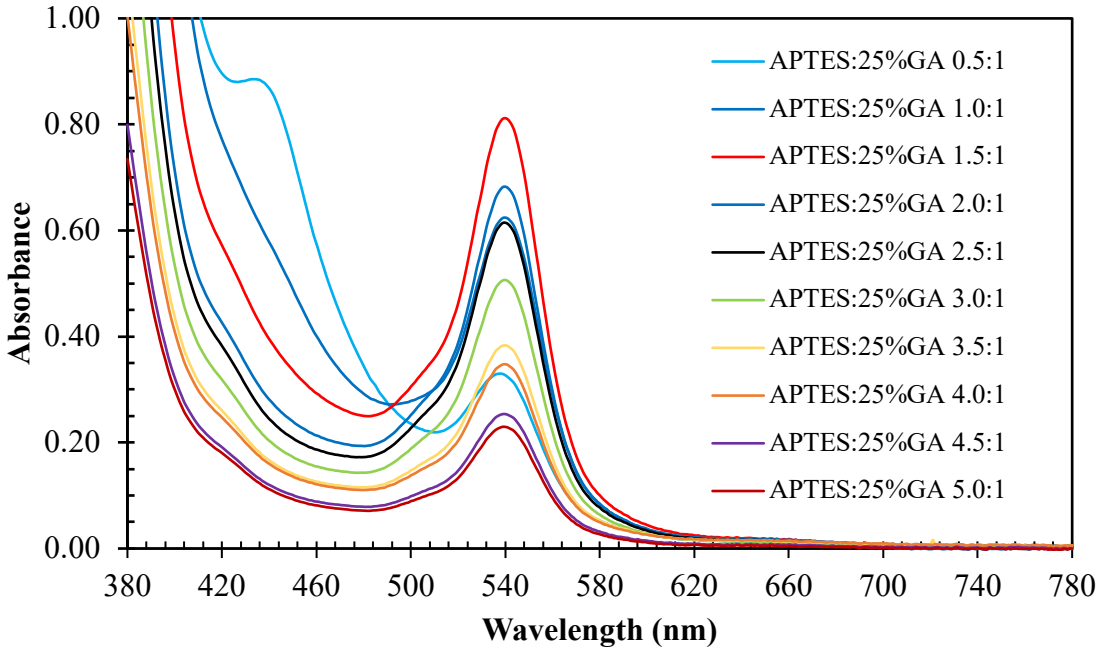


Figure 7 The absorption spectra of five-fold diluted APTES-GA complex solutions that were prepared with APTES : 25% GA volume ratios varying from 0.5:1 to 5.0:1 v/v.

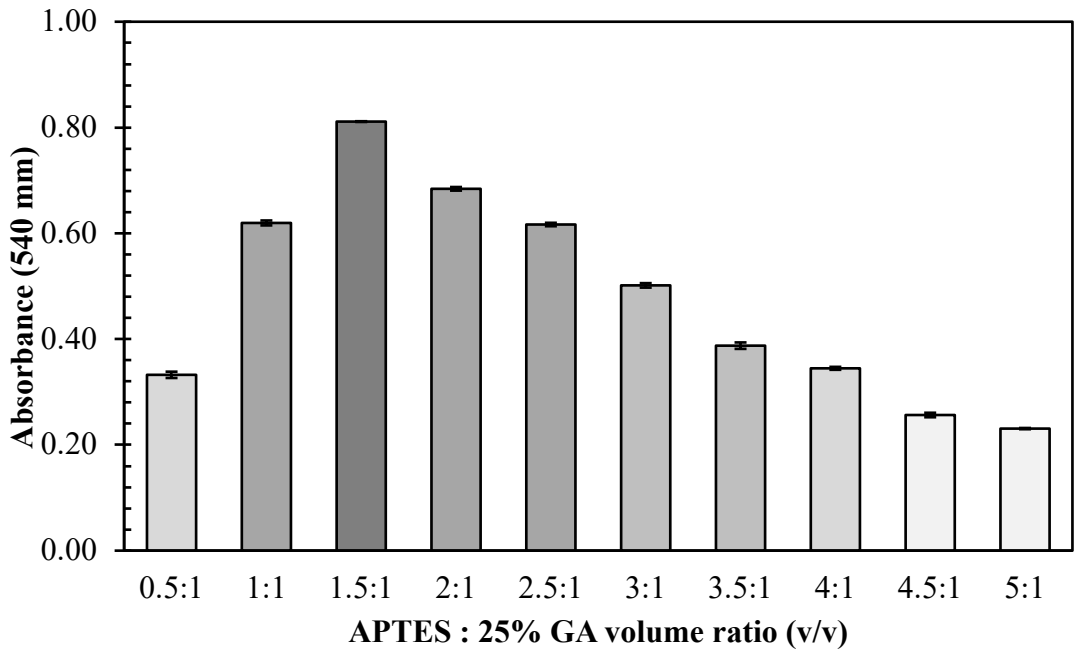


Figure 8 The absorbance at 540 nm of each APTES : 25% GA volume ratio. (n=3)

3.1.2 Incubation time

To our knowledge, the siloxane-glutaraldehyde complex that prepared from (3-aminopropyl)trimethoxysiloxane (APTMS) was brick-red color (Zhou et al., 2014). However, our observation showed that the red color APTES-GA complex did not appear instantly, but it was slowly developed overtime instead. The complex initially appeared as pale-yellow solution and it was gradually changed to red color in twelve hours. Afterward, the red color become tenser until the change was negligible to naked eyes (**Figure 9**).

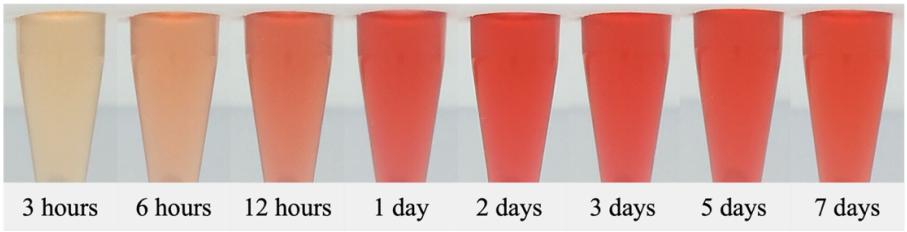


Figure 9 Digital images of APTES-GA probes at different incubation time.

The color intensity plot showed the red intensity was constant which was within our expectation, whereas both green intensity and blue intensity were decreasing until two days of incubation that it did not change significantly (**Figure 10**). The trend of absorbance at 540 nm corresponded to the green intensity. The probe showed low absorption at initial time of incubation as it was pale yellow, and the absorption increased higher until three days of incubation that it did not change significantly (**Figure 11**). Both results agreed that the suitable incubation time for APTES-GA preparation was three days. The color development of the APTES-GA complex was slow which might be attribute to large ethoxy groups on siloxane that hindered the complex formation.

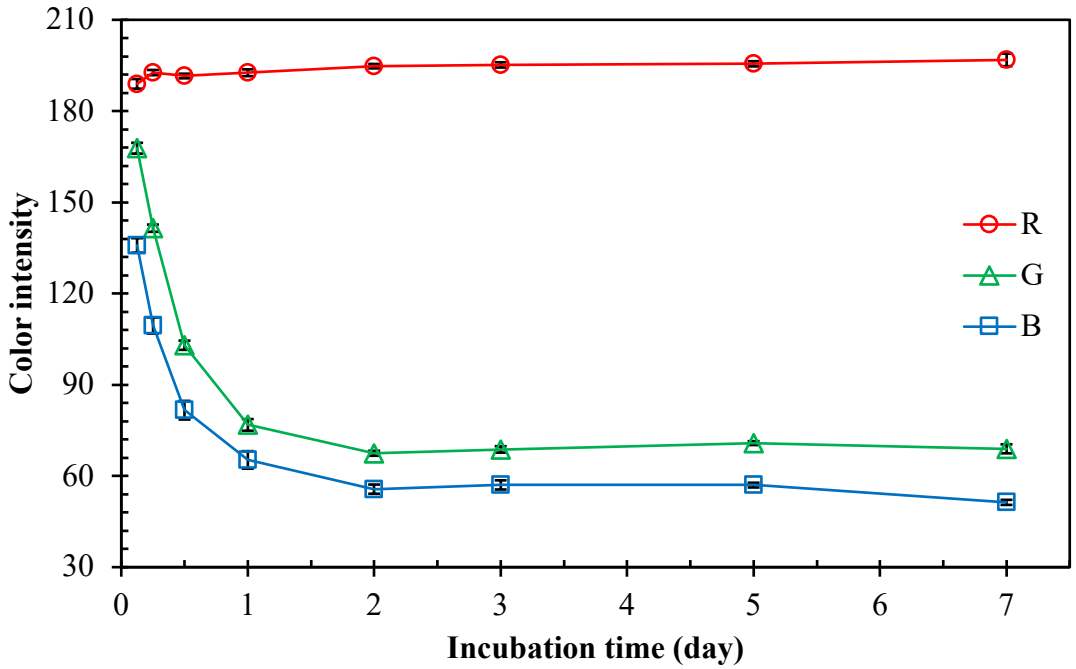


Figure 10 The plot of color intensity of APTES-GA probe against incubation time. (n=3)

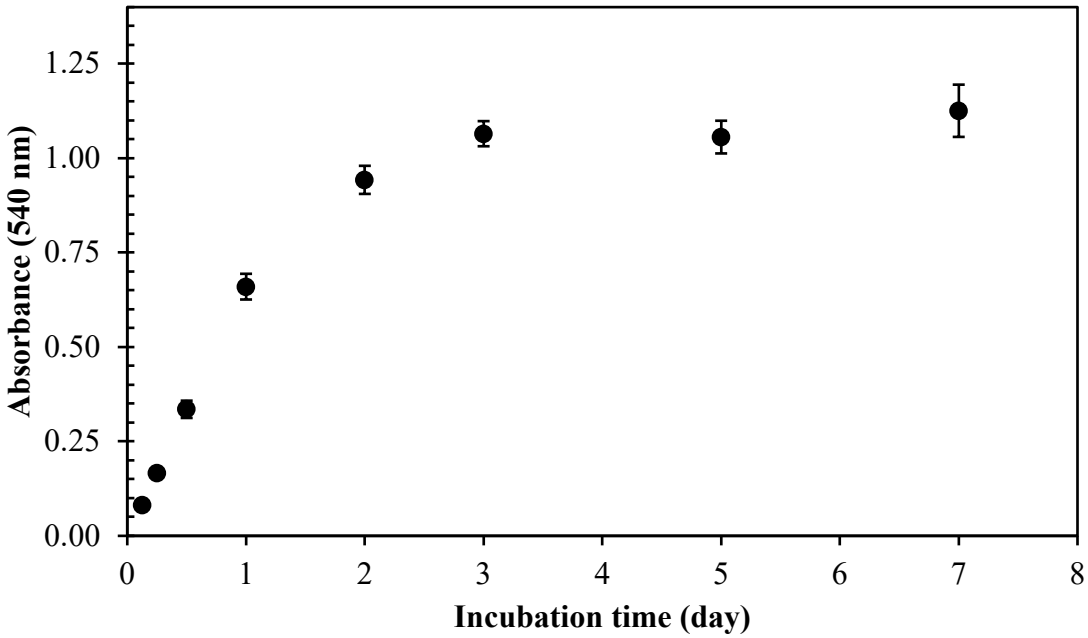


Figure 11 The plot of absorbance at 540 nm of APTES-GA complex against incubation time. (n=3)

3.2 Colorimetric determination of hydrogen peroxide

3.2.1 UV-visible absorption spectrophotometry measurement

The prepared APTES-GA probe, which was originally red color, would have the color bleached by the treatment with hydrogen peroxide solution. The color of probe and the color of resulting mixture could be distinguished by naked eyes (**Figure 12**).

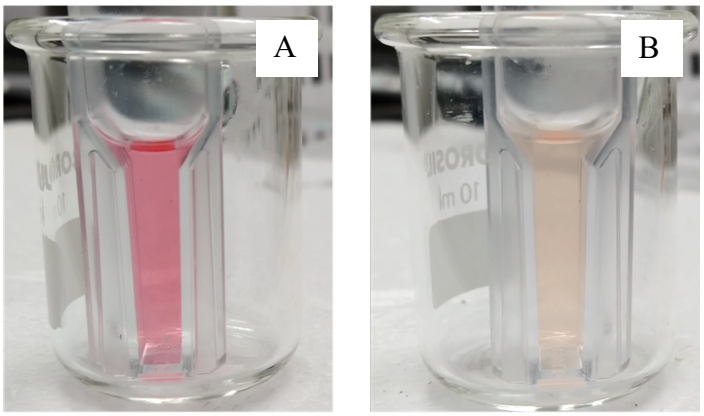


Figure 12 (A) The pictures of APTES-GA complex solution treated ethanol blank at a volume ratio of 3:7 v/v. (B) The picture of APTES-GA complex solution treated with 300 mmol L^{-1} (1.02 %w/v) hydrogen peroxide solution at a volume ratio of 3:7 v/v and the reaction time was 5 minutes.

UV-visible absorption spectra of APTES-GA and resulting mixture displayed a disparity of absorbance at wavelength 380 nm to 780 nm which indicated the weaken color in resulting mixture. The difference of absorbance at 540 nm was significant as it was in visible region and affected the degree of red color (**Figure 13**). Henceforth, the colorimetric strategy of hydrogen peroxide detection by color bleaching of APTES-GA complex was feasible.

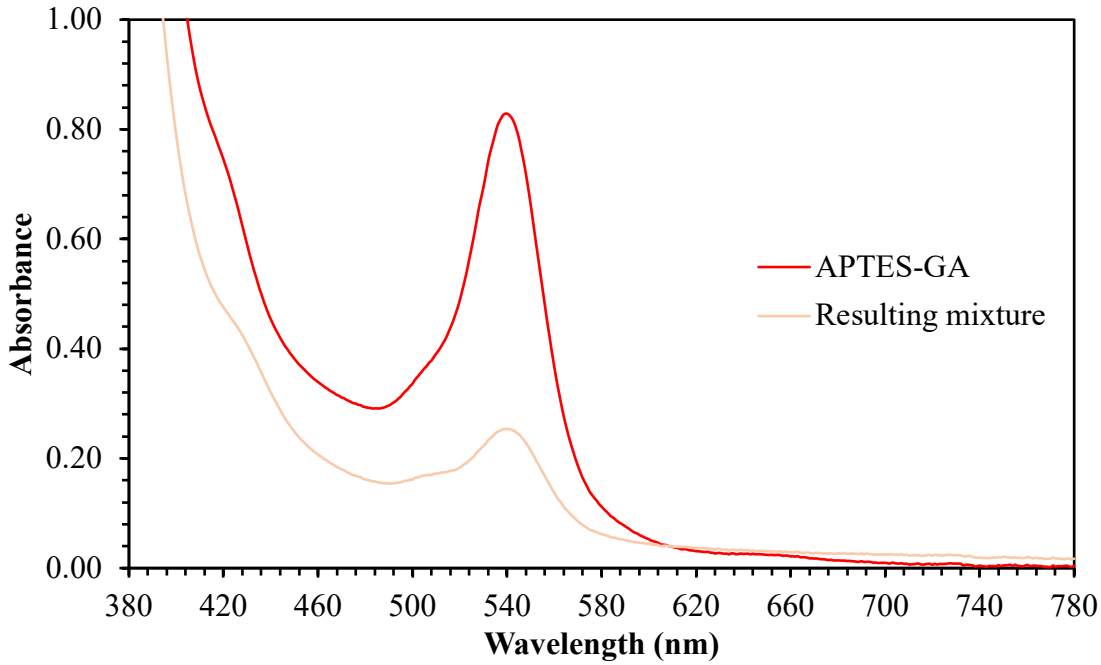


Figure 13 The UV-visible absorption spectra of APTES-GA solution after treatments with reagent blank (acetonitrile) and 300 mmol L^{-1} (1.02 %w/v) hydrogen peroxide solution for 5 minutes. The volume ratio of APTES-GA to treating solution was 3:7 v/v.

3.2.2 Fourier transform infrared spectroscopy measurement

To understand the reaction further, the Fourier transform infrared spectroscopy was utilized to observe the chemical interaction in both APTES-GA complex and colorimetric product. The FT-IR measurement was carried on KBr pellet which required the sample to be solid phase and it was expected that the chemical interaction in solid phase and solution phase were similar and not largely different.

The FT-IR spectra of solidified APTES-GA complex displayed medium absorption band around 3429 cm^{-1} (amine, N-H stretching) and 1650 cm^{-1} (imine, C=N stretching and amine, N-H bending) (**Figure 14**). A strong peak between 1000 - 1200 cm^{-1} in both compounds presented the Si-O-Si absorption band. A sharp and strong peak at 1700 cm^{-1} which represented carbonyl of aldehyde was not observed which clearly indicated that the APTES-GA formation was completed and there was no free aldehyde (-CHO) left. The FT-IR spectra of colorimetric product significantly showed higher absorption than APTES-GA at wavenumber of 1663 cm^{-1} and 1566 cm^{-1} (N-H

bending). This might attribute to the destruction of imine bond upon reacting with hydrogen peroxide and then increased the free -NH- in the mixture. As the result, this absorption band was stronger and sharper in colorimetric product than APTES-GA. According to literatures, a strong and sharp absorption at 1385 cm^{-1} belong to C-O stretching that indicated the C-O bond was formed after the reaction. Besides, the strong and broad band between 3200 - 3400 cm^{-1} clearly belong to the O-H bond which meant the addition of hydrogen peroxide to APTES-GA (Culler et al., 1985; Majoul et al., 2015).

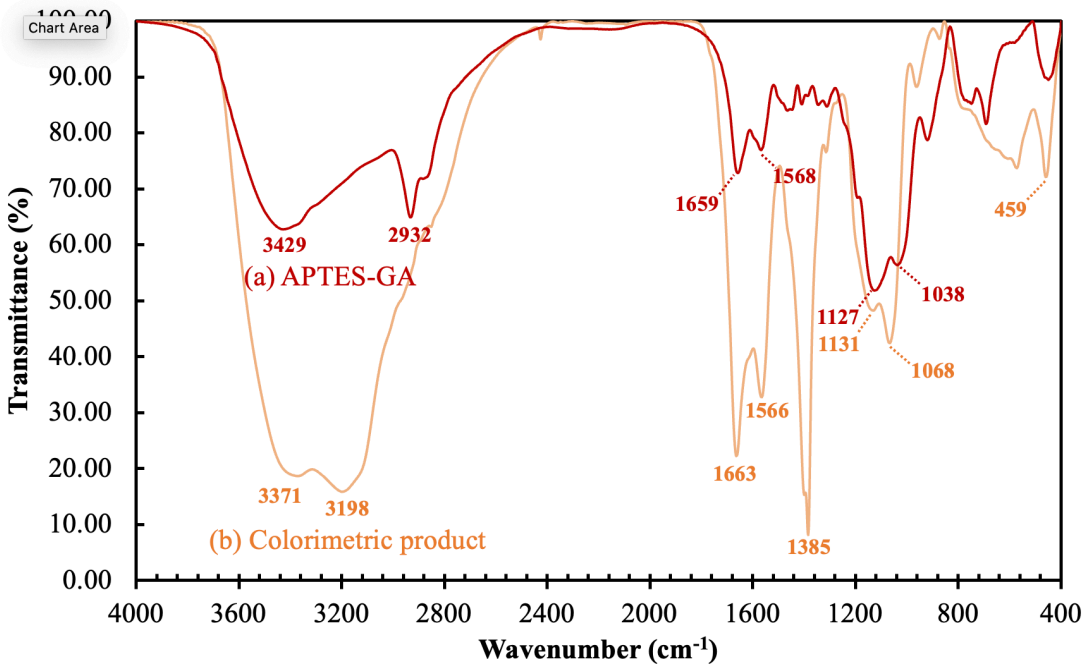


Figure 14 The FT-IR spectra of solid (a) APTES-GA complex and solid (b) colorimetric product.

3.3 Method development for hydrogen peroxide detection

3.3.1 Effects of the color detection mode

The digital image analysis of APTES-GA probe could provide various data depended on the digital system and application. The R-G-B system was utilized in this work because it was well-known and the most familiar system. The data obtained from digital image analysis by Image J software showed the linear relationship between the green intensity and concentrations of hydrogen peroxide (**Figure 15**). The blue

intensity also showed linear relationship similar to the green intensity, but the sensitivity was lower. The green intensity was, therefore, suitable as the response signal of the detection mode.

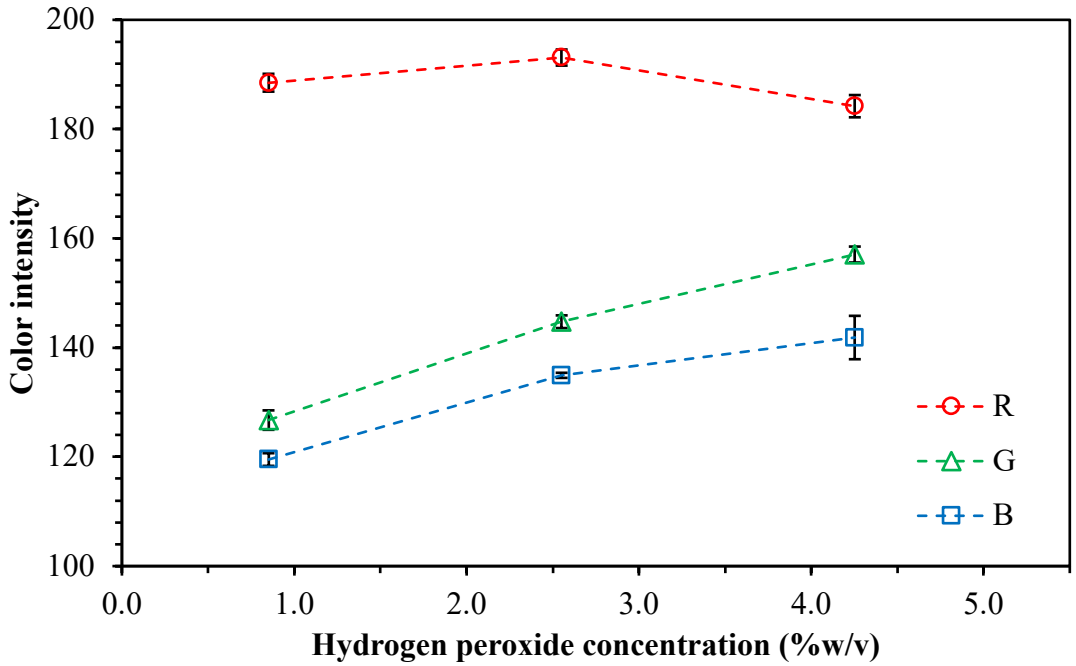


Figure 15 The plot of color intensity of APTES-GA probe after treatment with hydrogen peroxide solution. The volume ratio of APTES-GA : analyte solution was 1:1 v/v, and the reaction was 7 minutes. (n=3)

3.3.2 Volume ratio of APTES-GA solution to analyte solution

The change of color was vital to resulting mixture. The color bleaching was to reduce the intensity of original color of the probe to obtain the weaker resulting probe and the degree of color bleaching was influenced by the amount of analyte presented in sample. The volume ratio of APTES-GA to analyte solution could affect the degree of bleaching greatly. So, it was necessary to investigate the optimal the volume ratio used for detection. From the results of investigation, the green intensity of blank control decreased from volume ratio of 3:7 to 7:3 v/v as the fraction of APTES-GA increased. After the treatment with 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution, the resulting mixture had the red color weaken resulting to the increasing of green intensity (**Figure 16**).

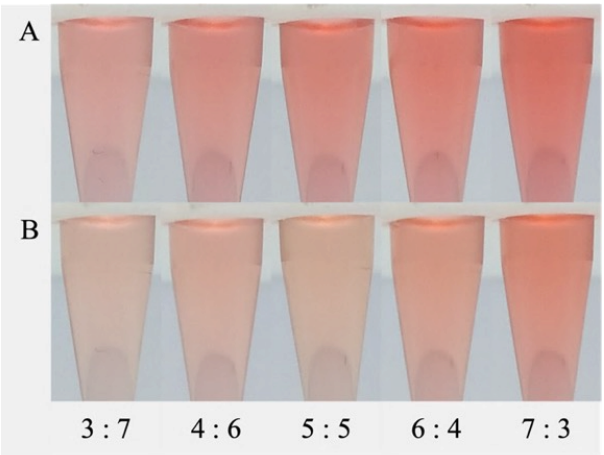


Figure 16 The APTES-GA probes after treatment with (A) blank (acetonitrile) and (B) 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution. The volume ratio of APTES-GA : hydrogen peroxide solution was ranged from 3:7 to 7:3 v/v (left to right). The reaction time was 7 minutes. (n=3)

The weakening green intensity for each replicate that represented how much the color was bleached, was calculated, and compared. The data showed that the volume ratio of 5:5 v/v, or 1:1 v/v, provided the best result as it had the most weakening green intensity (**Figure 17**). Therefore, the volume ratio of APTES-GA : analyte solution of 1:1 v/vwas selected and used for further investigation.

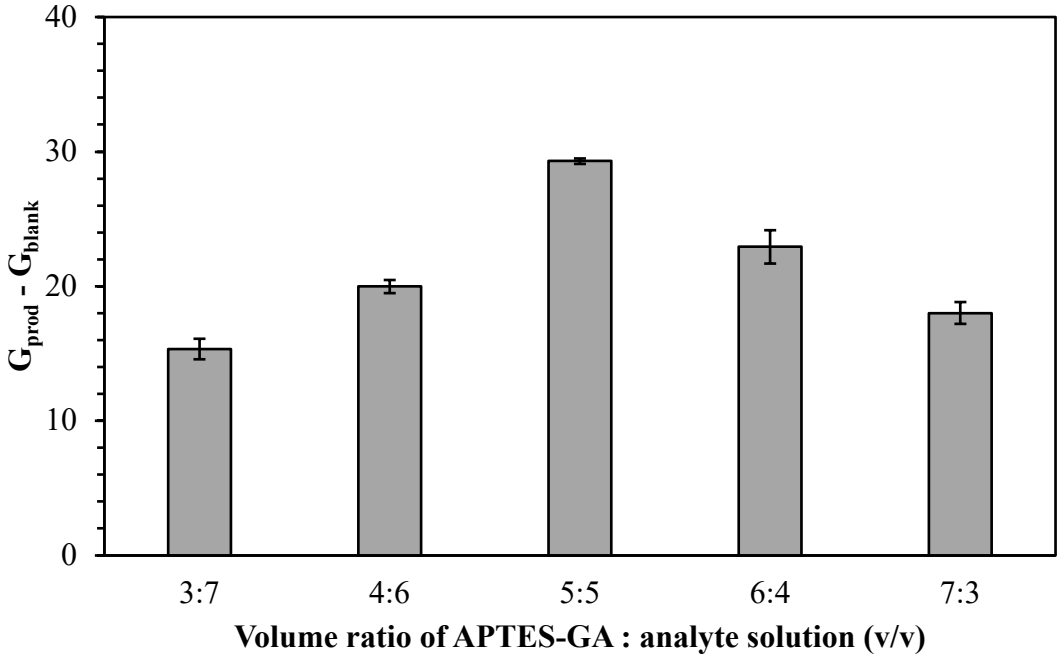


Figure 17 The difference of green intensity of APTES-GA probes that were treated with blank and 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution at volume ratio of APTES-GA : analyte solution ranged from 3:7 to 7:3 v/v. The reaction time was 7 minutes. G_{prod} referred to green intensity of resulting probes that were treated with 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution. G_{blank} referred to APTES-GA probes that were treated with blank. (n=3)

3.3.3 Effect of water content on colorimetric analysis

The antiseptic product containing hydrogen peroxide was sold in water solution. To our surprise, the preliminary experiment showed that the APTES-GA probe was aggregated when it mixed with ultrapure water. Acetone, ethanol, acetonitrile, and mixed solvent of acetonitrile/water were tested for detection media. The preliminary results showed that mixed solvent of acetonitrile/water gave the most satisfying result for detection media and negative control. The water content at a range of 0% (acetonitrile : water was 10:0 v/v) to 50% (acetonitrile : water was 5:5 v/v) in detection media was investigated. The data showed that the green intensity of colorimetric products was not largely different (**Figure 18**). Therefore, the analyte solution can be prepared in mixture of acetonitrile and water at volume ratio from 5:5

to 10:0. In other word, the analyte solution with at 0%v/v to 50%v/v of water was suitable for reaction media.

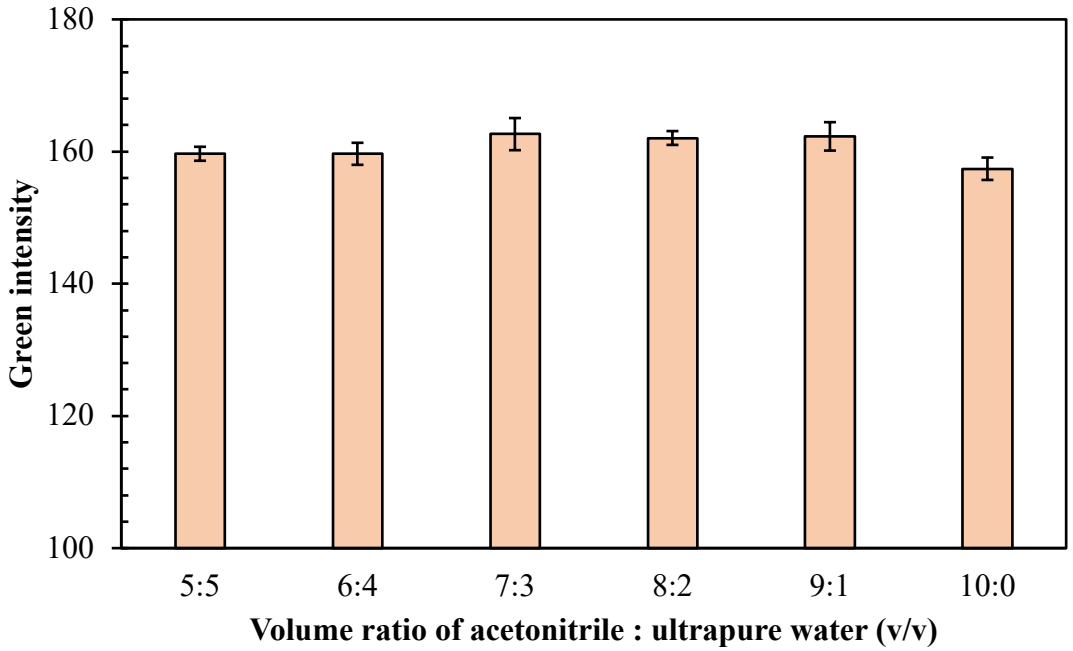


Figure 18 The green intensity of colorimetric product obtained by a treatment of 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution in medias that has acetonitrile : water ranged from 5:5 to 10:0 v/v. The volume ratio of APTES-GA : analyte solution was 1:1 v/v, and the reaction time was 7 minutes. (n=3)

3.3.4 Reaction time

The color of APTES-GA probe was weakening considerably after a treatment with 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution for a period of time. From the observation for 25 minutes, the red color at starting time was slowly weaken over the first 7 minutes and finally turned to pale yellow after 15 minutes (**Figure 19**). The plot of green intensity against the reaction time showed that the green intensity increased sharply from 1 minute to 10 minutes and the change was insignificant after 10 minutes (**Figure 20**). The independent t-test (unequal variance) was carried on comparing two datasets from reaction time of 10 minutes (mean = 176.533, SD = 2.718) and 15 minutes (mean = 178.871, SD = 3.006). The calculated results showed that the difference of analytical signal between two datasets was

insignificant (t(4) = 1.70, p = 0.10). Therefore, the duration of 10 minutes was suitable for detection method.

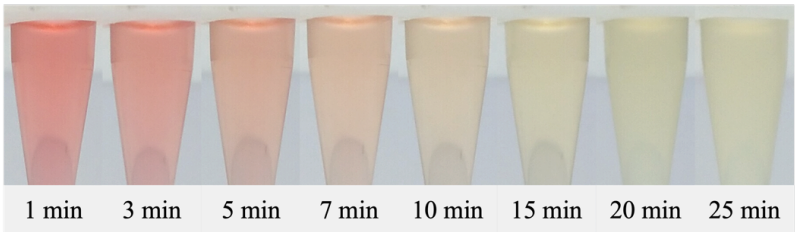


Figure 19 The pictures of APTES-GA probes after a treatment with 750 mmol L^{-1} (2.55 %w/v) hydrogen peroxide solution. The volume ratio of APTES-GA : analyte solution was 1:1 v/v and the observation time started from 1 minute to 25 minutes. (n=3)

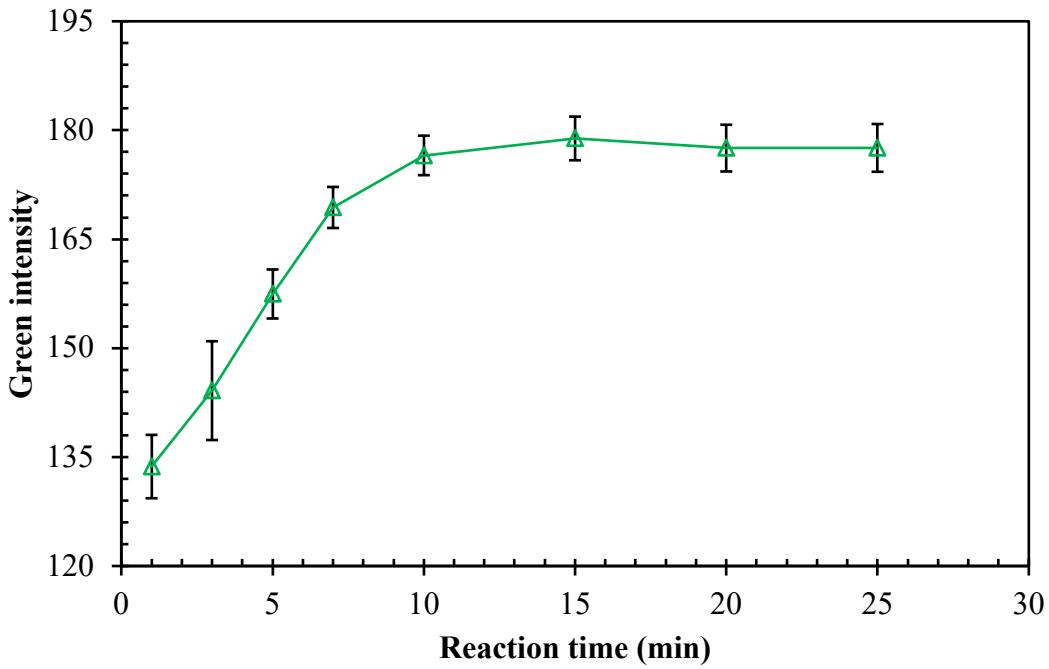


Figure 20 The plot of green intensity of resulting probe products against reaction time. (n=3)

3.4 Method validation

3.4.1 Linearity and range

The linearity of this detection method for determination of hydrogen peroxide was investigated in the concentration range of 1 - 1,500 mmol L^{-1} or 0.003% - 5.10% w/v. Based on naked-eye detection, the APTES-GA probe displayed color

variation from red to pale yellow when exposed to different concentrations of hydrogen peroxide, as demonstrated in **Figure 21**. The plots of various analytical signal against hydrogen peroxide concentrations were demonstrated (**Figure 22**, **Figure 23**, and **Figure 24**). The information of regression analysis obtained from different datasets was summarized in **Table 2**. These results indicated that the developed method have the potential for the determination of target analytes in antiseptic commercial products.

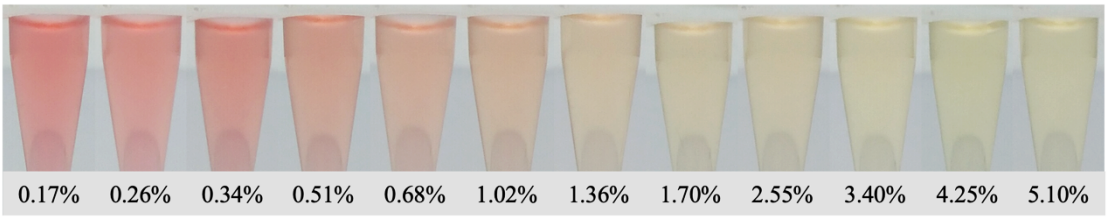


Figure 21 The pictures of APTES-GA probe after a treatment by hydrogen peroxide solution varying from 0.003 %w/v to 5.10 %w/v.

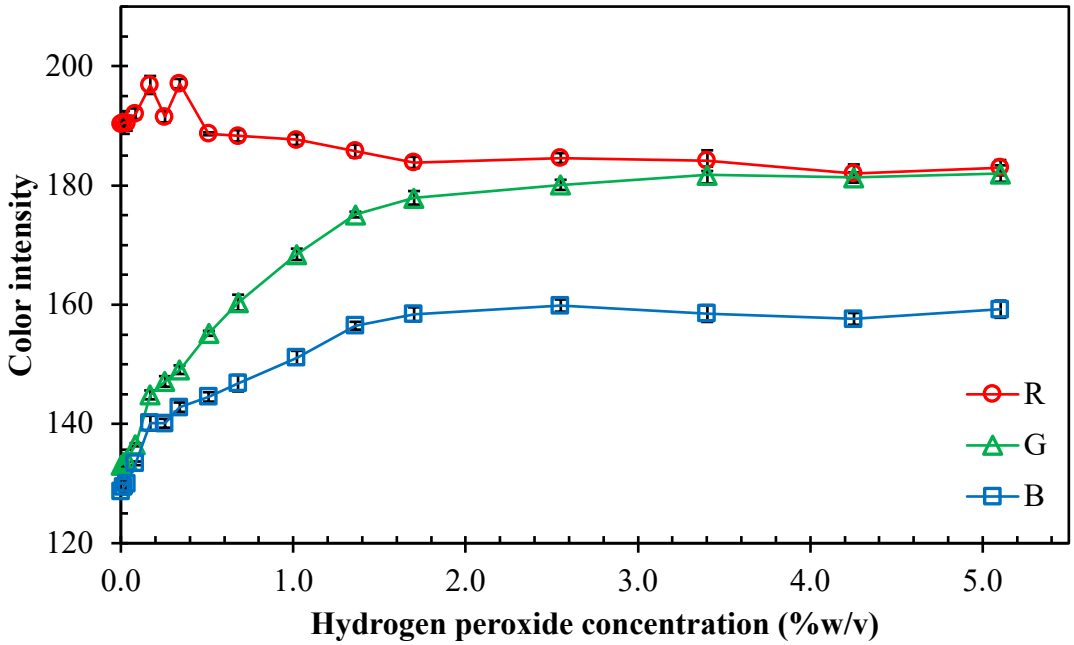


Figure 22 The plot of color intensity of resulting probe against hydrogen peroxide concentrations in a range of 0.003 %w/v - 5.10 %w/v. (n=7)

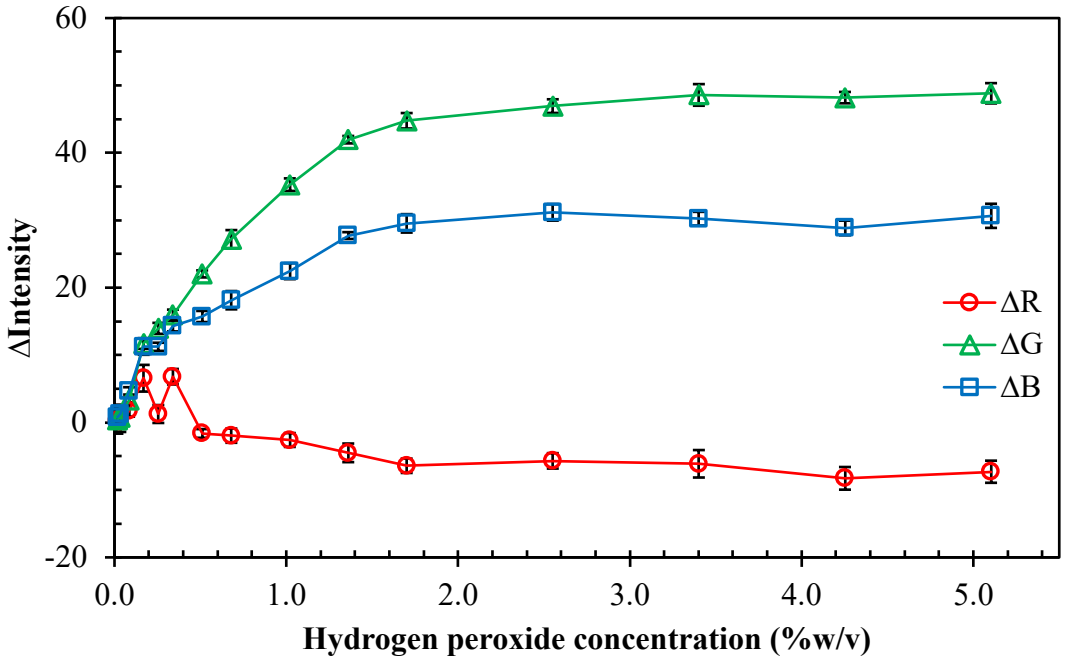


Figure 23 The plot of difference of color intensity (Δintensity) of resulting probe and blank against hydrogen peroxide concentrations in a range of 0.003 %w/v - 5.10% w/v. (n=7)

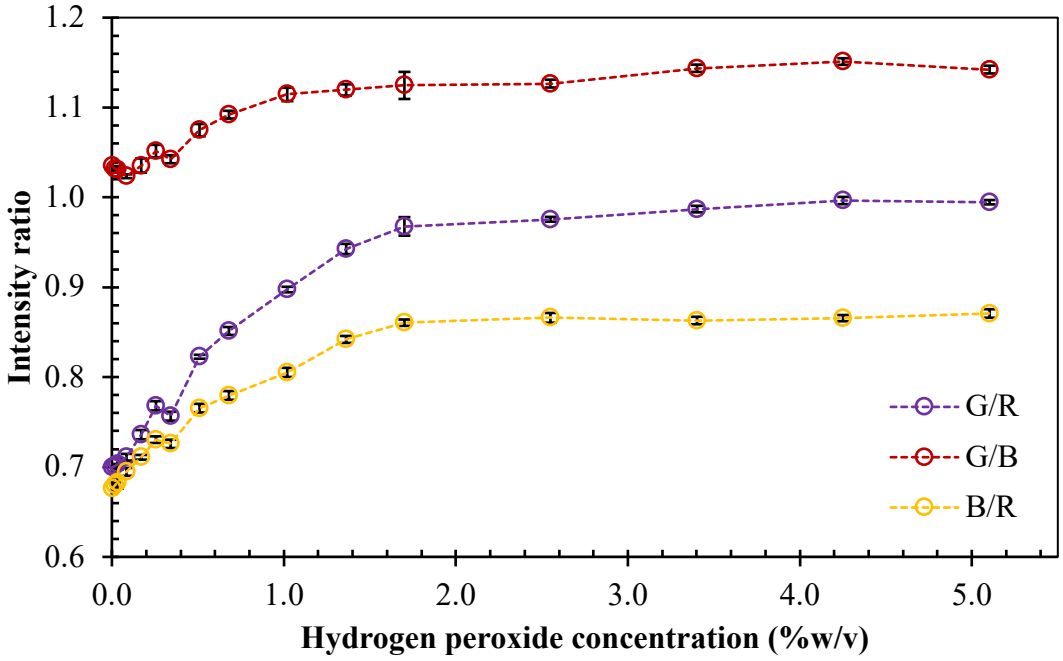


Figure 24 The plot of intensity ratio of resulting probe against hydrogen peroxide concentrations in a range of 0.003 %w/v - 5.10% w/v. (n=7)

Table 2 The information of regresion analysis of calibration plots.

Signal (y axis)	Range	Slope ± SD	y-intercept ± SD	R^2
G	0.17 - 1.36 %w/v	26.057 ± 1.117	141.026 ± 0.826	0.9909
B	0.17 - 1.36 %w/v	13.777 ± 0.520	137.466 ± 0.385	0.9929
ΔG	0.17 - 1.36 %w/v	26.057 ± 1.117	7.873 ± 0.826	0.9909
ΔB	0.17 - 1.36 %w/v	13.860 ± 0.605	8.686 ± 0.447	0.9906
G/R	0.17 - 1.36 %w/v	0.174 ± 0.015	0.717 ± 0.011	0.9658
G/B	0.17 - 1.36 %w/v	0.075 ± 0.010	1.029 ± 0.007	0.9131
B/R	0.17 - 1.36 %w/v	0.107 ± 0.008	0.699 ± 0.006	0.9725

3.4.2 Limit of detection (LOD) and limit of quantification (LOQ)

Hydrogen peroxide detection by APTES-GA probe provided the linearity at a ranged of 0.17 - 1.36 %w/v under optimized conditions. In this work, the LOD was calculated accorded to **Equation 2.1** and **Equation 2.2** whereas the LOQ was calculated accorded to **Equation 2.3** and **Equation 2.4**. The LOD and LOQ of the developed method were summarized in **Table 3**. Based on green intensity signal, which provided the best sensitivity for this study, the LOD and LOQ were 0.10 %w/v and 0.32 %w/v, respectively. The results implied that the developed methods have the potential to be applied for the determination of target analytes in antiseptic commercial products.

Table 3 The LOD and LOQ for each selected analytical signal calculated from datasets at a range of 0.17 %w/v - 1.36 %w/v.

Parameters	G	B	ΔG	ΔB	G/R	G/B	B/R
Slope	26.057	13.777	26.057	13.860	0.174	0.750	0.107
SDE of regression line	1.192	0.555	1.192	0.646	0.016	0.011	0.009
LOD[a] (%w/v)	0.14	0.12	0.14	0.14	0.28	0.44	0.25
LOQ[a] (%w/v)	0.46	0.40	0.46	0.47	0.92	1.47	0.84
SDE of y-intercept	0.826	0.385	0.826	0.447	0.011	0.007	0.006
LOD[b] (%w/v)	0.10	0.08	0.10	0.10	0.19	0.28	0.17
LOQ[b] (%w/v)	0.32	0.28	0.32	0.32	0.63	0.93	0.56

[a]based on standard error of regression line.

[b]based on standard error of y-intercept.

3.4.3 Precision and accuracy

The precision and accuracy experiment of this work was investigated in sample matrix. The analyte solution was fortified with hydrogen peroxide standard solution for three different concentration levels (low, medium and high concentrations) and observed the recovery of hydrogen peroxide detection. The intra- and inter-day precision of the developed method were summarized in **Table 4**. According to the results, the intra-day precisions of the proposed method for measurement of hydrogen peroxide at the concentrations of 50 mmol L^{-1} (0.17 %w/v), 100 mmol L^{-1} (0.34 %w/v), and 200 mmol L^{-1} (0.68 %w/v) were 0.074 - 0.363 %RSD, respectively while the inter-day precisions were 0.268 - 0.910 %RSD, respectively. The accuracy of this work was also satisfying as the recovery was 82.124 - 109.017 % for intra-day accuracy and 85.292 - 104.131 % for inter-day accuracy, as demonstrated in **Table 5**. All values met the acceptance criteria that precision and accuracy should be better than (i.e., less than) 15 %RSD and within a range of 80 - 115 % of percentage of recovery. The results support the precision and accuracy of determining hydrogen peroxide using APTES-

GA as a colorimetric probe in conjunction with smartphone-based digital image analysis.

Table 4 The information of inter-day and intra-day precision for the determination of hydrogen peroxide by APTES-GA probe.

Intra-day precision							
Day	Fortified concentrations (%w/v)	Green intensity			Mean	SD	%RSD
		1	2	3			
1st	0.17	159.652	160.017	160.103	159.924	0.239	0.150
	0.34	163.710	163.753	163.387	163.617	0.200	0.122
	0.68	169.801	169.872	168.845	169.506	0.574	0.338
2nd	0.17	158.900	159.325	159.505	159.243	0.311	0.195
	0.34	163.146	164.247	163.314	163.569	0.593	0.363
	0.68	170.314	170.278	170.547	170.380	0.146	0.086
3rd	0.17	159.683	160.553	159.871	160.036	0.458	0.286
	0.34	165.200	165.213	164.995	165.136	0.122	0.074
	0.68	172.285	172.544	172.750	172.526	0.233	0.135

Inter-day precision						
Fortified concentrations (%w/v)	Green intensity			Mean	SD	%RSD
	1st day	2nd day	3rd day			
0.17	159.924	159.243	160.036	159.734	0.429	0.268
0.34	163.617	163.617	165.136	164.123	0.877	0.534
0.68	169.506	170.380	172.526	170.804	1.554	0.910

Table 5 The information of inter-day and intra-day accuracy for determination of hydrogen peroxide by APTES-GA probe.

Intra-day accuracy							
Day	Fortified concentrations (%w/v)	Recovery (%)			Mean	SD	%RSD
		1	2	3			
1st	0.17	102.966	111.086	113.000	109.017	5.327	4.886
	0.34	96.622	97.100	93.029	95.584	2.225	2.328
	0.68	82.188	88.417	83.484	84.696	3.287	3.881
2nd	0.17	99.053	108.414	112.379	106.615	6.843	6.418
	0.34	96.289	108.414	98.139	100.947	6.532	6.471
	0.68	90.854	87.417	88.899	89.057	1.723	1.935
3rd	0.17	90.216	106.357	93.704	96.759	8.493	8.778
	0.34	96.286	96.407	111.964	101.552	9.017	8.879
	0.68	81.005	82.206	83.162	82.124	1.081	1.316
Inter-day accuracy							
Fortified concentrations (%w/v)		Recovery (%)			Mean	SD	%RSD
		1st day	2nd day	3rd day			
0.17		109.017	106.615	96.759	104.131	6.496	6.238
0.34		95.584	95.584	101.552	97.573	3.446	3.531
0.68		84.696	89.057	82.124	85.292	3.504	4.109

3.4.4 Interference study

The nine compounds that were selected for investigation comprised of low charge ion and high charge ion for both negative and positive charges, that were categorized as neutral, acidic, and basic based on their hydrolyzed capability. The results of the investigation of interferences revealed that $(NH_4)_2SO_4$, $Mg(NO_3)_2$, $NaHCO_3$, and K_2CO_3 caused an aggregation of APTES-GA complex (**Figure 25**).

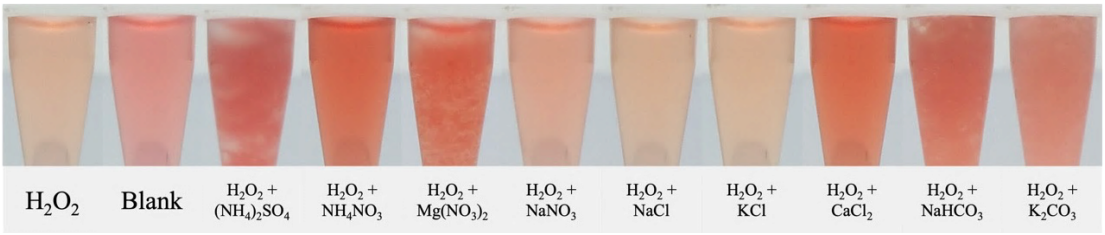


Figure 25 The colorimetric product obtained from APTES-GA probe that was treated with hydrogen peroxide detection in the presence of various interferences. The concentration of hydrogen peroxide and interferences in the respective mixture were 300 mmol L^{-1}.

Both of SO_4^{2-} ion and CO_3^{2-} ion is high negative charge ion and thus which could disrupt the dispersion of APTES-GA in the mixture. Apart from this, HCO_3^- and CO_3^{2-} ion also exhibits basic property which play a major role to deform the APTES-GA. For magnesium nitrate, the Mg^{2+} ion was high positive charge ion like Ca^{2+} ion. However, the nitrogen and oxygen atom within APTES-GA structure had a higher affinity toward Mg^{2+} ion than Ca^{2+} ion, resulting to an aggregation of APTES-GA. Ca^{2+} ion did not cause the aggregation of APTES-GA but, darkened the color of colorimetric product instead. The NH_4^+ ion that was a weak acid, also darkened the color of APTES-GA probe as well. As we could see from the result, Mg^{2+}, Ca^{2+}, HCO_3^-, CO_3^{2-}, SO_4^{2-}, and NH_4^+ at 300 mmol L^{-1} worsen the response of hydrogen peroxide detection. In another hand, small charge neutral ions like Na^+, K^+, Cl^-, and NO_3^- caused insignificant change of analytical signal at 300 mmol L^{-1} as the relative error was below 10% for $NaNO_3$, 5% for NaCl and KCl (**Figure 26**).

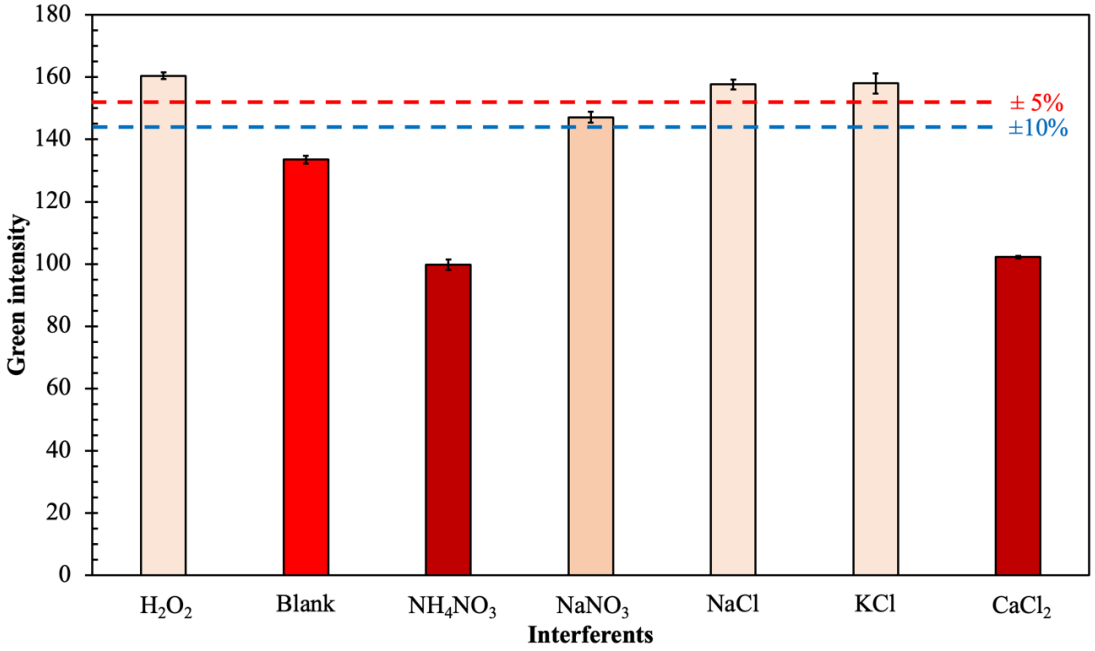


Figure 26 The green intensity of colorimetric product produced from hydrogen peroxide detection in the presence of various interferences, under optimized condition. The concentrations of hydrogen peroxide and interferences in the respective mixture were 300 mmol L^{-1}. (n=3)

Tolerances of APTES-GA for Ca^{2+} ($CaCl_2$) and NH_4^+ (NH_4NO_3) were elected to investigate further because these two substances did not cause an aggregation of APTES-GA and the mixture remained homogenous. Along with those two mentioned, the tolerance of Mg^{2+} ($Mg(NO_3)_2$) was also studied further to see the effect of concentration on the aggregation of APTES-GA. As shown in **Figure 27**, the response of APTES-GA in the presence of NH_4^+, Ca^{2+}, and $Mg^{2+}$was improved at low concentration. The relative error of APTES-GA response was below 10% at concentration of 1 mmol L^{-1} for of NH_4^+ and Mg^{2+} while the relative error of APTES-GA response in 1 mmol L^{-1} Ca^{2+} was approximately -5%.

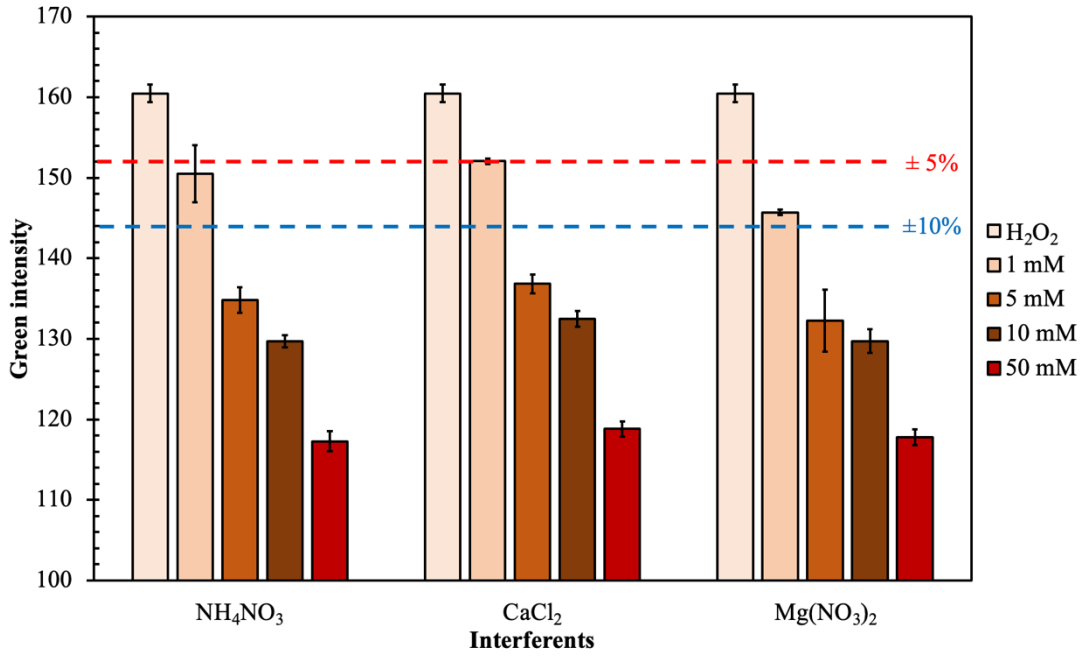


Figure 27 The green intensity of resulting probe obtained from hydrogen peroxide detection in various concentrations of interferences. (n=3)

To summarize, $Na^{+}$and Cl^{-} ion which could be found in saline solution (0.9 %w/v NaCl or approximately 154 mmol L^{-1} NaCl) that was used in medicine formula for antiseptic solution (Crna, 2018), did not interfere the detection by APTES-GA probe. The result support that APTES-GA probe was suitable for screening hydrogen peroxide medicinal solution.

3.4.5 Shelf-life evaluation of colorimetric probe

The APTES-GA probe was successfully prepared under optimized condition and stored at three different conditions for 6 weeks (**Figure 28**). The analytical signals of the three APTES-GA probes were investigated after several days of storage in different storage conditions; room temperature (25 ºC), domestic refrigerator (4 ºC), and freezer (-18 ºC).

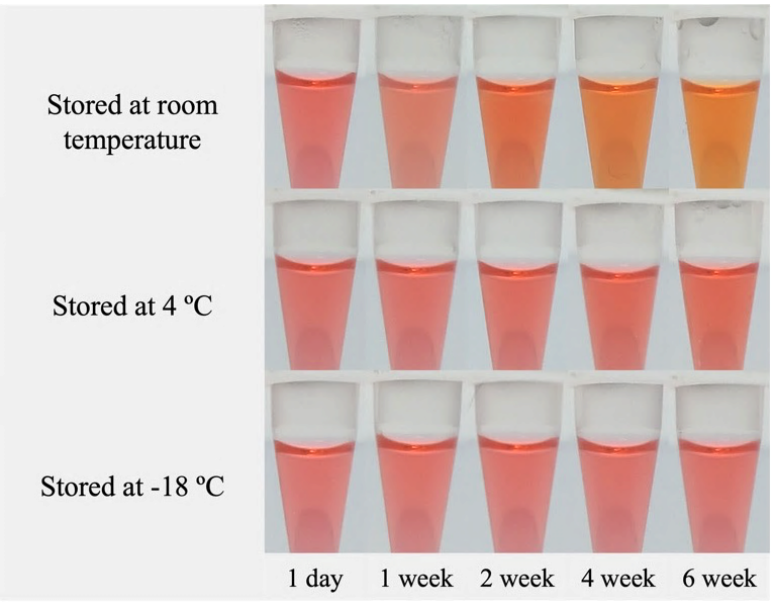


Figure 28 The APTES-GA probe after stored at three different conditions: room temperature, 4 ºC, and -18 ºC.

Figure 29 presents the time profiles of the green intensity of hydrogen peroxide detection after storage at room temperature, 4 ºC and -18 ºC. After storage at room temperature for 2 weeks, the red color of APTES-GA probe was slowly weaken and turned to orange. The green intensity of the hydrogen peroxide colorimetric product changed by -1.21% to -5.12% (**Figure 30**). The APTES-GA probe was stable for a period of 6 weeks at 4 ºC. The green intensity of hydrogen peroxide colorimetric product changed by -4.38% to +0.44% (**Figure 30**) after storage at 4 ºC for 6 weeks. The APTES-GA probe was also stable for a period of 6 weeks at -18 ºC. The green intensity of hydrogen peroxide colorimetric product changed by -0.50% to +1.15% (**Figure 30**) after storage at -18ºC for 6 weeks. This indicated that the APTES-GA probe could be stored at 4 ºC and -18 ºC for 6 weeks straight and remained an acceptable performance (%RE within ± 5%). Therefore, the most suitable storage condition that could preserve a good shelf-life of valuation was 4 ºC and -18 ºC for at least 6 weeks.

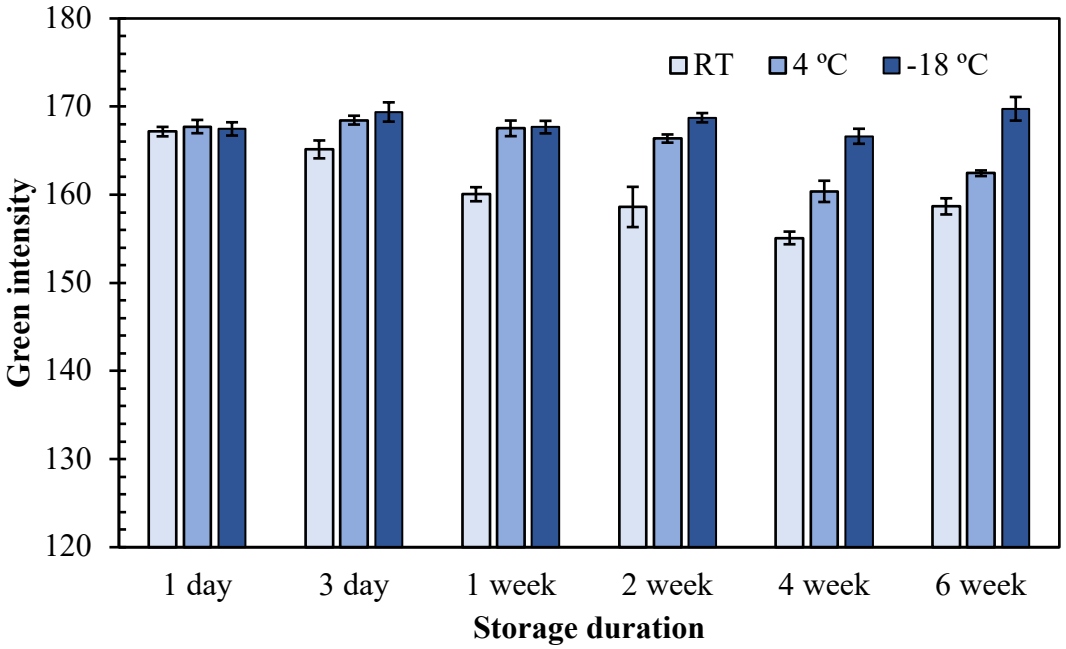


Figure 29 The green intensity of colorimetric products obtained from APTES-GA probes stored at three different conditions: room temperature, 4 ºC, and -18 ºC. (n=3)

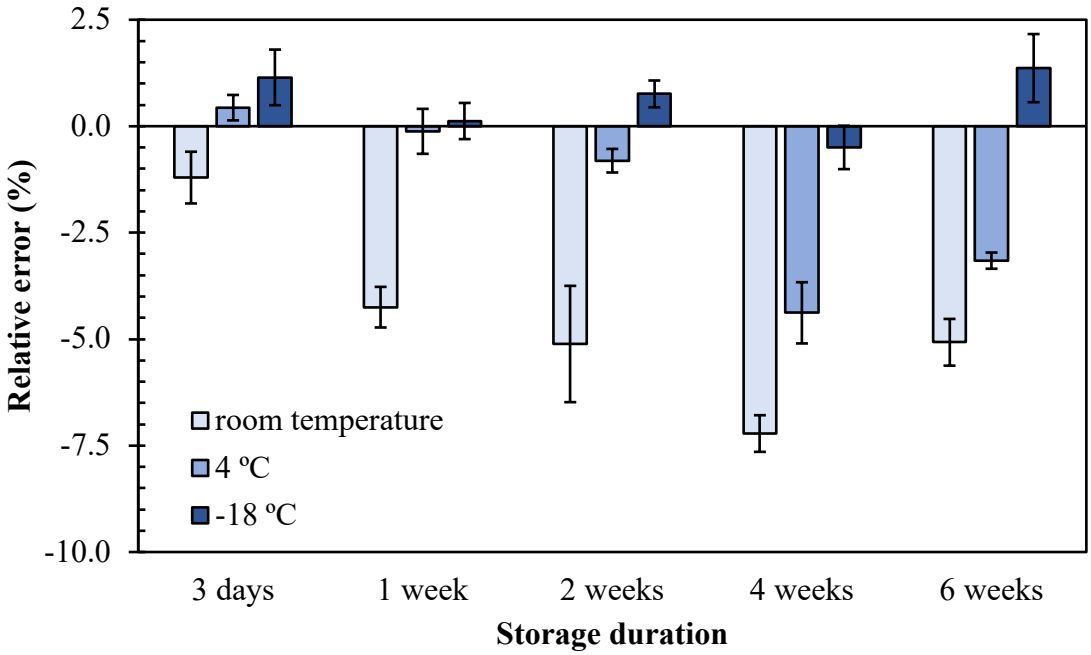


Figure 30 The relative error of green intensity of colorimetric products from APTES-GA probes stored at three different conditions: room temperature, 4 ºC, and -18 ºC. (n=3)

3.5 Determination of hydrogen peroxide in real samples

Three commercial brands of hydrogen peroxide medicine were investigated by APTES-GA probes with smartphone-based digital image analysis. The sample was diluted with acetonitrile to allow the concentration of hydrogen peroxide presented in calibration model of APTES-GA. Apart from this, the sample that was originally water matrix needed to be prepared into water-acetonitrile matrix prior to measurement by APTES-GA probe. All of samples were prepared as described in section 2.7 and 2.8 which were analyzed by the proposed method and the reference method ($TiOSO_4$ method). A spiked and recovery study was also conducted using hydrogen peroxide standard solutions of 50 mmol L^{-1} (0.17 %w/v), 100 mmol L^{-1} (0.34 %w/v), and 200 mmol L^{-1} (0.68 %w/v). The amounts of hydrogen peroxide content in different samples, determined by proposed method and $TiOSO_4$ method , are shown in **Table 6** and **Table 7**, respectively.

The level of hydrogen peroxide in three commercial products were found in the range 2.992 - 6.072 %w/v by proposed method. The percentage difference between the detected amounts of hydrogen peroxide obtained by the proposed approach and those obtained by the $TiOSO_4$ method was less than ±10% (**Table 8**), indicating that both methods are in good quantitative agreement. In addition, the accuracy of the proposed method was examined and reported in terms of the relative percentages of recovery. The obtained relative recoveries from the commercial hydrogen peroxide medicine ranged from 82.124% to 109.017%, which are in the acceptable range according to the ICH guidelines.

Table 6 The information of sample analysis of hydrogen peroxide antiseptic solution with APTES-GA probe. Brand A and Brand C were diluted 5-fold before measurement while Brand B was diluted 10-fold before measurement.

Sample	Hydrogen peroxide concentration (%w/v)		%Recovary
	Fortified	Found (n=3)	
Brand A	-	0.598 ± 0.022	-
	0.170	0.784 ± 0.009	103.027 ± 5.327
	0.340	0.924 ± 0.008	95.640 ± 2.225
	0.680	1.175 ± 0.022	84.746 ± 3.287
Brand B	-	0.607 ± 0.024	-
	0.170	0.789 ± 0.012	106.678 ± 6.847
	0.340	0.951 ± 0.022	101.007 ± 6.536
	0.680	1.213 ± 0.012	89.109 ± 1.725
Brand C	-	0.691 ± 0.027	-
	0.170	0.853 ± 0.014	95.071 ± 8.498
	0.340	1.034 ± 0.031	100.739 ± 9.022
	0.680	1.247 ± 0.007	81.736 ± 1.081

Table 7 The information of sample analysis of hydrogen peroxide antiseptic solution with $TiOSO_4$ spectrophotometry method. Brand A and Brand C were diluted 1000-fold before measurement while Brand B was diluted 2000-fold before measurement.

Sample	Hydrogen peroxide concentration (mmol L^{-1})		%Recovary
	Fortified	Found (n=3)	
Brand A	-	0.961 ± 0.009	-
	0.196	1.126 ± 0.006	84.158 ± 2.867
	0.490	1.381 ± 0.006	85.847 ± 1.318
	0.980	1.821 ± 0.040	87.787 ± 4.097
Brand B	-	0.852 ± 0.010	-
	0.196	1.050 ± 0.006	101.052 ± 3.017
	0.490	1.348 ± 0.023	101.115 ± 4.754
	0.980	1.853 ± 0.022	102.178 ± 2.263
Brand C	-	1.050 ± 0.009	-
	0.196	1.234 ± 0.006	93.857 ± 3.296
	0.490	1.504 ± 0.007	92.605 ± 1.421
	0.980	1.977 ± 0.033	94.607 ± 3.381

Table 8 The comparison of $TiOSO_4$ spectrophotometry method and APTES-GA probe on hydrogen peroxide determination in antiseptic samples.

Sample	Label	Found (%w/v, n=3)		%RE
		APTES-GA	$TiOSO_4$	
Brand A	3 %w/v	2.992 ± 0.111	3.268 ± 0.031	-8.446
Brand B	6 %w/v	6.072 ± 0.244	5.798 ± 0.066	+4.726
Brand C	3 %w/v	3.455 ± 0.133	3.573 ± 0.032	-3.303

CHAPTER 4

CONCLUSIONS

The APTES-GA complex was successfully prepared from (3-aminopropyl)triethoxysiloxane and 25% glutaraldehyde solution as a colorimetric probe. The investigation of an optimal condition for APTES-GA preparation revealed that the optimal volume ratio of APTES : 25% glutaraldehyde solution was 1.5:1 v/v in ethanol solvent as it provided the highest absorbance at 540 nm for spectrophotometric determination and the lowest green intensity for digital image analysis. The volume ratio of APTES : 25% glutaraldehyde solution could be reported as the optimal concentration of APTES and glutaraldehyde in mixture which were 3%v/v and 1%v/v, respectively. Apart from this, the optimal incubation time of APTES-GA preparation was three days before analysis. The optimal condition of the proposed method for hydrogen peroxide detection was for green intensity as the color detection mode. The volume ratio of APTES-GA solution and analyte solution was 1:1 v/v. The percentage of acetonitrile in water (0% to 50% v/v) is selected for dilution of the antiseptic commercial product. The optimal time was 10 minutes. Under the optimum conditions, a linear calibration curves were established at the range of 50 to 400 mmol L^{-1} or 0.17 %w/v to 1.36 %w/v using green intensity (R^2 = 0.9909) as analytical signal. The limit of detections (LOD) and limit of quantifications (LOQ) were calculated from standard error of y-intercept and slope of calibration curve, and obtained as 0.10 %w/v and 0.32 %w/v, respectively. Both LOD and LOQ was calculated based on the standard error of y-intercept and the slope of the calibration curve. The intra-day and inter-day precisions were 0.074 - 0.363 %RSD and 0.268 - 0.910 %RSD, respectively. The intra-day and inter-day accuracies were 82.124 - 109.017% and 85.292 - 104.131%, respectively. The APTES-GA probe displayed a good shelf-life of evaluation and can be stored at 4 ºC and -18 ºC up to 6 weeks. The proposed method was successfully applied for determination of hydrogen peroxide in antiseptic commercial products. The measured hydrogen peroxide in real samples were comparable with those obtained from a $TiOSO_4$ spectrophotometry that used as a reference method, reflecting the reliability of the

developed method. The device is convenient for on-site use because it is compact, disposable, and inexpensive. Apart from the application for the quality control of antiseptic commercial product, the developed probe has a potential to use as smart kit for detecting and identifying clandestine cocaine laboratories.

Table 9 The comparison of previous method and APTES-GA probe on hydrogen peroxide determination. (NR referred to no report)

Analytical method	Principal(reagent)	Sample type	Required procedure	Total time	LOD	Ref.
Spectrophotometry	Enzymatic oxidation (LCV/HRP)	-Marine water	-pH conditioning -Dilution	5 min	0.02 µM	Zang and Wong, 1994
Spectrophotometry	Enzymatic-mimic oxidation (TMB/CoS)	-River water -Tap water	-pH conditioning -Dilution	10 min	0.02 mM	Yang et al., 2016
Spectrophotometry	Enzymatic-mimic oxidation (TMB/IrNP)	NR	-pH conditioning -Incubation at 35 ºC	10 min	0.53 µM	Cui et al., 2017
Spectrophotometry	Enzymatic-mimic oxidation (TMB/α-$AgVO_3$ MC)	Antiseptic liquid	-pH conditioning -dilution (3000-fold)	2 min	2 µM	Wang et al., 2017
Spectrophotometry	Etching oxidation (*i*-AgNPS)	NR	-Dilution (10-fold)	60 min	90 nM	Zong et al., 2018
Digital image analysis	Color bleaching (APTMS-GA)	NR	NR	5 min	2.5 mM	Zhou et al., 2014
Digital image analysis	Enzymatic oxidation (Tyr/HRP)	Beverage	-pH conditioning -Dilution (200-fold)	2 min	51.2 µM	Hosu et al., 2019
Digital image analysis	Enzymatic-mimic oxidation (TMB/AgNPs)	NR	-pH conditioning	5 min	2 nM	Khachornsakkul & Dungchai, 2020
Digital image analysis	Color bleaching (APTES-GA)	Antiseptic liquid	-Dilution (5-fold to 10-fold)	10 min	0.10%w/v (27.9 mM)	This work

REFERENCES

Abdollahi, M., & Hosseini, A. (2014). Hydrogen Peroxide. In *Encyclopedia of Toxicology* (pp. 967-970). Elsevier.

AOAC international. (2016). Appendix F: Guidelines for Standard Method Performance Requirements. In *AOAC Official Methods of Analysis*. https://www.aoac.org/wp-content/uploads/2019/08/app_f.pdf

Choodum, A., Kanatharana, P., Wongniramaikul, W., & Nic Daeid, N. (2013). Using the iPhone as a device for a rapid quantitative analysis of trinitrotoluene in soil. *Talanta*, *115*, 143-149.

Christopher, L. P., Yao, B., & Ji, Y. (2014). Lignin Biodegradation with Laccase-Mediator Systems. *Frontiers in Energy Research*, *2*.

Crna, R. N. M. (2018, November 29). *How to make saline solution*. Medical News Today. https://www.medicalnewstoday.com/articles/323842

Cui, M., Zhou, J., Zhao, Y., & Song, Q. (2017). Facile synthesis of iridium nanoparticles with superior peroxidase-like activity for colorimetric determination of H2O2 and xanthine. *Sensors and Actuators B: Chemical*, *243*, 203-210.

Culler, S. R., Ishida, H., & Koenig, J. L. (1985). Structure of silane coupling agents adsorbed on silicon powder. *Journal of Colloid and Interface Science*, *106*(2), 334-346.

Farrugia, K. J., Savage, K. A., Bandey, H., Ciuksza, T., & Nic Daéid, N. (2011). Chemical enhancement of footwear impressions in blood on fabric - Part 2: Peroxidase reagents. *Science & Justice*, *51*(3), 110-121.

Harpaz, D., Eltzov, E., Ng, T. S. E., Marks, R. S., & Tok, A. I. Y. (2020). Enhanced Colorimetric Signal for Accurate Signal Detection in Paper-Based Biosensors. *Diagnostics*, *10*(1), 28.

Hosu, O., Lettieri, M., Papara, N., Ravalli, A., Sandulescu, R., Cristea, C., & Marrazza, G. (2019). Colorimetric multienzymatic smart sensors for hydrogen peroxide, glucose and catechol screening analysis. *Talanta*, *204*, 525-532.

Khachornsakkul, K., & Dungchai, W. (2020). Development of an ultrasound-enhanced smartphone colorimetric biosensor for ultrasensitive hydrogen peroxide detection and its applications. *RSC Advances*, *10*(41), 24463-24471.

Kiassen, N. V., Marchington, D., & McGowant, H. C. E. (1994). H_2O_2 Determination by the I_3^- Method and by $KMnO_4$ Titration. *Analytical Chemistry*, *66*(18), 2921-2925.

Lantam, A., Limbut, W., Thiagchanya, A., & Phonchai, A. (2020). A portable optical colorimetric sensor for the determination of promethazine in lean cocktail and pharmaceutical doses. *Microchemical Journal*, *159*, 105519.

Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells? *Trends in Biochemical Sciences*, *41*(3), 211-218.

Majoul, N., Aouida, S., & Bessaïs, B. (2015). Progress of porous silicon APTES-functionalization by FTIR investigations. *Applied Surface Science*, *331*, 388-391.

Murphy, E. C., & Friedman, A. J. (2019). Hydrogen peroxide and cutaneous biology: Translational applications, benefits, and risks. *Journal of the American Academy of Dermatology*, *81*(6), 1379-1386.

NATA. (2012). *Technical Note 17-Guidelines for the validation and verification of quantitative and qualitative test methods*. https://www.demarcheiso17025.com/document/Guidelines%20for%20the%20validation%20and%20verification%20of%20quantitative%20and%20qualitative%20test%20methods.pdf

National Center for Biotechnology Information. (2023). *PubChem Compound Summary for CID 784, Hydrogen Peroxide*. https://pubchem.ncbi.nlm.nih.gov/compound/Hydrogen-Peroxide

Periasamy, A. P., Ho, Y.-H., & Chen, S.-M. (2011). Multiwalled carbon nanotubes dispersed in carminic acid for the development of catalase based biosensor for selective amperometric determination of H2O2 and iodate. *Biosensors and Bioelectronics*, *29*(1), 151-158.

Qu, F., Yang, Q., Wang, B., & You, J. (2020). Aggregation-induced emission of copper nanoclusters triggered by synergistic effect of dual metal ions and the

application in the detection of H_2O_2 and related biomolecules. *Talanta*, *207*, 120289.

Rodríguez-López, J. N., Lowe, D. J., Hernández-Ruiz, J., Hiner, A. N. P., García-Cánovas, F., & Thorneley, R. N. F. (2001). Mechanism of Reaction of Hydrogen Peroxide with Horseradish Peroxidase: Identification of Intermediates in the Catalytic Cycle. *Journal of the American Chemical Society*, *123*(48), 11838-11847.

Tietje, C., & Brouder, A. (Eds.). (2010). International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use. In *Handbook of Transnational Economic Governance Regimes* (pp. 1041-1053). Brill | Nijhoff.

Urban, M. V., Rath, T., & Radtke, C. (2019). Hydrogen peroxide (H_2O_2): A review of its use in surgery. *Wiener Medizinische Wochenschrift*, *169*(9-10), 222–225.

Wang, Y., Zhang, D., & Wang, J. (2018). Metastable α-$AgVO_3$ microrods as peroxidase mimetics for colorimetric determination of H_2O_2. *Microchimica Acta*, *185*(1), 1.

West, M. (2021, July 21). *What to know about hydrogen peroxide*. What to Know about Hydrogen Peroxide. https://www.medicalnewstoday.com/articles/hydrogen-peroxide

Yang, H., Zha, J., Zhang, P., Xiong, Y., Su, L., & Ye, F. (2016). Sphere-like CoS with nanostructures as peroxidase mimics for colorimetric determination of H_2O_2 and mercury ions. *RSC Advances*, *6*(71), 66963-66970.

Yuan, J., Cen, Y., Kong, X.-J., Wu, S., Liu, C.-L., Yu, R.-Q., & Chu, X. (2015). MnO_2-Nanosheet-Modified Upconversion Nanosystem for Sensitive Turn-On Fluorescence Detection of H_2O_2 and Glucose in Blood. *ACS Applied Materials & Interfaces*, *7*(19), 10548-10555.

Zhang, L.-S., & Wong, G. T. F. (1994). Spectrophotometric determination of H_2O_2 in marine waters with leuco crystal violet. *Talanta*, *41*(12), 2137-2145.

Zhou, M., Yang, M., & Zhou, F. (2014). Paper based colorimetric biosensing platform utilizing cross-linked siloxane as probe. *Biosensors and Bioelectronics*, *55*, 39-43.

Zhou, Z., Yang, L., Huang, L., Liao, Y., Liu, Y., & Xiao, Q. (2020). A novel fluorescent probe for H_2O_2 detection based on CdSe@ZnS quantum dots/Ag nanocluster hybrid. *Analytica Chimica Acta*, *1106*, 176-182.

Zong, C., Li, B., Wang, J., Liu, X., Zhao, W., Zhang, Q., Nie, X., & Yu, Y. (2018). Visual and colorimetric determination of H_2O_2 and glucose based on citrate-promoted H_2O_2 sculpturing of silver nanoparticles. *Microchimica Acta*, *185*(3), 199.

Zor, E., Saglam, M. E., Akin, I., Saf, A. O., Bingol, H., & Ersoz, M. (2014). Green synthesis of reduced graphene oxide/nanopolypyrrole composite: Characterization and H_2O_2 determination in urine. *RSC Advances*, *4*(24), 12457.

APPENDIX

Table S1. The absorbance at 540 nm of APTES-GA solution that was incubated for 24 hours. The volume ratio of APTES : 25% GA ranged from 0.5:1 to 5.0:1 v/v. The solution was diluted five-fold before measurement.

APTES:25%GA (v/v)	Absorbance (540 nm)			Mean	SD	%RSD
	1	2	3			
0.5 : 1	0.3269	0.3316	0.3387	0.3324	0.0059	1.7972
1.0 : 1	0.6242	0.6196	0.6149	0.6196	0.0047	0.7505
1.5 : 1	0.8118	0.8113	0.8105	0.8112	0.0007	0.0808
2.0 : 1	0.6825	0.6818	0.6880	0.6841	0.0034	0.4964
2.5 : 1	0.6145	0.6203	0.6150	0.6166	0.0032	0.5213
3.0 : 1	0.5064	0.4992	0.4990	0.5015	0.0042	0.8406
3.5 : 1	0.3832	0.3850	0.3945	0.3876	0.0161	1.5666
4.0 : 1	0.3476	0.3421	0.3444	0.3447	0.0028	0.8013
4.5 : 1	0.2536	0.2544	0.2613	0.2564	0.0042	1.6510
5.0 : 1	0.2294	0.2313	0.2315	0.2307	0.0012	0.5023

Table S2. The red intensity of APTES-GA probe that was incubated for 24 hours. The volume ratio of APTES : 25% GA ranged from 0.5:1 to 5.0:1 v/v.

APTES:25%GA (v/v)	Red intensity			Mean	SD	%RSD
	1	2	3			
0.5 : 1	194.268	194.549	195.694	194.837	0.755	0.388
1.0 : 1	195.309	194.538	197.412	195.753	1.488	0.760
1.5 : 1	195.530	195.382	197.989	196.300	1.464	0.746
2.0 : 1	197.459	195.479	195.654	196.197	1.096	0.559
2.5 : 1	196.128	195.534	197.748	196.470	1.146	0.583
3.0 : 1	196.392	198.466	195.907	196.922	1.359	0.690
3.5 : 1	194.297	196.288	195.498	195.361	1.003	0.513
4.0 : 1	194.559	196.358	195.330	195.416	0.903	0.462
4.5 : 1	193.886	195.920	194.674	194.827	1.026	0.526
5.0 : 1	193.805	195.892	195.412	195.036	1.093	0.560

Table S3. The green intensity of APTES-GA probe that was incubated for 24 hours. The volume ratio of APTES : 25% GA ranged from 0.5:1 to 5.0:1 v/v.

APTES:25%GA (v/v)	Replicates			Mean	SD	%RSD
	1	2	3			
0.5 : 1	110.785	116.356	118.759	115.300	4.091	3.548
1.0 : 1	93.738	92.607	94.544	93.630	0.973	1.039
1.5 : 1	86.430	83.604	88.217	86.084	2.326	2.702
2.0 : 1	95.206	93.362	92.524	93.697	1.372	1.464
2.5 : 1	101.029	100.345	102.429	101.268	1.062	1.049
3.0 : 1	111.833	113.873	111.012	112.239	1.473	1.313
3.5 : 1	124.005	126.077	125.552	125.211	1.077	0.860
4.0 : 1	132.896	134.306	132.988	133.397	0.789	0.591
4.5 : 1	142.900	145.315	144.753	144.323	1.264	0.876
5.0 : 1	147.662	150.601	149.015	149.093	1.471	0.987

Table S4. The blue intensity of APTES-GA probe that was incubated for 24 hours. The volume ratio of APTES : 25% GA ranged from 0.5:1 to 5.0:1 v/v.

APTES:25%GA (v/v)	Replicates			Mean	SD	%RSD
	1	2	3			
0.5 : 1	35.603	37.049	38.980	37.211	1.694	4.553
1.0 : 1	61.439	61.220	62.538	61.732	0.706	1.144
1.5 : 1	87.197	83.443	88.745	86.462	2.726	3.153
2.0 : 1	108.508	105.562	105.432	106.501	1.740	1.633
2.5 : 1	118.853	118.240	120.869	119.321	1.375	1.153
3.0 : 1	129.623	132.021	138.491	133.378	4.587	3.449
3.5 : 1	139.018	141.699	140.103	140.273	1.349	0.961
4.0 : 1	145.026	146.554	145.682	145.754	0.767	0.526
4.5 : 1	151.224	153.798	151.894	152.305	1.335	0.877
5.0 : 1	153.936	157.247	156.083	155.755	1.680	1.078

Table S5. The absorbance at 540 nm of APTES-GA complex solutions that were prepared using APTES : 25% GA volume ratio of 1.5:1 v/v. The APTES-GA complex solution was measured at different time intervals starting from 3 hours to 7 days.

Incubation time	Replicates			Mean	SD	%RSD
	1	2	3			
3 hours	0.0819	0.0812	0.0826	0.0819	0.0007	0.8547
6 hours	0.1638	0.1633	0.1643	0.1638	0.0005	0.3053
12 hours	0.3384	0.3334	0.3317	0.3345	0.0035	1.0412
1 day	0.6659	0.6577	0.6611	0.6616	0.0041	0.6227
2 days	0.9150	0.9458	0.9512	0.9373	0.0195	2.0834
3 days	1.0463	1.0792	1.0657	1.0637	0.0165	1.5547
5 days	1.0409	1.0588	1.0211	1.0403	0.0189	1.8128
7 days	1.0590	1.1800	1.0280	1.0890	0.0803	7.3754

Table S6. The red intensity of APTES-GA probes that were prepared using APTES : 25% GA volume ratio of 1.5:1 v/v. APTES-GA probes were measured at different time intervals starting from 3 hours to 7 days.

Incubation time	Replicates			Mean	SD	%RSD
	1	2	3			
3 hours	190.169	187.141	189.523	188.944	1.595	0.844
6 hours	193.029	193.252	191.668	192.650	0.857	0.445
12 hours	192.347	191.343	190.869	191.520	0.755	0.394
1 day	191.949	191.939	193.882	192.590	1.119	0.581
2 days	193.980	195.424	194.870	194.758	0.728	0.374
3 days	194.184	195.790	195.544	195.173	0.865	0.443
5 days	194.645	196.346	195.614	195.535	0.853	0.436
7 days	198.387	194.477	197.553	196.806	2.059	1.046

Table S7. The green intensity of APTES-GA probes that were prepared using APTES : 25% GA volume ratio of 1.5:1 v/v. APTES-GA probes were measured at different time intervals starting from 3 hours to 7 days.

Incubation time	Replicates			Mean	SD	RSD
	1	2	3			
3 hour	168.800	165.733	168.811	167.781	1.774	1.057
6hour	140.122	141.927	142.364	141.471	1.189	0.840
12 hour	101.469	103.000	104.490	102.986	1.511	1.467
1 day	74.959	76.643	78.762	76.788	1.906	2.482
2 days	66.522	67.845	68.034	67.467	0.824	1.221
3 days	67.535	69.111	69.521	68.722	1.048	1.526
5 days	70.074	71.161	71.170	70.802	0.630	0.890
7 days	69.835	67.219	69.697	68.917	1.472	2.136

Table S8. The blue intensity of APTES-GA probes that were prepared using APTES : 25% GA volume ratio of 1.5:1 v/v. APTES-GA probes were measured at different intervals starting from 3 hours to 7 days.

Incubation time	Replicates			Mean	SD	%RSD
	1	2	3			
3 hour	135.759	133.446	138.223	135.809	2.389	1.759
6hour	106.576	109.995	111.825	109.465	2.664	2.434
12 hour	78.265	82.313	84.278	81.619	3.066	3.757
1 day	62.134	65.934	67.760	65.276	2.870	4.397
2 days	53.940	56.228	56.804	55.657	1.515	2.722
3 days	55.311	57.997	57.840	57.049	1.507	2.642
5 days	56.093	57.382	57.488	56.988	0.777	1.363
7 days	50.667	51.024	52.250	51.314	0.830	1.618

Table S9. The color intensity data of colorimetric product of APTES-GA probes that were treated with hydrogen peroxide solutions for 7 minutes. The volume ratio of APTES-GA : analyte solution was 1:1 v/v.

$[H_2O_2]$ (%w/v)	Replicates (Red intensity)			Mean	SD	%RSD
	1	2	3			
0	190.359	187.729	187.382	188.490	1.628	0.864
0.85	194.712	192.737	191.836	193.095	1.471	0.762
2.55	186.543	183.106	182.932	184.194	2.036	1.106
4.25	184.945	182.325	180.985	182.752	2.014	1.102
$[H_2O_2]$ (%w/v)	Replicates (Green intensity)			Mean	SD	%RSD
	1	2	3			
0	128.782	125.761	125.651	126.731	1.777	1.402
0.85	146.059	144.279	143.871	144.736	1.163	0.804
2.55	158.717	156.096	156.294	157.036	1.459	0.929
4.25	166.154	163.698	163.537	164.463	1.467	0.892
$[H_2O_2]$ (%w/v)	Replicates (Blue intensity)			Mean	SD	%RSD
	1	2	3			
0	120.845	118.764	118.918	119.509	1.160	0.970
0.85	135.430	134.518	134.814	134.921	0.465	0.345
2.55	146.402	139.137	139.988	141.842	3.972	2.800
4.25	146.099	144.920	145.549	145.523	0.590	0.405

Table S10. The green intensity data of colorimetric product of APTES-GA probes that were treated with 2.55 %w/v hydrogen peroxide solution for 7 minutes. The volume ratio of APTES-GA : analyte solution was investigated from 3:7 to 7:3 v/v.

Volume ratio (v/v)	Replicates (Blank)			Mean	SD	%RSD
	1	2	3			
3:7	147.174	143.539	143.217	144.643	2.198	1.519
4:6	133.874	131.030	131.339	132.081	1.560	1.181
5:5	128.208	124.387	124.405	125.667	2.201	1.751
6:4	119.681	116.253	116.439	117.458	1.928	1.641
7:3	111.208	107.761	108.547	109.172	1.806	1.655
Volume ratio (v/v)	Replicates (Resulting probe)			Mean	SD	%RSD
	1	2	3			
3:7	161.782	158.815	159.345	159.981	1.582	0.989
4:6	153.378	151.509	151.286	152.058	1.149	0.756
5:5	157.684	153.708	153.480	154.957	2.364	1.526
6:4	141.194	140.082	139.871	140.382	0.711	0.506
7:3	128.638	126.702	126.226	127.189	1.278	1.004
Volume ratio (v/v)	Replicates (Difference of green intensity)			Mean	SD	%RSD
	1	2	3			
3:7	14.608	15.276	16.128	15.337	0.762	4.967
4:6	19.504	20.479	19.947	19.977	0.488	2.444
5:5	29.476	29.321	29.075	29.291	0.202	0.690
6:4	21.513	23.829	23.432	22.925	1.239	5.403
7:3	17.430	18.941	17.679	18.017	0.810	4.497

Table S11. The green intensity data of colorimetric product of APTES-GA probes that were treated with 2.55 %w/v hydrogen peroxide solution for 7 minutes. The volume ratio of APTES-GA : analyte solution was 1:1 v/v. The acetonitrile (MeCN) : DI water (H_2O) volume ratio in analyte solution was studied from 5:5 to 10:0 v/v.

MeCN : H_2O (v/v)	Replicates			Mean	SD	%RSD
	1	2	3			
5:5	160.753	158.639	159.625	159.672	1.058	0.662
6:4	161.567	158.960	158.463	159.663	1.667	1.044
7:3	165.370	161.859	160.702	162.644	2.431	1.495
8:2	163.232	161.704	161.235	162.057	1.044	0.644
9:1	164.745	161.452	160.706	162.301	2.149	1.324
10:0	159.324	156.765	156.124	157.404	1.693	1.076

Table S12. The green intensity data of colorimetric product of APTES-GA probes that were treated with 2.55 %w/v hydrogen peroxide solution. The volume ratio of APTES-GA : analyte solution was 1:1 v/v. The measurement was carried on for over 25 minutes.

Reaction time (min)	Replicates			Mean	SD	%RSD
	1	2	3			
1	135.561	134.350	131.168	131.578	2.829	2.150
3	144.612	146.259	141.576	141.928	2.889	2.036
5	159.108	158.003	155.260	154.894	3.139	2.027
7	171.034	170.318	166.748	167.014	3.054	1.829
10	177.893	176.869	174.836	174.307	2.718	1.559
15	178.477	180.837	178.477	178.095	3.006	1.688
20	178.799	177.931	175.888	175.012	3.216	1.838
25	179.004	178.212	175.493	174.962	3.289	1.880

Table S13. The independent t-test data at 90% confidential level (α = 0.10) for the comparison of two datasets obtained from the colorimetric product at the reaction time of 10 and 15 minutes. The analysis was carried on by Microsoft Excel.

Independent T-test for unequal variances at 90% confidential level		
Parameters	10 min	15 min
Mean	176.533	178.871
Variance	2.421	3.245
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
T value	-1.701	
P value (two-tail)	0.164	
T critical (two-tail)	2.132	

Table S14. The green intensity data of colorimetric product of APTES-GA probes that were treated with hydrogen peroxide working solution at 1 - 1500 mmol L^{-1} or 0.003 %w/v to 5.103 %w/v. The volume ratio of APTES-GA : working solution was 1:1 v/v and the reaction time of measurement was 10 minutes.

$[H_2O_2]$ (%w/v)	Red intensity		Green intensity		Blue intensity	
	Mean	SD	Mean	SD	Mean	SD
0.003	190.275	0.699	133.153	0.221	128.696	0.478
0.017	190.559	1.904	133.547	1.039	129.498	1.171
0.034	190.434	1.254	133.975	1.705	129.954	1.431
0.085	192.029	0.885	136.513	0.266	133.396	0.306
0.170	196.852	1.507	144.875	0.745	140.140	1.229
0.255	191.545	0.951	147.127	0.882	140.119	0.745
0.340	197.076	0.794	149.096	0.719	142.814	0.781
0.510	188.659	0.241	155.206	0.412	144.540	0.788
0.680	188.329	0.959	160.339	1.337	146.831	1.374
1.021	187.688	0.907	168.443	0.954	151.102	1.162
1.361	185.784	1.050	175.117	0.471	156.476	0.655
1.701	183.862	0.939	177.922	1.135	158.393	1.164
2.552	184.594	0.832	180.095	0.853	159.855	1.017
3.402	184.159	1.738	181.756	1.409	158.518	1.406
4.253	182.002	1.521	181.355	0.828	157.625	0.991
5.103	182.980	1.285	181.999	1.377	159.242	1.436

Table S15. The green intensity data of resulting product obtained from APTES-GA probes that stored at three conditions. The stored APTES-GA were treated with 1.02% w/v hydrogen peroxide solution (1:1 v/v). The reaction time of measurement was 10 minutes.

Storage duration	Room temperature		4 ºC		-18 ºC	
	Mean G	SD	Mean G	SD	Mean G	SD
1 day	167.160	0.535	167.733	0.753	167.469	0.753
3 day	165.142	1.015	168.463	0.501	169.388	1.094
1 week	160.055	0.797	167.530	0.886	167.672	0.711
2 week	158.610	2.283	166.374	0.465	168.739	0.527
4 week	155.095	0.719	160.381	1.205	166.633	0.853
6 week	158.677	0.917	162.437	0.319	169.752	1.340

Table S16. The relative error of analytical signal of resulting product obtained from APTES-GA probes that stored at three conditions. The stored APTES-GA were treated with 1.02% w/v hydrogen peroxide solution (1:1 v/v). The reaction time of measurement was 10 minutes.

Storage duration	Room temperature		4 ºC		-18 ºC	
	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)
3 days	-1.207	0.607	0.435	0.299	1.146	0.653
1 week	-4.250	0.477	-0.121	0.528	0.121	0.425
2 weeks	-5.115	1.366	-0.810	0.277	0.758	0.315
4 weeks	-7.217	0.430	-4.383	0.719	-0.499	0.509
6 weeks	-5.075	0.549	-3.158	0.190	1.363	0.800


KU KASETSART UNIVERSITY
KAMPHAENG SAEN CAMPUS
The 19th KU KPS National Conference ครั้งที่ 19
การประชุมวิชาการระดับชาติ
มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน
ระหว่างวันที่ 8-9 ธันวาคม 2565
เกษตรศาสตร์อัจฉริยะ สุขภาวะคนไทย สู้ภัยเศรษฐกิจ
Proceedings
ผลงานทางวิชาการ 8 สาขา
1.สาขาพืชและเทคโนโลยีชีวภาพ
2.สาขาสัตว์และสัตวแพทย์
3.สาขาวิศวกรรมศาสตร์
4.สาขาศึกษาศาสตร์และพัฒนศาสตร์
5.สาขามนุษยศาสตร์ สังคมศาสตร์
และอุตสาหกรรมบริการ
6.สาขาวิทยาศาสตร์สุขภาพและการกีฬา
7.สาขาวิทยาศาสตร์ เทคโนโลยี สิ่งแวดล้อม
และความหลากหลายทางชีวภาพ
8.สาขาส่งเสริมการเกษตร

การประชุมวิชาการระดับชาติ ครั้งที่ 19 มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน วันที่ 8-9 ธันวาคม 2565

อุปกรณ์ตรวจวัดไฮโดรเจนเปอร์ออกไซด์แบบตรวจวัดสี จากสารเชิงซ้อนไซลอกเซน-กลูตารัลดีไฮด์

A colorimetric probe for hydrogen peroxide detection by siloxane-glutaraldehyde complex

เพชราวุธ รามศิริ[1] อภิชัย พลชัย[1*] และ อารีย์ ชูดำ[2]
Petcharawut Ramsiri[1], Apichai Phonchai[1*] and Aree Choodum[2]

บทคัดย่อ

ไฮโดรเจนเปอร์ออกไซด์เป็นสารตั้งต้นสำคัญสำหรับเตรียมสารระเบิดเปอร์ออกไซด์ ซึ่งถูกนำมาใช้ก่อเหตุระเบิดในช่วงหลายปีที่ผ่านมา การตรวจวัดไฮโดรเจนเปอร์ออกไซด์ในของเหลวสังเคราะห์สารระเบิดเปอร์ออกไซด์เป็นวิธีทางเลือกที่สามารถตรวจพิสูจน์การสังเคราะห์สารระเบิดเปอร์ออกไซด์เบื้องต้นได้ งานวิจัยนี้จึงได้พัฒนาอุปกรณ์ตรวจวัดจากวัสดุโครมาโทกราฟีแบบแผ่นบางที่ดูดซับสารเชิงซ้อนของ 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซนและกลูตารัลดีไฮด์ (APTES-GA) อุปกรณ์ตรวจวัดจะถูกฟอกจางสีจากสีแดงเป็นสีเหลืองอ่อน เมื่อทำปฏิกิริยากับไฮโดรเจนเปอร์ออกไซด์ ซึ่งสังเกตได้ด้วยตาเปล่า ภาพของอุปกรณ์ตรวจวัดถูกบันทึกภาพด้วยโทรศัพท์มือถือและวิเคราะห์ค่าสัญญาณความเข้มสีด้วยซอฟต์แวร์ ภายใต้สภาวะที่เหมาะสมของวิธีที่พัฒนาขึ้น อุปกรณ์ตรวจวัดสามารถวิเคราะห์ไฮโดรเจนเปอร์ออกไซด์ในตัวอย่างของเหลวสังเคราะห์สารระเบิดเปอร์ออกไซด์จำลองได้จริง อุปกรณ์ตรวจวัดมีความแม่นยำที่ดี และขีดจำกัดการตรวจวัดและขีดจำกัดการตรวดวัดเชิงปริมาณเท่ากับ 12.8 mM และ 42.8 mM ตามลำดับ

คำสำคัญ: อุปกรณ์ตรวจวัดสี ไฮโดรเจนเปอร์ออกไซด์ สารระเบิด ไตรอะซิโตนไตรเปอร์ออกไซด์

ABSTRACT

Hydrogen peroxide is an essential precursor for peroxide explosives which have been responsible for a number of terrorist attacks. Hydrogen peroxide detection in the synthetic mixture of peroxide explosives is an alternative method to screen peroxide explosive mixtures. In this work, the proposed probe was developed using the complex of (3-aminopropyl)triethoxysiloxane-glutaraldehyde (APTES-GA) entrapped on a thin layer chromatography sheet. The brick-red color of the probe was bleached to pale yellow by hydrogen peroxide, which was qualitatively detectable by the naked eye. A smartphone with available software was utilized to photograph the probe in order to obtain RGB values simultaneously. Under the optimized condition, the developed method can be used as an alternative approach to detect hydrogen peroxide in simulated explosive mixture samples. It provided good precision with a limit of detection and a limit of quantification of 12.8 mM and 42.8 mM, respectively.

Keyword: colorimetric probe, hydrogen peroxide, explosives, triacetone triperoxide
*Corresponding author; email address: apichai.ph@psu.ac.th

[1]สาขาวิทยาศาสตร์สุขภาพและวิทยาศาสตร์ประยุกต์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่ จ. สงขลา 90112
Department of Health and Applied Sciences, Faculty of Science, Prince of Songkhla University, Hat Yai campus, Songkhla 90112 Thailand
[2]คณะเทคโนโลยีและสิ่งแวดล้อม มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตภูเก็ต จ. ภูเก็ต 83102
Faculty of Technology and Environment, Prince of Songkhla University, Phuket campus, Phuket 83102 Thailand

คำนำ

สารระเบิดเปอร์ออกไซด์ เช่น ไตรอะซิโตนไตรเปอร์ออกไซด์ (triacetone triperoxide; TATP) และ เฮกซะเมทิลลีนไตรเปอร์ออกไซด์ไดเอมีน (hexamethylene triperoxide diamine; HMTD) เป็นสารประกอบเปอร์ออกไซด์อินทรีย์ที่มีพันธะเดี่ยวระหว่างออกซิเจนกับออกซิเจน (O-O) ภายในโมเลกุลจำนวนมาก มีความเสถียรต่ำ ว่องไวต่อแรงกระทำภายนอก จึงเป็นสารระเบิดง่ายและมีพลังระเบิดสูง (Matyáš and Pachman, 2013) หลายปีที่ผ่านมา สารระเบิดไตรอะซิโตนไตรเปอร์ออกไซด์ถูกใช้ก่อเหตุระเบิดในหลายประเทศ สร้างความเสียหายแก่ทรัพย์สินและมีผู้บาดเจ็บตลอดจนผู้เสียชีวิตจำนวนมาก (Bergen and Sterman, 2018) เหตุผลส่วนหนึ่งที่ไตรอะซิโตนไตรเปอร์ออกไซด์ ถูกตรวจพบเป็นวัตถุระเบิดบ่อยครั้ง เป็นเพราะสารตั้งต้นหลักในการผลิตสารระเบิดไตรอะซิโตนไตรเปอร์ออกไซด์ ได้แก่ ไฮโดรเจนเปอร์ออกไซด์และอะซิโตน (Matyáš and Pachman, 2013) ต่างเป็นสารเคมีที่สามารถเข้าถึงได้ง่ายและจัดหาได้สะดวก จึงมีความเสี่ยงที่ผู้ก่อการร้ายจะเข้าถึงสารเคมีเหล่านี้และลักลอบเตรียมสารระเบิดเปอร์ออกไซด์ภายในเคหะสถานหรือบ้านพักได้ ดังนั้น การตรวจพิสูจน์สารเคมีในการผลิตสารระเบิดเปอร์ออกไซด์จึงเป็นทางเลือกสำคัญที่สามารถช่วยตรวจสอบคัดกรองกระบวนการผลิตสารระเบิดเปอร์ออกไซด์เพื่อใช้เป็นหลักฐานทางด้านนิติวิทยาศาสตร์ ตลอดจนป้องกันและป้องปรามการนำสารระเบิดเปอร์ออกไซด์ไปก่อเหตุการณ์ความไม่สงบได้

การตรวจวิเคราะห์สารระเบิดเปอร์ออกไซด์ทางตรงด้วยเครื่องมือมาตรฐานทำได้หลายวิธี เช่น โครมาโทกราฟีของเหลว (liquid chromatography) (Widmer *et al*., 2002) แก๊สโครมาโทกราฟี (gas chromatography) (Kende *et al*., 2008) และ แมสสเปกโตรสโกปี (mass spectroscopy) (Almeida Assis *et al*., 2019) แต่ด้วยข้อจำกัดของเครื่องมือ การตรวจพิสูจน์ด้วยวิธีมาตรฐานจึงไม่เหมาะสมกับการตรวจวิเคราะห์ในสถานที่เกิดเหตุที่ต้องการความสะดวกและเวลาตรวจพิสูจน์ที่รวดเร็ว ในปัจจุบัน การตรวจวิเคราะห์สารระเบิดเปอร์ออกไซด์โดยอ้อมได้รับความนิยมเป็นอย่างมาก โดยวิเคราะห์สารไฮโดรเจนเปอร์ออกไซด์ที่เป็นผลิตภัณฑ์จากการสลายตัวของสารระเบิดเปอร์ออกไซด์ในสภาวะกรด (Matyáš and Pachman, 2013) หรือภายใต้การฉายแสงอัลตร้าไวโอเลต (Stambouli *et al*., 2004) ซึ่งทำได้ง่ายกว่าการตรวจวัดสารระเบิดเปอร์ออกไซด์โดยตรง พร้อมทั้งในของเหลวผสมที่สังเคราะห์สารระเบิดเปอร์ออกไซด์มีความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์ในปริมาณที่สูง ดังนั้น สารไฮโดรเจนเปอร์ออกไซด์จึงเป็นสารเคมีสำคัญที่ดีสำหรับใช้เป็นตัวชี้วัดในการวิเคราะห์เบื้องต้นของสารระเบิดเปอร์ออกไซด์ได้

ปัจจุบันมีการศึกษาและสังเคราะห์สารเชิงซ้อนของไซลอกเซน-กลูตารัลดีไฮด์ มีสีแดงเข้มเคลือบบนวัสดุต่างๆ เช่น กระดาษ (Zhou *et al*., 2014) และ อินเดียมดีบุกเคลือบออกไซด์ (ITO) (Ren *et al*., 2018) เป็นต้น เพื่อใช้เป็นโพรบสำหรับตรวจวัดสารไฮโดรเจนเปอร์ออกไซด์ เนื่องจากสารเชิงซ้อนของไซลอกเซน-กลูตารัลดีไฮด์ สามารถถูกออกซิไดซ์ด้วยโมเลกุลไฮโดรเจนเปอร์ออกไซด์ โดยโครงสร้างพันธะคู่ของสารเชิงซ้อนของไซลอกเซน-กลูตารัลดีไฮด์ถูกทำลายด้วยปฏิกิริยาออกซิเดชัน (Rani *et al*., 2021) และเกิดการเปลี่ยนแปลงสีจากสีแดงเปลี่ยนเป็นไม่มีสี ซึ่งสังเกตเห็นได้ด้วยตาเปล่า และสามารถวิเคราะห์ความสัมพันธ์ของความเข้มสีที่จางลงกับความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์ ด้วยแอพพลิเคชันหรือซอฟต์แวร์ที่สามารถอ่านค่าสัญญาณสีในโหมดสีแดง เขียวและน้ำเงิน หรือโหมดสีเทาของภาพถ่ายดิจิทัล

งานวิจัยนี้ได้พัฒนาอุปกรณ์ตรวจวัดไฮโดรเจนเปอร์ออกไซด์ในของเหลวสังเคราะห์สารระเบิดไฮโดรเจนเปอร์ออกไซด์ ด้วยสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ที่เคลือบลงบนแผ่นโครมาโทกราฟีแผ่นบางหรือแผ่นทีแอลซี (thin layer chromatography strip, TLC strip) และตรวจวัดการเปลี่ยนแปลงสีของอุปกรณ์ตรวจวัดด้วยการวิเคราะห์ภาพถ่ายดิจิทัลจากโทรศัพท์มือถือด้วยซอฟต์แวร์ Image J โดยทำการศึกษาสภาวะที่เหมาะสมของการตรวจวิเคราะห์และพิสูจน์การใช้ได้ของวิธี เพื่อให้ได้อุปกรณ์ตรวจวัดไฮโดรเจนเปอร์ออกไซด์ใน

ของเหลวสังเคราะห์สารระเบิดเปอร์ออกไซด์ ที่พกพาง่าย ราคาถูก มีความแม่นยำ และตรวจวิเคราะห์เพื่อคัดกรองของเหลวสังเคราะห์สารระเบิดเปอร์ออกไซด์ได้จริง

อุปกรณ์และวิธีการ

การเตรียมสารละลายสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์

ปิเปตเอทานอล (บริษัท Supelco.) ปริมาตร 9.50 mL ลงในขวด vial ที่มีขนาด 20 mL จากนั้นเติม 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน (บริษัท Tokyo Chemical Industry) ปริมาตร 300 µL แล้วคนด้วยแท่งแม่เหล็กที่ความเร็ว 500 รอบต่อนาที เป็นเวลา 1 นาที จึงเติมสารละลายกลูตารัลดีไฮด์เข้มข้น 25% ในน้ำ (บริษัท Sigma-Aldrich เกรด II) ปริมาตร 200 µL คนสารละลายผสมด้วยแท่งแม่เหล็กที่ความเร็ว 500 รอบต่อนาที ต่ออีก 1 นาที ปิดฝาขวด vial และพันด้วยพาราฟิน ตั้งสารละลายผสมที่อุณหภูมิห้องเป็นเวลา 1 วัน ก่อนจะถูกนำไปศึกษาขั้นต่อไป

การเตรียมสารละลายมาตรฐานไฮโดรเจนเปอร์ออกไซด์

ปิเปตสารละลายไฮโดรเจนเปอร์ออกไซด์ เข้มข้น 30% (บริษัท Sigma-Aldrich) ปริมาตร 1.00 mL ถ่ายลงในขวดวัดปริมาตรขนาด 10 mL แล้วปรับปริมาตรด้วยอะซิโตไนไตรล์ (บริษัท Macron Fine Chemicals™) จนได้สารละลายไฮโดรเจนเปอร์ออกไซด์มาตรฐาน 10.00 mL จากนั้นนำไปเจือจางด้วยตัวทำละลายอะซิโตไนไตรล์ ให้มีความเข้มข้นของโดรเจนเปอร์ออกไซด์ ตั้งแต่ 0 mM ถึง 500 mM (หรือ mmol/L)

การศึกษาการเกิดปฏิกิริยาของสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์และไฮโดรเจนเปอร์ออกไซด์

ปิเปตสารละลายสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ ปริมาตร 300 µL ลงในหลอดทดลอง จากนั้นเติมสารละลายมาตรฐานไฮโดรเจนเปอร์ออกไซด์ที่มีความเข้มข้น 300 mM ปริมาตร 700 µL เขย่าหลอดทดลองให้สารละลายผสมเข้ากัน แล้วถ่ายของผสมลงในคิวเวทท์ (cuvette) ชนิดพอลีสไตรีน (บริษัท VWR European) และวัดค่าการดูดกลืนคลื่นแสงในช่วงความยาวคลื่น 380 nm ถึง 780 nm ด้วยเครื่องสเปกโตรโฟโตมิเตอร์ (บริษัท Thermo Scientific, รุ่น GENESYS™ 10S UV-Vis) ที่เวลาเกิดปฏิกิริยา 5 นาที และทำการทดลองชุดควบคุมผลทางลบ (แบลงค์) ด้วยวิธีเดียวกันโดยใช้ตัวทำละลายอะซิโตไนไตรล์ปริมาตร 700 µL

การศึกษาการเตรียมอุปกรณ์ตรวจวัดไฮโดรเจนเปอร์ออกไซด์จากสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ บนแผ่น TLC

ตัดแผ่น TLC (ซิลิกาเจล 60 F_{254} บนแผ่นอะลูมิเนียม บริษัท Merck) ให้มีขนาด 10 × 10 mm นำแผ่นตัด TLC จุ่มลงในสารละลายเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์เป็นเวลา 5 วินาทีเพื่อให้สารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ถูกดูดซับบนแผ่นตัด TLC ใช้กระดาษทิชชูซับสารละลายส่วนเกินออกจากแผ่นตัด TLC แล้วผึ่งแห้งที่อุณหภูมิห้อง เป็นเวลา 10 นาที เพื่อให้ตัวทำละลายระเหยจนหมด จะได้อุปกรณ์ตรวจวัด TLC สำหรับศึกษาการดูดซับสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ 1 ครั้ง ทำซ้ำจนได้อุปกรณ์ตรวจวัด TLC ที่ดูดซับสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ตั้งแต่ 2 ครั้ง จนถึง 5 ครั้ง ถ่ายรูปอุปกรณ์ตรวจวัดแผ่น TLC ด้วยโทรศัพท์มือถือ (Android, Vivo Y50) และวิเคราะห์ค่าความเข้มสัญญาณสีเขียวด้วยซอฟต์แวร์ Image J (ทำการทดลอง 5 ซ้ำ) ทดสอบการเปลี่ยนแปลงสัญญาณความเข้มสีเมื่อทำปฏิกิริยากับไฮโดรเจนเปอร์ออกไซด์โดยนำอุปกรณ์ตรวจวัดแผ่น TLC แช่ลงในสารละลาย

มาตรฐานไฮโดรเจนเปอร์ออกไซด์ที่มีความเข้มข้น 200 mM ปริมาตร 1 mL ที่บรรจุในบีกเกอร์ขนาด 5 mL ปิดคลุมปากบีกเกอร์ด้วยเทปอะลูมิเนียม จับเวลา 20 นาที จากนั้นผึ่งอุปกรณ์ตรวจวัดให้แห้งสนิทที่อุณหภูมิห้อง แล้วถ่ายรูปด้วยโทรศัพท์มือถือเพื่อวิเคราะห์ค่าความเข้มสัญญาณสีเขียวด้วยซอฟต์แวร์ Image J (ทำการทดลอง 5 ซ้ำ)

การศึกษาเวลาที่เหมาะสมของการตรวจวัดไฮโดรเจนเปอร์ออกไซด์ด้วยอุปกรณ์ตรวจวัดไฮโดรเจนเปอร์ออกไซด์จากสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ บนแผ่น TLC

จุ่มอุปกรณ์ตรวจวัดแผ่น TLC ที่เตรียมได้ลงในสารละลายมาตรฐานไฮโดรเจนเปอร์ออกไซด์ที่มีความเข้มข้น 200 mM ปริมาตร 1 mL ที่บรรจุในบีกเกอร์ขนาด 5 mL ปิดคลุมปากบีกเกอร์ด้วยเทปอะลูมิเนียม ศึกษาระยะเวลาที่ใช้ทดสอบ (exposure time) ที่เวลา 5 10 20 30 40 50 และ 60 นาที จากนั้นผึ่งอุปกรณ์ตรวจวัดให้แห้งที่อุณหภูมิห้อง ถ่ายรูปด้วยโทรศัพท์มือถือและวิเคราะห์ค่าความเข้มสัญญาณสีเขียวด้วยซอฟต์แวร์ Image J (ทำการทดลอง 5 ซ้ำ)

การศึกษาช่วงความเป็นเส้นตรงของวิธีวิเคราะห์ ขีดจำกัดการตรวจวัด และขีดจำกัดการตรวจวัดเชิงปริมาณ

แช่อุปกรณ์ตรวจวัดแผ่น TLC ที่เตรียมได้ในสารละลายมาตรฐานไฮโดรเจนเปอร์ออกไซด์ปริมาตร 1 mL ที่มีความเข้มข้น ตั้งแต่ 1 mM จนถึง 500 mM ที่บรรจุในบีกเกอร์ขนาด 5 mL ปิดคลุมปากบีกเกอร์ด้วยเทปอะลูมิเนียม หลังจากระยะเวลาทดสอบที่เหมาะสม ผึ่งอุปกรณ์ตรวจวัดให้แห้งที่อุณหภูมิห้อง ถ่ายรูปด้วยโทรศัพท์มือถือและวิเคราะห์ค่าความเข้มสัญญาณสีเขียวด้วยซอฟต์แวร์ Image J (ทำการทดลอง 5 ซ้ำ) (NATA., 2012)

การศึกษาการตรวจวิเคราะห์ตัวอย่างจำลองของเหลวสังเคราะห์สารระเบิดเปอร์รอกไซด์

การจำลองการสังเคราะห์สารระเบิดเปอร์ออกไซด์ไตรอะซิโตนไตรเปอร์ออกไซด์อ้างอิงจาก Dubnikova *et al*. (2005) ทำได้โดยผสมอะซิโตน (บริษัท RCI Labscan) ปริมาตร 5.6 g (0.1 mol, 7 mL) กับสารละลายไฮโดรเจนเปอร์ออกไซด์เข้มข้น 30% ปริมาตร 10.2 mL (0.1 mol) แล้วค่อย ๆ เติมกรดซัลฟิวริกเข้มข้น 98% (บริษัท RCI Labscan) จำนวน 5 หยด ที่อุณหภูมิ 0 - 5 ºC

งานวิจัยนี้ศึกษาตัวอย่างจำลองในสถานการณ์ต่าง ๆ ที่อาจตรวจพบ ณ ที่เกิดเหตุ ดังนี้

1. ของเหลวผสมอะซิโตนและไฮโดรเจนเปอร์ออกไซด์ที่ยังไม่เติมกรดแก่
2. ของเหลวผสมอะซิโตนและไฮโดรเจนเปอร์ออกไซด์ที่เติมกรดแก่แล้วแต่ปฏิกิริยายังไม่สมบูรณ์
3. ของเหลวผสมอะซิโตนและไฮโดรเจนเปอร์ออกไซด์ที่เติมกรดแก่แล้ว และปฏิกิริยาเกิดได้สมบูรณ์
4. สารละลายไฮโดรเจนเปอร์ออกไซด์เข้มข้น 30% ที่จะถูกใช้สังเคราะห์สารระเบิดเปอร์ออกไซด์

ทำการทดสอบตัวอย่างจำลองโดยปิเปตของเหลวปริมาตร 1 mL ตามสถานการณ์จำลองที่ 1 ถึง สถานการณ์จำลองที่ 4 มาทดสอบกับอุปกรณ์ที่พัฒนาขึ้น โดยใช้สภาวะที่เหมาะสมของวิธีทดสอบได้ศึกษามาก่อนหน้านี้ ผึ่งอุปกรณ์ตรวจวัดให้แห้งสนิทที่อุณหภูมิห้อง ถ่ายรูปด้วยโทรศัพท์มือถือและวิเคราะห์ค่าความเข้มสัญญาณสีเขียวด้วยซอฟต์แวร์ Image J (ทำการทดลอง 3 ซ้ำ) เปรียบเทียบผลการทดลองกับการวิเคราะห์ผลทางบวกและผลทางลบ

ผลการทดลองและวิจารณ์

ผลการศึกษาการเกิดปฏิกิริยาของสารเชิงซ้อน APTES-GA และไฮโดรเจนเปอร์ออกไซด์

รูปที่ 1. แสดงสเปกตรัมการดูดกลืนคลื่นแสงในช่วงความยาวคลื่น 380 nm ถึง 780 nm ของสารละลายผสมสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์กับสารละลายแบลงค์ และสารละลายผสมสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์กับสารมาตรฐานไฮโดรเจนเปอร์ออกไซด์ที่ความเข้มข้น

300 mM ที่เวลา 5 นาที ผลการทดลองพบว่า สเปกตรัมการดูดกลืนคลื่นแสงของสารละลายผสมสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์กับสารละลายแบลงค์ (เส้นสีแดง) มีค่าการดูดกลืนสูงสุดในช่วงแสงขาวที่ความยาวคลื่น 540 nm (λ_{max}) จึงสังเกตเห็นสารละลายเป็นสีแดง และสเปกตรัมการดูดกลืนคลื่นแสงของสารละลายผสมสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์กับสารมาตรฐานไฮโดรเจนเปอร์ออกไซด์ที่ความเข้มข้น 300 mM (เส้นสีเหลือง) ค่าการดูดกลืนที่ความยาวคลื่น 540 nm ลดลง ซึ่งน้อยกว่าค่าการดูดกลืนในช่วงความยาวคลื่น 430 nm ทำให้ผลิตภัณฑ์ที่เกิดขึ้นมีสีเหลือง สอดคล้องกับทฤษฎีวงล้อสี (Algar *et al.*, 2015) จากผลการทดลอง แสดงให้เห็นว่าสารละลายผสมสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์เกิดการฟองจางสีเมื่อมีสารไฮโดรเจนเปอร์ออกไซด์อยู่ในตัวอย่าง และสนับสนุนให้การวิเคราะห์ค่าสัญญาณความเข้มสีเขียวของอุปกรณ์ตรวจวัด มีความสำคัญมากกว่าการวิเคราะห์สัญญาณความเข้มสีแดงและสัญญาณความเข้มสีน้ำเงิน

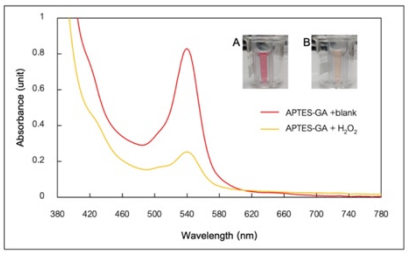


Figure 1 UV-Visible absorption spectra of reagent blank (700 µL) with APTES-GA complex solution (300 µL) (red line) and the oxidation reaction of 300 mM hydrogen peroxide solution (700 µL) with APTES-GA complex solution (300 µL) (yellow line). The spectra were measured at 5 min after mixing.

ผลการศึกษาการเตรียมอุปกรณ์ตรวจวัดไฮโดรเจนเปอร์ออกไซด์จากสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ด้วยแผ่น TLC

จากผลการศึกษาพบว่าเมื่อเพิ่มจำนวนครั้งของการดูดซับสารละลายเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์บนแผ่นตัด TLC (1 ถึง 5 ครั้ง) จะได้อุปกรณ์ตรวจวัด TLC ที่มีสีแดงเข้มขึ้น เนื่องจากสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ถูกดูดซับบนแผ่น TLC มากขึ้น เมื่อวิเคราะห์ภาพถ่ายดิจิทัลโดยใช้สัญญาณความเข้มสีเขียวด้วยซอฟต์แวร์ Image J พบว่ามีค่าความเข้มสีเขียวมีค่าลดลงตามจำนวนครั้งการดูดซับสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ (รูปที่ 2a) และเมื่อนำแผ่นตัด TLC ที่ดูดซับสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์มาทดสอบกับสารละลายมาตรฐานไฮโดรเจนเปอร์ออกไซด์ที่ความเข้มข้น 200 mM พบว่าแผ่นตัด TLC ที่ถูกเตรียมโดยการดูดซับสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์จำนวน 4 ครั้ง ให้ค่าความต่างสัญญาณความเข้มสีเขียวสูงที่สุด (รูปที่ 2b) และเมื่อเปรียบเทียบสัญญาณค่าความเข้มสีเขียวระหว่างการใช้แผ่นตัด TLC ที่ถูกเตรียมโดยการดูดซับสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์จำนวน 3 และ 4 ครั้ง ไม่มีค่าแตกต่างกันอย่างมีนัยสำคัญทางสถิติที่ความเชื่อมั่น 95% ดังนั้น จึงเลือกใช้แผ่นตัด TLC ที่ถูกเตรียมโดยการดูดซับสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์จำนวน 3 ครั้ง ในการเตรียมอุปกรณ์สำหรับตรวจวัดไฮโดรเจนเปอร์ออกไซด์

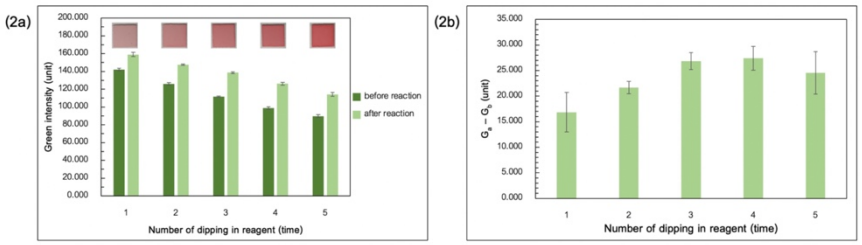


Figure 2 (2a) Green intensity values of APTES-GA probes which adsorbed APTES-GA complex at different amounts (dark green column) and green intensity values of APTES-GA probes after immersion in 1 mL of 200 mM hydrogen peroxide solution (pale green column). (2b) The differences of green intensity values of APTES-GA probes before (G_b) and after reaction (G_a). Note: The difference between the green values of the APTES-GA probes before and after reaction was G_a - G_b.

ผลการศึกษาเวลาที่เหมาะสมของการตรวจวัดไฮโดรเจนเปอร์ออกไซด์ด้วยอุปกรณ์ตรวจวัดไฮโดรเจนเปอร์ออกไซด์จากสารเชิงซ้อน 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซน-กลูตารัลดีไฮด์ บนแผ่น TLC

ผลการศึกษาระยะเวลาการทดสอบที่เหมาะสมแสดงดังรูปที่ 3 อุปกรณ์ตรวจวัดที่ใช้เวลาทดสอบ 5 นาทีให้ค่าสัญญาณความเข้มสีเขียวน้อยที่สุด แต่ยังคงแตกต่างจากชุดควบคุมผลลบ เมื่อเวลาทดสอบมากขึ้น สีของอุปกรณ์ตรวจวัดจะถูกฟอกจางลงได้มาก ทำให้ค่าสัญญาณความเข้มสีเขียวเพิ่มขึ้นตามลำดับ เมื่อใช้เวลาทดสอบมากกว่า 10 นาที พบว่าค่าสัญญาณความเข้มสีเขียวของอุปกรณ์มีค่าไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ดังนั้น ระยะเวลาทดสอบที่เหมาะสมคือ 10 นาที

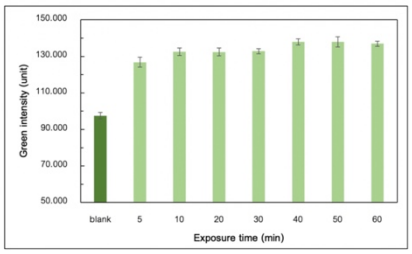


Figure 3 Green intensity values of APTES-GA probes after immersion in 1 mL of 200 mM hydrogen peroxide solution for 5, 10, 20, 30, 40, 50 and 60 minutes.

ผลการศึกษาช่วงความเป็นเส้นตรงของวิธีวิเคราะห์ ขีดจำกัดการตรวจวัด และขีดจำกัดการตรวจวัดเชิงปริมาณ

ผลการศึกษาความสัมพันธ์ของค่าสัญญาณความเข้มสีเขียวกับความเข้มข้นของสารละลายมาตรฐานไฮโดรเจนเปอร์ออกไซด์ในช่วงความเข้มข้น 1 mM ถึง 500 mM พบว่า วิธีวิเคราะห์ให้ช่วงความเป็นเส้นตรงที่ความเข้มข้น 50 mM ถึง 500 mM โดยมีสมการเส้นตรงที่แสดงความสัมพันธ์ของค่าสัญญาณความเข้มสีเขียว (y) กับความ

เข้มข้นของสารละลายมาตรฐานไฮโดรเจนเปอร์ออกไซด์ (x) คือ y = (0.0597 ± 0.001)x + (117.631 ± 0.256) โดยที่มีค่า R^2 เท่ากับ 0.9956 มีค่าขีดจำกัดการตรวจวัดและขีดจำกัดการตรวจวัดเชิงปริมาณเท่ากับ 12.8 mM และ 42.8 mM ตามลำดับ อุปกรณ์ตรวจวัดให้ความแม่นยำที่ดี มีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ (relative standard deviation; RSD) ของการทดสอบที่ความเข้มข้น 100 mM 250 mM และ 500 mM ในช่วง 0.5% ถึง 1.9%

ผลการศึกษาการตรวจวิเคราะห์ตัวอย่างจำลองของเหลวสังเคราะห์สารระเบิดเปอร์รอกไซด์

ตัวอย่างของเหลวสังเคราะห์สารระเบิดเปอร์ออกไซด์ถูกจำลองขึ้นโดยการทำปฏิกิริยาของอะซิโตนและไฮโดรเจนเปอร์ออกไซด์ เข้มข้น 30% โดยมีกรดซัลฟิวริกเป็นตัวเร่งปฏิกิริยา จากการทดสอบตัวอย่างจำลอง ทั้ง 4 สถานการณ์ พบว่า อุปกรณ์ตรวจวัดสามารถตรวจวิเคราะห์ของเหลวสังเคราะห์สารระเบิดเปอร์ออกไซด์เชิงคุณภาพได้ และให้ผลการทดสอบที่ชัดเจน สามารถสังเกตได้ด้วยตาเปล่า (ตารางที่ 1)

Table 1. Results of hydrogen peroxide detection in simulated peroxide explosive mixture by APTES-GA probes.

	Experiments	Results (n=3)	Resulted probe
	Negative control (blank, Acetone:H_2O 1:1)	-	
1	Hydrogen peroxide solution (30%)	+	
2	Mixture of acetone and hydrogen peroxide solution (30%) without acid catalyst	+	
3	Mixture of acetone and hydrogen peroxide solution (30%) with acidic catalyst	+	
4	Mixture of acetone and hydrogen peroxide solution (30%) with acidic catalyst, after 1 day of reaction	+	

สรุปและเสนอแนะ

จากการศึกษาการพัฒนาอุปกรณ์ตรวจวัดสารไฮโดรเจนเปอร์ออกไซด์จากสารเชิงซ้อนของ 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซนกับกลูตารัลดีไฮด์ โดยใช้วัสดุแผ่นโครมาโทกราฟีแผ่นบางเป็นส่วนรองรับการตรวจวัด พบว่าสามารถตรวจวัดไฮโดรเจนเปอร์ออกไซด์ในของผสมสังเคราะห์สารระเบิดเปอร์ออกไซด์เชิงคุณภาพ โดยอาศัยการฟอกจางสีของสารเชิงซ้อนไซลอกเซน-กลูตารัลดีไฮด์ ซึ่งสามารถวิเคราะห์ผลการทดลองเบื้องต้นได้ด้วยตาเปล่า และวิเคราะห์ค่าสัญญาณความเข้มสีเขียวของภาพดิจิทัลโดยใช้ซอฟต์แวร์ Image J ร่วมกับการใช้สมาร์ทโฟนเป็นอุปกรณ์สำหรับถ่ายรูป อุปกรณ์ตรวจวัดที่พัฒนาขึ้น สามารถเตรียมได้ง่าย ราคาถูก พกพาสะดวก และมีความแม่นยำ โดยวิธีทดสอบมีค่าขีดจำกัดการตรวจวัดสารไฮโดรเจนเปอร์รอกไซด์เท่ากับ 12.8 mM และสามารถนำไปประยุกต์ใช้การตรวจวัดไฮโดรเจอนเปอร์ออกไซด์ในของผสมสังเคราะห์สารระเบิดเปอร์ออกไซด์นอกห้องปฏิบัติการหรือสถานที่เกิดเหตุ

กิตติกรรมประกาศ

ผู้วิจัยขอขอบคุณทุนสนับสนุนจากโครงการพัฒนาและส่งเสริมผู้มีศักยภาพทางวิทยาศาสตร์และเทคโนโลยี (พสวท.) และสาขาวิทยาศาสตร์สุขภาพและวิทยาศาสตร์ประยุกต์ หลักสูตรนิติวิทยาศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่ ตลอดจนผู้ช่วยวิจัยในกลุ่มวิจัยนิติเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่ ที่ให้การสนับสนุนจนผลงานวิจัยได้เสร็จสมบูรณ์

เอกสารอ้างอิง

Algar, W. R., De Jong, C. A. G., Maxwell, E. J., and Atkins, C. G. 2015. Demonstration of the Spectrophotometric Complementary Color Wheel Using LEDs and Indicator Dyes. Journal of Chemical Education 93(1): 162–165.

Almeida Assis, A. C., Caetano, J., Florêncio, M. H., and Cordeiro, C. 2019. Triacetone triperoxide characterization by FT-ICR mass spectrometry: Uncovering multiple forensic evidence. Forensic Science International 301: 37–45.

Bergen, P., and Sterman, D. 2018. Jihadist Terrorism 17 Years After 9/11. New America. Available source: https://www.newamerica.org/international-security/reports/jihadist-terrorism-17-years-after-911/key-trends-in-terrorism/, Retrieved by October 2, 2022,

Dubnikova, F., Kosloff, R., Almog, J., Zeiri, Y., Boese, R., Itzhaky, H., Alt, A., and Keinan, E. 2005. Decomposition of Triacetone Triperoxide Is an Entropic Explosion. Journal of the American Chemical Society 127(4): 1146–1159.

Kende, A., Lebics, F., Eke, Z., and Torkos, K. 2008. Trace level triacetone-triperoxide identification with SPME–GC-MS in model systems. Microchimica Acta 163(3–4): 335–338.

NATA. 2012. Technical Note 17 - Guidelines for the validation and verification of quantitative and qualitative test methods. http://www.demarcheiso17025.com/document/Guidelines%20for%20the%20validation%20and%20verification%20of%20quantitative%20and%20qualitative%20test%20methods.pdf.

Matyáš, R., and Pachman, J. 2013. Charpte10: Organic peroxide, 255-287, Primary Explosives (2012th ed.). Springer.

Rani, B., Agarwala, A., Behera, D., Verma, V. P., Singh, A. P., and Shrivastava, R. 2021. A highly sensitive coumarin-thiophene hybrid chemosensor for sensing of H_2O_2 and aluminium. Dyes and Pigments 194: 109596.

Ren, X., Lu, P., Feng, R., Zhang, T., Zhang, Y., Wu, D., & Wei, Q. 2018. An ITO-based point-of-care colorimetric immunosensor for ochratoxin A detection. Talanta 188: 593–599.

Stambouli, A., El Bouri, A., Bouayoun, T., and Bellimam, M. 2004. Headspace-GC/MS detection of TATP traces in post-explosion debris. Forensic Science International 146: S191–S194.

Widmer, L., Watson, S., Schlatter, K., and Crowson, A. 2002. Development of an LC/MS method for the trace analysis of triacetone triperoxide (TATP). The Analyst 127(12): 1627–1632.

Zhou, M., Yang, M., and Zhou, F. 2014. Paper based colorimetric biosensing platform utilizing cross-linked siloxane as probe. Biosensors and Bioelectronics 55: 39–43.

อุปกรณ์ตรวจวัดไฮโดรเจนเปอร์ออกไซด์แบบตรวจวัดสี จากสารเชิงซ้อนไซลอกเซน-กลูตารัลดีไฮด์

เพชราวุธ รามศิริ[1] อภิชัย พลชัย[1*] และ อารีย์ ชูดำ[2]

[1]หลักสูตรนิติวิทยาศาสตร์ สาขาวิทยาศาสตร์สุขภาพและวิทยาศาสตร์ประยุกต์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่ จ. สงขลา 90112

[2]คณะเทคโนโลยีและสิ่งแวดล้อม มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตภูเก็ต จ. ภูเก็ต 83102

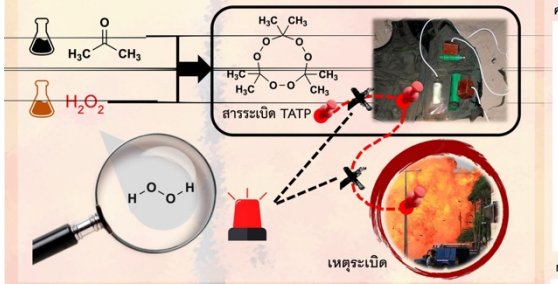
*อีเมลล์: apichai.ph@psu.ac.th

บทคัดย่อ

ไฮโดรเจนเปอร์ออกไซด์เป็นสารตั้งต้นสำคัญสำหรับเตรียมสารระเบิดเปอร์ออกไซด์ ซึ่งถูกนำมาใช้ก่อเหตุระเบิดในช่วงหลายปีที่ผ่านมา การตรวจวัดไฮโดรเจนเปอร์ออกไซด์ในของเหลวสังเคราะห์สารระเบิดเปอร์ออกไซด์เป็นวิธีทางเลือกที่สามารถตรวจพิสูจน์การสังเคราะห์สารระเบิดเปอร์ออกไซด์เบื้องต้นได้ งานวิจัยนี้จึงได้พัฒนาอุปกรณ์ตรวจวัดจากวัสดุโครมาโทกราฟีแบบแผ่นบางที่ดูดซับสารเชิงซ้อนของ 3-อะมิโนโพรพิลไตรเอทอกซีไซลอกเซนและกลูตารัลดีไฮด์ (APTES-GA) อุปกรณ์ตรวจวัดจะถูกฟอกจางสีจากสีแดงเป็นสีเหลืองอ่อนเมื่อทำปฏิกิริยากับไฮโดรเจนเปอร์ออกไซด์ ซึ่งสังเกตได้ด้วยตาเปล่า ภาพของอุปกรณ์ตรวจวัดถูกบันทึกภาพด้วยโทรศัพท์มือถือและวิเคราะห์ค่าสัญญาณความเข้มสีด้วยซอฟต์แวร์ ภายใต้สภาวะที่เหมาะสมของวิธีที่พัฒนาขึ้น อุปกรณ์ตรวจวัดสามารถวิเคราะห์ไฮโดรเจนเปอร์ออกไซด์ในตัวอย่างของเหลวสังเคราะห์สารระเบิดเปอร์ออกไซด์จำลองได้จริง อุปกรณ์ตรวจวัดมีความแม่นยำที่ดี และขีดจำกัดการตรวจวัดและขีดจำกัดการตรวดวัดเชิงปริมาณเท่ากับ 12.8 mM และ 42.8 mM ตามลำดับ

คำสำคัญ: อุปกรณ์ตรวจวัดสี ไฮโดรเจนเปอร์ออกไซด์ สารระเบิด ไตรอะซิโดนไตรเปอร์ออกไซด์

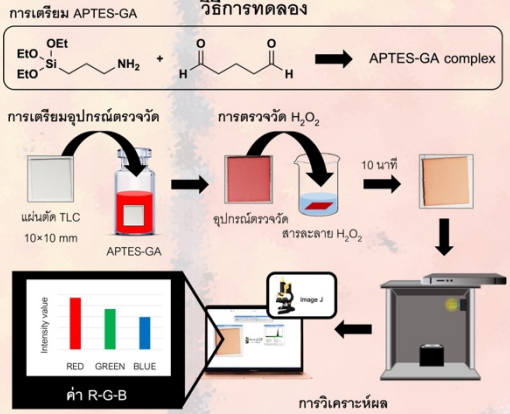
ที่มาและความสำคัญ



จุดประสงค์

เพื่อพัฒนาอุปกรณ์ตรวจวัดจากสารเชิงซ้อน APTES-GA โดยมีแผ่น TLC รองรับ ในการตรวจวิเคราะห์สารไฮโดรเจนเปอร์ออกไซด์เบื้องต้น ในของเหลวสังเคราะห์ของสารระเบิดเปอร์ออกไซด์

วิธีการทดลอง



สรุปและข้อเสนอแนะ

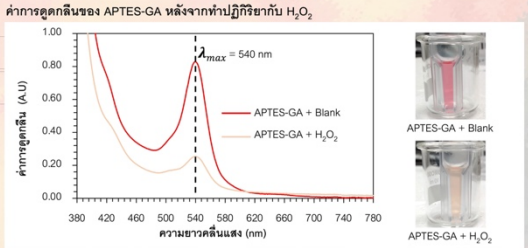
อุปกรณ์ตรวจวัดสารไฮโดรเจนเปอร์ออกไซด์จาก APTES-GA ที่ถูกพัฒนาขึ้น โดยใช้วัสดุแผ่น TLC เป็นส่วนรองรับการตรวจวัด สามารถตรวจวัดไฮโดรเจนเปอร์ออกไซด์ได้โดยอาศัยการฟอกจางสีของ APTES-GA และวิเคราะห์ค่าสัญญาณความเข้มสีเขียวของภาพดิจิทัลโดยใช้ซอฟต์แวร์ Image J ร่วมกับการใช้สมาร์ทโฟนเป็นอุปกรณ์ถ่ายภาพ อุปกรณ์ตรวจวัดที่พัฒนาขึ้น สามารถเตรียมได้ง่าย ราคาถูก พกพาสะดวก และมีความแม่นยำ โดยวิธีทดสอบมีค่าขีดจำกัดการตรวจวัดสารไฮโดรเจนเปอร์ออกไซด์เท่ากับ 12.8 mM และสามารถนำไปประยุกต์ใช้การตรวจวัดไฮโดรเจนเปอร์ออกไซด์ในของผสมสังเคราะห์สารระเบิดเปอร์ออกไซด์นอกห้องปฏิบัติการหรือสถานที่เกิดเหตุ

เอกสารอ้างอิงสำคัญ

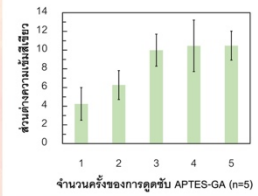
Zhou, M., Yang, M., and Zhou, F. 2014. Biosensors and Bioelectronics 55: 39–43.

ผลการทดลอง

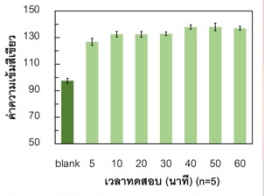
ค่าการดูดกลืนของ APTES-GA หลังจากทำปฏิกิริยากับ H_2O_2



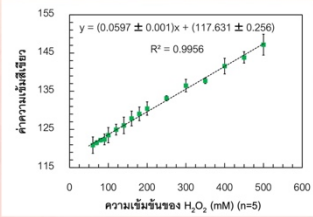
การดูดซับ APTES-GA บนแผ่น TLC



เวลาทดสอบที่เหมาะสม



ช่วงความเป็นเส้นตรงของวิธีวิเคราะห์



ตัวอย่างจำลอง

- ❑ ช่วงความเป็นเส้นตรง ; 50 ถึง 500 mM
- ❑ ขีดจำกัดการวิเคราะห์เชิงปริมาณ ; 42.8 mM
- ❑ ขีดจำกัดการตรวจวัด ; 12.8 mM
- ❑ ความเที่ยงของวิธี ; RSD = 0.5% ถึง 1.9% (n=5)

ตัวอย่างทดสอบ	ผลการทดลอง*	อุปกรณ์ตรวจวัด
ชุดควบคุมผลทางลบ (ตัวทำละลายผสม อะซิโตน:น้ำ = 1:1)	-	
สารละลาย H_2O_2 เข้มข้น 30%	+	
ของเหลวผสม H_2O_2 และอะซิโตน	+	
ของเหลวผสม H_2O_2 และอะซิโตน ที่สภาวะกรด	+	
ของเหลวผสม H_2O_2 และอะซิโตน ที่สภาวะกรด (หลังเกิดปฏิกิริยา 1 วัน)	+	

*(+) หมายถึง ตรวจพบ H_2O_2 และ (-) หมายถึง ตรวจไม่พบ H_2O_2

กิตติกรรมประกาศ

มหาวิทยาลัยสงขลานครินทร์ คณะวิทยาศาสตร์ Department of Applied Science

VITAE

Name Mr. Petcharawut Ramsiri

Student ID 6310220034

Educational Attainment:

Degree	Name of Institution	Year of Graduation
Bachelor of Chemistry	Khon Kaen University	2020

Scholarship and Awards during Enrolment:

1. The Development and Promotion of Science and Technology Talents Project (DPST) (Thailand government scholarship)

List of Publications and Proceeding:

1. Ramsiri, P., Choodum, A., Phonchai, A., A colorimetric probe for hydrogen peroxide detection by siloxane-glutaraldehyde complex. 19th Kasetsart University Kamphaeng Saen Campus National Conference, Nakhonpathom, Thailand, 2022.