POLYVINYL ALCOHOL/STARCH MODIFIED COTTON THREAD FOR GLUCOSE DISTANCE-BASED COLORIMETRIC DETECTION

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ในงานวิจัยนี้ด้ายฝ้ายถูกนำมาดัดแปรด้วยพอลิไวนิลแอลกอฮอล์และแป้ง เพื่อใช้เป็นอุปกรณ์ตรวจวัดความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์และกลูโคสซึ่งสามารถอ่านผลได้ง่ายด้วยเทคนิคเชิงสีฐานระยะทาง โดยนำพอลิไวนิลแอลกอฮอล์และแป้งซึ่งเป็นพอลิเมอร์ที่มีความเข้ากันได้ทางชีวภาพมาดัดแปรด้ายฝ้ายเพื่อเพิ่มสมบัติในการคงประสิทธิภาพของเอนไซม์และรีเอเจนต์ที่ใช้ในการตรวจวัดกลูโคสโดยอาศัยเอนไซม์ โดยใช้เอนไซม์ 2 ชนิดในการตรวจวัดกลูโคสได้แก่กลูโคสออกซิเดส และฮอสแรดิชเปอร์ออกซิเดส ร่วมกับโพแทสเซียมไอโอไดด์ซึ่งเป็นอินดิเคเตอร์รีเอเจนต์ ในปฏิกิริยาที่เกิดขึ้นนั้นโพแทสเซียมไอโอไดด์ถูกออกซิไดซ์โดยไฮโดรเจนเปอร์ออกไซด์ ได้ผลิตภัณฑ์เป็นสารประกอบเชิงซ้อนระหว่างแป้งและโพแทสเซียมไอโอไดด์ซึ่งมีสีน้ำเงินเข้มออกดำ โดยความยาวของเส้นด้ายที่เกิดการเปลี่ยนสีมีความสัมพันธ์กับความเข้มข้นของกลูโคส ทำให้สามารถตรวจวัดความเข้มข้นของกลูโคสในเชิงปริมาณได้ง่ายและรวดเร็วด้วยตาเปล่าโดยไม่ต้องใช้เครื่องมือในการแปลผลสัญญาณสี หลังจากศึกษาภาวะที่เหมาะสมอุปกรณ์ตรวจวิเคราะห์ฐานเส้นด้ายดัดแปรแสดงการตรวจวัดไฮโดรเจนเปอร์ออกไซด์และกลูโคสด้วยช่วงความเป็นเส้นตรงระหว่าง 1.0 ถึง 6.0 มิลลิโมลาร์ และ 0.1 ถึง 5.0 มิลลิโมลาร์ ตามลำดับ นอกจากนี้ยังพบว่าไม่มีการรบกวนจากสารอื่นที่มักพบในน้ำตามนุษย์ ได้แก่ โพแทสเซียมคลอไรด์ โซเดียมคลอไรด์ ยูเรีย กรดแอสคอร์บิก กรดยูริก คลอเรสเตอรอล และโบวายซีรั่มอัลบูมิน อุปกรณ์ตรวจวิเคราะห์ที่ถูกพัฒนาขึ้นสามารถนำไปตรวจวัดกลูโคสเชิงปริมาณในน้ำตาเทียมได้

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Pornchanok Punnoy : POLYVINYL ALCOHOL/STARCH MODIFIED COTTON THREAD FOR GLUCOSE DISTANCE-BASED COLORIMETRIC DETECTION. Advisor: Prof. PRANUT POTIYARAJ, Ph.D. Co-advisor: Nipapan Ruecha, Ph.D.

In this thesis, polyvinyl alcohol (PVA) and starch modified cotton thread was developed as a cotton thread-based device for distance-based colorimetric detection of hydrogen peroxide (H_2O_2) and glucose. PVA and starch, the biocompatible polymers, were modified on a cotton thread to enhance the enzymatic stability and reagent immobilization efficiency. The colorimetric glucose detection of the device was based on a bienzymatic reaction involving glucose oxidase (GOx) and horseradish peroxidase (HRP), incorporated with potassium iodide (KI) as an indicator, which was oxidized by H_2O_2 to provide blue-black color band of an iodine–starch complex. The length of color band on the cotton thread is proportional to H_2O_2 and glucose concentrations, allowing for a simple and rapid quantitative detection observed by the naked eyes without any signal readout instrument. For the best performance of the proposed device, various parameters were studied, such as the ratio of PVA/starch, KI volume, HRP volume and sample volume. Under the optimal conditions, the cotton thread-based device was used to detect H_2O_2 and glucose providing linear ranges of 1.0-6.0 mM and 0.1-5.0 mM with detection limits of 0.25 mM and 0.1 mM, respectively. Furthermore, the interference effect of glucose detection was also investigated and the obtained results showed no interfering from common compounds in human tear (*i.e.* potassium chloride, sodium chloride, urea, ascorbic acid, uric acid, cholesterol and bovine serum albumin). Eventually, the developed device could be successfully applied for the quantitative determination of glucose in artificial human tear.

Field of Study:	Petrochemistry and Polymer Science	Student's Signature .....
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CHAPTER I

INTRODUCTION

1.1 Introduction

Nowadays, non-invasive diagnosis has become an interesting and alternative tool for point-of-care testing (POCT) owing to blood-free measurement which can avoid the user's painfulness during examination and prevent an infection risk and other complications compared to traditional invasive diagnosis [1-3]. Therefore, a non-invasive sensor has been developed as a portable device for simple, rapid and on-site measurement [4, 5]. Various materials have been used as the sensing substrates for non-invasive sensor fabrication [6] , such as nitrocellulose membrane [7], filter paper [8], textile [9] and cotton thread [10]. Among all, a cotton thread has gained much attention to be a sensing substrate material due to its inexpensiveness, biocompatibility and requirement of low sample volume. Furthermore, the properties of the cotton thread, consisting of twisting strands of cellulosic fiber and the gaps between strands, can generate microfluidic channels for liquid transport along the thread via capillary force without the use of external pump. Therefore, the development of a cotton thread-based device is one of an interesting prospect which deserves further investigation and would be used as a desirable analytical sensor for point-of-care testing, such as early stage diagnosis in developing countries [11-13].

Glucose is a common biomarker used for identifying and monitoring prediabetes and diabetes. Normally, the glucose levels in human body are found to be 2.78–5.55 mM in urine and 0.05–0.50 mM in human tear, and the abnormal high level of glucose is known as diabetes risk [14]. The development of glucose monitoring system is the most important factor for management and treatment of diabetes. Currently, self-diagnosis device of blood glucose still need a finger-stick blood sample with an invasive needle, which can cause painfulness and infection. Therefore, a non-invasive glucose sensor is an alternative tool for overcoming such drawbacks [15].

Several analytical methods have been coupled with a non-invasive glucose sensor including electrochemical and colorimetric detections [16]. Among all, colorimetric detection has received considerable attention for POCT due to its simple, user-friendly, cost-effective, rapid, flexible and sensitive method for various analyte detection. Furthermore, the measurement of this method is achieved by readily observing of the color intensity/hue change signal via the naked eye [17, 18].

Recently, Li et al. [19] reported the cotton thread-based colorimetric device as a platform for glucose assay by evaluating the change of color intensity. However, the observed color change can indicate only qualitative or semi-quantitative analysis. Consequently, for accurate quantitative analysis, the optical instruments (e.g. camera or scanner) and software are still required. To overcome this limitation, the cotton thread-based device combined with the visual distance-based colorimetric detection has been developed for glucose quantitative analysis. Distance-based colorimetric detection relies on the measurement of the length of a color change band which are correlated with the analyte concentration. In this approach, analyte can be measured by the naked eye and eliminated the need for optical devices or external instruments. To improve the distance-based colorimetric signal, a variety of biopolymers have been used for the surface modification such as chitosan, starch and cellulose. Among them, starch is a biopolymer of interest due to its high abundance, biodegradability, and biocompatibility. However, the pure starch film generally lacks the mechanical strength and process ability [20, 21]. To improve these properties, starch is blended with polyvinyl alcohol (PVA) in order to improve its mechanical properties. Furthermore, PVA can also stabilize the enzymatic activity and indicator reagent immobilization efficiency which has been entrapped on the cotton thread, allowing for the improvement of color length differentiation [22].

In this work, a cotton thread-based device coupled with the distance-based colorimetric readout has been proposed for quantitative determination of hydrogen peroxide (H_2O_2) and glucose, providing a non-invasive glucose sensor for clinically relevant POCT. PVA/starch was modified on the cotton thread aimed to enhance the detection sensitivity. A bienzymatic reaction including glucose oxidase (GOx) and horseradish peroxidase (HRP) was cooperated with potassium iodide (KI) to be

oxidized by H2O2 and produce a colorimetric signal. This device displayed a simple, reliable, and rapid measurement leading to a great potential application for the non-invasive glucose sensor in human tear.

1.2 Objectives

- To fabricate a cotton thread-based device modified with polyvinyl alcohol (PVA) and starch
- To measure hydrogen peroxide (H_2O_2) and glucose using the PVA/starch modified cotton thread-based device coupled with distance-based colorimetric detection
- To apply the developed device for glucose detection in human tear

Scope of the research

This study focuses on the development of cotton thread-based device coupled with distance-based colorimetric detection of hydrogen peroxide (H_2O_2) and glucose. The cotton thread was modified by PVA/starch and the surface morphology of the modified cotton thread was characterized. The systematic optimizations and the analytical performances of the developed device were investigated. Finally, the PVA/starch modified cotton thread-based device were applied for the determination of glucose in human tear.

CHAPTER II

THEORY AND LITERATURE SURVEYS

In this chapter, the theory and basic principle of bio/chemical sensor, including colorimetric sensor and enzyme-based colorimetric sensor, are described in the first part. As for the second part, the substrate materials of sensor are explained. The thread surface modification by using PVA and starch is described in the third part. Finally, glucose and non-invasive sensor are discussed.

2.1 Bio/Chemical sensor

The definition of bio/chemical sensors is ‘‘a device that transforms the chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal.’’ defined by The International Union of Pure and Applied Chemistry [23]. Generally, two basic functional units have been identified, including a recognition receptor and a transducer part. Types of sensor can be classified based on target analyte. If the recognition receptor is coupled with a biological analyte (e.g. enzyme, antibody, DNA, etc.), the device called biosensor (Figure 2.1). After the analyte coupled with the recognition receptor, the receptor converts the analyte concentration into a measurable signal which the transducer can be measured [24], and then it was processed and analyzed to a readable value.

Nowadays, biosensor is an attractive and useful analytical device applied for various fields of applications. Biosensor can be distinguished according to transducer part, for instance, optical sensor, electrochemical sensor, thermometric sensor, piezoelectric sensor and so on [25]. However, this study primarily focuses on a colorimetric sensor.

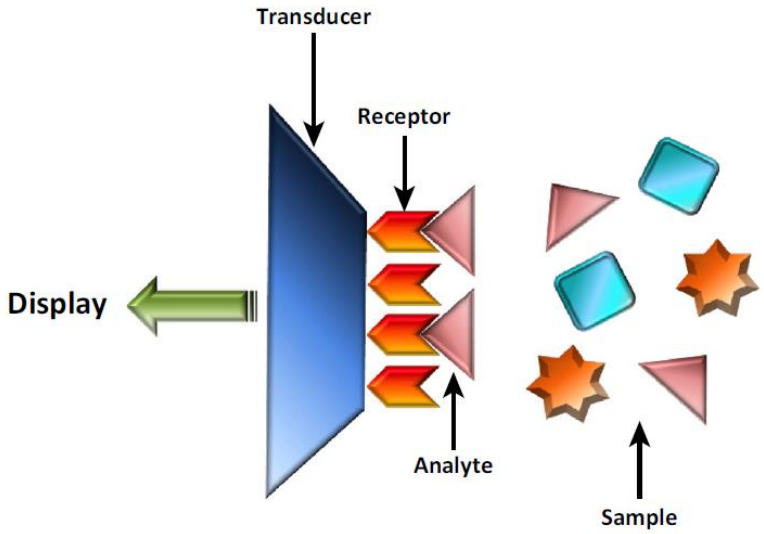


Figure 2.1 Schematic representation of important component of the chemical sensor [25].

2.1.1 Colorimetric sensor

Colorimetric sensor is the most popular analytical scheme of optical sensor, which is used to instantaneously detect an analyte by distinguishing color change of the product from a chemical reaction with reagent [26]. Colorimetric sensor has attracted a special interest because of their numerous advantages as following. Firstly, colorimetric sensor allows simple detection based on color change which can be easily read via the naked eye without the expensive instrumentation requirement. Moreover, with the help of spectrophotometer or optical instrument, colorimetric sensor provides more reliable and precise result for chemical analysis. Next, interpretation of the color change signal using only the naked eye displays an attractive option in terms of portability and ease of operation. Therefore, colorimetric sensor can be used for field-based side application, which is often required in resource-limited area and poorly equipped laboratories [27]. As previous mentions, colorimetric sensor can be performed outside laboratories, leading to well suitable application for point-of-care testing (POCT) [28], which can be defined as real-time diagnosis approach in home healthcare, accident site, and emergency situation. Furthermore, colorimetric sensor has proved to be fast, adaptable, cost-effective and

specific for the detection of a wide range of various analytes [29]. The basic signal readouts of colorimetric sensor via naked eyes are described as following.

2.1.1.1 Intensity-based colorimetric sensor

Selective and specific detection of analytes associated with a specific chemical reaction between the targeted analytes and sensing materials includes complex formation between metal ions and chelate reagents [30], nanoparticles aggregation [31], and enzymatic reactions [32]. For the colorimetric measurement, the popular method for the interpretation of color change signal is intensity-based method, which is based on various color intensities/hue of detection region. The users can read the result and determine the analyte concentration by comparing the appeared color intensities to color reference chart related to the standard chart of known sample concentration [33-35] as shown in Figure 2.2. The precision of this method relies on user interpretation and ambient light condition (*i.e.* illumination angle, intensity), resulting in the problem of user error because different users may obtain different interpretations of the same color. To overcome this problem, the optical instruments (*e.g.* camera or scanner) and software must be required for facile and accurate quantitative analysis [36].

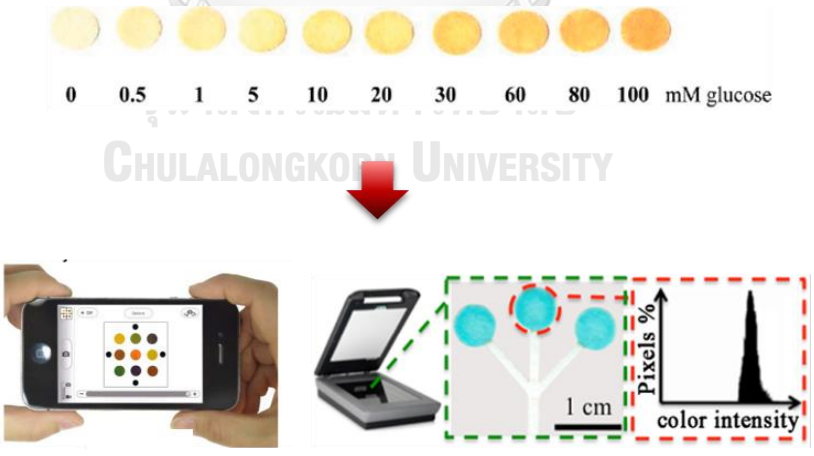


Figure 2.2 Intensity-based colorimetric measurement with optical instrument [37], [38].

With the purpose of achieving simple colorimetric sensor that can be quantitatively detected the analyte by naked eye without optical instruments,

several research groups have reported other alternative instrument-free detection approached by using distance-readout [39], time-readout [40], and counting of the zones colored by a chemical reaction [41] (Figure 2.3). In this study, distance-based colorimetric detection has been focused.

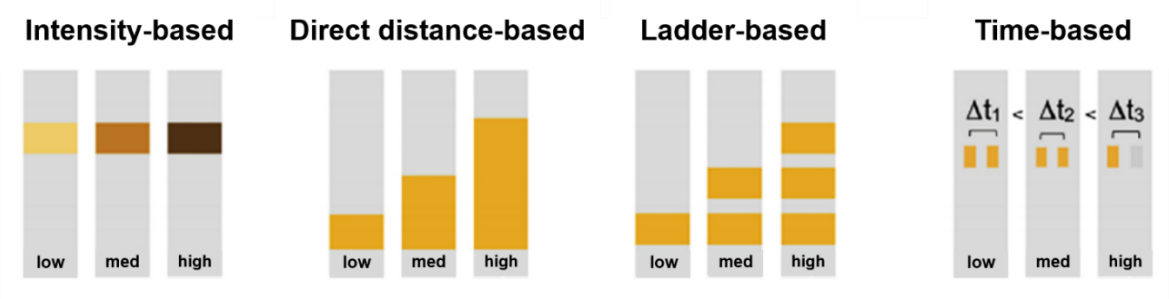


Figure 2.3 Semi-quantitative/quantitative colorimetric measurement without optical instrument [42].

2.1.1.2 Distance-based colorimetric detection

Distance-based colorimetric detection is an alternative simplified technique for quantitative analysis. Compared to the intensity-based measurement, the distance-based colorimetric detection relies on the measurement of length signal instead of color intensity without the requirement of optical device or instruments leading to fewer personal bias and errors from user interpretation [43]. Because of its characteristic of ease to use without the need for any instruments, the distance-based colorimetric detection displays a great potential for integrated into portable analytical devices [44]. The mechanism of quantitative analysis relies on capillary action within a microfluidic channel. In this approach, colorimetric reagents deposited on the substrate react with the flowing analyte, and then produce color band along the capillary flow path of the device substrate which the color band occurs from enzymatic reaction [45], aggregation [46], precipitation [39] or complexation [47] (Figure 2.4). The measurement could be accomplished when the sample reaches to the end of the flow path. Quantification of the analyte is achieved by measuring the length of color band, similar to reading temperature on a thermometer [36]. The color length is related to the analyte concentration, and the high concentration of

analyte shows longer color length than the low concentration. The distance-based measurement offers several advantages, such as cost-effective, user-friendly, easy to use, and disposable with quantitative analysis. Additionally, it can provide instrument-free readout for quantitative analysis by the naked eye [48, 49].

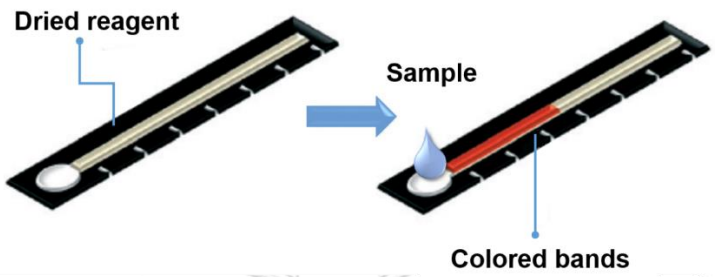


Figure 2.4 Translation of molecular signals to distance-based readout based on capillary action [44].

2.1.2 Enzyme-based colorimetric sensor

Enzymatic-based colorimetric sensor is a biosensor incorporated with the optical transducers. Enzyme acts as a bioreceptor molecule which specifically recognizes the analyte and converts to measurable signal by transducer. The enzymatic detection process is conducted by the chemical reactions using a specific enzyme and substrate to form an enzyme-substrate complex, resulting in a distinctive color change [50]. Colorimetric detection mostly uses oxidase-family and peroxidase enzymes with indicators reagent which provides measurable color change between the oxidized and reduced forms.

The oxidase-family and peroxidase are the most common used enzymes in bienzymatic system. The oxidase-family enzyme oxidizes its substrate to produce an oxidized product and hydrogen peroxide (H_2O_2). After that, the amount of H_2O_2 product is detected by the peroxidase enzyme with indicator reagent to generate a visual color change (Figure 2.5).

Figure 2.5 Schematic illustration of the enzymatic reaction based colorimetric method.

The bienzymatic system can be integrated into medical diagnostics of various biomarkers, such as glucose, lactate, uric acid, cholesterol and sarcosine [51-53], owing to the specificity of the enzyme. Enzyme significantly improves the selectivity of the detection and provides more accuracy result. Additionally, it can reduce or eliminate interference effect in analytical measurement. The common enzymatic reactions that can be utilized for analytical application are presented in Table 2.1.

Table 2.1 Most common enzymatic assays for colorimetric detection.

Enzymatic system	Target analyte	Application	Ref.
Oxidoreductases	Glucose, lactate, cholesterol, uric acid and sarcosine	Clinical analysis (multiple)	[54, 55]
Transferases	Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities	Clinical analysis (liver function)	[56]
Hydrolases	Acetylcholinesterase activity	Neurological disorders and organophosphate poisoning	[57, 58]

The efficiency of enzyme is influenced by pH and the work of each enzyme is suitable within specific pH ranges. Thus, the detection using bienzymatic system needs to be optimized, in order to avoid a dysfunctional system. Enzyme is commonly prepared in a buffer with an optimal pH. For example of glucose detection, Martinez and coworker [59] prepared a solution containing glucose oxidase (optimal pH: 5.5), horseradish peroxidase (optimal pH: 6.0–6.5), potassium iodide and trehalose for glucose determination by using phosphate saline buffer (PBS) with pH 6.0, which is a compromising pH value between the optimal pH for each enzyme in the solution.

2.1.2.1 Indicator

Normally, indicator is the importance for enzyme-based colorimetric assay because color change signal of enzymatic reactions can be occurred via redox chemical reaction using the indicator reagents that are capable to present or change a visible color due to a chemical stimulus. Potassium iodide (KI), a salt of stable iodine with a K(+) counter ion, is one of the commonly used as the indicator reagents [60]. A visual color change of KI produces from the oxidation of iodide (colorless) to iodine (yellow–brown color) by hydrogen peroxide with horseradish peroxidase as a catalyst [61] (Figure 2.6).

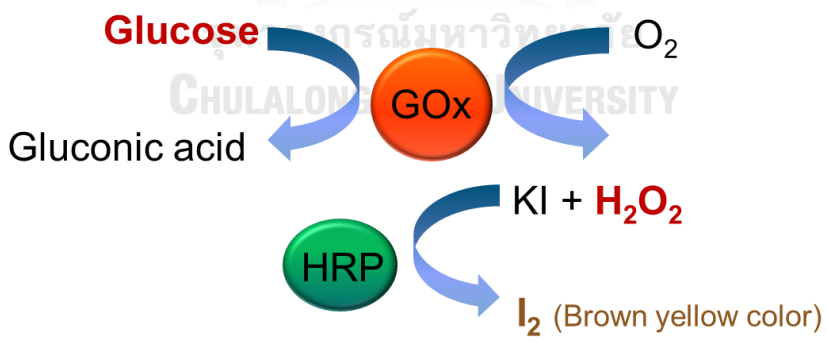
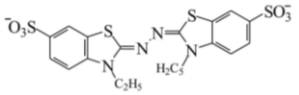
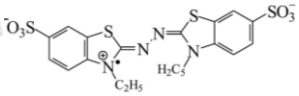
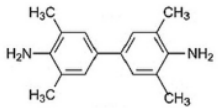
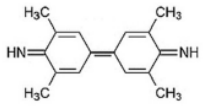
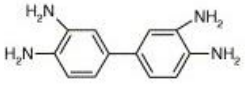
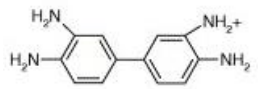


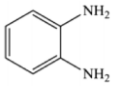
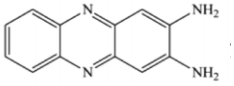
Figure 2.6 Visual color change of KI produced from the oxidation of iodide (colorless) to iodine (yellow-brown color).

KI is a preferable reagent for the development of a colorimetric detection. Compared to the other common indicators, such as 2,2'-azino-bis(3-

ethylbenzothiazoline-6-sulphonic acid) (ABTS), 3,3' ,5,5'-Tetramethylbenzidine (TMB), o-Phenylenediamine (OPD) and 3,3'-Diaminobenzidine (DAB) [62], KI provides less expensive, very stable and low toxic. The common indicator reagents for colorimetric detection are presented in Table 2.2.

Table 2.2 Common redox indicators for peroxide assay coupled with oxidase-based systems [62].

Indicator	Reduced form	Oxidized form	Ref.
Potassium iodide (KI)	I^- λ_{max} = 193 nm ε_{193} = 1.42 10^4 M^{-1} cm^{-1} λ_{max2} = 226 nm ε_{226} = 1.34 10^4 M^{-1} cm^{-1}	I_3^- λ_{max} = 353 nm ε_{353} = 2.6 10^4 M^{-1} cm^{-1} λ_{max2} = 287.5 nm ε_{226} = 4.0 10^4 M^{-1} cm^{-1}	[64]
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)	 λ_{max2} = 340 nm ε_{340} = 3.6 10^4 M^{-1} cm^{-1}	 λ_{max} = 414 nm ε_{414} = 3.6 10^4 M^{-1} cm^{-1} λ_{max2} = 340 nm ε_{340} = 5.4 10^4 M^{-1} cm^{-1}	[65]
3,3',5,5'-Tetramethylbenzidine (TMB)	 λ_{max} = 285 nm ε_{285} = 2.0 10^4 M^{-1} cm^{-1}	 λ_{max} = 450 nm ε_{450} = 7.2 10^4 M^{-1} cm^{-1}	[66]
3,3'-Diaminobenzidine (DAB)	 (does not absorb significantly in the visible)	 λ_{max} = 454 nm ε_{454} = 3.1 10^4 M^{-1} cm^{-1} λ_{max2} = 510 nm ε_{510} = 2.3 10^4 M^{-1} cm^{-1}	[67]

Indicator	Reduced form	Oxidized form	Ref.
o-Phenylenediamine (OPD)	 λ_{max} = 289 nm ε_{289} (not available)	 λ_{max} = 417 nm ε_{417} = 1.6 10^4 M^{-1} cm^{-1}	[68]

Paula, B. and co-workers [69] developed glucose colorimetric assay using toner-based microzone plates. Toner-based microzones were printed on polyester film and filled with a cellulose paste, which the colorimetric measurements were performed. KI was manually pipetted inside each microzone as indicator reagent. The proposed approach exhibited high accuracy for glucose detection in serum and urine samples. A linear range and limit of detection were found to be 0 to 10 mmol/L and 0.6 mmol/L, respectively.

Andres, W. M. and co-workers [59] developed paper-based microfluidic devices for multiple colorimetric detection. The devices were fabricated on a paper using photolithography and were functionalized with enzyme and KI as indicator reagents for glucose and protein colorimetric assays.in artificial urine. The linear range for glucose detection was 0–5 mM, with a limit of detection of 0.5 mM glucose. Moreover, the range for detecting protein was accurated down to 4 μM.

Elizabeth, E. and co-workers [70] synthesized a silica nanoparticle-modified microfluidic paper-based analytical device (μPAD) with the improvement of color intensity for three different enzymatic assays (lactate, glucose, and glutamate). The μPAD was fabricated using nanoparticles modified with 3-aminopropyltriethoxysilane. The selected enzymes and KI indicator reagent were used for colorimetric reactions. Furthermore, the addition of silica nanoparticles yielded a significant improvement in color intensity and provided the high sensitivity with limits of detection (LODs) of 0.63 mM, 0.50 mM, and 0.25 mM for lactate, glucose, and glutamate, respectively. The devices could be accepted as a strong platform for point-of-care testing in the limited resources area.

Scott, A. K. and co-workers [71] fabricated paper-based microfluidic devices using a novel polymer blend for monitoring urinary ketones, glucose, and salivary nitrite. The chromogenic reagents were used for colorimetric reaction, such as KI, sodium nitroprusside and sulfanilamide Color change of the paper substrate was captured using a scanner, and then images were converted to CMYK format in Adobe Photoshop for analyzing the color intensity. The linear ranges for glucose, nitrite and ketone were found to be 3 to 50 mM, 5 to 250 μM and 5 to 16 mM, respectively.

2.2 Substrate materials of sensor

Different types of materials including glass, polydimethylsiloxane (PDMS), paper, and thread have been utilized as the substrate materials for the fabrication of sensor [43]. In this study, cellulose based materials including paper and thread are focused because they are the more popular than other materials in term of cost, availability and fabrication process.

2.2.1 Cellulose

Nowadays, the natural polymers have gained great attention as the substrate materials due to their accessibility, biodegradability, biocompatibility and low cost. Some examples of natural polymers are cellulose, natural rubber, silk, and wool. Among them, cellulose is one of the most widely used [72, 73], because cellulose is the most abundant natural polymer and high efficiency in industrial technology as the composite materials, textiles, drug delivery systems and personal care products [74]. Besides, cellulose is a linear polysaccharide polymer which is formed by β-1,4 linked d-glucose units. There are a large number of polar (–OH) hydroxyl groups in its structure, which can form extensive hydrogen bond networks including H-bonds between the main chains and also H-bonds within the chains [75]. According to its molecular structure, hydroxyl groups are the chemical characteristics of cellulose which are the main functional groups responsible for the reactivity, such as chemical modification and functionalization leading to the desirable properties of cellulose, including the ability to absorb moisture, biodegradability, biocompatibility, chemical stability and nontoxicity. Additionally, three-dimensional fibrous membrane of cellulose can be used as matrix for transporting of the chemical reagents. From the above description about their properties, cellulose-based materials as a platform for

detection devices demonstrates greatly potential for applications in colorimetric sensing, such as self-diagnostics and biosensors [76]. Among various products derived from cellulose, paper and textile including thread have received attention through their suitable properties as the substrates used in colorimetric sensors.

2.2.1.1 Paper

Paper is a well-known material usually produced from cellulose fiber which is highly attractive for the development of paper based analytical device in the field of analytical and clinical chemistry [77]. This simple cellulosic substrate introduces an outstanding material for fluid handling and analysis because paper is produced from cellulose fibers which can store reagent and liquid to generate microfluidic flow system via capillary action without the requirement of external force [78]. The microfluidic channels are created by patterning hydrophobic barrier on hydrophilic paper [79]. To produce paper-based analytical platform, paper is commonly printed using various printing methods including photolithography, inkjet printing, screen printing, wax printing and stencil printing [80]. For the desired paper-based device, different types of paper are investigated such as cellulose, cation-exchange and anion-exchange papers. The choice of paper material depends on the fabrication method required in device developing. Additionally, cellulose fibers of paper can be functionalized using a variety of functional groups for preferable properties, including chromatographic separation, uniformity of color development, binding of immobilized molecule. Furthermore, the paper based material properties, such as abundant, low-cost, lightweight, portable and equipment-free [81], are particularly important for integrated into material for distance-based analytical device for a wide range of applications. The previous reports about distance-based analytical device using paper as material display as following.

David, M. C. and co-workers [39] developed paper-based analytical devices (PADs) combined with distance-based colorimetric detection of different biological or environmental samples. This method provided simple technique to perform PADs with quantitative and straightforward detection. Furthermore, the broad applicability was demonstrated by measuring glucose, nickel, and glutathione using three different detection chemistries including enzymatic reactions, metal complexation, and

nanoparticle aggregation, respectively. The results showed an excellent quantitative results and inexpensive device for point-of-use applications.

Xiaofeng, W. and co-workers [44] developed a microfluidic distance readout sweet hydrogel integrated paper-based analytical device (μDiSH-PAD) for portable and quantitative detection of different target analytes. The platform relied on a target-responsive aptamer-cross-linked hydrogel for target recognition, cascade enzymatic reactions for signal amplification, and a microfluidic paper-based analytical device (μPAD) for visual distance-based quantitative readout. The developed distance-based colorimetric quantitative method could be effectively applied for cocaine measurement in urine with high selectivity and sensitivity and provided excellent linear relationship ranging of 10–400 μM and a low detection limit of 3.8 μM. Moreover, the distance-based method provided instrument-free readout for quantitative detection via the naked eye, with less influence of user interpretation.

David, M. C. and co-workers [36] developed microfluidic paper-based analytical devices coupled with a distance-based detection method for quantifying metal (Ni, Cu, and Fe) concentrations from aerosolized particulate matter. The visual quantification of analytes was based on the distance of a colorimetric reaction. The results were recorded with half-millimeter resolution by printing rulers beside each detection channel. Metals (Ni, Cu, and Fe) were individually detected in separated sample loading zone with a single detection channel and co-sample loading zone with a multi-detection channel. The limit of detection for Ni, Cu, and Fe in single and multi-channel devices was 0.1, 0.1, 0.05 μg and 5, 5, 1 μg, respectively.

Ying, C. and co-workers [82] developed a distance-based immunoassay based on microfluidic paper-based analytical devices (μPADs) for semi-quantitative detection of carcinoembryonic antigen (CEA) via TMB-H_2O_2 chromogenic reaction. The results indicated that H_2O_2 induced the precipitated TMB to form precipitation polymer with the cooperation of HRP and produce the length of color bar negatively correlated to the concentration of CEA. CEA levels in the human serum could be detected providing a linearity ranging of 3×10^{-3} to 3×10^{-7} M with the detection limit of 5×10^{-8} M. This system was efficiency for biomarker detection in point-of-care (POC) diagnostics field.

From above mentioned literatures, a paper platform is effectively used as material for distance-based colorimetric detection, however it still requires a hydrophobic barrier and long analysis time and large sample volume.

2.2.1.2 Thread

Recently, thread, consisting of twisting strands of cellulosic fiber, has gained attention as microfluidic device substrate. The cellulosic fiber can be divided into three parts, including the primary wall, the secondary wall and the lumen. The primary wall is covered with natural plant waxes. While, the secondary wall is almost pure cellulose that influences the swelling behavior. The lumen or cavity is the channel in the center of the fiber which affects contributing to fluid flow [74]. Additionally, these structural characteristics provide a tiny gap between strands of each fiber to create the microfluidic channels form and facilitate fluid flow by capillary force. Due to the capillary force, the fluid can wick along the thread without the need of external power source. This property exhibits lower sample consumption than paper material. Therefore, thread is widely used as substrate material for distance-based analytical device due to its several advantages, such as low cost, low sample volume requirement, ubiquity, and environmental-friendly material. Moreover; it also provides a high flexibility, lightness, and toughness. For the selection of type of thread as analytical device, thread can functionalize by different chemical groups [83]. For instance, Reches and co-workers [84] demonstrated a wicking rate and enabled thread-based device by investigating the different types of threads (cotton, rayon, hemp, nylon, polyester, wool, 50% cotton, 50% acrylic, acrylic, and natural silk) as the candidate materials for thread-based microfluidic devices. Among candidate materials, cotton thread was selected because of its advantages of inexpensive and biodegradable materials leading to available and widespread manufactured in almost all region worldwide, especially developing region. Moreover, cotton thread has a good wicking ability without the need of plasma oxidation for improve wicking rate.

Compared to paper-based analytical device, thread based analytical device provides rapid analysis because liquid wicks only one dimensionally along a thread result in fast flow rate of wicked fluid, whereas paper presents the radial flow

because of its two-dimensional material and requires a pattern to control the fluid flow direction [32]. In addition, thread also has different properties such as the length of the fibers, the inter-fiber bonding, as well as differences in the characteristic porous channel structures. The applications of thread-based analytical device coupled with distance-based colorimetric detection have been demonstrated for various fields, including environmental monitoring, food quality control and especially, personal health care and clinical diagnosis. Therefore, thread is a good candidate material for the preparation of a distance-based colorimetric detection.

For as example of the thread-based microfluidic devices based on distance-based colorimetric detection, Yingshuai, L. and co-workers [45] developed thread-based microfluidic devices for semi-quantitative detection of biomedical and environmental analytes by measuring the length of color change. The thread was tied a knot in the central of the thread and fixed to a supporting polymer film. The indicator reagent was deposited in the knot and flowed along the thread via capillary force. After that, the sample was introduced onto the threads and generated different lengths of color change correlated to the concentrations of the analytes in the samples (Figure 2.7). This study demonstrated a successful method for measuring two different bioassays, including protein and nitrite in simulated human urine. Additionally, this method could be used for the detection of Ni^{2+} in waterway.

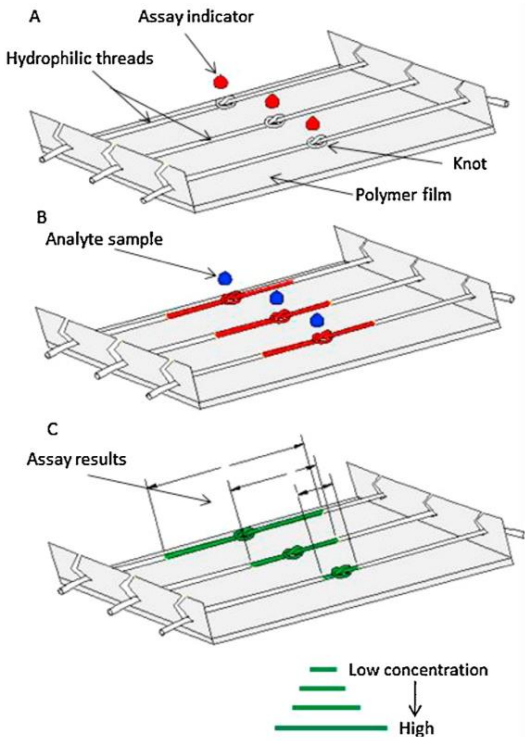


Figure 2.7 Schematic illustration of the microfluidic thread-based analytical device using length measurement method.

2.3 Surface modification

For the preparation of thread-based colorimetric sensor, surface modification is usually required to improve sensitivity and enhance both physical and analytical performances. The interesting chemicals used in this study are polyvinyl alcohol (PVA) and starch.

2.3.1 Polyvinyl alcohol

Polyvinyl alcohol (PVA) is a synthetic vinyl polymer with a chemical formula of $[CH_2CH(OH)]_n$. The structure of commercial PVA is a copolymer of vinyl alcohol and vinyl acetate [85] as shown in Figure 2.8. PVA exhibits high solubility in water and excellent biodegradability. These features enable applications of PVA as an emulsifier, colloidal particles stabilizer, adhesive, and coating agent in the textile and paper industries. Additionally, the biocompatibility of PVA and its non-toxicity are considered as the great advantages for biomedical applications, such as

manufacturing of contact lenses, artificial heart surgery, drug delivery systems, wound dressings and medical biosensors [86, 87].

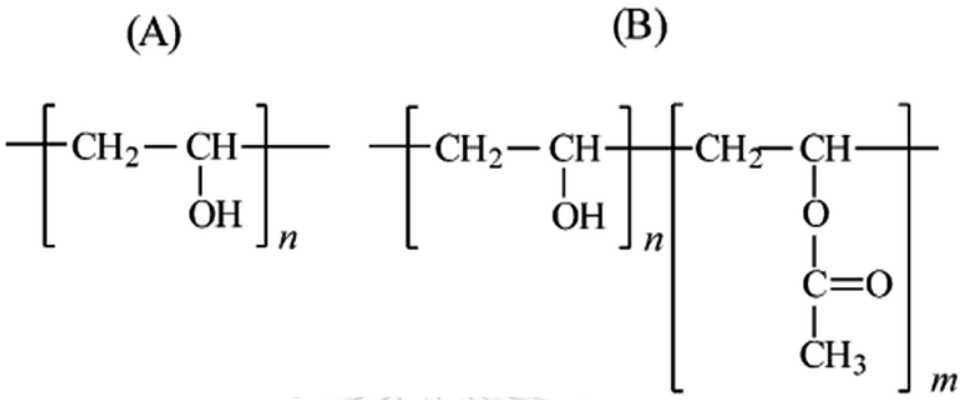


Figure 2.8 Structure of commercial PVA : (A) copolymer of vinyl alcohol and (B) vinyl acetate [85].

PVA has been widely used for biological and chemical stabilizations of enzyme assay in many studies because it provides complete stabilization of analytical enzyme, including glucose oxidase (GOx) and peroxidase [22, 88]. Moreover, PVA significantly improves the stability of the hydrogen peroxide (H_2O_2), which is a useful reagent in enzymatic assays [89]. Therefore, PVA can extend the usefulness of analytical enzyme for various enzymatic assays and promote stabilization of enzyme substrates for analysis outside of a laboratory [90], which is important for achieving point-of-care testing. Generally, PVA is widely used by combination with other polymers to improve its properties, such as hydrophilic polymers and natural materials (biopolymers), which can inspire green chemistry and low cost leading to being a useful polymer in various applications.

Youngung. S. and co-workers [91] developed nucleic acid testing (NAT) based on a paper platform using loop-mediated isothermal amplification (LAMP). Paper platform consisted of four reaction pads which contained target-specific LAMP primers and reagents for the LAMP reaction. PVA was selected as the optimal stabilizing reagent because it yielded the highest intensity of the reaction pad compared to the control pad and enhanced accuracy of LAMP readout. LAMP-based

multiple diagnostic device showed a potential for real-time analysis with quantitative detection of 10^2-10^5 copies of genomic DNA.

Juuso O. and co-workers [92] developed a simple method based on flexographic printing of polystyrene to form liquid guiding boundaries and layers on paper substrate for glucose colorimetric detection. Chromatographic paper for glucose detection consisted of five channel of reactions spots. GOx was applied into reaction spots in PVA-based solutions. The use of PVA exhibited a positive effect on the long-term stability of GOx.

Kamlesh S. and co-workers [93] developed a plasmonic colorimetric sensing strategy using PVA modified silver nanoparticles (AgNPs) for selective detection of lead (Pb). AgNPs as a reducing agent and polyvinyl alcohol (PVA) were used as capping agents. A filter paper fabricated with AgNPs/PVA was used for quantitative determination of Pb(II) by measuring color developed which interpreted via smartphone, followed by analysis in ImageJ software to determine the color intensity of the analyte.

Yangxi Zhang and co-workers [94] developed a fast-response colorimetric humidity sensor based on AgNPs enhanced PVA thin-film interferometer. PVA film was used to enhance the color of a humidity sensing. When the humidity of the external environment increased, the PVA film absorbed moisture from air, resulting in increased thickness of PVA film and slight reduction of refractive index. Then, color change was observed and taken by a commercial camera. After image processing, the change of color hue with respect to time was obtained, and the measured humidity curve was calculated.

2.3.2 Starch

Starch is one of the most favorable natural polymers due to its structure and properties. Starch consists of two main structural polysaccharide polymers including linear amylose and a highly branched amylopectin which join together with **α**-D-(1–4) and/or **α** -D-(1–6) bond [95]. The structure of starch is shown in Figure 2.9. The difference of proportions of amylose and amylopectin depends on the source (e.g. corn, potato, tapioca, wheat) which largely influences physicochemical properties of

starch. High amylopectin content contributes toward more crystallinity structure, whereas high amylose content tends to be amorphous structure [96].

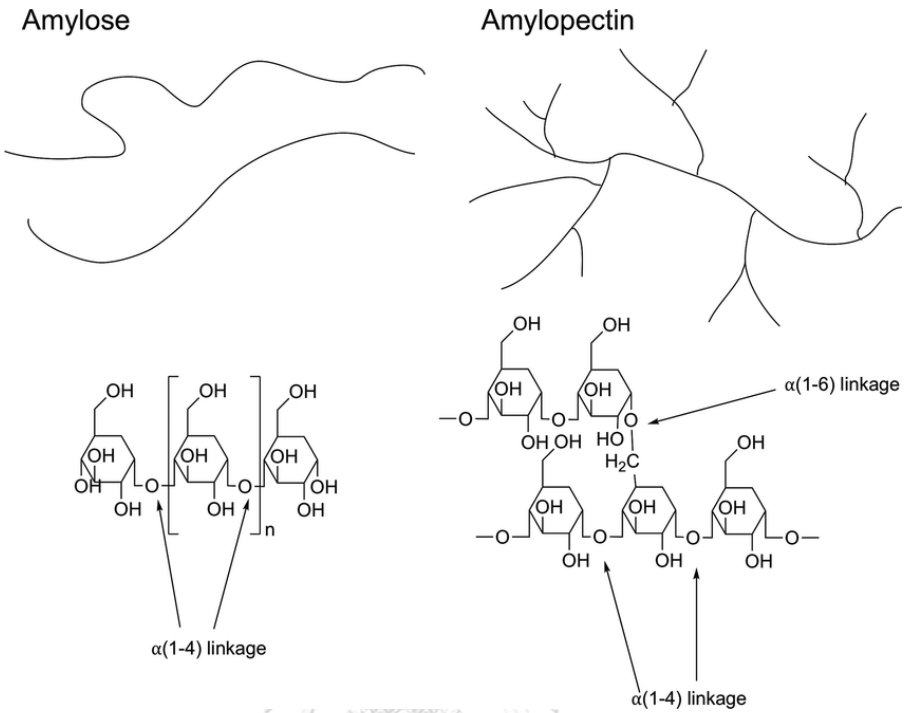


Figure 2.9 Structures of starch components a) amylose and b) amylopectin [97].

Starch can be transformed into gel via three steps of thermal reaction. In the first step, the swelling of the hydrophilic starch granules occurs by adsorption. In the second step, the gelatinization is observed after dissolving starch in high temperature condition, resulting in the destruction of the granule structure. The third step is called the retrogradation which is the reassociation or recrystallization of the polysaccharides into a gel. The properties of formed gel rely on two main parameters, such as the amount of amylose content and gelatinization temperature. The high amylose content promotes retrogradation leading to high gel strength. Therefore, starch with higher amylose content is generally selected for better film forming properties and used as aqueous based coating materials [21, 98]. The amylose contend of different sources presents in Table 2.3.

Table 2.3 Amylose and amylopectin content of starch from different sources [99].

Type of starch	Amylose (%)	Amylopectin (%)
Amylomaize	48-77	23-52
Banana	17-24	76-83
Corn	17-25	75-83
High-amylose corn	55-70	30-45
Potato	17-24	76-83
Rice	15-35	65-85
Sorghum	25	75
Cassava	19-22	28-81
Wheat	20-25	75-80
Waxy	<1	>99
Yam	9-15	85-91

From the properties of starch, it has been widely used in the various applications due to its ready availability, inexpensive, biodegradability and good film-forming properties. Furthermore, the presence of many hydroxyl groups in the starch structure can enhance the surface attachment of the enzyme [100]. The applications of starch are shown as following.

Inyoung, C. and co-workers [101] developed a new colorimetric pH indicator film using agar, potato starch, and natural dyes extracted from purple sweet potato. Color changes of the pH indicator film presented a simple and visual method to detect quality changes of food products, since the food pH changed under the spoilage processes. This method improved that large quantities of anthocyanins and its natural dyes could be immobilized into starch film and provided pH indicator films for food packaging materials. This film showed non-toxic and reliable responses toward pH variations.

Jalal, I. and co-workers [102] prepared starch films containing a chemodosimeter probe based on a quinolinium merocyanine dye for cyanide detection in pure water. Immersion of this functionalized film in an aqueous solution of cyanide induced a color change that could be used for the detection of cyanide. This easy-to-use material also showed high degree of cyanide selectivity in the aqueous medium.

Chanita, B and co-workers [103] prepared curcumin nanoparticles entrapped within starch as a colorimetric reagent for the detection of boron in the wastewater. Starch was selected as the natural polymer for the entrapment due to its film-forming properties, complete biodegradability and low cost.

Aree, C. and co-workers [104] developed phenanthroline-tapioca starch thin film for the colorimetric detection of ferrous ions in aqueous solution. Starch was introduced as the environmentally friendly substrate to entrap standard chromogenic reagent (1, 10-phenanthroline) for the fabrication of a ferrous colorimetric sensor. This sensor showed wide linear range with low detection limit and a relative high stability of over 3 months storage either in a refrigerator or in an ambient condition.

Moreover, starch can also be applied for colorimetric detection due to its ability to form iodine–starch complex. The iodine–starch complex can occur via simple reaction by mixing starch with iodine in an aqueous solution, then the color of solution is immediately changed from yellow–brown to dark blue–black color because iodine–starch complex is formed. The iodine–starch complex is a water soluble, non-toxic, and observable by naked eye [105, 106]. Therefore, the iodine–starch reaction has been widely used for colorimetric detection of different analytes, such as adenosine, glucose, amylase, hydrogen sulphide.

Jinfang, N. and co-workers [106] developed a new type of colorimetric assays in term of 2D LPCAs (two dimensional liquid-phase colorimetric assays) using old iodine–starch complex reaction. The term “2D” could be measured not only color intensity but also color length as the qualitative information. The formed iodine–starch complex provided blue—black color that could be easily observed by naked-eye. The results of the analysis required only the ability to notice the color and to count the color length-related marked bars on test tubes used to transport the mixture. This sensor showed good sensitivity for glucose detection with the linear range of 0.37–25 mM and lower limit of detection of 0.37 mM.

Meng, M. L. and co-workers [105] developed paper-based analytical devices for the detection of hydrogen peroxide and glucose coupled with starch—iodide—gelatin colorimetric assay. The analytical performance of colorimetric detection was

operated by the starch—iodine color reaction. The developed device exhibited a good linear relationship with glucose concentration ranging from 0.5 to 5mM and hydrogen peroxide concentration ranging from 0.5 to 6 mM, with the detection limit of 0.05 mM and 0.1 mM, respectively.

Liangqia, G. and co-workers [107] developed colorimetric biosensor for on-site assay of paraoxon in environmental water samples by employing the iodine—starch color reaction coupled with multi-enzyme (AChE, ChO and HRP) cascade catalytic reactions. This colorimetric biosensor showed high sensitivity for paraoxon detection with the limit of detection of 4.7 ppb. Furthermore, the colorimetric biosensor was successfully applied for the assay of paraoxon in vegetable irrigation water samples.

Sudeok, K. and co-workers [108] prepared starch modified paper-based colorimetric iodide sensor (PBCIS). The oxidant in the starch modified paper oxidized iodide ions to iodine and the iodine reacted with starch to form a blue–violet complex. The paper could be scanned in a flatbed scanner, and Adobe Photoshop was used for the measurement of iodide concentration. This iodide sensor provided reliable values of the conversion of aryl iodides.

Yingshuai, L. and co-workers [109] developed colorimetric immunoassay for ultrasensitive quantitation of prostate specific antigen (PSA) based on glucose oxidase (GOx) catalyzed cascade formation of blue-black iodine–starch complex. UV–vis spectrophotometer was considered as an instrument for quantitative detection. This immunoassay showed an ultralow limit of detection of 0.46 pg/mL and a wide linear range of 1 pg/mL-1 μg/mL.

2.4 Glucose sensor

2.4.1 Glucose

Glucose, a monosaccharide (or simple sugar), is an important metabolite substrate for energy production in the human body. Blood glucose is a sugar transported via the blood stream to supply energy. Levels of glucose in blood are tightly regulated in the human body. Therefore, glucose has been most commonly used as an important biomarker for screening diabetes and can be potentially useful for diagnosing prediabetes [105, 110]. Normally, glucose levels existing in normal

human body are ranging from 4.9-6.9 mM in blood, 2.78-55 mM in urine, 0.06-0.11 mM in sweat, 0.23-0.38 mM in saliva and 0.05-0.5 mM in ocular fluid [14]. The high abnormal levels of glucose in human body can cause of *diabetes,* resulting in a high risk of complications, including kidney damage, nerve damage, amputations and blindness [111]. The recommended way of efficiency controlling diabetes is self-monitoring of blood glucose combined with appropriate medication. Overview of technologies for glucose detection in human is presented in Figure 2.10.

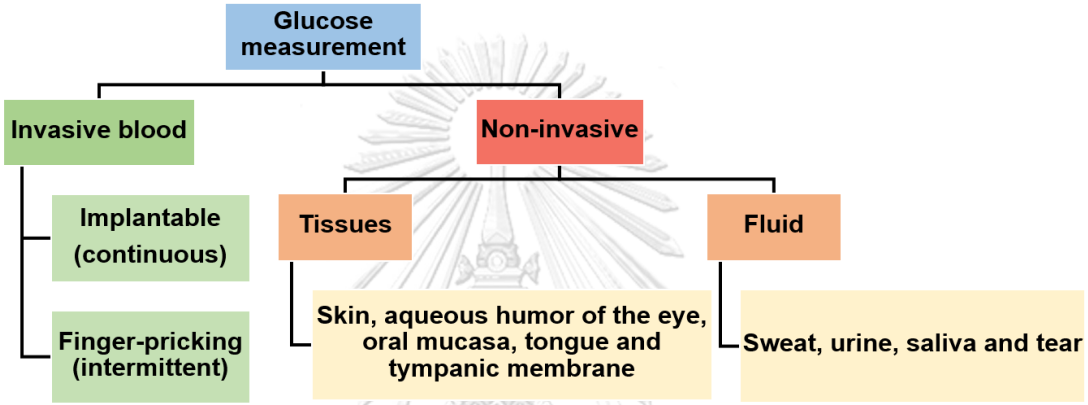


Figure 2.10 Overview of technologies for invasive and non-invasive glucose detection.

Currently, self-diagnostic device of blood glucose for diabetes diagnosis still need a finger-stick blood sample with an invasive needle (Figure 2.11), which puncture the skin for monitoring glucose concentration. This invasive procedure not only causes painfulness after repeated use for several times a day but also creates risks of tissue damage at the site of multiple punctures [112, 113]. Therefore, non-invasive glucose detection has been an interesting device providing painless measurement process.

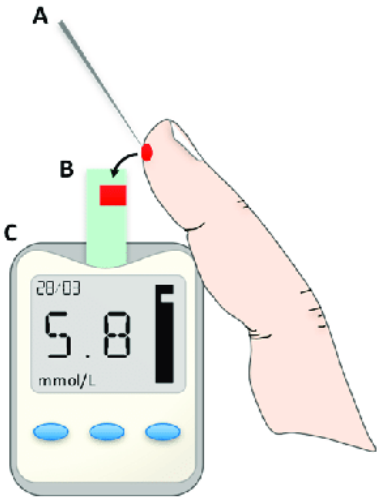


Figure 2.11 Finger pricking device consist of A. Lancet needle; B. Blood sample on test-strip; C. Glucose meter displaying glucose concentration in mmol/L.

2.4.2 Non-invasive sensor

Non-invasive sensor is a medical diagnostic device that does not require the bio fluids obtained by puncturing the body such as incision or injection. Therefore, the procedure of non-invasive sensor can reduce the user's painfulness during examination and provide lower risk of wound infection and other complications compared to the conventional invasive diagnosis. Due to its advantages, non-invasive sensor can enable more complete assessment of the health status of the person by measuring biomarkers in human fluids, such as tear, sweat, saliva and interstitial fluid [25, 114]. The targeted biomarkers can be used as measurable indicators for some biological process or condition. Therefore, the biomarkers are often measured and evaluated for early diagnosis of many diseases. Non-invasive sensor can be measured a various types of biomarkers such as protein (*e.g.* albumin, fibrinogen, hemoglobin), lipids (*e.g.* triglyceride), electrolytes (*e.g.* heavy metal ions, calcium, sodium, chlorine, potassium) and metabolites (*e.g.* glucose, lactate, uric acid) [115]. Among biomarkers, this study focuses on glucose detection as non-invasive glucose sensor which is the criteria as a key biomarker for diabetes.

The preferable way for painless measurement of glucose concentration is the replacement of blood with other body fluids that is capable for measuring glucose

levels, such as saliva, urine, sweat or tears [116]. In case of urine and saliva, the technical drawback comes from the variable water content, or dilution effect. Therefore, tear is chosen as sample for glucose detection in this study. Tear fluid is continuously replenished by the production of the lacrimal gland and other accessory glands at a rate of production in a range of 0.5–2.2 μL/min [117-119]. The literature reports relating to non-invasive glucose biosensor are shown as below.

Ellen, F. M. G. and co-workers [120] developed a paper-based colorimetric biosensor modified with chitosan for measuring glucose concentration in human tear samples. TMB and enzymatic mixture containing GOx and HRP were entrapped in chitosan modified paper. Colorimetric detection was performed using a scanner. After images were converted to Red-Green-Blue (RGB) scale, the result was analyzed in Corel Photo-PaintTM software. A wide linear range was obtained between 0.1 and 1.0 mM with the limit of detection (LOD) of 50 μM. Moreover, this device provided low-cost, pain-free and non-invasive biosensors in glucose monitoring.

Byoung-Hoon, K. and co-workers [121] developed microfluidic paper-based analysis for a non-invasive diagnosis of diabetes using a colorimetric Schirmer strip. The design of patterned wax barrier on Schirmer strip could be useful to integrate collection and detect tears. Additional solvent was injected on a sampling site after tear collection, which accumulated the dispersed glucose into active site by capillary-driven flow for signal amplification. Glucose concentration was quantitatively detected in a range of 0.1-2 mM. The colorimetric Schirmer strip provided quick, cheap, straightforward glucose colorimetric device. Moreover, it can lead to the development of point-of-care test for noninvasive diagnosis of the diverse disease-related biomarkers

Ellen F. M. Gabriel. and co-workers [54] modified paper-based analytical devices (μPADs) with chitosan to improve the analytical performance of colorimetric measurements associated with enzymatic bioassays. Quantitative analysis of glucose and UA in biological samples was performed using a chromogenic solution composed of 4-aminoantipyrine and sodium 3, 5-dichloro-2-hydroxy-benzenesulfonate (4-AAP/DHBS). The enhanced analytical performance of modified μPADs led to obtain

LODs of 23 μM for glucose and 37 μM for UA. Therefore, the proposed modified μPAD can be an alternative device for non-invasive glucose monitoring

CHAPTER III

EXPERIMENTAL

This chapter includes the information of chemical and reagent, material, instrument, experiment procedure, distance-based colorimetric detection and real sample analysis.

3.1 Chemicals and reagents

- D-(+)-Glucose (Sigma-Aldrich, St. Louis, MO, USA)
- D-(+)-Trehalose dehydrate (Sigma-Aldrich, St. Louis, MO, USA)
- Glucose Oxidase (GOx) from Aspergillus niger (5,000 units/g, Type II, ≥ 10000 units/g solid, Sigma-Aldrich, St. Louis, MO, USA)
- Hydrogen peroxide (H_2O_2) (30%; Merck, Schuchardt, Hohenbrunn Germany)
- Horseradish peroxidase (HRP) (296 units/g, (Sigma-Aldrich, St. Louis, MO, USA)
- Lubricant eye drop (Cellufresh, Waco, Texas, USA)
- Phosphate buffered saline tablet (PBS) (Sigma-Aldrich, St. Louis, MO, USA)
- Polyvinyl alcohol (PVA) (Mw 13,000, Sigma-Aldrich, St. Louis, MO, USA)
- Potassium iodide (KI) (Sigma-Aldrich, St. Louis, MO, USA)
- Rice starch (Sigma-Aldrich, St. Louis, MO, USA)

3.2 Materials

- Cotton thread (D·M·C/BLANC, Bangkok, Thailand)
- Filter paper No.1 (Whatman-GE Healthcare, Pittsburgh, PA, USA)
- Fusion 5 (Whatman-GE Healthcare, Pittsburgh, PA, USA)
- Plastic backing card (Serve Science, Bangkok, Thailand)

3.3 Instruments

- ColorQube 8570 solid ink color printer (Fuji Xerox Co., Ltd., Tokyo, Japan)
- Fourier transform infrared spectroscopy (FTIR) (PerkinElmer, Waltham, MA, USA)

- Scanning electron microscope (SEM) (JSM-6400; Japan Electron Optics Laboratory Co., Ltd, Tokyo, Japan)
- Ultrasonic bath (Branson CPX5800H, Danbury, CT, USA)
- Digital ceramic hot plate stirrer (VELP scientific, Usmate, MB, Italy,)

3.4. Preparation of stock solutions

3.4.1 Preparation of 20 mg/mL starch

0.2 g of starch was dissolved in 10 mL of deionized water and stirred by a magnetic stirrer at 150 °C in a water bath. Then, the mixture was degassed in an ultrasonicator for 15 min at a room temperature.

3.4.2 Preparation of 10 mg/mL polyvinyl alcohol (PVA)

0.1 g of PVA was dissolved in 10 mL of deionized water and stirred by a magnetic stirrer at 150 °C in a water bath. Then, the mixture was degassed in an ultrasonicator for 15 min at a room temperature.

3.4.3 Preparation of 0.5 M potassium iodine (KI)

0.1660 g of KI was dissolved in 2 mL of 0.1 M PBS.

3.4.4 Preparation of 296 unit/mL horseradish peroxidase (HRP)

1.0 mg of HRP was dissolved in 1 mL of 0.1 M PBS.

3.4.5 Preparation of 100 mM hydrogen peroxide (H_2O_2)

10 µL of H_2O_2 was dissolved in 990 µL of 0.1 M PBS.

3.4.6 Preparation of 0.3 M trehalose

0.2054 g of trehalose dehydrate was dissolved in 2 mL of 0.1 M PBS.

3.4.7 Preparation of 5,000 unit/mL glucose oxidase (GOx)

29.6 mg of GOx was dissolved in 1 mL of 0.1 M PBS.

3.4.8 Preparation of 100 mM glucose

0.0360 g of glucose was dissolved in 2 mL of 0.1 M PBS.

3.5 Modification of cotton thread

Cotton thread was firstly cleaned by plasma cleaner for 5 min to remove a non-cellulosic component of a superficial waxy layer covering on the cotton thread. The mixture solution of PVA and starch was prepared for cotton thread coating. Cotton thread was soaked and passed through a pinhole of a needle tip containing 4 µL of

PVA/starch mixture, followed by dried at room temperature. The modified cotton thread was kept in the desiccator as a ready-to-use modified cotton thread. The experimental parameters for modified cotton thread preparation were systematically optimized, such as polymers and ratio of PVA and starch. The efficiency of modified cotton thread was tested by detecting the H2O2 that is product generated by the reaction between glucose and GOx. The modified cotton thread was coated with the mixture of KI and HRP and dried at a room temperature. The volume of KI and HRP were optimized. Then, the cotton thread-based device was achieved and ready to use.

3.5.1 Effect of polymer for cotton-thread modification

The effect of polymeric composition was investigated by modifying cotton threads with pure starch, pure PVA and mixture of PVA/starch and compared to an unmodified cotton thread.

3.5.2 Effect of ratio of PVA and starch

The effect of ratio of PVA and starch for modifying the cotton thread was studied at 2:1, 1:1, 1:2, 1:3 and 1:5 (PVA: Starch).

3.5.3 Effect of KI volume

The effect of volume of KI as indicator reagent was investigate at 4, 6, 8 and 10 µL.

3.5.4 Effect of HRP volume

The effect of volume of HRP as catalytic enzyme was studied at 4, 6, 8 and 10 µL.

3.5.5 Effect of sample volume

The effect of sample volume was investigated by applying food coloring additive as an example of sample at 5, 7, 10, 15, 20, 25 and 30 µL in to sample loading zone.

3.5.6 Characterization of PVA/starch modified cotton thread

The surface morphologies of unmodified and modified threads were investigated by SEM. The chemical composition of modified thread was also

chemically characterized by FTIR. The spectra were recorded over the range 4000—400 cm^{-1}.

3.6 Device design

3.6.1 Cotton thread-based device for H_2O_2 detection

The cotton-thread based device for H2O2 detection was designed consisting of a sample loading zone (Ø = 6 mm of a filter paper) for color development and a detection zone (40 mm length of the PVA/starch modified thread) for color measurement (Figure 3.1). For the preparation, the modified thread was soaked and passed through a pinhole of a needle tip containing 4 µL of the mixture of 0.5 M KI and HRP and followed by dried at room temperature. The reagent coating process was repeated two times. Finally, the thread was ready to use for further experiment.

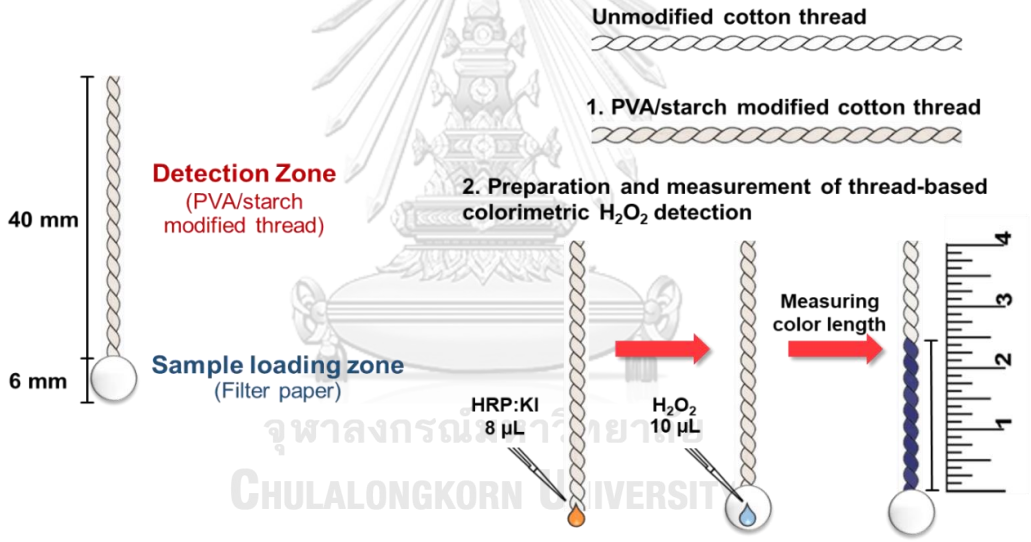


Figure 3.1 The design and the modification step of cotton thread-based device for distance-based colorimetric detection of H_2O_2.

3.6.2 Cotton thread-based device for glucose detection

To increase the analytical performances of glucose colorimetric detection, two different designs of the sample loading zone were prepared and studied. The first design was as same as the cotton thread-based device for H_2O_2 detection using a filter paper as the sample loading zone (Figure 3.2a). The second design of sample loading zone is a multilayer consisting of three layers including wax-printed filter

paper with 3 mm diameter of hydrophilic zone, double adhesive tape with 3 mm diameter of a hole and sample pad with 5 mm diameter were used to prepare sample loading zone instead of the use of only filter paper. These two designs of sample loading zone were attached on the same size of the modified cotton thread.

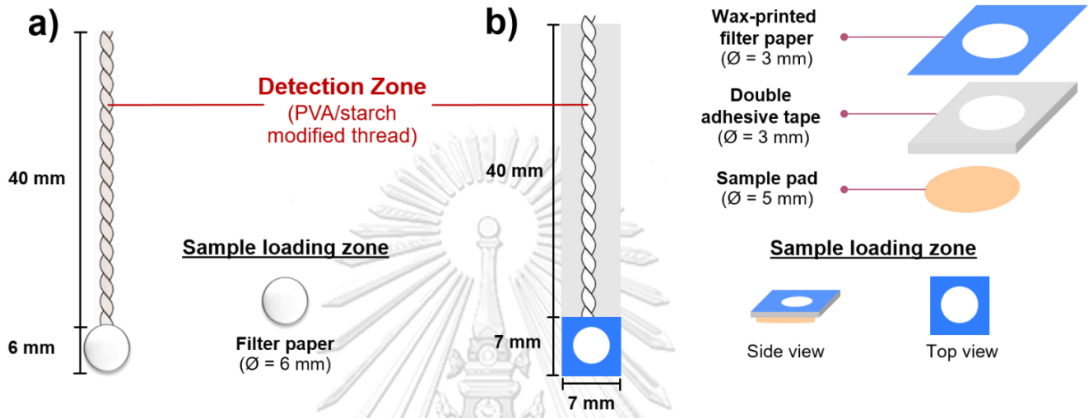


Figure 3.2 The design of cotton thread-based device for glucose detection, a) 1st design and b) 2nd design.

3.6.2.1 Preparation of sample loading zone

To prepare a sample loading zone, the design pattern was firstly printed on a filter paper using ColorQube 8570 solid ink color printer. Then, the pattern paper was placed on a hot plate at 170 °C for 40 s for melting wax into the paper to create hydrophobic and hydrophilic zones. After obtaining wax-printed filter paper, 1 µL of trehalose was spotted the paper and dried at a room temperature. After that, 0.5 µL of 5000 unit/mL GOx was spotted on the paper and dried at a room temperature. After that, the wax-printed filter paper coated with 0.3 M trehalose and GOx was immobilized on the top side of double adhesive tape with 3 mm diameter of a hole. Next, the sample pad with 5 mm diameter was also attached on the bottom side of the double adhesive tape (Figure 3.3).

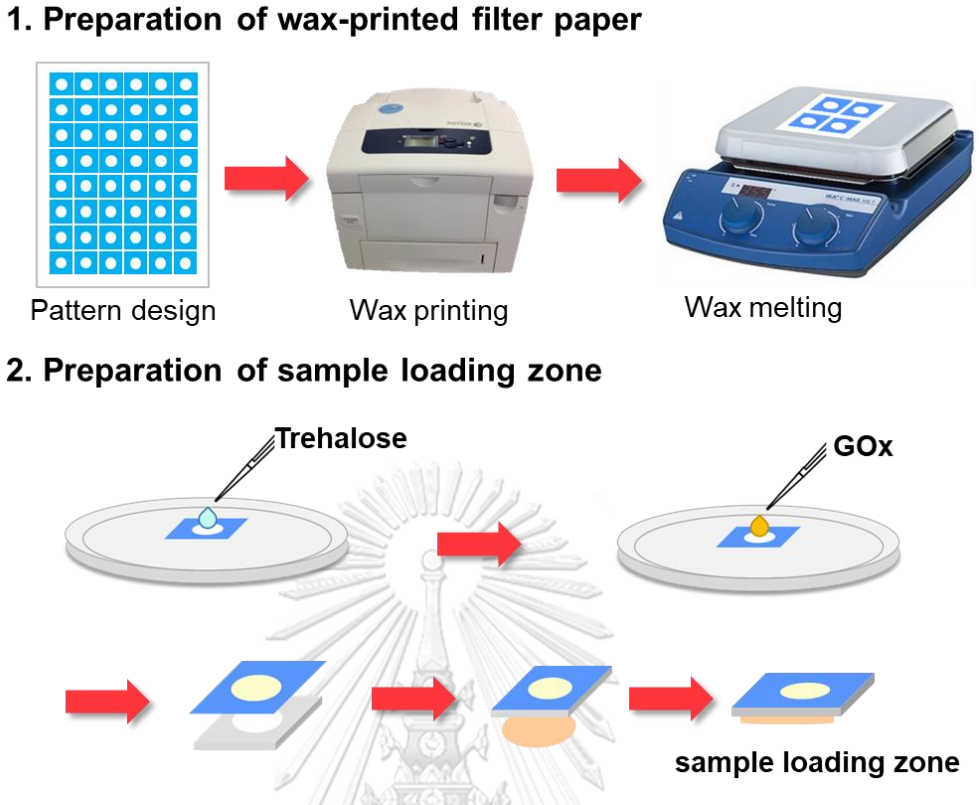


Figure 3.3 Preparation of sample loading zone of cotton thread-based device.

3.6.2.2 Preparation of cotton thread-based device for glucose detection with multilayers of sample loading zone

A plastic backing card was cut with a size of 7 mm width and 47 mm length. The detection zone was first attached on the top of the backing card followed by attaching with sample loading zone on the bottom of the backing card to be ready for glucose detection (Figure 3.4).

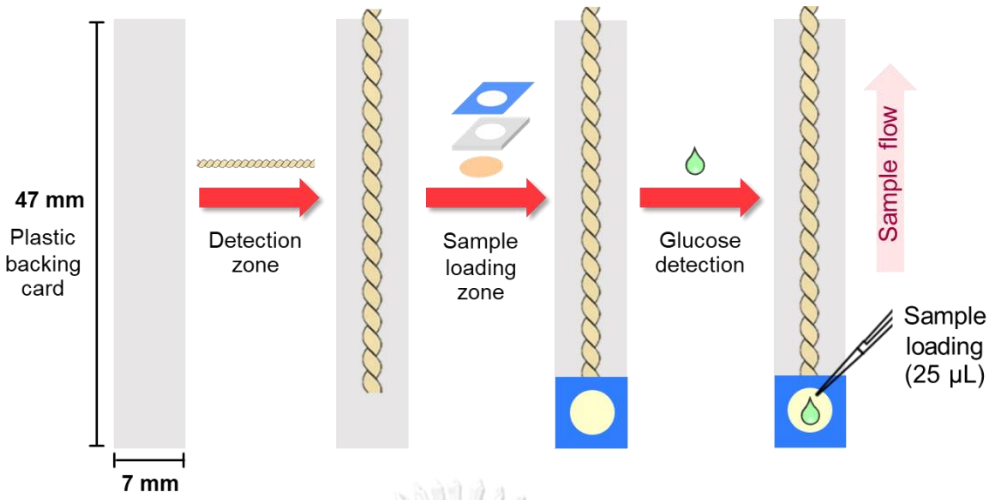


Figure 3.4 Preparation of cotton thread-based device for glucose detection with multilayers of sample loading zone

Various parameters for the preparation of sample loading zone of the second design of cotton thread-based device for the detection of glucose were optimized, such as thickness of double adhesive tape, length of modified cotton thread and diameter of hydrophilic zone of wax-printed filter to obtain a good performance for distance-based colorimetric glucose detection.

3.6.2.3 Effect of the thickness of double adhesive tape

The effect of the thickness of double adhesive tape was examined using 1.5 mm and 1 mm of double adhesive tape.

3.6.2.4 Effect of the length of the modified cotton thread

The effect of the length of the modified cotton thread was considered using different length of modified cotton thread at 4, 5 and 6 cm.

3.6.2.5 Effect of the diameter of hydrophilic zone of wax-printed filter paper

The effect of the diameter of hydrophilic zone of wax-printed filter paper was studied at 3 and 4 mm.

3.6.2.6 Effect of glucose sample volume

The effect of sample volume were investigated by applying food coloring additive as an example of sample at 10, 15, 20, 25 and 30 µL in to sample loading zone.

3.7 Detection of analytes

3.7.1 Detection of hydrogen peroxide

The different concentrations of H_2O_2 from 0.25 to 10 mM were prepared from the stock solution of 100 mM H_2O_2. Then, 10 µL of H_2O_2 was dropped on the sample loading zone. The color change band was observed and measured in centimeter (cm). Then, the calibration curve between color length (cm) and H_2O_2 concentration (mM) was plotted and a linear range was observed. The limit of detection using the device was also evaluated by the naked eye.

3.7.2 Detection of glucose

The different concentrations of glucose between 0.1 and 5 mM were prepared from stock solution of 100 mM glucose. 25 µL of glucose was dropped on sample loading zone. The color change band was observed and measured in cm. Then, a calibration curve between color length (cm) and glucose concentration (mM) was plotted to obtain linear range. The LOD of glucose detection was observed via the naked eye.

3.8 Modification of the detection zone

Previously, three-step modification of the modified cotton thread was time-consuming to prepare and perform. To reduce the preparation time, the appropriate volume of PVA/starch, KI and HRP were mixed together. Then, cotton thread was soaked in the mixture and passed through a pinhole of a needle tip followed by dried at room temperature as shown in Figure 3.5. The modified cotton thread was kept in the desiccator as a ready-to-use modified thread.

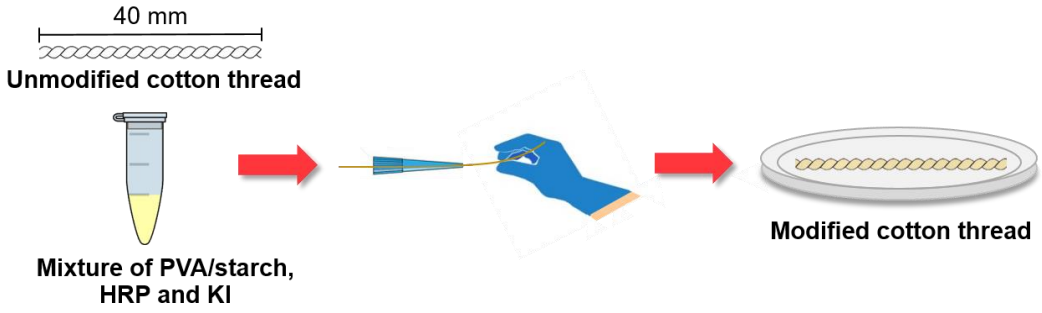


Figure 3.5 Preparation of detection zone of cotton thread-based device.

3.9 Interferece study

To study interference effect of glucose detection, 5 mM of KCl, NaCl, urea, aascorbic acid, uric acid, and cholesterol and 1.0% BSA were investigated. The selectivity of the cotton thread-based device for glucose detection was evaluated by measuring the intense dark blue–black color length compared to 5 mM of glucose.

3.10 Storage stability study

The shelf storage time-dependent stability of cotton thread-based device was studied over a period of 7 days. The cotton thread-based devices were kept at 4 °C with the close system and then used to acquire color length response for 0.5 mM of glucose.

3.11 Real sample analysis

3.11.1 Real sample preparation

The standard calibration method was selected for the detection of glucose in tear samples. The tear samples used in this study was lubricant eye drop as an artificial tear. The known concentration of glucose (0.25, 0.5 and 1.0 mM) were added to the samples. After mixing of the solution, 25 µL of solution was spotted on the sample loading zone of the device. After completed reaction, the color change band was observed and measured.

3.11.2 Recovery

The applicability of the device for real sample analysis was assessed by recovery percentage. Recovery study was used for validating the performance of a standard addition method. The equation for percent of recovery was given as following:

$$\%\ \text{Recovery} = \frac{\text{Measured value of spiked sample} - \text{Measured value of unspiked sample}}{\text{Known value of spiked sample}} \times 100$$

CHAPTER IV

RESULTS AND DISCUSSION

For this chapter, the results including optimization of cotton thread-based device for distance-based colorimetric detection of H_2O_2 and glucose, physical characterization and chemical characterization of the modified cotton thread, analytical performance of the cotton thread-based device, interference study, and real sample analysis were systematically investigated and discussed.

4.1 Optimization of cotton thread-based device

To enhance the efficiency and performance of the proposed device, several parameters, such as effect of polymer for cotton thread modification, ratio of PVA and starch, KI volume, HRP volume, and sample volume were investigated by evaluating and measuring intense dark blue–black color length.

4.1.1 Effect of polymers for cotton thread modification

Different polymer composition, including pure PVA, pure starch and PVA/starch, were modified on a cotton thread in order to enhance color signal efficiency. The unmodified, starch modified, PVA modified and PVA/starch modified cotton threads were prepared, followed by spotting KI and HRP and measuring 5 mM H_2O_2. As shown in Figure 4.1, the light-yellow color was occurred for the unmodified cotton thread, and then the apparent color change quickly faded to colorless, while yellow color was observed for the PVA modified cotton thread. Both starch and PVA/starch modified cotton thread displayed the dark blue–black color lengths, and the longest length of color and the highest intense dark blue-black color was clearly observed on the PVA/starch modified cotton thread. The modification of PVA on the cotton thread promoted the ability of the enzyme immobilization and reagent entrapment leading to substantial improvement of the color length differentiation. Furthermore, the modification of starch provided dark blue–black color length due to the formation of the iodine—starch complex. Hence, PVA/starch was selected as a modified polymer on the cotton thread which can significantly improve the color change signal, leading to a simple and rapid assay by direct readout via the naked eye.

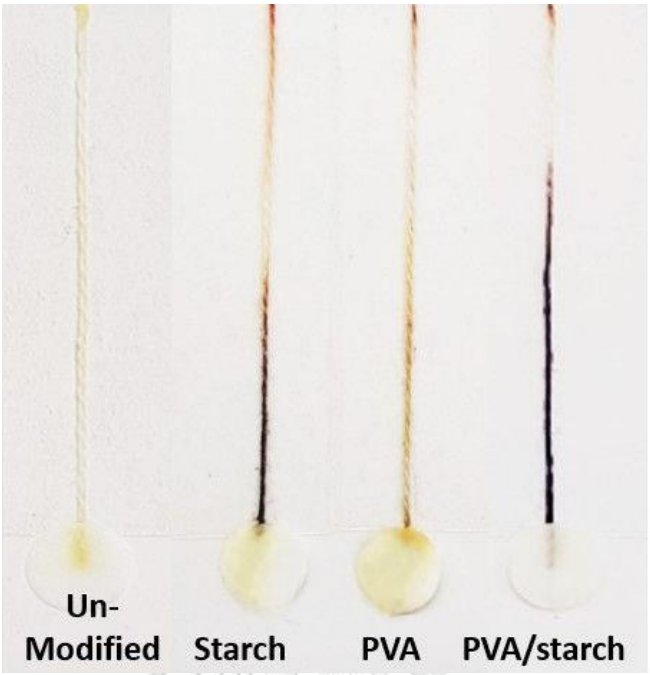


Figure 4.1 Color change of the different modifications of the cotton thread after the measurement of 5 mM H_2O_2.

4.1.2 Effect of ratio of PVA and starch

From the previous study of the effect of polymer, the mixture of PVA and starch modified on the cotton thread can significantly improve the colorimetric assay for H_2O_2 detection by enhancing the color change signal. The effect of ratio of PVA and starch (PVA/starch) was also optimized for the best performance of the detection by varying the PVA/starch ratio at 2:1, 1:1, 1:2, 1:3 and 1:5 as shown in Figure 4.2. At the ratio of 2:1 and 1:1, the measurement of the color length was difficult due to the pale color change. At the ratio of 1:3 and 1:5 with the high weight ratio of starch, short color length was observed due to the high viscosity of starch leading to difficult flowing. At the ratio of 1:2, the color signal was appropriate for both color length and color intensity. Thus, PVA/starch at the ratio of 1:2 was selected as an optimal value because the results can be clearly observed by the naked eye.

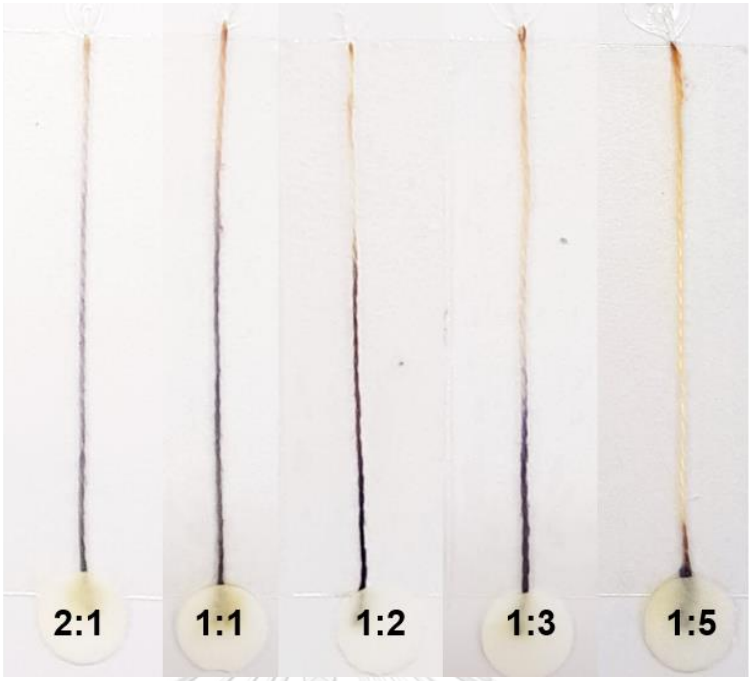


Figure 4.2 Color signal on the cotton thread modified by different PVA/starch ratios after the measurement of 5 mM H_2O_2.

4.1.3 Effect of KI volume

In this work, KI is an important substance for the detection because H_2O_2 induce the oxidation of KI (colorless) to iodine (yellow brown) via catalytic agent (HRP). Then, the intense colored iodine–starch complex is formed by reacting with starch from PVA/starch modified on the cotton thread, resulting in the appearance of dark blue–black length on the modified thread. From the importance of KI towards the detection, an effect of KI volume was studied between 4 and 10 µL. As shown in Figure 4.3, from naked-eye evaluation, 8 µL of KI volume displayed the highest color length compared to other KI volumes.

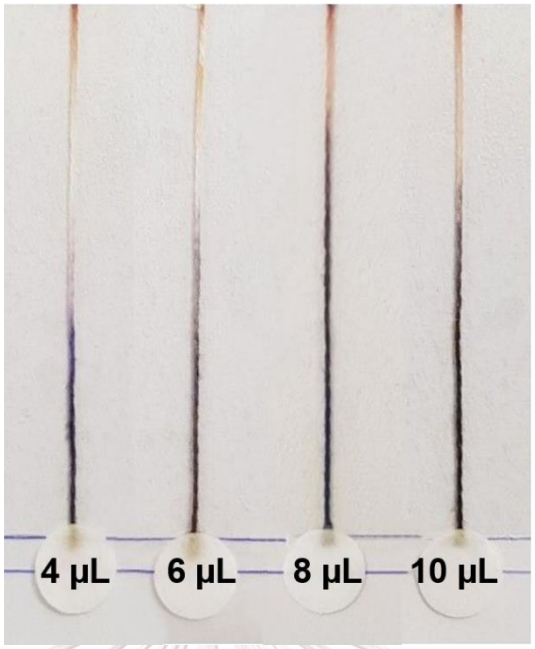


Figure 4.3 Color length on the modified thread using the different volumes of KI after the measurement of 5 mM H_2O_2.

Furthermore, the color length was also measured in centimeter (cm) and the bar graph between color length (cm) and volume of KI (µL) was plotted for more precision of the selected KI volume. As shown in Figure 4.4, the highest color length was still observed at 8 µL of KI volume. Therefore, 8 µL was selected as an optimal KI volume.

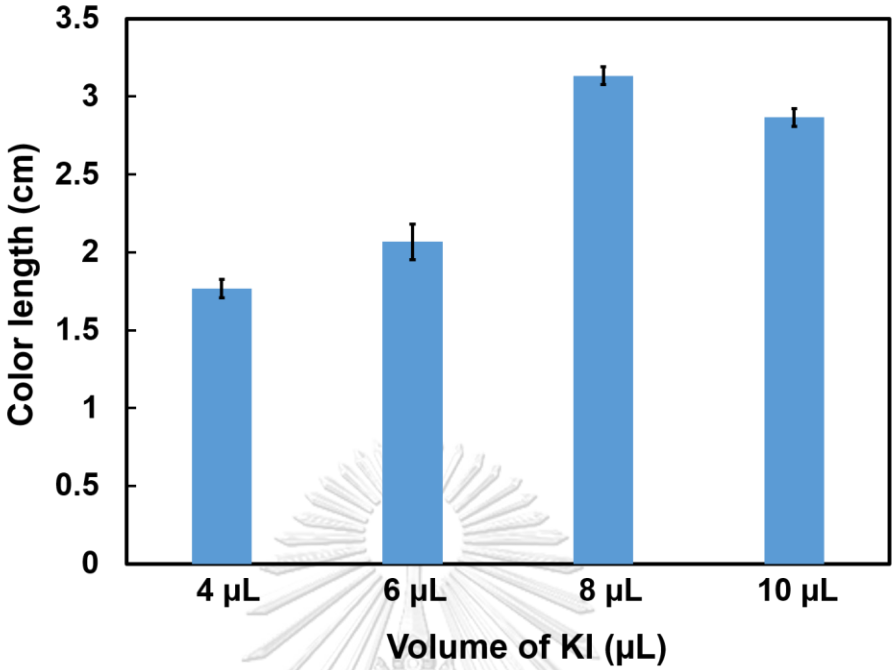


Figure 4.4 The bar graphs plotted between color length (cm) and different volumes of KI (µL).

4.1.4 Effect of HRP volume

Due to the importance of HRP as catalytic reagent for the detection, an effect of HRP volume was investigated between 2 and 10 µL for enhancing the efficiency and performance of glucose detection. From naked-eye measurement as shown in Figure 4.5, both 6 and 8 µL of HRP presented the longest color length for measuring 5 mM H_2O_2.

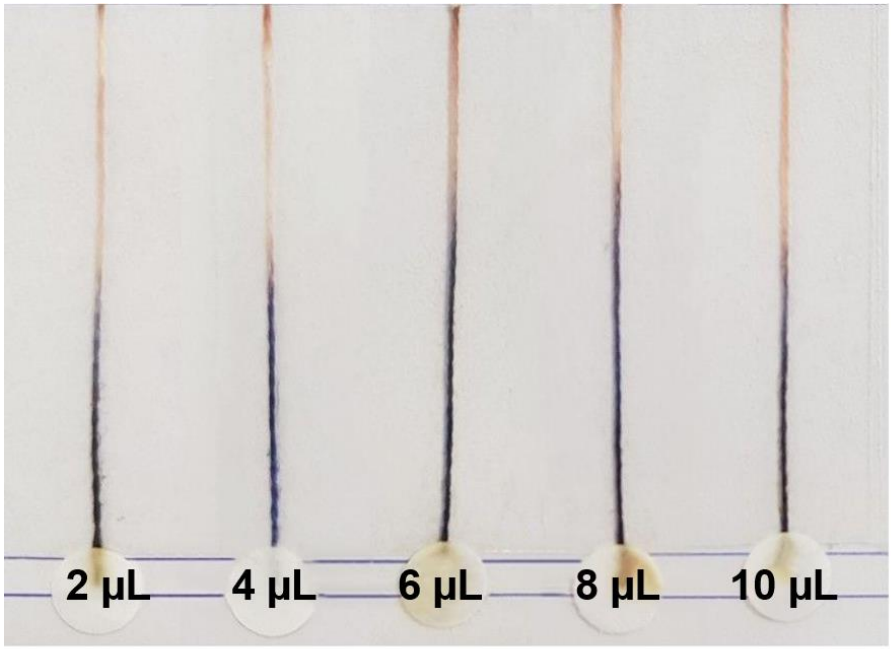


Figure 4.5 Color length on the modified thread using the different volumes of HRP after the measurement of 5 mM H_2O_2.

Furthermore, the color length was measured and the bar graph between color length (cm) and volume of HRP (µL) was plotted as shown Figure 4.6. The longest color length was still observed at 6 µL and 8 µL of HRP.

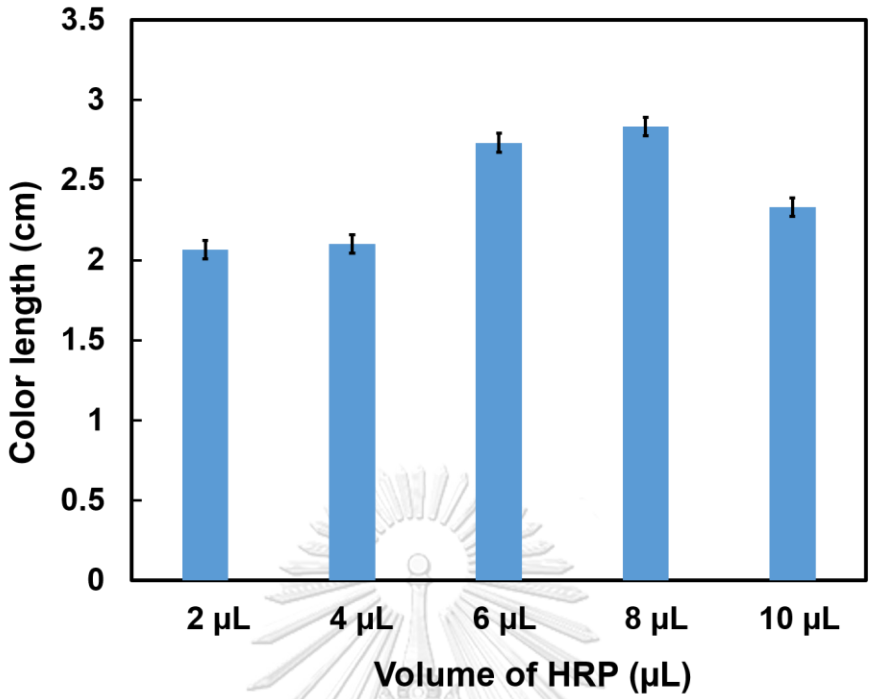


Figure 4.6 The bar graph plotted between color length (cm) and different volumes of HRP (µL).

To select the HRP volume, different concentrations of H_2O_2 between 1 and 5 mM were measured for the comparison between the use of 6 and 8 µL of HRP. As shown in Figure 4.7, 8 µL of HRP provided higher linearity than 6 µL. Therefore, 8 µL of HRP was chosen as an optimal volume for the further experiment.

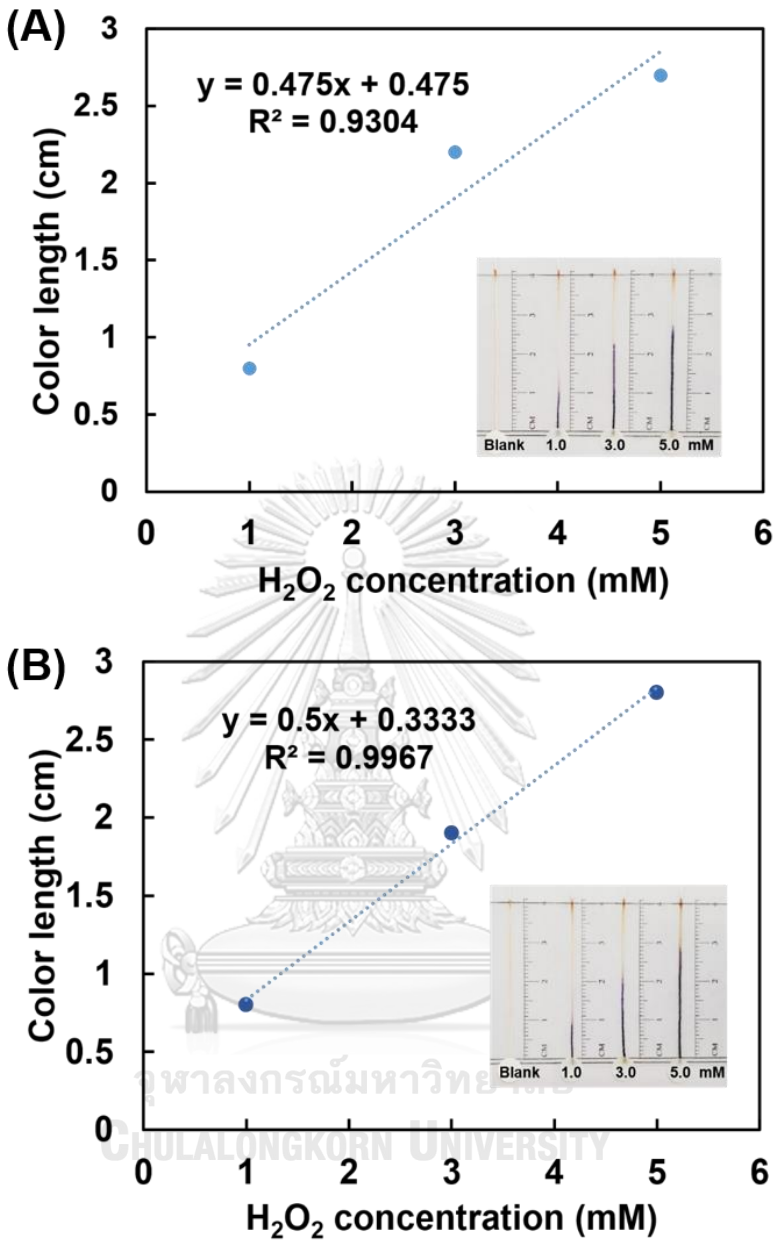


Figure 4.7 Measurement of H_2O_2 in a concentration range of 1—5 mM using different HRP volumes at (A) 6 µL and (B) 8 µL.

4.1.5 Effect of sample volume

The effect of sample volume was optimized by applying different volumes of a food coloring additive as an example of sample, followed by evaluating color length to observe an appropriate volume for H_2O_2 detection. As shown in Figure 4.8, color length on the device increased with the increasing of volume from 5—10 µL and

then the higher volume than 10 µL displayed the constant color length and the excess solution on the thread. Thus, 10 µL of the aqueous solution was chosen as an optimal sample volume for wicking along the modified thread and filling the device completely.

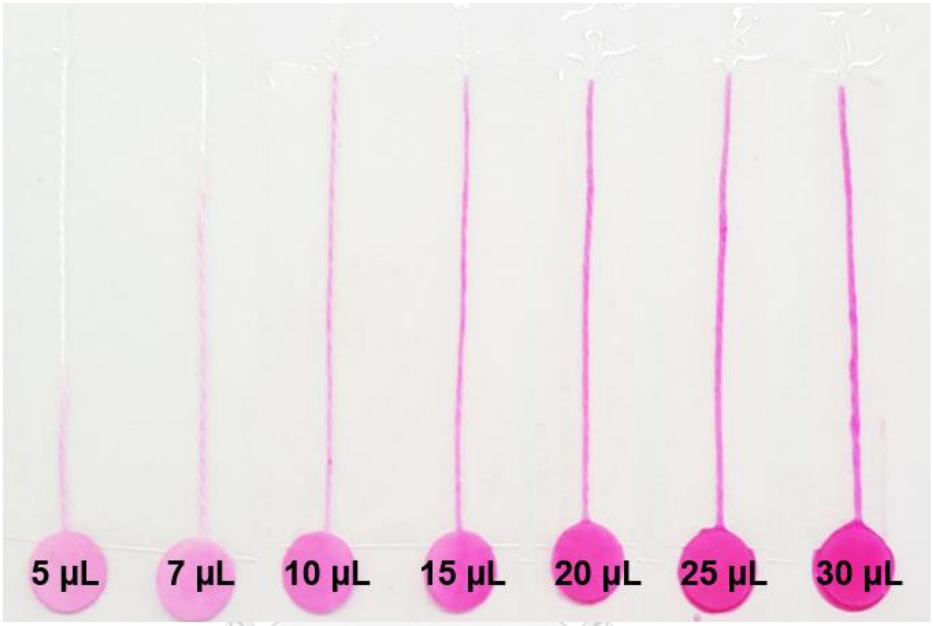


Figure 4.8 Color length of the modified thread after applying different sample volumes of a food coloring additive.

4.1.6 Characterization of PVA/starch modified cotton thread

The surface morphology of PVA/starch modified thread was investigated via SEM compared to an unmodified thread. As shown in Figure 4.9A, SEM image of the unmodified thread presented a smooth fiber surface. After the modification of the cotton thread with PVA/starch as shown in Figure 4.9B, the rough surface of the thin film was observed. These results confirmed that the surface of the modified cotton thread was well-covered with PVA/starch leading to the improvement of hydrophilicity of the cotton thread.

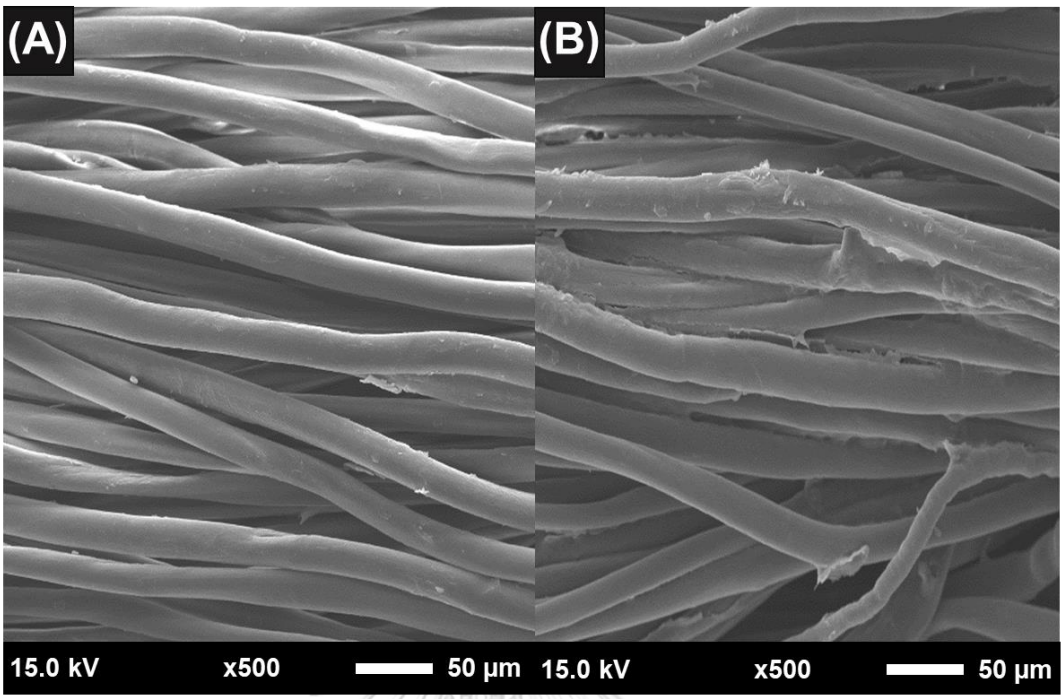


Figure 4.9 The SEM micrographs of (A) unmodified thread and (B) PVA/starch modified thread with a magnification of 500×.

For chemical characterization, FT-IR spectroscopy was used to identify the PVA/starch modified cotton thread in a range of 400 – 4000 cm^{-1} (Figure 4.10). FT-IR spectrum of the unmodified cotton thread showed the characteristic band of cellulose presented in the Table 4.1. The peak corresponding to 1724 cm^{-1} represented the carbonyl group in aldehyde or carboxylic acid. From FT-IR spectrum of PVA/starch modified cotton thread, the intensity of the carbonyl absorption peak was lower than an unmodified one due to the absence of carbonyl group (1724 cm^{-1}). This absence occurred because pectin and wax in PVA/starch, containing carboxylic group, maybe overlaid the carbonyl group [122].

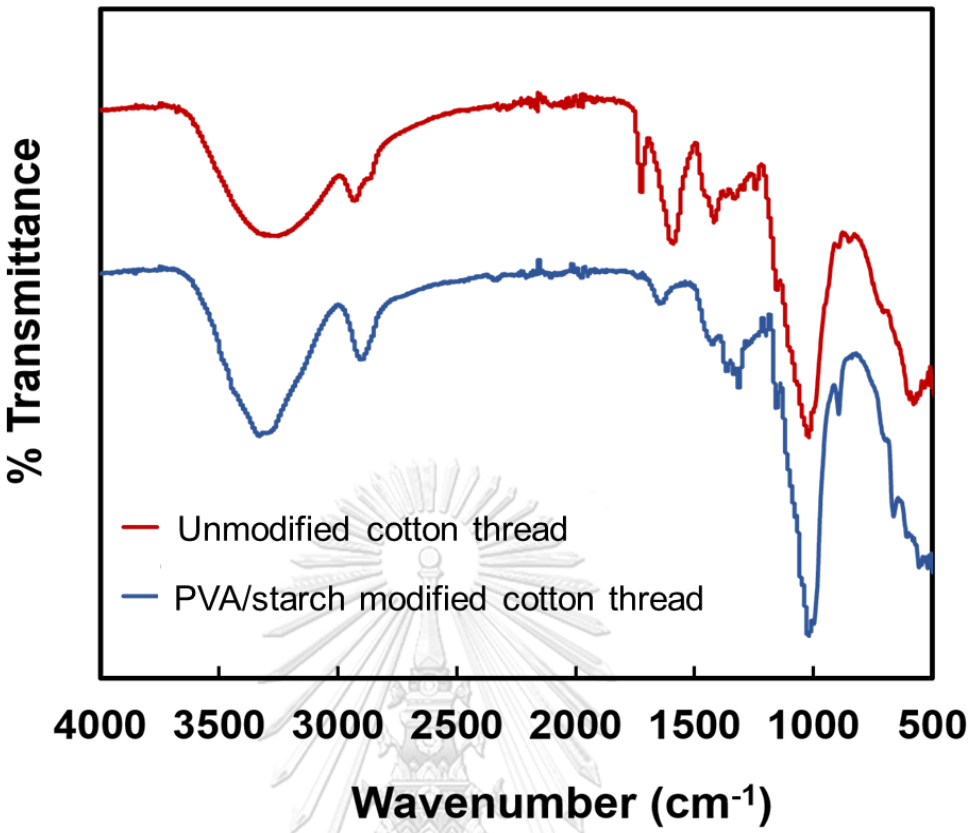


Figure 4.10 FT-IR spectra of unmodified (red line) and PVA/starch (blue line) modified cotton threads.

Table 4.1 The summary information from FT-IR spectra of unmodified and PVA/starch modified cotton threads

Literature (cm^{-1}) [123]	Experimental peaks obtained (cm^{-1})		Peak characteristics
	Unmodified cotton thread	PVA/starch modified cotton thread	
3570-3200	3270	3333	H-bonded OH stretch
3000-2800	2910	2901	C–H stretching
1728	1724	-	C=O stretch in –COOH
1650-1500	1594	1652	Adsorbed H_2O, Carboxylate (COO^-) stretch

4.2 Device design

4.2.1 Design of sample loading zone of device for H_2O_2 and glucose detection

In this experiment, two different designs of the sample loading zone were prepared and compared to obtain a suitable design in the fabrication of the cotton thread-based device for distance-based colorimetric detection of glucose according to Figure 3.2. The first design of sample loading zone contained only filter paper (∅ = 6 mm) as same as the previous device for H_2O_2 detection. The second design was multilayer of sample loading zone including wax-printed filter paper, double adhesive tape and sample pad. The aim of the fabrication of multilayer was to prolong the reaction time between glucose and GOx on sample loading zone for producing the H2O2 product to promote glucose detection performance. As shown

in Figure 4.11A, after loading sample on the first design, sample immediately wicked into the detection zone, and the color length of the detection at low concentration of glucose cannot be measured due to an incomplete binding reaction between glucose and GOx before flowing on the modified cotton thread. For the second design, after applying sample on the sample loading zone, the solution was held in sample loading zone for 30 sec, leading to the complete binding reaction between glucose and GOx before wicking into the detection zone. As shown in Figure 4.11B, the results of the use of second design indicated that both color intensity and color length significantly increased. In addition, the boundary of color length signal of the second design was easily interpreted compared to the first design. Thus, the second design of sample loading zone was selected as a well-suited device for glucose detection.

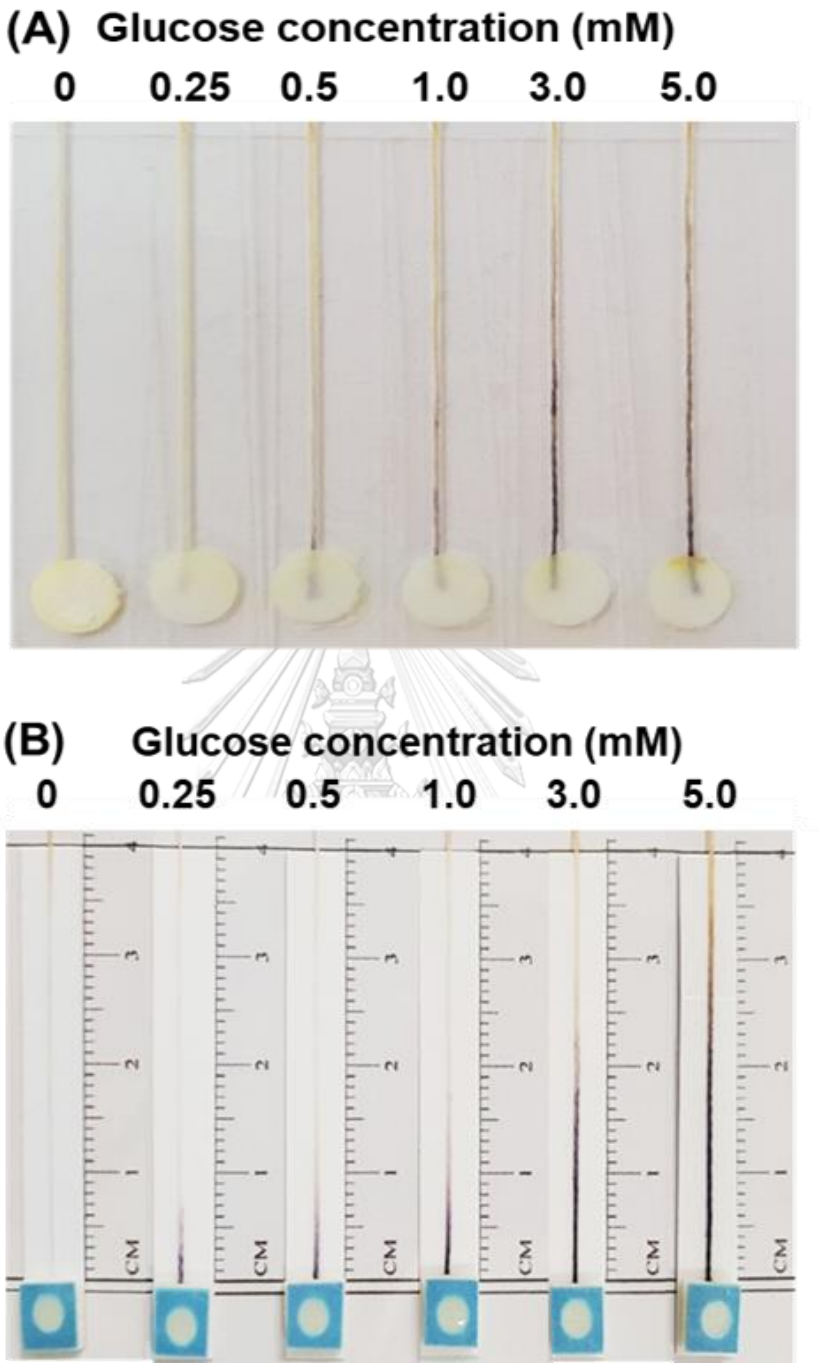


Figure 4.11 Detection of glucose in the concentration range of 0-5 mM using different designs of sample loading zone; (A) the first design and (B) the second design.

To find the optimal set of conditions for distance-based colorimetric detection of glucose using cotton thread-based device, several parameters were examined, such as thickness of double adhesive tape, length of the modified cotton thread and

diameter of hydrophilic zone of the sample loading zone. The influence of such parameters on the color development was investigated by measuring of the intense dark blue–black color length.

4.2.2 Effect of the thickness of double adhesive tape

The influence of the thickness of double adhesive tape on the color length signal of the device was investigated by using 1.0 and 1.5 mm double adhesive tape. The use of double adhesive tape aimed to slow down the sample flow for eliminating the error from incomplete binding reaction between glucose and GOx. As shown in Figure 4.12, 1.5 mm of the thickness displayed more linearity in color length signal than 1.0 mm after the measurement of different concentrations of glucose because they provided an appropriate delay time for glucose detection. The sample solution could be held on the 1.0 and 1.5 mm double adhesive tape for 25 and 30 sec, respectively. Therefore, 1.5 mm thickness of double adhesive tape was chosen for the further study.

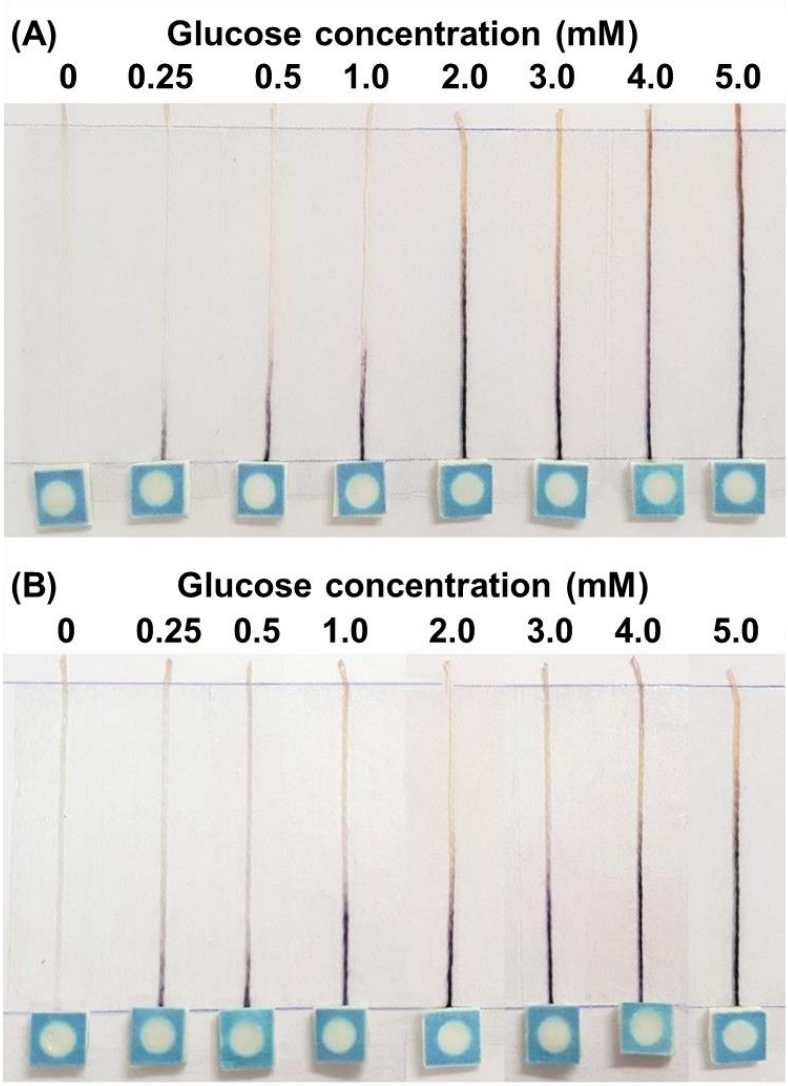


Figure 4.12 Detection of glucose using different thicknesses of double adhesive tape at (A) 1.0 mm and (B) 1.5 mm.

4.2.3 Effect of the length of the modified cotton thread

The efficiency of PVA/starch modified thread-based device as detection zone in different lengths were evaluated by comparing color length differentiation and linearity. The influence of detection zone length was studied at 4, 5 and 6 cm by using different volumes of sample at 25, 35 and 40 µL, respectively. The different lengths of the detection zone were used to measure glucose concentration ranging from 0—5 mM as shown in Figure 4.13.

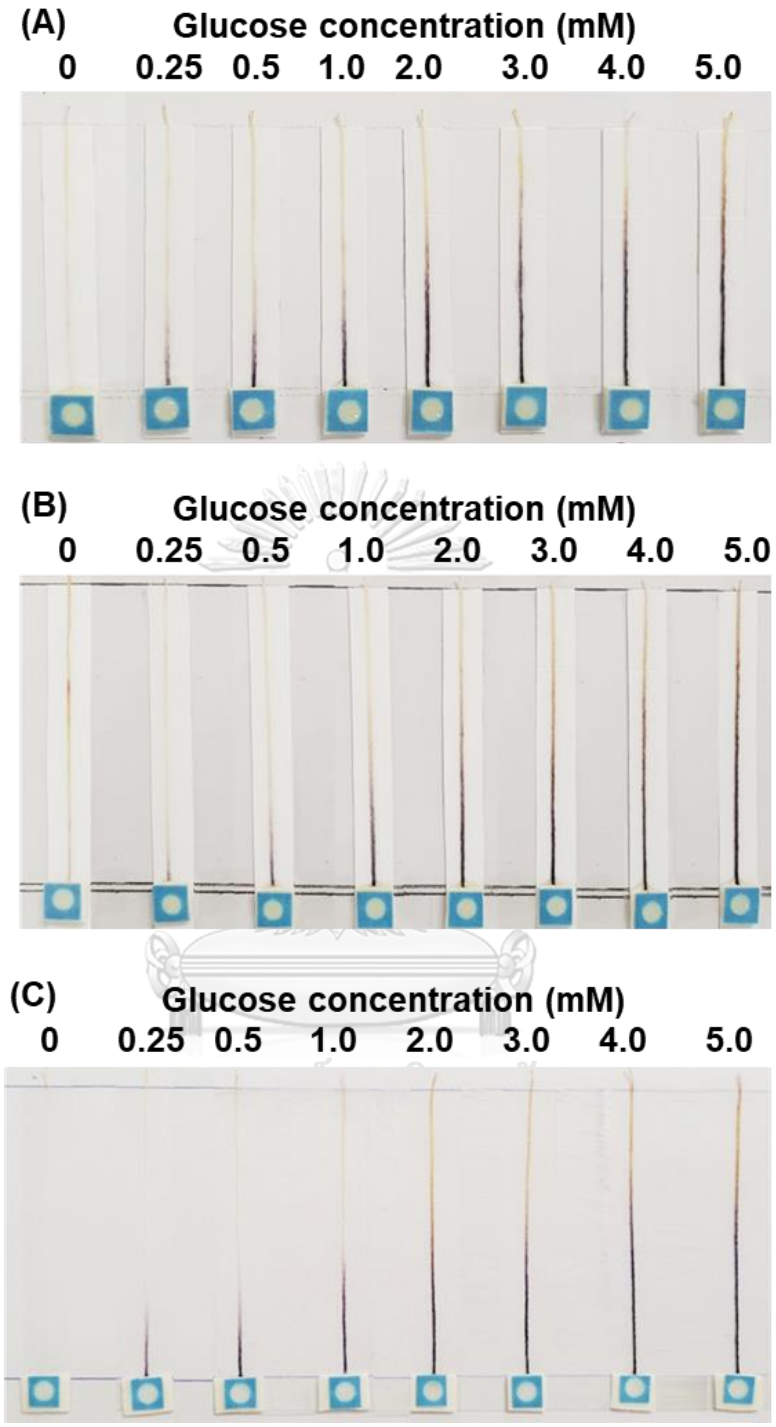


Figure 4.13 Detection of glucose using different lengths of detection zone at (A) 4 cm, (B) 5 cm and (C) 6 cm with the sample volume of 25, 35 and 40 µL, respectively.

As shown in Figure 4.14, calibration curves, in different lengths of detection zone at 4 cm, 5 cm and 6 cm, showed correlation coefficients of 0.9991 (Figure 4.14A), 0.9650 (Figure 4.14B) and 0.9086 (Figure 4.14C), respectively. The length of detection zone at 4 cm provided better efficiency than 5 and 6 cm and can be minimized the usage of sample and reagent. Therefore, 4 cm length of the modified cotton thread was chosen as an optimal length of detection zone.

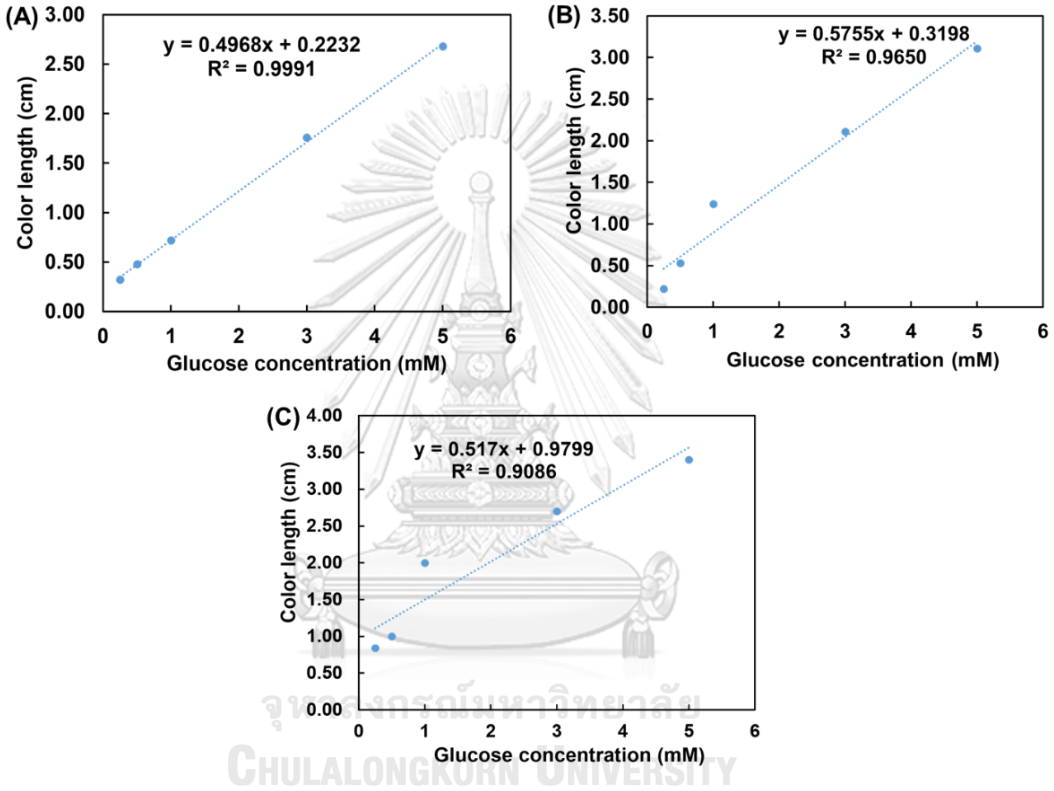


Figure 4.14 Calibration curves using the different lengths of the modified cotton thread at (A) 4 cm, (B) 5 cm and (C) 6 cm.

4.2.4 Effect of the diameter of hydrophilic zone

The effect of diameter of hydrophilic zone was studied at 3 and 4 mm as shown in Figure 4.15. After measuring glucose concentration ranging from 0—5 mM, there were no significant differences in detection efficiency between hydrophilic zones with the diameter of 3 mm and 4 mm. However, the use of hydrophilic zone with the diameter of 3 mm required smaller sample volume and faster overall reaction time (25 µL, 120 sec) than 4 mm (30 µL, 125 sec). Therefore, the hydrophilic

zone with the diameter of 3 mm was chosen as an optimal diameter size for sample loading zone.

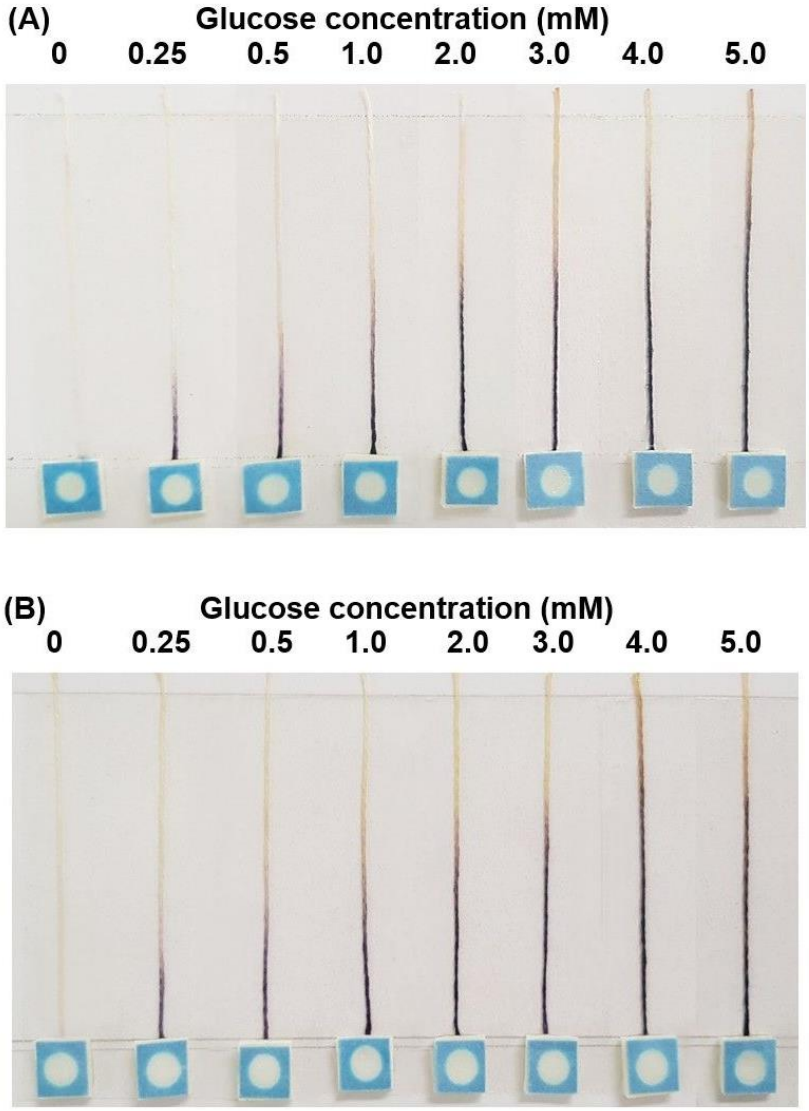


Figure 4.15 Detection of glucose using different diameters of hydrophilic zone of sample loading zone at (A) 3mm and (B) 4 mm.

4.2.5 Effect of sample volume

An effect of sample volume was optimized by applying various volumes of a food coloring additive as an example of sample, followed by evaluating color length to observe an appropriate volume for glucose detection. As shown in Figure 4.16, color length on the device increased with the increasing of volume from 10—30 µL

and then the higher volume than 25 µL displayed the constant color length and the excess solution on the thread. Thus, 25 µL of the aqueous solution was chosen as an optimal sample volume for wicking along the modified thread and filling the device completely.

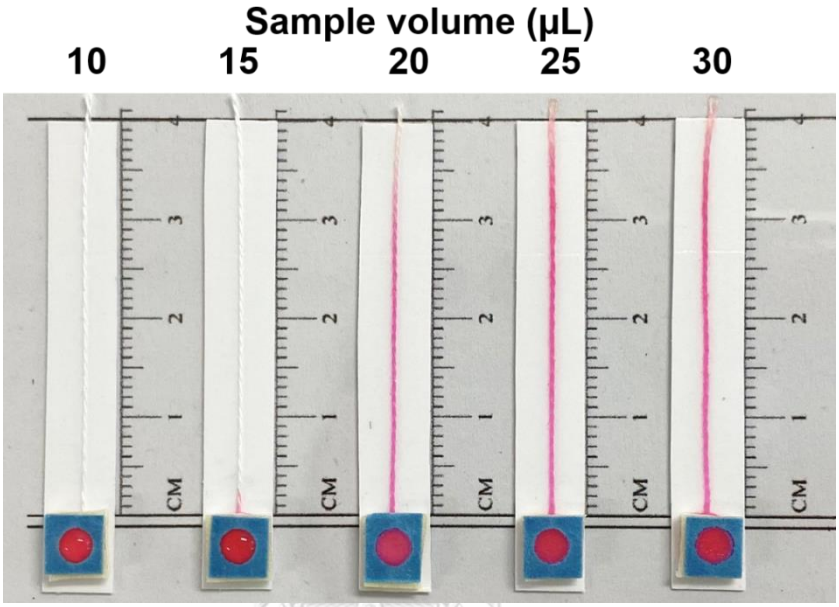


Figure 4.16 Color length of the modified thread after applying different sample volumes of a food coloring additive using 3 mm diameter of hydrophilic zone and 4 mm length of detection zone.

4.3 Detection of analyte

4.3.1 Detection of hydrogen peroxide

H_2O_2 detection was performed using cotton thread-based device based on the color length of the iodine–starch complex and the ruler-like scale marks were used for indicating color length of the detection. After applying different concentrations of H_2O_2 ranging from 0-9 mM on the device, sample wicked along the threads and filled the device within 30 sec, and the results of color length can be visually assessed by the naked eye as shown in Figure 4.17. The lowest concentration of H_2O_2 observed by naked eyes was determined to be 0.25 mM.

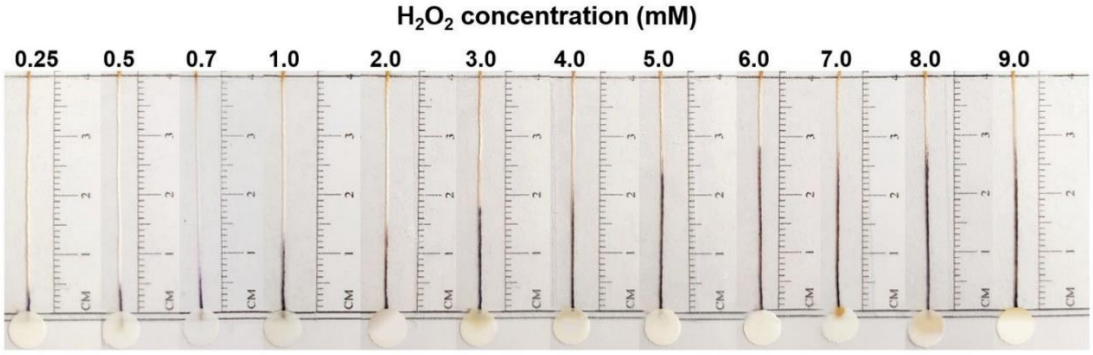


Figure 4.17 Cotton thread-based device for distance-based colorimetric detection of H_2O_2 (0.25—9 mM).

From the abovementioned results, the developed device displayed linear response in a range of 1-6 mM (Figure 4.18A) and a calibration curve between color length (cm) and concentration of H_2O_2 was plotted (Figure 4.18B) providing a high correlation coefficient (R^2) of 0.9987.

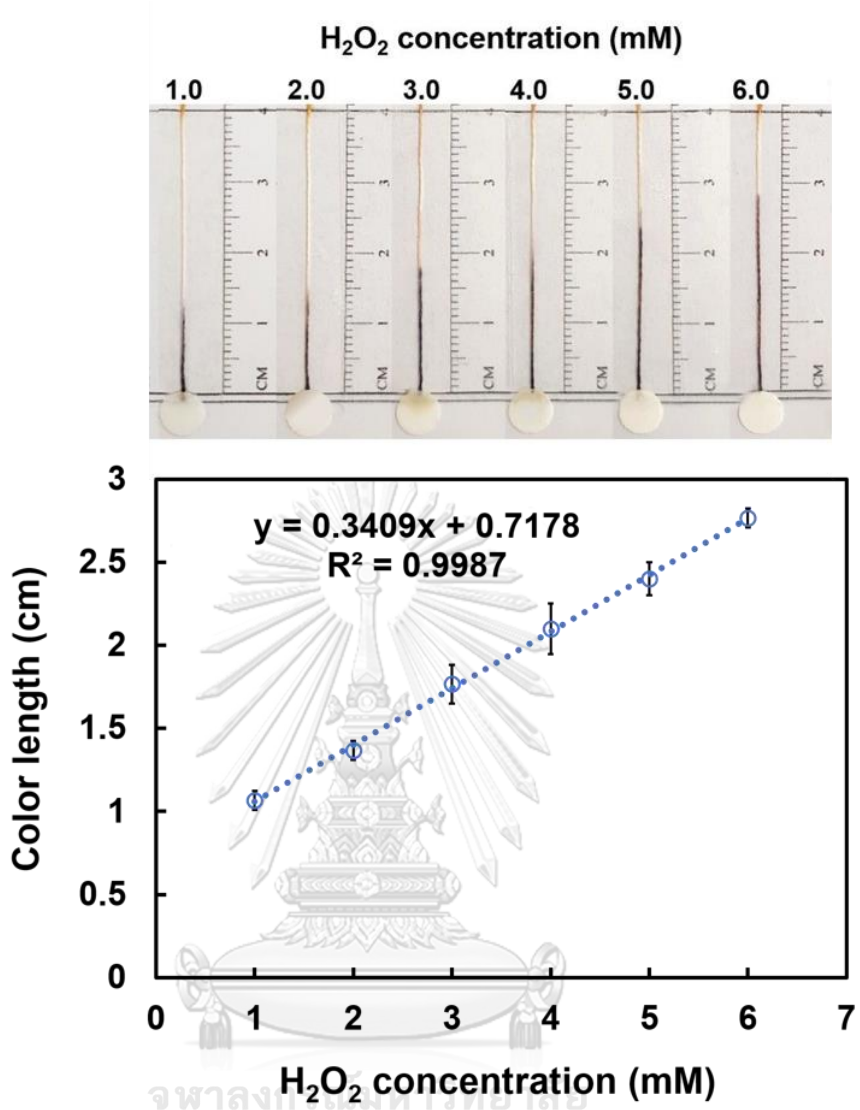


Figure 4.18 (A) Photograph image of the cotton thread-based device upon H_2O_2 detection and (B) A calibration curve between color length (cm) and H_2O_2 concentration ranging from 1 to 6 mM (n = 5).

4.3.2 Detection of glucose

The detection of glucose on the cotton thread-based device was performed by using the specific binding reaction between glucose and GOx to produce H_2O_2. Then, the reaction of the produced H_2O_2 occurred to form iodine–starch complex, resulting in the appearance of the dark blue–black length on the modified thread. For the best performance of glucose detection, the sample loading and detection zones

were attached on a plastic backing card for the easy interpretation of the color boundary, and the ruler-like scale marks were used for the naked-eye evaluation of color length. Under the optimal conditions, the glucose quantification was investigated by applying 25 µL of glucose on the sample loading zone, follow by wicking of the sample along the thread and the overall reaction was completed within 120 sec. As shown in Figure 4.19A, the color length linearly increased with the increasing of glucose concentration ranging from 0.1 to 5.0 mM and a calibration curve between color length (cm) and glucose concentration (mM) was plotted in Figure 4.19B. A linear range of glucose was observed from 0.1-5.0 mM with a correlation coefficient (R^2) of 0.9906 with the visual limit of detection of 0.1 mM. The quantification limit value and linear range were enough to differentiate between the normal (<0.5 mM) and the abnormal (≥ 0.5) glucose level in the human tear, which is an important cut-off value to indicate diabetes.

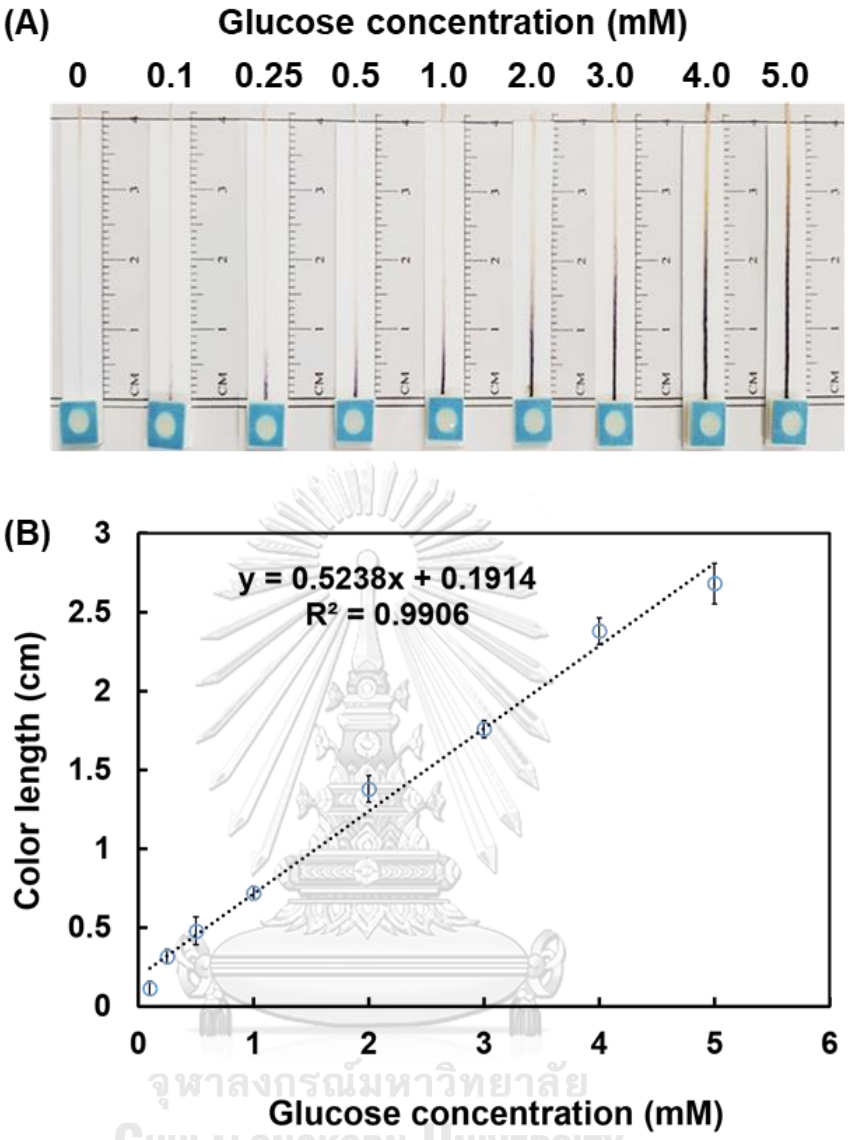


Figure 4.19 (A) Photograph image of the device upon analyzing different concentrations of glucose and (B) Calibration curve between color length (cm) and glucose concentration ranging from 0.1 to 5 mM (n = 5).

4.4 Interference study

The selectivity of the device was determined by testing the response of the device to different interference consisting of 5 mM of KCl, NaCl, urea, ascorbic acid, uric acid, and cholesterol and 1% BSA which are the overconcentration of substances existing in the human tear. As shown in Figure 4.20, the presence of the interfering substances did not interfere the detection of glucose because there are no color

changes observed in the presence of interferences. Hence, the developed device might be promoted as a high selective device for glucose detection due to the specific chemical reaction between GOx and glucose.

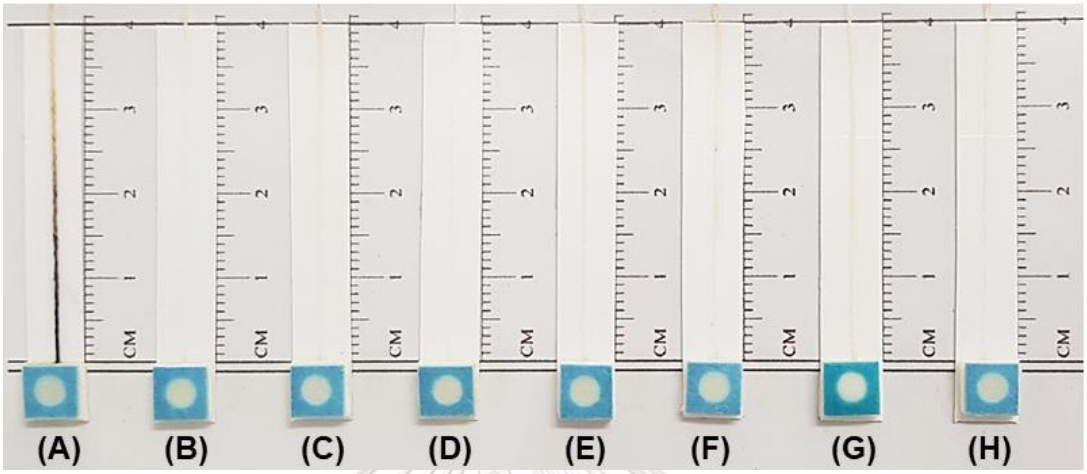


Figure 4.20 Interference study of the developed device by measuring (A) 5 mM glucose, (B) 5 mM uric acid, (C) 5 mM urea, (D) 5 mM ascorbic acid, (E) 5 mM NaCl, (F) 5 mM KCl, (G) 5mM cholesterol and (H) 1% BSA.

4.5 Stability of the cotton thread-based sensor

The stability of the cotton thread-based device was investigating by measuring the presence color length response to 0.5 mM of glucose in PBS solution multiple times (n = 5). The developed device was stored at 4°C for 7 days, and the color signal remained above 88% compared to their initial values as shown in Figure 4.21. Therefore, the stability of the developed device might be further improved.

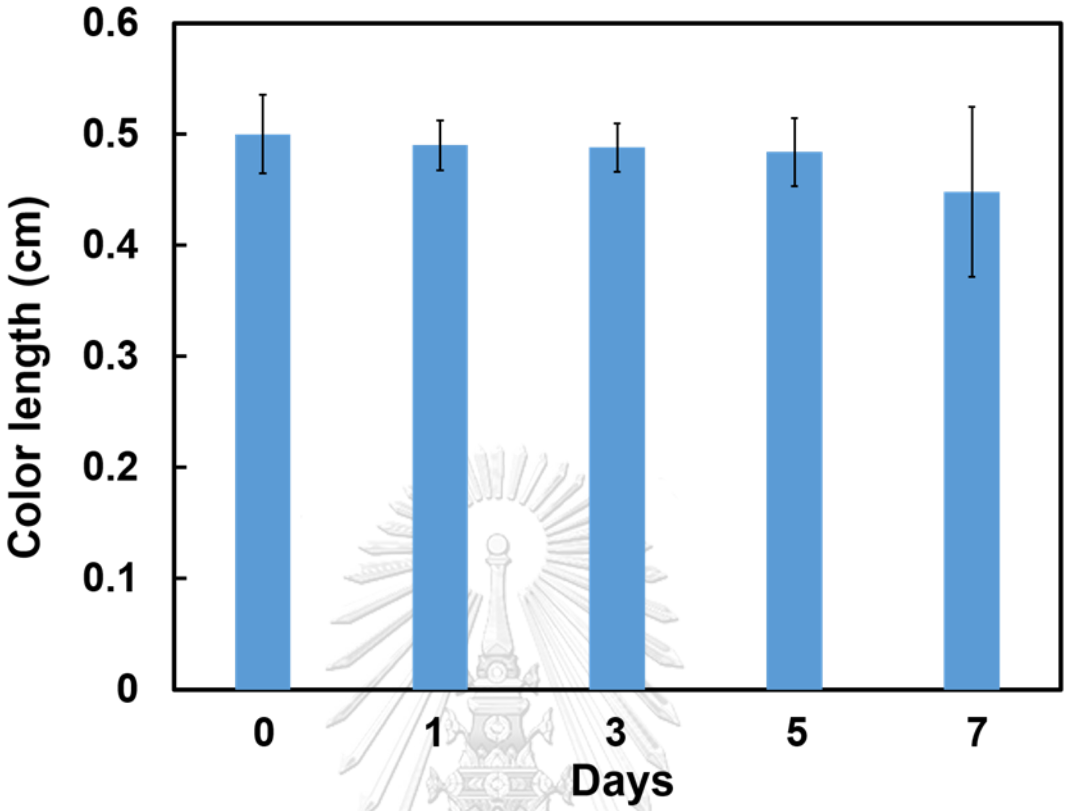


Figure 4.21 Time-dependent color length response of 5.0 mM glucose using individual cotton thread-based devices (n =5).

4.6 Real sample analysis

The inlet of the device was designed to absorb small volume of fluid. This benefit could be useful for the analysis of a small volume of sample, especially human tear fluid. Therefore, quantification of glucose in human tear fluid was investigated using the cotton thread-based device coupled with distance-based colorimetric assay. For the measurement, artificial tears as a human tear fluid were spiked with the known concentrations to be 0.25, 0.5 and 1.0 mM glucose and measured using the device as shown in Figure 4.21. The recovery percentage of the detection was calculated to be 97—104 as shown in Table 4.2. These results indicated that the developed cotton thread-based device for distance-based colorimetric detection of glucose possesses the potential for glucose detection in real sample.

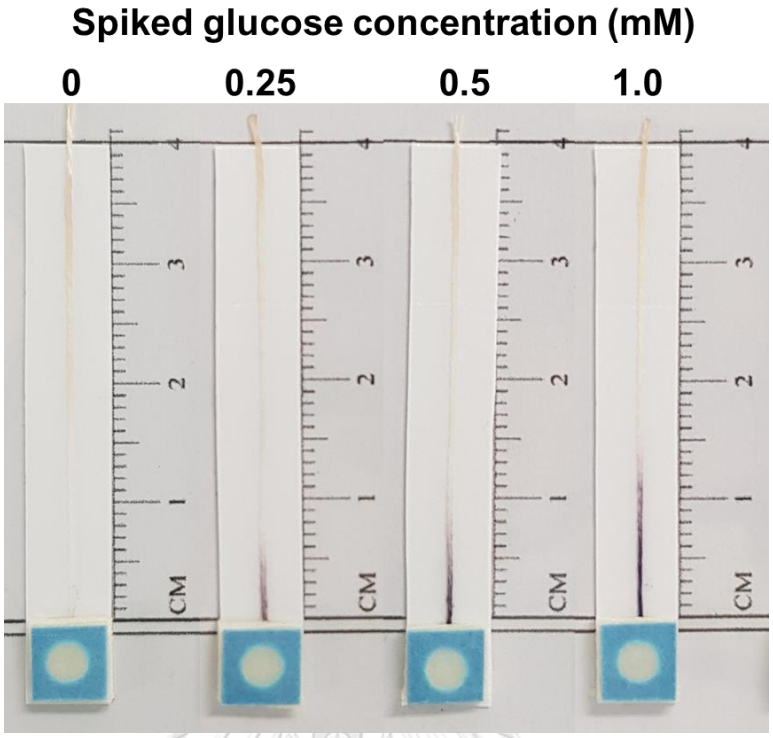


Figure 4.22 Cotton thread-based device for distance-based colorimetric detection of glucose spiked in artificial tear at concentration ranging from 0—1 mM

Table 4.2 Recovery analysis using PVA/starch modified cotton thread-based device for distance-based colorimetric detection of glucose spiked in artificial tear (n = 5)

Spiked glucose concentration (mM)	Measured glucose concentration (mM)	Recovery (%)
0	-	-
0.25	0.24 ± 0.05	97
0.5	0.51 ± 0.08	102
1	1.04 ± 0.08	104

4.7 3D printed device holder coupled with the cotton thread-based device.

3D printed device was prepared to combine with the cotton thread-based device as shown in Figure 4.23. This new platform exhibited could be suited to apply as alternative device for on-site diabetes screening.

Figure 4.23 A new platform of 3D printed device holder coupled with the cotton thread-based device.

CHAPTER V

CONCLUSION

5.1 Conclusion

In this study, the cotton thread-based device was successfully created and used as visual distance-based colorimetric readout for non-invasive detection of hydrogen peroxide (H_2O_2) and glucose. The obtained device can quantitatively detect both H_2O_2 and glucose based on the length of color change observed by the naked eye, directly correlated to the targeted analyte concentration. Since the thread can be self- microfluidic channel, liquid can wick along the thread by capillary force without an external pump requirement, applicable for be able to low sample volume measurement.

The design of the cotton thread-based device consisting of a sample loading zone and 4 cm length of a cotton thread as a detection zone for color development and measurement, respectively. For the detection zone preparation, the cotton thread was firstly modified by PVA/starch with the ratio of 1:2 to improve the enzyme and reagent stabilization leading to high detection sensitivity. Then, 8 µL of KI and 8 µL of HRP were applied on the modified cotton thread, respectively. After modification with PVA/starch, the modified cotton thread was characterized by SEM and FT-IR compared to the unmodified thread, verifying the rough surface of the thin film of PVA/starch film covered on the cotton thread.

The sample loading zone for glucose detection differed from H_2O_2 detection (one layer of sample loading zone) because of the requirement of an additional step of the interaction between glucose and glucose oxidase (GOx) to form H_2O_2 prior to measurement. Thus, the sample loading zone of the device was specially designed into multilayer including a wax-printed paper with 3 mm diameter of hydrophilic zone, a double adhesive tape with 3 mm diameter of a hole, and 5 mm diameter of sample pad. The sample solution could be held on the sample loading zone for 30 s leading to completed interaction between glucose and GOx prior to flowing to the detection zone.

For the measurement, the sample volumes of 10 and 25 µL were dropped on the sample loading zone, and the reaction completed within 30 and 120 s for H_2O_2 and glucose detection, respectively. The blue-black color band can be observed in the presence of H_2O_2 and glucose, whereas there was no band in the absence of H_2O_2 and glucose. Under the optimal conditions, this device provided a linear range of 1.0—6.0 mM and 0.1—5.0 mM with a detection limit of 0.25 mM and 0.1 mM for H_2O_2 and glucose detections, respectively. The normal range of glucose in human tear of healthy person is 0.05–0.5 mM and the exceeding concentration range can cause prediabetes or diabetes. Hence, the developed device could be differentiated the abnormal of glucose in human tear for screening diseases. Moreover, the device also exhibited excellent selectivity for glucose detection from several various interferences in human tear owing to the enzyme specificity. Eventually, the proposed thread-based colorimetric device was successfully applied for the detection of glucose in human tear with the recovery percentages from 97 to 104 %.

5.2 Suggestion works

This PVA/starch modified cotton thread-based colorimetric detection could be developed as an alternative test kit for on-site diabetes screening. The planar form of thread on the backing card in the fabrication process might affect the efficiency of glucose detection. Furthermore, this developed device might be applied for further applications such as clinical diagnosis and environmental monitoring.

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