DEVELOPMENT OF COLORIMETRIC AND ELECTROCHEMICAL METHODS FOR THE DETERMINATION OF HISTAMINE

PRANGTHIP NAKTHONG

Graduate School Srinakharinwirot University

2020

การพัฒนาวิธีการตรวจวัดเชิงสีและเชิงเคมีไฟฟ้า สำหรับวิเคราะห์ปริมาณสารฮีสตามีน

ปรางค์ทิพย์ นาคทอง

ปริญญานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตร
ปรัชญาดุษฎีบัณฑิต สาขาวิชาเคมีประยุกต์
คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
ปีการศึกษา 2563

DEVELOPMENT OF COLORIMETRIC AND ELECTROCHEMICAL METHODS FOR THE DETERMINATION OF HISTAMINE

PRANGTHIP NAKTHONG

A Dissertation Submitted in Partial Fulfillment of the Requirements

for the Degree of DOCTOR OF PHILOSOPHY

(Applied Chemistry)

Faculty of Science, Srinakharinwirot University

2020

THE DISSERTATION TITLED

DEVELOPMENT OF COLORIMETRIC AND ELECTROCHEMICAL METHODS FOR THE DETERMINATION OF HISTAMINE

BY

PRANGTHIP NAKTHONG

HAS BEEN APPROVED BY THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DOCTOR OF PHILOSOPHY
IN APPLIED CHEMISTRY AT SRINAKHARINWIROT UNIVERSITY

(Assoc. Prof. Dr. Chatchai Ekpanyaskul, MD.)
Dean of Graduate School

ORAL DEFENSE COMMITTEE

..... Major-advisor
(Assoc. Prof. Dr.Weena Siangproh, Ph.D.)

..... Chair
(Prof. Dr.Orawon Chailapakul)

..... Committee
(Assoc. Prof. Dr.Apinya Chaivisuthangkura)

Title	DEVELOPMENT OF COLORIMETRIC AND ELECTROCHEMICAL METHODS FOR THE DETERMINATION OF HISTAMINE
Author	PRANGTHIP NAKTHONG
Degree	DOCTOR OF PHILOSOPHY
Academic Year	2020
Thesis Advisor	Associate Professor Dr. Weena Siangproh , Ph.D.

Rapid and simple determination of histamine is a key requirement for freshness evaluation of fish. In this work, we developed two effective methods for histamine detection. First, we demonstrated a new paper-based colorimetric device for screening of histamine using label-free silver nanoparticles (AgNPs). The measurement is based on the oxidation of silver nanoparticles (AgNPs), which induces the etching of AgNPs and leads to the changing of color. In the presence of histamine in 0.1 M phosphate buffer solution (PBS) pH 5, the color of the AgNPs was rapidly changed from pink to orange-yellow that can be visualized in 1 min. The semi-quantitative read-out by naked-eye over a broad histamine concentration range (20-100 ppm) can be obtained using standard color chart. However, the proposed colorimetric sensor showed low selectivity because of the interference from other biogenic amines. To solve this major problem, we kept developing a novel electrochemical method for selectively quantitative analysis of histamine using modifier-free screen-printed graphene electrodes (SPGEs). In alkaline media (0.2 M NaOH), histamine detection obtained from SPGE showed a well-defined oxidation peak of histamine at +0.55 V (vs. Ag/AgCl) without any enzyme immobilization. Under the optimized conditions, the developed method can be performed with excellent selectivity, sensitivity (the limit of detection of 0.62 ppm) and wide linear range (5-100 ppm). Moreover, the proposed electrochemical method was successfully applied to detect histamine in canned fish samples with recovery values ranging from 90.72 to 101.21% and the results obtained were agreed with the standard HPLC-FLU. Therefore, the proposed methods could be alternative choices for fast screening and for the determination of histamine depending on the purpose of user. These new findings are expected to apply for practical test in the food industry in the future.

Keyword : histamine, silver nanoparticles, paper-based analytical device, screen-printed graphene electrode

ACKNOWLEDGEMENTS

First and foremost, I would like to express my very great appreciation to my advisor, Associate Professor Dr. Weena Siangproh, for guidance, support, and encouragement throughout my Ph.D. study. She helped and recommended me in all the time of research, including the journal publication and the dissertation. I feel motivated and encouraged whenever I was suffered by problems from the experiments. Without her invaluable advices, this dissertation would not have accomplished. It has been a great pleasure and honor to be under the supervision of her as my advisor.

My deepest acknowledgment goes to Associate Professor Dr. Takeshi Kondo, who invited me to join the "Sakura exchange program in science" at Tokyo University of Science in 2017. I enjoyed about the learning of both research and Japanese culture throughout 20 days in Japan. Thank you for giving me good memories and opportunities.

I would like to give special thanks to my dissertation committees, Professor Dr. Orawon Chailapakul and Associate Professor Dr. Apinya Chaivisuthangkura for investing time and providing valuable suggestions. Moreover, I would like to thank you for letting my defense be an enjoyable moment.

I greatly appreciate the support received through the collaborative work undertook with Professor Dr. Orawon Chailapakul and others members at Electrochemistry and Optical Spectroscopy Center of Excellence (EOSCE) from Chulalongkorn University. They continuously supported of chemicals and tools in the procedure of research.

The mostly important, I gratefully acknowledge the funding received towards my Ph.D. study from the Science Achievement Scholarship of Thailand (SAST) and the National Research Council of Thailand (NRCT).

Also, I would like to thank all members of the Electroanalytical and Imaging Sensor Research Group (EISRG) for their support and discussion how to improve the qualities of my research and paper. Thanks for putting up with me during my Ph.D. period and always giving me words of encouragement.

Lastly, I am deeply and sincerely grateful to my family for their love,

encouragement, patience, and understanding me. They allowed me to spend most of the time on this dissertation. I may not make it happen without their support. Thanks for listening to my problems and providing perspective.

PRANGTHIP NAKTHONG

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1
INTRODUCTION

Background

Nowadays, fish and their products are gradually becoming the favored food of people in many countries. Due to their richness in premium protein, vitamins, minerals, and low-fat food. Hence, the quality control of fish and their products must be highly concerned because of theirs effect on human health. Freshness is one of the main factor used to determine the quality of fish products. Generally, after the death of fish, there is a gradual loss of freshness because of a series of complicated chemical reactions such as proteolysis, glycolysis, and lipolysis by enzymatic reactions of microorganisms. The consumption of spoiled fish is a health risk for the consumer. Therefore, the freshness of fish becomes an important factor used to evaluate the safety of fish products. From literature, various indexes of freshness including trimethylamine, volatile amine, histamine, hypoxanthine, and hydrogen sulfide were employed to evaluate the fish freshness. Among them, the level of histamine has been proposed as an important freshness index of fish (Chena et al., 2017).

Histamine is known as an important biogenic amine which found in many food products. It is derived from the bacterial decarboxylation of amino acid histidine by histidine decarboxylase (Kumar & Goyal, 2018) . Most histamine produce during microbial decomposition of Scombroid fish such as tuna and mackerel because these fish species contain high levels of histidine (Halasz, Barath, Sarkadi, & Holzapfel, 1994). A hazardous level of histamine is produced by the microbial decarboxylation of the free histidine present in the muscular tissue of fish. For these reasons, the United States Food and Drug Administration (FDA) has established at 50 ppm of histamine as the chemical index for fish spoilage (Shahzad, Zaidi, & Koo, 2017). Furthermore, consuming in excess histamine can cause toxicity that so-called histamine fish poisoning or scombroid poisoning. There are several appearances of symptoms such as nausea, vomiting, diarrhea, and allergies (Hashemi, Nazari, & Noshirvani, 2017). Moreover, the histamine content is used as a biomarker for quality control to monitor during food production,

storage, and transportation. Hence, the determination of histamine is an important topic in food safety, clinical diagnosis, and the storage of fish products.

To estimate histamine levels for fish freshness, various analytical methods have been reported on histamine detection including thin-layer chromatography (TLC) (Tao et al., 2011), colorimetric assay (Patange, Mukundan, & Kumar, 2005), fluorometric assay and enzyme-linked immunosorbent assay (ELISA) (Leszczynska, Wiedlocha, & Pytasz, 2004). Among these mentioned, chromatographic techniques such as gas chromatography (GC) (Hwang, Wang, & Choong, 2003), high-performance liquid chromatography (HPLC) (Saaid, Saad, Hashim, Mohamed Ali, & Saleh, 2009) and capillary electrophoresis (CE) (N. Zhang, Wang, Zhang, Deng, & Zhang, 2008) are mostly reported as a precisely and reliably quantitative analysis for histamine. Although these techniques exhibit a high sensitivity and selectivity, they still have some limitations such as requirement of derivatization and sample preparation, time consumption, expensive instrumentation and the most important is that they cannot be able to reach the portability. To overcome these problems, it is a challenge to continuously develop a novel methodology for histamine detection to fulfill a role of field analysis in food supply chain.

Currently, paper-based analytical devices (PADs) are considerably attractive because they are portable, easy to use, inexpensive, small sample volume, and disposable. As a result, they have been widely used for point of care testing (POCT), food safety assurance, and environmental monitoring (Rattanarat et al., 2014). The most common detection method which used with PADs is colorimetric assay. Nowadays, colorimetric sensors based on noble metal nanoparticles (NPs) such as silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) have been widely used in PADs. Due to their excellent optical properties enable brilliant color display for sensing applications which can be visualized with the naked eyes (Yakoh, Rattanarat, Siangproh, & Chailapakul, 2018). Only a few further reports of paper-based histamine detection have been presented in the literature (Huang et al., 2017; Luo et al., 2015). In previous work, the proposed sensors were suitable for screening of histamine in real

samples. However, the main drawbacks of these methods are need for derivatization of histamine, complicated process and time-consuming of analysis. Besides, from the literature study, it was found that AgNPs have never been used as a colorimetric reagent for colorimetric detection of histamine. AgNPs have some advantages compared with AuNPs. For example, the extinction coefficients of AgNPs are higher than AuNPs of the same size and a lower cost compared to AuNPs (Jiang & Yu, 2008). For this reason, it prompts us to propose a simple paper-based colorimetric sensor for semi-quantitative analysis of histamine using label-free AgNPs.

Although the paper-based colorimetric sensor can provide a simple and rapid visual analysis method, its quantitative detection ability is poor with relatively low sensitivity and selectivity. For the quantification of histamine, the electrochemical method offers a promising alternative choice due to high sensitivity, simplicity, rapid analysis, and low cost of instrumentation in comparison to chromatography and spectrophotometry. The main drawbacks for the detection of histamine by electrochemical method are poor electrochemical properties (the high oxidation potential of histamine at 1.2-1.4 V vs. SCE (Sarada, Rao, Tryk, & Fujishima, 2000), electrode surface fouling, and the effect of interferences leading to the limitation of selectivity. To solve this problem, enzyme-based biosensors with immobilized amine oxidases (Niculescu et al., 2000), diamine oxidase (Keow et al., 2012), methylamine dehydrogenases (Zeng, Tachikawa, Zhu, & Davidson, 2000) and quinohemoprotein amine dehydrogenase (Yamamoto, Takagi, Kano, & Ikeda, 2001) have been reported in the past few decades. However, these biosensors still have drawbacks such as lifetime and cost of enzymes, complicated procedure, time consumption, and poor reproducibility. Furthermore, several reports published described the outstanding performance of electrochemical sensor for detection of histamine by modification of working electrode surface using various nanoparticles such as metal nanoparticles (Carralero, González-Cortés, Yáñez-Sedeño, & Pingarrón, 2005; Gajjala & Palathedath, 2018; Svarc-Gajic & Stojanovic, 2010) and carbon nanoparticles (Geto, Tessema, & Admassie, 2014; Shahzad et al., 2017). Recently, graphene electrodes, especially screen-printed

graphene electrode (SPGE), has been received much attention for the development of electrochemical sensors. Because of its properties such as high electrical conductivity, high electron mobility, large surface area, and high sensitivity (Panraksa, Siangproh, Khampieng, Chailapakul, & Apilux, 2018). Such benefits of SPGE, it prompts us to develop a new electrochemical sensor for analysis of histamine. And one of the most important is that as revealed by literature searches there are no research reported on the dealing with direct electrochemical oxidation of histamine using SPGE. Therefore, the second part of this research is first to develop a new electrochemical sensor for directly quantitative analysis of histamine using SPGE without any modifiers.

As mentioned, in this work, we presented two simply alternative analytical assay for histamine detection. In the first part, a simple and rapid colorimetric method was developed using label-free silver nanoprisms (AgNPrs) as a chromogenic agent for rapid screening of histamine on PADs. These devices were fabricated by wax-printing on the filter paper substrate. Subsequently, the electrochemical method was performed using modifier-free SPGE for the determination of histamine in alkaline media (NaOH). These devices were designed by screen-printed technology on the transparency film substrate. The conditions of electrochemical detection were studied and optimized using square wave voltammetry (SWV). Under optimized conditions, SPGE showed a very low oxidation potential of histamine (+0.58 V vs. Ag/AgCl) avoiding perturbations from other biogenic amines. Finally, a dual colorimetric/electrochemical detection was developed and applied for the determination of histamine in canned fish samples with high sensitivity and selectivity.

Objectives of the research

1. To develop a new colorimetric sensor for semi-quantitative analysis of histamine based on PADs using silver nanoprisms (AgNPrs).

2. To develop a novel electrochemical sensor for quantitative analysis of histamine using modifier-free SPGE.

3. To apply the proposed sensors for histamine detection in canned fish samples.

Significance of the research

1. This proposed colorimetric sensor can fabricate to be a test-kit for rapid screening of histamine.

2. This proposed electrochemical sensor can exhibit good performance with excellent sensitivity and selectivity for the quantitative analysis of histamine.

3. This proposed sensor can apply for monitoring the freshness of fish.

Scope of the research

In this research can be divided into 2 parts which are;

1. The colorimetric method was developed using AgNPrs for semi-quantitative of histamine on PADs. These PADs are fabricated by wax-printing on filter paper substrate. The effects of experimental parameters are investigated, including pH, type and concentration of AgNPrs, the ratio of AgNPrs and histamine, reaction time and interferences.

2. The electrochemical method was developed using unmodified SPGE for quantitative determination of histamine. This device is designed by screen-printed technology on transparency film. The conditions of electrochemical detection are studied and optimized using square wave voltammetry. The experimental parameters are investigated including pH, concentration of electrolyte, step potential, amplitude, and frequency. Moreover, the analytical performances of this method are also determined.

Finally, the proposed methods are applied to detect histamine in canned fish samples with high sensitivity, selectivity, accuracy, and precision.

CHAPTER 2

THEORY AND LITERATURE REVIEWS

1. Formation and intoxication of histamine

Histamine (2-[4-imidazoly]ethylamine) is known as a neurotransmitter, which is produced in the body by the decarboxylation of histidine in the presence of *histidine decarboxylase* (Kumar & Goyal, 2018). It is mainly produced in the mast cells, basophils, platelets, histaminergic neurons, and enterochromaffin cells. After that, it is stored into vesicles until it is released upon stimulation. In the normal level (20-200 nM), histamine plays an important role in different physiological functions such as the secretion of gastric acid, cell growth and cellular differentiation, the day-night rhythm, neurotransmission and immunomodulation (Comas-Baste, Latorre-Moratalla, Bernacchia, Veciana-Nogues, & Vidal-Carou, 2017). Generally, histamine can be metabolized in 2 pathways by *diamine oxidase* (DAO) and *histamine-N-methyl transferase* (HNMT) shown in Figure 1 (Wifling, 2015). Although, histamine is metabolized by enzymatic activity in the human gut and converted into less physiologically active products. However, the detoxification process becomes insufficient when excess histamine is consumed.

In addition, histamine is known as an important biogenic amine which can be found in a variety of food such as seafood, cheese, sauerkraut, beer, wine, and processed meat. In general, it is formed in food by the decarboxylation of histidine with bacterial histidine decarboxylase (Yadav, Nair, Sai, & Satija, 2019). The level of histamine in food depends on the enzyme activity, the temperature and time during food processing and storage. Histamine concentration in food is directly relevant to human health and food quality. Moreover, histamine has been proposed as a chemical freshness index of fish, due to *Scombridae* species of fish such as tuna and mackerel contain a high level of histidine (Halasz et al., 1994). The levels of histamine at 50 mg/kg or higher can cause spoilage of fish. Furthermore, consuming in excess histamine can cause toxicity that so-called histamine fish poisoning or scombroid poisoning. There are several appearances of symptoms such as nausea, vomiting, diarrhea, and allergies

(Hashemi et al., 2017). The severity of symptoms depends on both the amount of histamine ingested and the sensitivity of the individual to histamine poisoning.

To prevent the potential risk provoked by histamine in foods, the United State Food and Drug Administration (FDA) has established at level of 50 ppm of histamine as the chemical index for fish spoilage. And the guidance levels suggested for histamine content in seafood are: for safe consumption < 50 ppm; possibly toxic 50-200 ppm; probably toxic 200-1000 ppm, and toxic and unsafe for human consumption >1000 ppm (Shahzad et al., 2017). In addition, the European Union (EU) has established Commission Regulation No. 2073/2005 to limit the amount of histamine in food must lower than 200 ppm (Lin, Chen, & Lin, 2018). Hence, the detection of histamine is an important topic in food safety, clinical diagnosis, and storage of fish products.

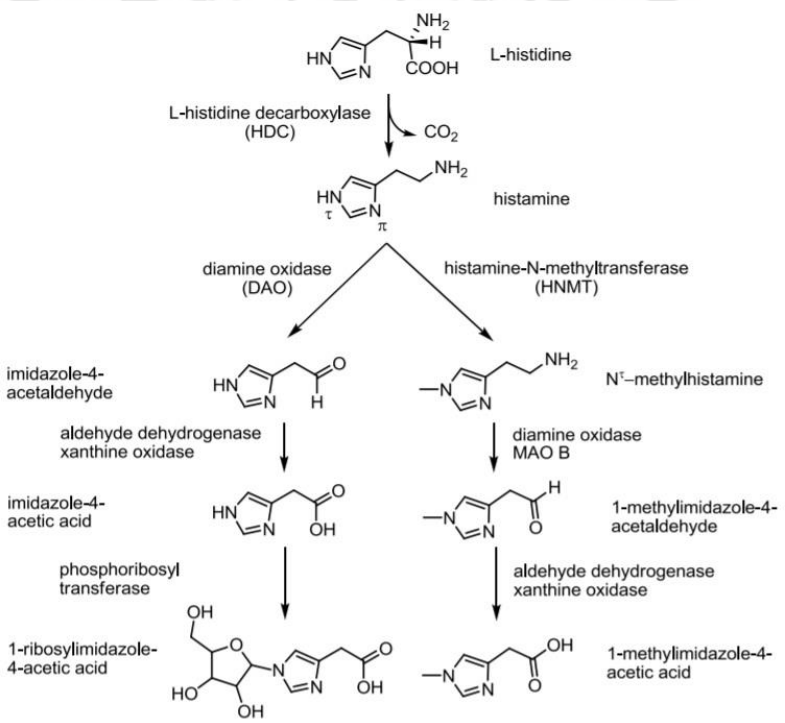

Figure 1 Synthesis and metabolism of histamine

From: Wifling, D. (2015). Constitutive activity of the human histamine H_4 receptor: Molecular modelling, binding and functional studies on wild-type and mutant H_4R orthologs. Dissertation. University Regensburg. Germany. p 15.

2. Methodology for histamine analysis

In fact, there are two main reasons for the determination of histamine in fish including the toxicity on human health and the possibility used as an index for fish freshness. For these reasons, numerous analytical methods have been developed for the detection of histamine. The most common analytical methods for determination of histamine consisted of the well-accepted Association of Official Analytical Chemists (AOAC) fluorometric method (AOAC 977.13), the spectrofluorometric method, enzyme-linked immunosorbent assay (ELISA) methods, the colorimetric enzyme test, and high-performance liquid chromatography (HPLC) methods which summarized in Table 1 (FAO/WHO, 2013).

Table 1 Comparison of the commonly used test methods for histamine detection.

	AOAC method	Spectrofluorometric method	ELISA	Colorimetric method	HPLC method
Time needed for one test	1-2 h	1-2 h	1 h	1 h	1 h
Equipment	fluorometer	spectrofluorometer	spectrophotometer	spectrophotometer	HPLC
Limit of quantification	1-5 ppm	1.5 ppb	2-5 ppm	20 ppm	1.5-5 ppm
Range	1-150 ppm	1.5 ppb - 100 ppm	0-500 ppm	0.8-300 ppm	5-2,500 ppm
General advantages	-robust -repeatable -accurate -precise	-accuracy -precision -good recovery -not expensive	-Easy (kit) -fast -low equipment costs -possibility of multiple tests simultaneously	-Easy (kit) -fast -low equipment costs -possibility of multiple tests simultaneously -semi-quantitative evaluation by visual colorimetry	-quantification of all biogenic amines -accuracy -precision

From: FAO/WHO. (2013). Joint FAO/WHO expert meeting on the public health risks of histamine and other biogenic amines from fish and fishery products. p 17.

As mentioned, there are several methods proposed for the determination of histamine, however, these methods still have limitations such as requirement derivatization step, need for sample preparation, time consumption, expensive instrumentation, use of unstable and high-cost of enzymes, and cannot meet to the portability. To overcome these problems, it is a challenge to continuously develop a novel method for histamine detection.

3. Paper-based analytical devices

In recent years, the development of paper-based analytical devices (PADs) has grown exponentially with significant impact on academia and industry. PADs have been widely used for point of care testing (POCT), food analysis, and environmental monitoring (Rattanarat et al., 2014). For creating PADs, the use of paper as a substrate is so attractive due to it has many unique advantages as shown in Figure 2 (Akyazi, Basabe-Desmonts, & Benito-Lopez, 2018). As can see below, PADs have a number of merits such as easy to use, portable, inexpensive, low sample volume, and disposable.

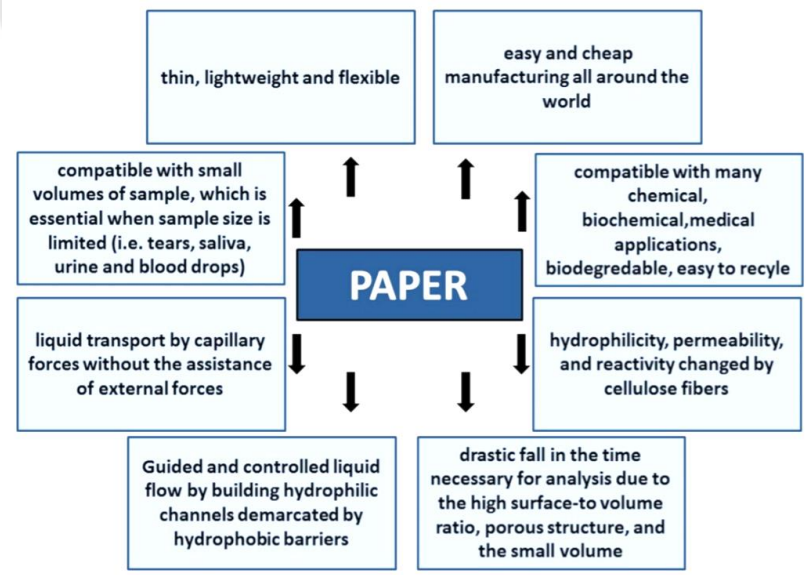

Figure 2 Key points of paper for paper-based analytical devices (PADs)

From: Akyazi, T., Basabe-Desmonts, L., & Benito-Lopez, F. (2018). Review on microfluidic paper-based analytical devices towards commercialisation. p. 3.

3.1 Fabrication method

The main goal of fabricating PADs is to create hydrophobic barriers and hydrophilic area on paper substrates. In 2007, PADs platform is first invented by Whiteside group at Harvard University. This device was created by photolithography method on paper for glucose and protein detection (Martinez, Phillips, Butte, & Whitesides, 2007) . Since then, various techniques for PADs fabrication have been developed such as wax printing, inkjet printing, photolithography, flexographic printing, plasma treatment, laser treatment, wet etching, screen-printing and wax screen-printing (Xia, Si, & Li, 2016) . In this dissertation, wax printing was selected for fabrication of PADs because this technique is simple, fast, low cost, while it needs only computer designed pattern. Wax printing uses wax as a blocking material to form hydrophobic barriers that aqueous solutions cannot cross. The fabrication of PADs by wax printing on paper substrate consist of two steps:

1. Printing patterns of wax on the surface of the paper using a wax printer.

2. Melting the wax into the paper to form hydrophobic barriers using a hot plate. Printing procedure was shown in Figure 3 (Carrilho, Martinez, & Whitesides, 2009).

Figure 3 Wax printing techniques for fabrication of PADs

From: Carrilho, E., Martinez, A. W., & Whitesides, G. M. (2009). Understanding wax printing: a simple micropatterning process for paper-based microfluidics. p. 7092.

3.2 Detection in PADs

With the development of PADs, various conventional detection techniques, such as colorimetry (H. Wang et al., 2014), electrochemistry (Rattanarat et al., 2014), fluorescence (Thom, Lewis, Yeung, & Phillips, 2014), chemiluminescence (Yu, Wang, Ge, & Ge, 2011), and electrochemiluminescence (Wu et al., 2015) have been applied to test both single and multiplex analytes. However, colorimetry is one of the most widely employed techniques for PADs because of the advantages of visual readout, rapid detection, feasibility for remote area applications, easy operation and high stability (Fu & Wang, 2018). Typically, the colorimetric assay can be detected by visually observing the change color, intensity, or brightness caused by the reaction between analytes and chromogenic agents. The color intensity is proportional to the analyte concentration. This technique can be monitored data obtained by the scanner, smartphone, or digital camera. Nevertheless, the important challenge for the development of colorimetric PADs is achieving a low detection limit with high sensitivity and selectivity.

Colorimetric method based on silver nanoparticles

In the past decade, colorimetric assays based on noble metal nanoparticles (NPs) such as gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) have been widely used in PADs, due to unique optical, electrical, and photothermal properties. Basically, when the dimensions of the metal are reduced to the nanoscale, the optical properties are dominated by a collective oscillation of conduction electrons in resonance with incident electromagnetic radiation. This phenomenon is termed surface plasmon resonance (SPR). For noble NPs exhibit a strong plasmon resonance band in the visible region. The SPR band intensity and wavelength depended on the size and shape of the particles, the type of metal, the dielectric properties of the medium, and the distance between particles (Schofield, Haines, Field, & Russell, 2006). Therefore, they can be used as a color-reagent instead of using chemical reagent.

For optical sensors, the color changes associated with NPs aggregation have been exploited in the development of colorimetric assays. The colloidal solutions of AuNPs and AgNPs have high extinction coefficients and a different color in the visible

region. The visualization of color change between an individual and well-spaced NPs compared to aggregate ones is easy to observe, such as red to blue for AuNPs and yellow to brown for AgNPs. Hence, a well-designed chemical interaction between the analyte and NPs surrounding leads to a change of color and the visual detection of the target analyte (Vilela, Gonzalez, & Escarpa, 2012). For example, Yakoh et al. proposed a simple paper-based colorimetric sensor for detection of chloride ions (Cl^-) using label-free silver nanoprism (AgNPrs) as shown in Figure 4. The measurement is based on the oxidative etching of the AgNPrs to smaller silver nanospheres (AgNSs) by Cl^-. In the presence of Cl^-, it can catalyst the oxidative etching of AgNPrs. The color change of the AgNPrs from dark-violet to red (AgNSs) was visualized by the naked eye in 5 min (Yakoh et al., 2018).

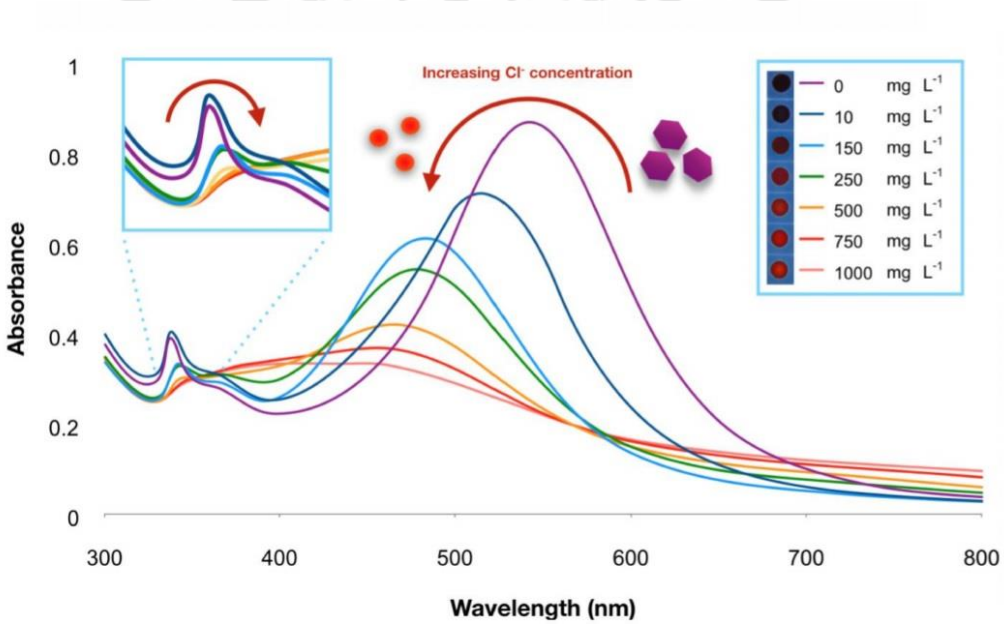

Figure 4 Paper-based colorimetric sensors for Cl^- detection using AgNPrs

From: Yakoh, A., et al. (2018). Simple and selective paper-based colorimetric sensor for determination of chloride ion in environmental samples using label-free silver nanoprisms. p. 134-140.

4. Electrochemical method

Electrochemistry is a branch of analytical chemistry concerned with the relationship between electrical and chemical effects. A large part of this field deals with the study of chemical changes caused by the passes of an electric current and the production of electrical energy by chemical reactions (Bard & Faulkner, 2001). The measured electrical quantity such as current, potential, and charge, will be directly detected proportional to the amount of analyte. Generally, electrochemical methods can be divided into two major techniques including potentiometry and voltammetry. In this research, the voltammetry technique is selected as an electrochemical method for the determination of histamine.

4.1 Voltammetry

Voltammetry is a class of electrochemistry which measures the current (*i*) as a function of potential (*E*) or voltage applied to a working electrode. The resulting plotted a graph between current versus applied potential is called voltammogram. Voltammetry is a useful technique to study the electrochemical behavior of electroactive species that can be oxidized or reduced at the working electrode. When the potential is applied to more negative potential, it becomes more strongly oxidizing agent, and the reduction reaction occurs. In contrast, when the potential is applied to more positive potential, it becomes more strongly reducing agent, and the oxidation reaction occurs. Voltammetry can be classified as many different types depended on the potential waveform that applied to the working electrode, such as linear sweep voltammetry (LSW), cyclic voltammetry (CV), normal pulse voltammetry (NPV), differential pulse voltammetry (DPV), square wave voltammetry (SWV) and stripping voltammetry. In this research, cyclic voltammetry and square wave voltammetry were used to investigate the electrochemical behavior and the electrochemical determination of histamine.

4.2 Cyclic voltammetry

Cyclic voltammetry (CV) is a common technique for investigation of the redox reaction to characterize the electrochemical behavior of analyte. Because it offers a rapid location of redox potentials of the electroactive species. Moreover, this technique is the most widely used for acquiring qualitative information about the electrochemical reactions such as the thermodynamics of redox processes, the kinetics of electron-transfer reactions, and coupled chemical reactions or adsorption processes (Heinze, 1984).

In an experiment, the equipment required to perform cyclic voltammetry consists of a potentiostat connected to three-electrodes (working, reference and counter electrodes immersed in the stagnant solution (supporting electrolyte). Then, the potential is applied linearly versus time to the working electrode as a triangular potential waveform as shown in Figure 5 (Fiala). During the potential is scanned backward and forward past the formal potential (E°) of an analyte. At the same time, the potentiostat measures the current resulting from an electrochemical reaction that occurred at the working electrode surface. The current density is proportional to the concentration of the analyte in solution. From the results, plotting of currents versus applied potentials is called a cyclic voltammogram.

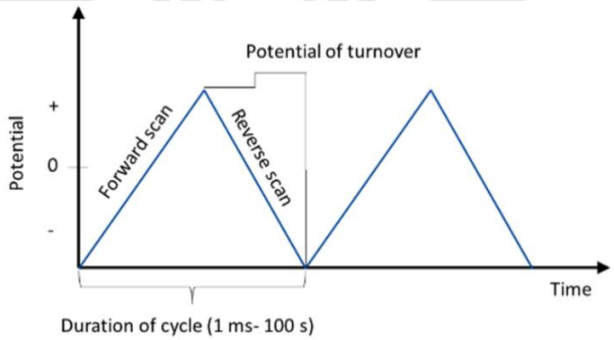

Figure 5 illustration of cyclic voltammetry waveform

From: Fiala, R. Cycling Voltammetry: principle. Retrieved May 18, 2019, from https:// physics.mff.cuni.cz/kfpp/povrchy/method/cv-principles.

Typically, the cyclic voltammogram of reversible redox reaction in a single potential was showed in Figure 6. In forward scan, the potential is applied in the positive direction leading to an oxidation reaction. The electroactive species loses an electron at the working electrode giving rise to an anodic peak current ($i_{p,a}$) that provides an oxidation peak at anodic peak potential ($E_{p,a}$). Afterward, the potential is applied in the negative direction (reverse scan) leading to a reduction reaction that can be observed the cathodic currents ($i_{p,c}$) and the reduction peak at a cathodic peak potential ($E_{p,c}$) (J. Wang, 2000).

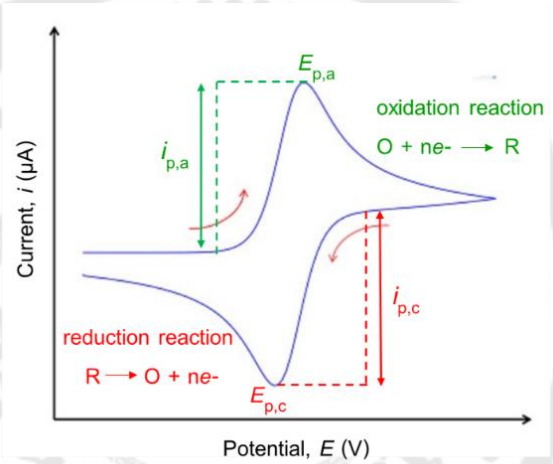

Figure 6 Typical cyclic voltammogram for a reversible redox reaction

According to a reversible redox reaction, it can be characterized by Randles-Sevcik equation 1:

$$I_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} \upsilon^{1/2} \quad \text{.....eq. 1}$$

Where:

I_p = peak current

n = number of electrons that participated in redox reaction

A = electrode area (cm^2)

C = concentration of analyte (mol/L)

D = diffusion coefficient (cm^2/s)

υ = scan rate (V/s)

From equation 1, the peak currents will increase linearly as a function of the square root of scan rate. A plot of the peak currents versus the square root of scan rate is linear indicating that the electrochemical process occurred at the electrode surface is diffusion controlled. On the other hand, the analyte is adsorbed on the electrode surface, the peak current is proportional to the scan rate rather than the square root of scan rate.

Nevertheless, cyclic voltammetry is commonly used for characterization and qualitative analysis of the analyte. This technique is not suitable for quantitative analysis due to this technique provides low sensitivity and difficulty for detection of multiple analytes. Thus, in this research, square wave voltammetry is selected for quantitative detection of histamine.

4.3 Square-wave voltammetry

Square-wave voltammetry (SWV) is a powerful electrochemical technique. This technique has received growing attention for quantitative detection of organic and inorganic compounds. The square-wave voltammetric technique has proven to be highly sensitive for the direct evaluation of concentrations. Thus, this technique can be widely used for trace and ultra-trace analysis. The waveform of square-wave voltammetry was shown in Figure 7a. The excitation signal in square-wave voltammetry consists of a symmetrical square-wave pulse of amplitude (E_{SW}) is superimposed on a staircase waveform of step height (ΔE). The current is sampled twice during each square wave cycle, one at the end of the forward pulse (t_f) and again at the end of the reverse pulse (t_r). The forward current (i_f) occurs from the first pulse per cycle, which is in the direction of the staircase waveform. The reverse current (i_r) arises from the end of the second pulse, which is in the opposite direction. For the net current (i_{net}) is obtained by taking the difference between the forward and reverse current ($i_{net} = i_f - i_r$). This technique provides excellent sensitivity due to the net current is larger than either the forward or reverse components. When the difference current is plotted versus the potential staircase, the shape of square wave voltammogram is obtained as shown in Figure 7b (Nantaphol, 2016). The peak height is directly proportional to the concentration of the

electroactive species. The main advantages of this technique are high sensitivity and easy to identify peak separation (Kounaves, 1997).

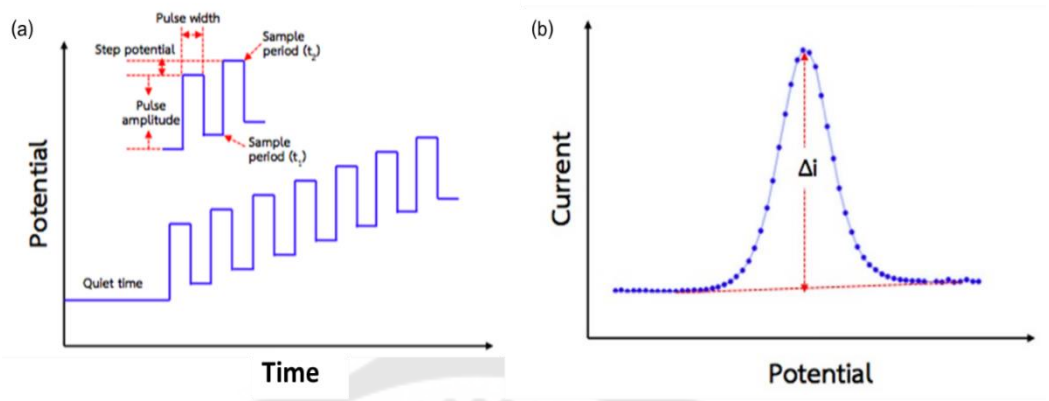

Figure 7 (a) Excitation waveform of square-wave voltammetry and
(b) The shape of square wave voltammogram

From: Nantaphol, S. (2016). Electrochemical detection of organic and biological compounds with modified electrode. p 16.

4.4 Screen-printed graphene electrodes

Over the past few years, the designs of electrochemical sensors have been developed to improve the analytical performance in terms of sensitivity, selectivity, reliability, facilitate of fabrication and use, as well as lower costs. Disposable sensors have received attention in several applications such as diagnostic, environmental and industrial analysis. Nowadays, screen-printed electrodes (SPEs) have been extensively employed for developing novel electrochemical sensing platforms. The screen printing method is an alternative technology used for fabricating disposable sensors instead of using the traditional electrode due to its economic substrate, low cost, rapid response, simple, capable of mass production and suitable for on-site analysis (Charoenkitamorn, Chaiyo, Chailapakul, & Siangproh, 2018).

The fabrication of SPEs consists of layer-by-layer deposition of ink upon a substrate by using a homemade screen-printed method or screen printing machine. SPEs usually include a three-electrode configuration (working, counter and reference

electrodes) printed on the various types of substrates (paper, plastic or ceramic). For example, in Figure 8 shows the commercial screen-printed electrodes (Dropsens). During the printing process of SPEs, the pastes most commonly used are silver ink and carbon ink. Silver ink is printed as a reference electrode or conductive track, whereas carbon ink is printed as a working and counter electrode. Besides, the working electrodes can be printed with other materials such as gold, platinum, graphite, and graphene inks. This method has many advantages such as design flexibility, process automation, good reproducibility, and a wide choice of materials (Taleat, Khoshroo, & Mazloum-Ardakani, 2014).

Recently, screen-printed graphene electrodes (SPGE) have been received much attention for the development of electrochemical sensors. Because of its properties such as high electrical conductivity, high electron mobility, large surface area, and high sensitivity (Panraksa et al., 2018). Therefore, this research focused on the development of an electrochemical sensor for quantitative analysis of histamine using SPGE.

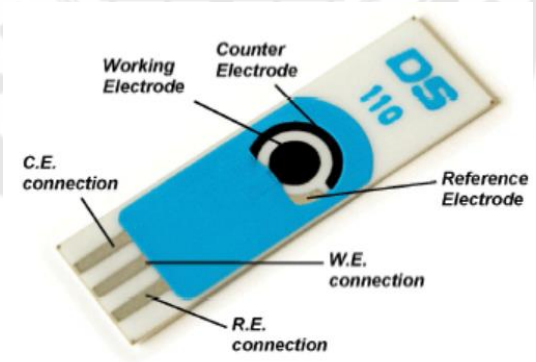

Figure 8 Commercial screen-printed electrode

From: Dropsens. Screen-printed electrodes. Retrieved May 20, 2019 from http://www.dropsens.com/en/screen_printed_electrodes_pag.html.

5. Literature reviews

Current advances in nanoparticles-based sensing of histamine for assuring the safety of food products have grown exponentially. The unique physical and chemical properties of nanoparticles make them are extremely suitable for designing new devices and also improved the sensitivity of devices. Therefore, in this section, the review about the development of nanoparticles-based histamine sensing were focused on. The literature reviews can be divided into 2 part: colorimetric sensor and electrochemical sensor.

5.1 Colorimetric sensor for histamine detection

In 2015, Luo et al. presented an immunochromatographic assay strip (ICA strip) using colloidal AuNPs-antibody for rapid detection of histamine. In this approach, the AuNPs labeled with anti-*p*-nitrobenzoylated histamine antibody (anti-NPHA-mAb-AuNPs) were used to develop lateral flow assay kits. After that, the coating antigen (hapten 2-ovalbumin) and sheep anti-mouse IgG were dispensed onto the nitrocellulose membrane as the test (T) and control (C) lines, respectively. If the sample solution has the histamine, it will compete the binding sites of mAb-AuNPs with the coating antigen on T line. Then the T line will be weak or absent, indicating that the test result was weakly positive or positive. Or the other hand, if histamine absences in the sample solution, anti-NPHA-mAb-AuNPs will bind to the coating antigen and the T line will display the red line, demonstrating the result was negative. This ICA strip exhibited the excellent detection ability with a visual detection limit of 6.0 ppm in the qualitative experiment, while a detection limit of 1.0 ppm in the semi-quantitative experiment for fish samples (Luo et al., 2015).

In 2016, Mattsson et al. developed on-chip binding inhibition immunoassay for histamine detection using modified carbon nanoparticles (CNPs) as a detection label. CNPs were oxidized and further modified with different functional end groups of silanes (thiol, epoxy, and amine). In addition, 1-pyrenebutanoic acid *N*-succinimidyl ester (PSE) was used as a conjugation agent. The prepared CNPs-antibody conjugates were used in a binding inhibition assay for histamine. In this assay, histamine immobilized onto the solid surface (via carrier protein) competes for the anti-histamine antibody binding sites with the analyte in solution. Afterward, the bound anti-histamine

antibody is detected with a CNPs-antibody, which displays a grey to black color. The semi-quantitative read-out by naked-eye over a broad concentration range (44-293 ppm). A detection limit was found to be 22 ppm (Mattsson, Jungmann, Lieberzeit, & Preininger, 2016).

In 2017, Huang et al. reported an aggregation based colorimetric method for monitoring of histamine using citrate-capped AuNPs. Under experimental conditions, the aggregation of AuNPs is induced by electrostatic interaction between the positive changes on the protonated histamine and the negative changes of citrate-capped AuNPs. This accelerates the replacement of citrate ions by the imidazole ring of histamine by virtue of a strong affinity between imidazole ring and AuNPs. This induces the aggregation of AuNPs, thereby triggering a color change from red to blue. However, this assay was not specific for histamine detection. Thus, the researcher suggested pretreatment the sample with Cu^{+} ions due to these ions can block 20 amino acids found in proteins. The minimum visually detectable histamine concentration was found to be 1.81 μM, while a limit of detection was calculated to be 38 nM with UV-Vis spectro photometer (Huang et al., 2017).

A few earlier reviews can be found in the literature. In previous work, the proposed sensors were suitable for screening of histamine in real samples. However, the main drawbacks of these methods are need for derivatization of histamine, complicated process and time-consuming of analysis. Besides, from the literature study, it was found that AgNPs have never been used for colorimetric detection of histamine before. In particular, AgNPs have some advantages compared with AuNPs. Specifically, the extinction coefficients of AgNPs are higher than AuNPs of the same size and a lower cost compared to AuNPs (Jiang & Yu, 2008). Thus, AgNPs have been widely utilized for the colorimetric sensor of various substances.

Hence, the first part of this research is developed for the first time new, simple, and rapid paper-based colorimetric sensor for semi-quantitative analysis of histamine using label-free AgNPs.

5.2 Electrochemical sensor for histamine detection

The main drawbacks for the detection of histamine by the electrochemical method are histamine poor electrochemical properties, electrode surface fouling, and the effect of interferences. In the past few decades, several reports have been published, describing the outstanding performance of the electrochemical sensor for detection of histamine by modification of working electrode surface, especially various nanoparticles such as metal nanoparticles and carbon nanoparticles. Hence, the objective of this review is an overview of the development of the electrochemical sensor for histamine detection using various modified electrode surface.

In 2000, Sarada et al. studied the electrochemical oxidation of histamine using boron-doped diamond electrode (BDDE) coupled to flow injection analysis (FIA) with amperometric detection. In addition, comparison experiments were carried out using a glassy carbon electrode (GCE). At the GCE showed an ill-defined oxidation peak of histamine at + 1.20 V and high background current. In contrasts, at the BDDE showed a well-defined oxidation peak of histamine at +1.40 V and very low background current. The results indicated that the BDDE provided higher sensitivity than comparison to the GCE, even though the applied potential is so high. The developed method displayed the linear range of 0.5 -100 μM and the limit of detection of 0.5 μM (Sarada et al., 2000).

In 2005, Carralero et al. developed the electrochemical sensor for detection of histamine using gold nanoparticles-modified glassy carbon electrodes (AuNPs-GCE). In this research, the GCE modified by electrodeposition of AuNPs has been prepared and used for the determination of histamine by FIA and HPLC with pulsed amperometric detection (PAD) as the detection mode. As can be observed, at the AuNPs-GCE showed much higher the histamine peak current than at a conventional Au disk electrode. The results showed the oxidation peak of histamine at + 0.55 V. The developed sensor provided high sensitivity and reproducibility. A limit of detection of 0.6 μM histamine was obtained (Carralero et al., 2005).

In 2010, Svarc-Gajic and Stojanovic studied the electrochemical oxidation of histamine using thin film nickel electrode. In this work, the glassy carbon disc electrode modified by electrodeposition of nickel has been prepared and used for the determination of histamine by chronopotentiometric detection. Before each analysis the nickel electrode was transferred to alkaline solution (0.5 M NaOH) and pretreated by CV technique (E = -0.4 to +0.5 V, 20 cycles) in order to activate the electrode and to form $Ni(OH)_2$/NiOOH couple. The formation of a redox couple $Ni(OH)_2$/NiOOH which shows very excellent properties as a catalyst in an electron transfer. Electrochemically formed catalyst can be exploited for histamine oxidation on nickel electrode. The results showed the oxidation peak of histamine at +0.4 V. The developed method can be performed with good sensitivity (the limit of detection of 0.11 ppm) and in wide concentration range (0.5-110 ppm) (Svarc-Gajic & Stojanovic, 2010).

In 2014, Geto et al. proposed a new electrochemical sensor based on a multi-walled carbon nanotubes (MWCNTs) coated conducting polymer (*p*-(AHNSA) or poly(4-amino-3-hydroxynaphthalene sulfonic acid)) modified on the glassy carbon electrode. The results revealed that the both of MWCNTs and conducting polymer not only enhance the oxidation current but also reduce the oxidation potential of histamine (at + 1.0 V). The high performance of the conducting polymer modified can be explained by the existence of electrostatic interaction between the positive changes on the protonated histamine and the negative changes of the polymer film functional units (sulfonate groups). While, the catalytic effect of MWCNTs from the unique properties such as large specific surface area, strong adsorptive ability and subtle electronic properties. The developed sensor showed the linear range of 0.1-100 μM and the limit of detection of 0.0762 μM (Geto et al., 2014).

In 2016, Apetrei and Apetrei proposed a novel biosensor for quantification of histamine based on diamine oxidase (DAO) immobilized into a nanostructured platinum nanoparticles/graphene/chitosan thin film modified on screen-printed carbon electrode. The amperometric measurements with the biosensor have been implemented in buffer solution (pH 7.4) and applied potential at +0.4 V. The electrochemical signal of

the proposed biosensor is associated with the oxidation reaction of hydrogen peroxide (H_2O_2), which is the enzymatic product of the interaction between DAO and histamine. The proposed biosensor provided high sensitivity, low detection limit (0.0254 μM) and a wide linear range from 0.1 to 300 μM (Apetrei & Apetrei, 2016).

In 2017, Shahzad et al. developed the modification of GCE with fluorine-doped reduced graphene oxide (FrGO) for histamine detection. This sensor showed a well-defined irreversible oxidation peak of histamine at +0.7 V. Moreover, the oxidation peak current of histamine greatly increased when the content of fluorine increased. Because of fluorine doping provides a synergistic effect of improving electrical conductivity. The proposed sensor offered an excellent sensitivity (the limit of detection of 7 nM), a wide linear range (0.2-80 μM), and good selectivity. However, the disadvantage of this work is the preparation of this sensor is complicated (Shahzad et al., 2017).

In 2018, Kumar and Goyal developed the electrochemical sensor for detection of histamine using AgNPs decorated graphene nanoribbon (GNRs) modified on the pyrolytic graphite electrode. In this research, AgNPs were prepared by the reduction of silver nitrate and the GNRs were prepared by chemical method. Then, the composite of AgNPs and GNRs was drop casted onto the surface of edge plane of pyrolytic graphite (EPPG). The results showed that the GNRs-AgNP exhibited superior catalytic activity towards the oxidation of histamine. The square wave voltammetry has been used for the determination of histamine. The proposed sensor demonstrated a linear range of 1-50 and 60-500 μM and the limit of the detection was found to be 0.049 μM. The fabricated sensor showed good performance with excellent selectivity and sensitivity for quantification of histamine in real samples (Kumar & Goyal, 2018).

In 2019, Hassan et al. proposed a novel, rapid and cost-effective approach for the electrochemical sensing of histamine based on magnetic molecularly imprinted polymer (magnetic-MIP). In this research, the histamine magnetic-MIP was synthesized by the core-shell method using histamine as a template, and 2-vinylpyridine as functional monomer. The direct electrochemical detection of histamine was carried out

by preconcentration of histamine on the magnetic-MIP with further magnetic actuation on the surface of magneto-actuated graphite-epoxy composite (m-GEC) electrode. The magnetic-MIP showed high binding capacity towards the adsorption of histamine on the surface electrode. The results showed that the oxidation peak of histamine was observed at +0.94 V. The developed sensor exhibited high sensitivity and selectivity. A limit of detection was found to be 1.6 10^{-6} ppm (Hassan et al., 2019).

Overall, many research works on various types of electrode modifications have been performed in the quantification of histamine. The electrochemical nanosensor has significantly enhanced sensitivity and selectivity. However, few papers dealing with direct electrochemical oxidation of histamine on conventional electrodes such as platinum, gold, nickel, thin-film mercury, boron-doped diamond (BDD), glassy carbon electrodes have been published due to the high oxidation potential of histamine at +1.2 to + 1.4 V (Geto et al., 2014). Recently, graphene electrodes, especially screen-printed graphene electrodes (SPGE), have been received much attention for the development of electrochemical sensors. Because of its properties such as high electrical conductivity, high electron mobility, large surface area, and high sensitivity (Panraksa et al., 2018). Therefore, the second part of this research is to develop an electrochemical sensor for quantitative analysis of histamine using SPGE.

CHAPTER 3

METHODOLOGY

1. Equipment, materials and chemicals

1.1 Equipment and materials

- PalmSens handheld potentiostat (model Palm Instruments BV operate by PSTrace5.4, Netherlands)
- Computer notebook (HP Pavilion x360)
- Wax printer (Xerox Color Qube 8570, Japan)
- Digital camera
- Oven from Memmert
- Hotplate stirrer from Laboratory & Medical Supplies (LMS)
- Microcentrifuge tube from Hettich
- Ultrasonicator from Mettler electronic
- Syringe pump (NE-1000 Programmable Single Syringe Pump, New Era Pump System, Inc.)
- High performance disperser (IKA® T25 ULTRA-TURRAX®)
- Vortex from Scientific Industries
- Filter paper (Whatman no.1)
- Transparency film (polypropylene, purchased from local stationery store)
- Micropipette 100 and 1000 µL from Labnet

1.2 Materials and chemicals

- Silver nitrate from Merck
- Hydrogen peroxide from Merck
- Sodium borohydride from Merck
- Soluble starch from Merck
- Graphene ink from Serve Science Co., Ltd., Thailand
- Silver/silver chloride (Ag/AgCl) ink from Sun Chemical Ltd., UK

- Histamine dihydrochloride from Sigma Aldich
- 1,4-Diaminobutane dihydrochloride (putrescine) from Sigma Aldich
- Cadaverine dihydrochloride from Sigma Aldich
- Tyramine hydrochloride from Sigma Aldich
- Tryptamine from Sigma Aldich
- Sodium dihydrogen orthophosphate from Ajax Finechem
- di-Sodium hydrogen orthophosphate from Ajax Finechem
- Sodium hydroxide from Ajax Finechem
- Acetone from Fisher Chemical

2. Experimental

There are two parts for this chapter including;

2.1 Paper-based sensor for semi-quantitative of histamine using AgNPrs.

2.1.1 The preparation of the AgNPs

AgNPs were prepared according to the procedure described in previous work (Parnklang et al., 2013) . Briefly, The AgNPs were synthesized by reduction of $AgNO_3$ using $NaBH_4$ and the shape transformation using a 30% H_2O_2 solution. The starch solution was used as a stabilizer. First, a solution of silver salt was prepared by dissolving 0.63 g $AgNO_3$ in 500 mL of the 2% (w/v) starch solution. Then, a reducing agent was prepared by dissolving 0.21 g $NaBH_4$ in 500 mL of the 2% (w/v) starch solution. To obtain nanoparticles, the $NaBH_4$ solution was added immediately into the $AgNO_3$ solution under a vigorous stir. The colorless solution turned light yellow, indicating the formation of small AgNPs. Afterword, the synthesized AgNPs was boiled for 2 h and cooled down at room temperature to ensure the elimination of residual $NaBH_4$. Then, the final volume of the AgNPs was adjusted to 1000 mL with DI water in order to obtain the final concentration of 400 ppm AgNPs. Finally, the shape transformation of AgNPs was done by an injection of 30% H_2O_2 solution at the rate of 13.45 mL/min into a 200 mL of AgNPs solution under a vigorous stir for 10 min to ensure a complete reaction. The AgNPs solution turned color from light yellow to pink, violet, and blue that depended on the molar ratio between H_2O_2 and AgNPs.

2.1.2 Design and fabrication of the PADs

The pattern of the PADs was created as an array containing a circular area with a diameter of 6 mm using Adobe illustrator CS6 software. The devices were fabricated using a wax printing method on filter paper substrate (Whatman no.1). There are two steps of printing and can be done within 2 min:

1. Printing of the wax pattern on the filter paper by using the wax printer.

2. Melting of the wax-printed paper on a hot plate at 175 °C for 40 s. This device was heated to create the hydrophobic area (blue area), while the area without wax was the hydrophilic area (detection zone).

All fabrication procedures of the PADs are shown in Figure 9.

Figure 9 Design and fabrication of the PADs by wax printing method.

2.1.3 Colorimetric assay of histamine on PADs

Before use, PADs were sealed with packing tape in the bottom of paper to prevent the solution leak. For colorimetric assay of histamine, 10 µL of the pink of AgNPrs was firstly dropped onto the detection zone. Next, 10 µL of histamine solution in 0.1 M phosphate buffer solution (pH 5) was added into the detection zone. The color change can be monitored by the naked eyes within 1 min to screen the concentration range of histamine. In the presence of histamine, the color of AgNPrs changed from pink to orange-yellow. All procedures of the colorimetric assay are shown in Figure 10. The photograph of PADs was recorded by a digital camera in a light control box. Then, the color intensity was measured using ImageJ. In this work, we utilized red scale values to evaluate the detection performance.

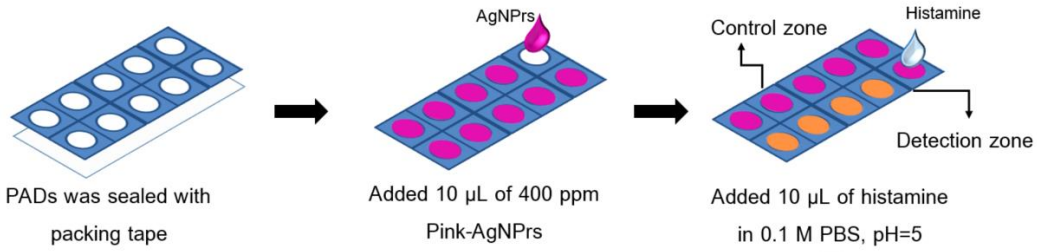

Figure 10 A simple colorimetric assay for histamine detection on PADs using AgNPrs.

2.1.4 The study of parameters in colorimetry

The optimal parameters were focused on

- Effect of the pH of phosphate buffer solution in the range of 3 - 9.
- Effect of the type of AgNPs: violet, blue, pink, and yellow.
- Effect of the concentration of AgNPrs in the range of 100 - 400 ppm.
- Effect of the ratio of AgNPrs and histamine; 1:1, 1:2, 1:3, 2:1 and 3:1.
- Effect of the detection time in the range of 1 - 9 min.

2.1.5 Interference study

The selectivity of the proposed method for histamine detection using label-free AgNPrs was studied in the presence of some biogenic amines such as putrescine, cadaverine, tyramine, and tryptamine. By adding various interfering substances into a pink of AgNPs solution. If the color change of AgNPrs from pink to orange-yellow, this result indicates that this method exhibited low selectivity.

2.2. Electrochemical sensor based on modifier-free SPGE for quantitative analysis of histamine.

2.2.1 Design and fabrication of the SPGE

The proposed SPGE was designed using Adobe illustrator CS6 software. The SPGEs were fabricated using the home-made screen-printing method on the transparency sheet substrate. All fabrication procedures of the SPGE printing are shown in Figure 11. First, graphene ink was screened to obtain the counter and working electrode, respectively. Then, this sensor was placed into an oven at 55 °C for 30 min. After that, silver/silver chloride (Ag/AgCl) ink was screened to obtain the reference electrode and conducting pads. Finally, these sensors were placed into an oven at 55 °C for 30 min to complete the process of electrode fabrication. The obtained electrodes containing 20 devices per time were kept in dry place. For screen-printed carbon electrode (SPCE), they were fabricated using the same screen-printed template block (geometrical area of electrode = 0.126 cm^2) as screen-printed graphene electrodes by replacing graphene ink with carbon ink. All steps were performed as same as SPGE.

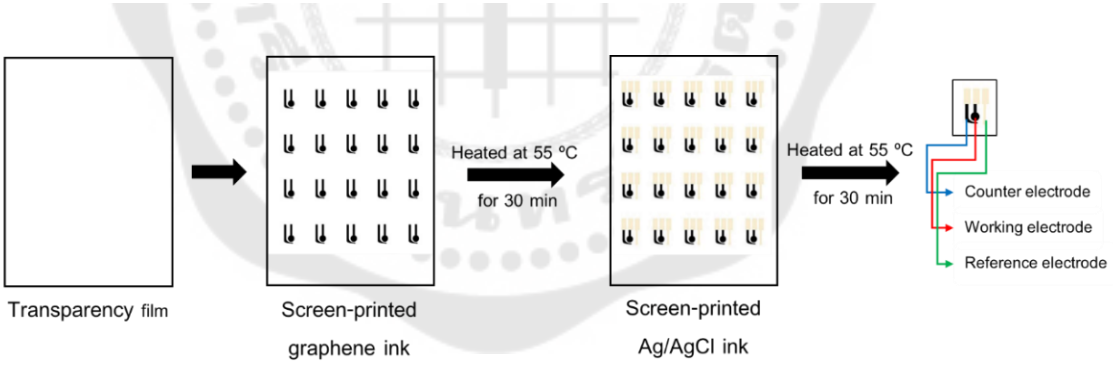

Figure 11 Screen-printing method for fabrication of the SPGE.

2.2.2 The electrochemical measurement of histamine using SPGE

Voltammetric measurements were performed at room temperature using a PalmSens handheld potentiostat. The electrochemical behavior of the histamine was investigated at the surface of SPGE using cyclic voltammetry (CV). The cyclic voltammograms were recorded from -1.0 V to +1.0 V at a scan rate of 100 mV/s. Square-wave voltammetry (SWV) was used for the direct determination of histamine. For the

analytical procedures (Figure 12), 100 µL of histamine solution in 0.2 M NaOH was added to cover onto the electrochemical detection zone of the device. Then, the potential was applied to the system from 0.0 V to +0.8 V by using the setup as follow: step potential = 0.025 V; amplitude = 0.075 V; frequency = 25 Hz.

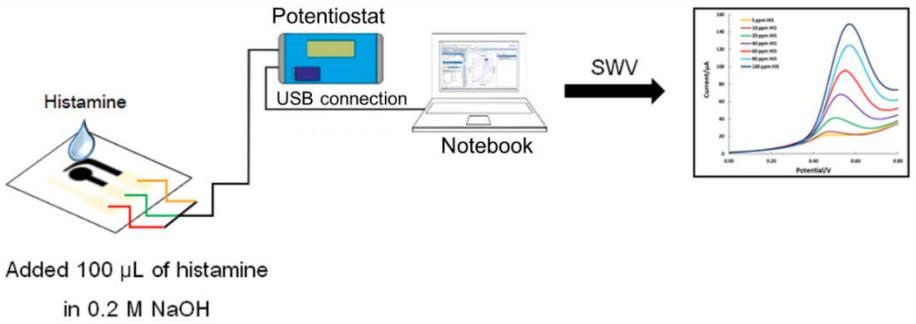

Figure 12 Schematic of the determination of histamine on SPGE using SWV.

2.2.3 Optimization of experimental parameters using SWV

The optimal parameters in SWV were focused on

- Effect of pH of phosphate buffer solution in the range of 3 - 9.
- Effect of concentration of NaOH in the range of 0.05 - 0.5 M.
- Effect of the step potential in the range of 0.01 - 0.03 V.
- Effect of the amplitude in the range of 0.01 - 0.1 V
- Effect of the frequency in the range of 10 - 30 Hz

All parameters were investigated by using 100 µL of 100 ppm histamine in 0.2 M NaOH.

2.2.4 The study of analytical performances

The analytical performance of the proposed method was investigated. Under the optimal conditions, the analytical performance parameters were studied including linear range, the limit of detection (LOD), the limit of quantification (LOQ), accuracy, and precision.

2.2.5 Interference study for histamine detection

The selectivity of the proposed sensor for histamine detection was studied in the presence of some biogenic amines such as putrescine, cadaverine, tyramine, and tryptamine.

2.2.6 Analysis of histamine in canned fish samples

To demonstrate the benefit and capability of the proposed method, the electrochemical assay was applied to detect histamine in canned fish samples. The canned fish samples were purchased from a supermarket. The samples were prepared following in previous work (Yilmaz & Inan, 2015). Briefly, five grams of muscle tissue was homogenized with 40 mL of distilled water. This mixture was stirred for 5 min and then placed in an ultrasound bath and sonicated for 20 min. The resulted mixture was centrifuged for 10 min at 6,000 rpm. Next, the supernatant was filtered through a Whatman filter paper. Before measurement, the filtrate solution was diluted to 1000 µL with 0.2 M NaOH for electrochemical detection of histamine. The determination of histamine was measured with SPGE, followed by topic 2.2.2. For spiked samples, the standard histamine was added into sample before performing the sample preparation as same as previous. The samples were spiked with different concentrations of standard histamine (10.00, 20.00 and 40.00 ppm). The spiked samples were used to study the recovery to check the accuracy of the proposed method.

2.2.7 Validation of the proposed method with HPLC method

To confirm the accuracy and reliability of the approach, the developed method was validated using the traditional HPLC method (Nakthong & Muangthai, 2015). By calculating a paired t-test value at 95 % confidence level was performed. The statistics revealed that $t_{calculated}$ was below $t_{critical}$ which suggested no significant difference between the two methods. The results indicated that this proposed method was reliable for histamine determination in real samples.

CHAPTER 4
RESULTS AND DISCUSSION

In this chapter, the results of histamine detection were presented following their proposed method. First is colorimetric detection and second is electrochemical detection. Each method contained their principle and steps to obtain the optimal conditions. In final, the electrochemical method was applied for the determination of histamine in selected real samples and results obtained were compared to those obtained from the standard HPLC-FLU method.

1. Colorimetric detection of histamine

1.1 Principle of the colorimetric assay

Based on literature review (Parnklang et al., 2013) for synthesis of silver nanoparticles, the researcher have learnt that shape transformation of silver nanospheres (AgNSs) to silver nanoprisms (AgNPrs) can be controlled by the amount of injected H_2O_2 during the procedure. Typically, the colloidal solution of AgNSs is yellow due to its surface plasmon resonance (SPR) absorption band (λ_{max} = 402 nm). After injection of H_2O_2 solution into the AgNSs colloidal solution, the aggregation of the AgNSs occurred to obtain the AgNPrs. Thus, the size of AgNPrs is bigger than AgNSs leading to the color of the AgNSs was changed immediately from yellow to pink, violet, or blue depending on the molar ratio between added H_2O_2 and AgNSs. From the knowledge obtained, it prompted us to develop a simple paper-based colorimetric sensor for histamine detection using label-free pink-AgNPrs which provided the remarkable color-change in the absence and presence of histamine. In the presence of histamine, it was found that the color of AgNPs was changed from pink (AgNPrs) to orange-yellow (AgNSs) immediately and observing can be rapidly visualized by the naked eyes within 1 min as shown in Figure 13. The researcher proposed that in the presence of histamine, it can catalyst the oxidative etching of the pink-AgNPrs to smaller yellow-AgNSs by dissolved oxygen (O_2), resulting in a reduction in the particle size of the AgNPrs. In addition, the trend of color change from pink to orange-yellow was directly proportional to the

concentration of histamine in the sample. Therefore, this finding could be applied for the determination of histamine in real samples.

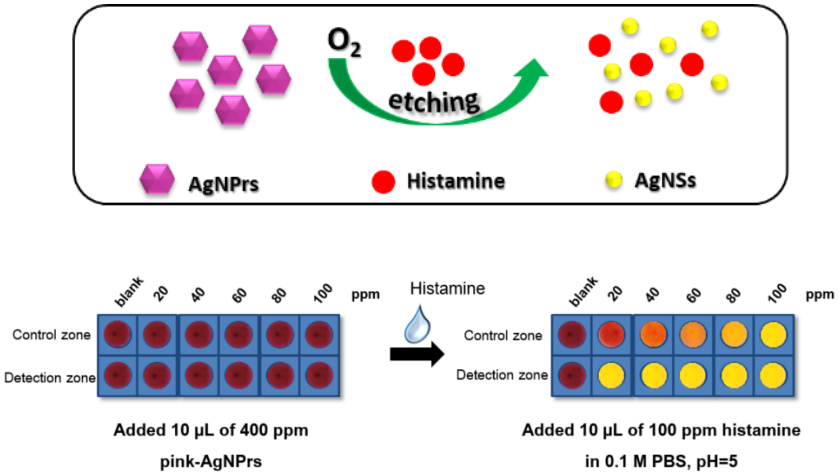

Figure 13 Schematic representation of the colorimetric assay for histamine screening

Generally, silver (Ag^0) is a noble metal that is stable toward the oxidation by water. Because of its relatively high standard reduction potential of Ag^0 (E^o Ag^+/Ag^0 = + 0.799 V), AgNPs could not be oxidized by dissolved oxygen in water (E^o O_2/OH^- = +0.401 V). On the other hand, AgNPs can be oxidized by dissolved oxygen to release Ag^+ under the oxygen-rich condition. Moreover, the releasing of Ag^+ is more favorable in acidic media (pH < 5.5) since a slightly acidic pH will increase the solubility of O_2 and allow a faster Ag^+ release. Besides, AgNPs could be oxidized in acidic media to generate Ag_2O that adsorbs onto the surface of AgNPs to form a core (AgNPs)-shell (Ag_2O) structure as shown in Figure 14 (W. Zhang, Xiao, & Fang, 2018). Thus, the oxidative transformation of AgNPs to release Ag^+ requires dissolved O_2 (as an oxidizer) as shown in equation (1). The oxidative process of AgNPs by dissolved O_2 is slow. However, in the presence of histamine (in phosphate buffer solution, pH 5.0), it accelerates the oxidative etching of AgNPs. Due to protonated histamine played role an intermediate that more reactive and stronger than O_2 as oxidant as shown in equation (2). Therefore, the oxidative dissolution of AgNPs process can be represented by a two-step reaction. The second step is much faster than the first one. Hence, the first step is the rate-limiting step (C. Zhang, Hu, & Deng, 2016).

$$Ag^{0} + \frac{1}{4}O_{2\,(aq)} + H^{+}_{(aq)} \xrightarrow{\text{slow}} Ag^{+}_{(aq)} + \frac{1}{2}H_2O_{(l)} \qquad \text{Eq. (1)}$$

$$\frac{1}{4}O_{2\,(aq)} + H^{+}_{(aq)} \xrightarrow[\text{(slow)}]{\text{AgNPs}} Ag^{+}_{(aq)} + \text{Histamine}^{+} \xrightarrow[\text{(fast)}]{\text{AgNPs}} Ag^{+}_{(aq)} + \frac{1}{2}H_2O_{(l)} \qquad \text{Eq. (2)}$$

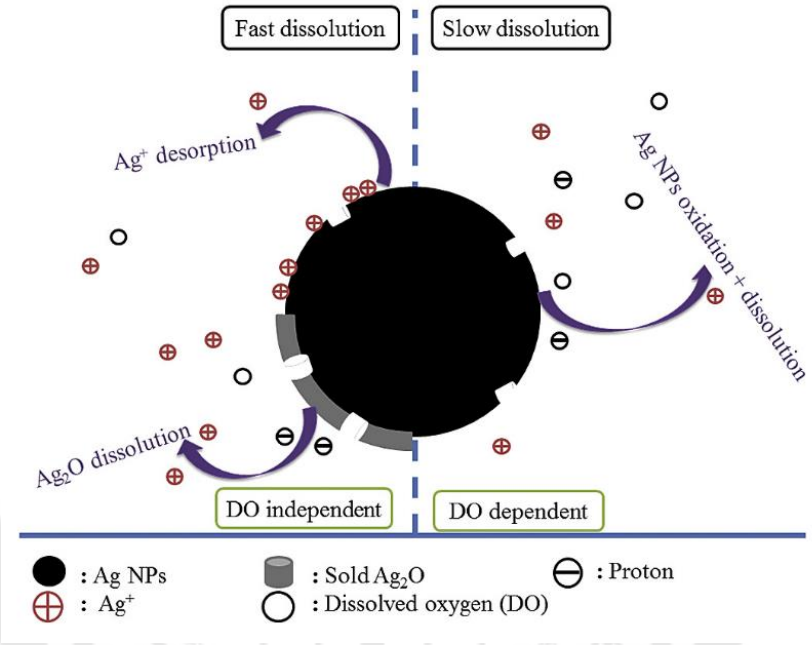

Figure 14 Oxidative dissolution of AgNPs by dissolved O_2 and H^+

1.2 Optimization of colorimetric detection

To achieve the best performance of paper-based colorimetric sensor, all factors related to the color change of AgNPs was investigated. Since the color change of AgNPs depended on the size and the surroundings. Therefore, the optimal factors effected on the size and the surroundings of AgNPs were focused including the effect of pH of buffer solutions, type and concentration of AgNPs, the ratio between AgNPs and histamine, detection time and interferences. All factors were optimized using 100 ppm of histamine solution. The red intensity values of the AgNPs before and after the addition of histamine solution ($\Delta I = I_{sample} - I_{blank}$) was determined and the highest ΔI obtained from each parameter was considered to use as the optimal values for the proposed method.

1.2.1 Effect of pH of buffer solutions, type and concentration of AgNPs

First, the influence of the pH (0.1 M phosphate buffer solution) on this proposed method was investigated including pH 3, 5, 7, 8 and 9. Concurrently, the type of AgNPs colloid (yellow, pink, violet, and blue) was also studied. The results were observed that only the use of 0.1 M phosphate buffer solution at pH 5 allowed the color change in the presence of histamine as shown in Figure 15. The change was rapidly observed within 1 min and it was found that the use of pink-AgNPrs provided the highest ΔI value. Thus, 0.1 M phosphate buffer solution at pH 5.0 was selected for use in further experiments to detect the histamine at various concentrations. Additionally, the pink-AgNPrs was selected as a suitable colorimetric agent for histamine detection.

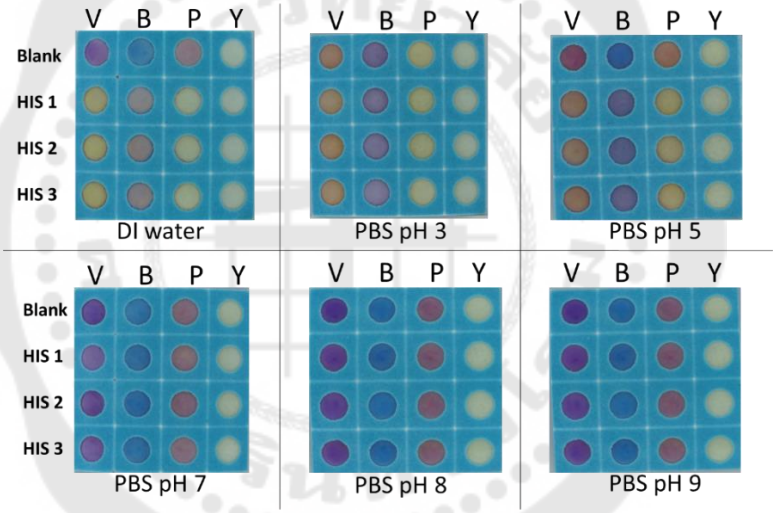

Figure 15 Effect of pH (0.1 M phosphate buffer solution, PBS: pH 3-9) and type of AgNPs colloid (V = violet, B = blue, P = pink, and Y = yellow)

Moreover, the effect of concentration of pink-AgNPrs was studied in the range from 100 ppm to 400 ppm. It was observed that when the concentration of pink-AgNPrs was increased, the ΔI value was also increased. The maximum value of ΔI was determined at 400 ppm of pink-AgNPrs (Figure 16A). This may be concern to the high concentration of reactant leading to fast kinetic reaction. Hence, 400 ppm was chosen as the optimal concentration of the pink-AgNPrs.

1.2.2 Effect of the ratio between pink-AgNPrs and histamine

The optimal ratio between pink-AgNPrs and histamine (1:1, 1:2, 1:3, 2:1, and 3:1 (v/v)) was investigated as shown in Figure 16B. It was found that the ratio between pink-AgNPrs and histamine at 1 to 1 (red bar graph) provided the possibility to detect histamine due to the ΔI value increased proportionally to the concentration of histamine. Therefore, the ratio between pink-AgNPrs and histamine of 1:1 (10 µL: 10 µL) was selected for the subsequent work.

1.2.3 Effect of the detection time

The detection time was next examined in the range of 1-9 min (Figure 16C). As can see in the results obtained, the ΔI value gradually increased from 1 min to 3 min and remained steady after 5 min. However, in this work, the detection time was chosen at 1 min because the color intensity obtained from this detection time is proportional to the concentration of histamine.

1.2.4 Effect of other biogenic amines

The selectivity is one of the most important parameter for development of the new method. Therefore, the selectivity of the proposed paper-based sensing was studied using various biogenic amine compounds (cadaverine, putrescine, tyramine, and tryptamine) possibly found in real samples. It was found that in the presence of these biogenic amines, the color of the pink-AgNPrs was changed to yellow as well as histamine (shown in Figure 17). This indicated that these interferences can interfere the detection of histamine using the proposed method. Fortunately, the concentration of these biogenic amine found in fish sample are very low levels (Yen & Hsieh, 1991) in comparison to histamine content. Thus, these interferences can be negligible in order to detect histamine in real samples.

Under the optimal conditions, the semi-quantitative read-out by the naked eye over a broad concentration range (20-100 ppm) can be obtained (Figure 13). The proposed method enables us to detect histamine by naked-eye with the detection limit of 20 ppm which is below the permissible limit by FDA (50 ppm).

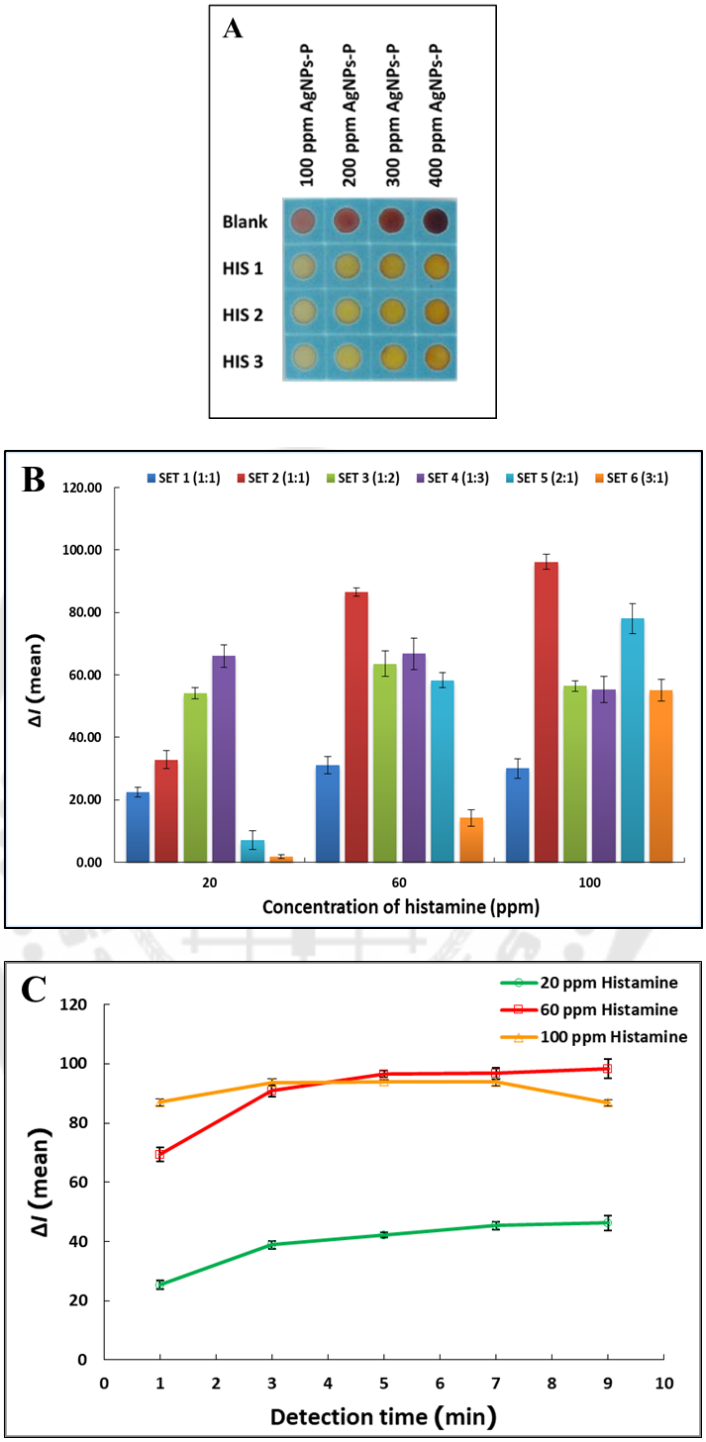

Figure 16 Effect of experimental conditions for histamine detection on PADs;

(A) Concentration of pink-AgNPrs (AgNPs-P)

(B) The ratio between AgNPrs and histamine

(C) Detection time.

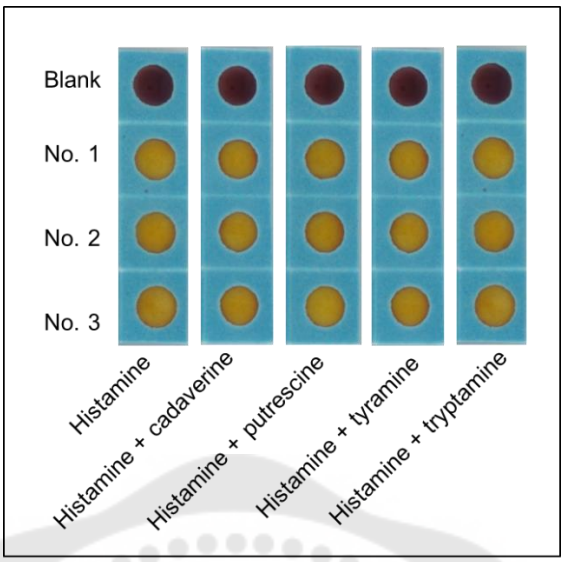

Figure 17 Effect of other biogenic amines

Although the paper-based colorimetric sensor performed a simple and rapid visual analysis method, however, its quantitative detection ability was poor selectivity. To solve this problem, the electrochemical method was considered as a promising alternative choice due to high selectivity and sensitivity. Next section, the development of a new electrochemical method for the selective quantification of histamine was demonstrated.

2. Electrochemical detection of histamine

2.1 Effect of different type of supporting electrolyte on the electrochemical behavior of histamine at SPGE

A systematic study of the influence of the type of supporting electrolyte on the electrochemical behavior of histamine at SPGE was first investigated. Preliminary experiments were carried out by SWV using 0.1 M phosphate buffer solution at different pHs (pH 3, 5, 7, and 9) as supporting electrolyte. These experiments revealed that the highest peak current and well-defined peak shape was obtained when using a 0.1 M phosphate buffer solution at pH 7 (Figure 18C) as supporting electrolyte. However, the oxidation peak of histamine occurred at a high potential of +1.20 V (vs. Ag/AgCl). The results implied that the use of phosphate buffer solution at pH 7 as a supporting electrolyte still provided a low selectivity because another biogenic amine can be oxidized as well at high potential. Therefore, the researcher tried to review literatures and it was found that besides buffer solutions, NaOH solution was used as a supporting electrolyte for histamine detection at a thin film nickel electrode (Svarc-Gajic & Stojanovic, 2010) . As results demonstrated, the chronoamperometric response of histamine showed the oxidation process at lower oxidative potential of + 0.55 V (vs. Ag/AgCl). This means that histamine was easier to be oxidized in alkaline medium. This finding also implied that the enhancement of selectivity by avoiding perturbations from interferences will be obtained. From this work, the researcher was motivated to change a supporting electrolyte from phosphate buffer solution to NaOH solution to reduce an oxidative peak potential of histamine and to increase the selectivity of the proposed method. As shown in Figure 18E, as expected, using a NaOH solution as a supporting electrolyte can reduce the oxidative potential of histamine from +1.20 V to +0.58 V (vs. Ag/ AgCl) which corresponding to previous work. In addition, the current signal of histamine detection can also be enhanced. From this behavior, it can suggest that NaOH plays the significant role toward the oxidation of histamine using the proposed SPGE. This may be concerned that histamine is a strong base with containing two N-groups of pK_a ~9.4 for the aliphatic amino group and pK_a ~5.8 for the imidazole ring. In strong alkaline medium, the researcher believed that the imidazole ring of histamine is

deprotonated to form a negative charge which is easier to be oxidized at the SPGE. Moreover, the researcher has preliminarily performed the experiment using other biogenic amines that have a similar chemical structure to histamine such as tryptamine and tyramine. It is clearly observed that there is no effect from these amines because their oxidation peak appeared at + 0.12 V and + 0.22 V for tryptamine and tyramine, respectively. Thus, a NaOH solution was selected to use as a suitable supporting electrolyte for this work.

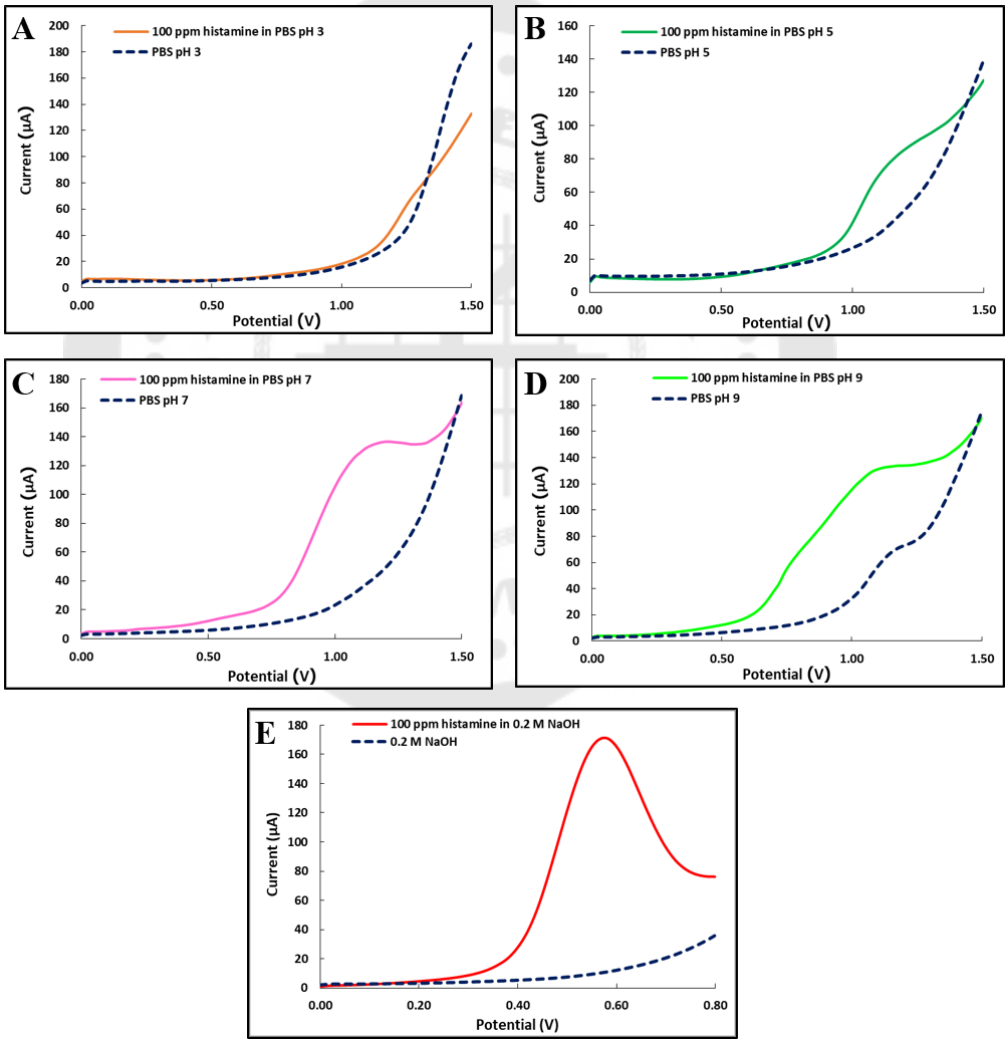

Figure 18 Square-wave voltammograms of histamine in different type of supporting electrolytes obtained from using SPGE: (A) 0.1 M phosphate buffer solution (PBS) pH = 3, (B) PBS pH = 5, (C) PBS pH = 7, (D) PBS pH = 9, and (E) 0.2 M NaOH solution

As results obtained from using a NaOH solution, it deeply prompted us to specifically study the effect of alkaline concentration to the oxidative peak current of histamine by using the proposed SPGE. The experiment was examined using various concentrations of NaOH solution in the range of 0.05-0.5 M, which have pH values from 12.7 to 13.7 corresponding to their concentrations. It can be observed that, the oxidative peak current was gradually increased until it reaches 0.2 M NaOH (pH 13.3). When the concentration of NaOH solution was greater than 0.2 M, the oxidative current responses did not change significantly (Figure 19). It implied that this concentration of a supporting electrolyte was high enough for compensation of IR drop in electrochemical cell and pH of this solution was enough for complete conversion of histamine to deprotonated form which it is easy to be oxidized at the SPGE surface. Therefore, 0.2 M NaOH solution was selected as the optimal concentration of a supporting electrolyte for this proposed method and used for the subsequent work.

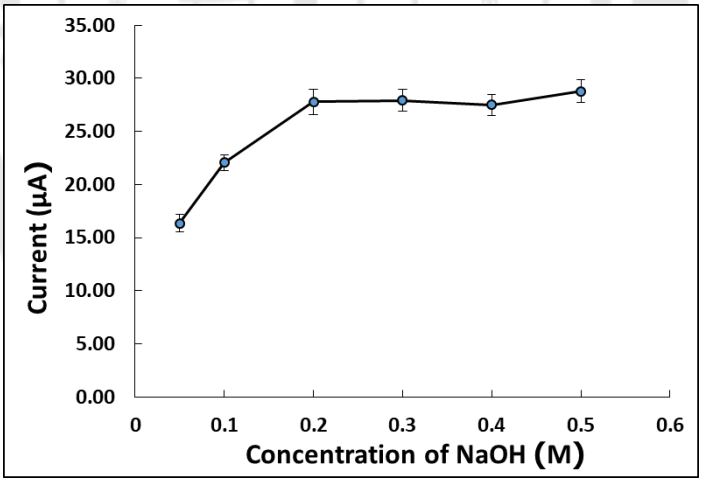

Figure 19 The effect of NaOH concentration

To confirm that SPGE is the most suitable electrode for the direct oxidation of histamine in alkaline media. Comparison was performed with the experiment set-up using screen-printed carbon electrode (SPCE), which have the same geometrical area of SPGE, instead of SPGE at the best electrolyte condition (0.2 M NaOH solution). It was found that the oxidative current of histamine obtained from SPGE was 2 times higher

than that obtained from SPCE as shown in Figure 20. This may be concerned to the fact that the SPGE provided a rough and porous surface with lots of graphene sheets leading to larger electroactive surface area (0.342 cm^2 for SPGE and 0.191 cm^2 for SPCE) and higher electrical conductivity which is corresponding to the previous reported by our group (Thangphatthanarungruang et al., 2020). In addition, the use of SPGE provided a lower oxidative peak potential than using SPCE. Therefore, it is verified that SPGE is a suitable electrode material for the determination of histamine using the proposed conditions.

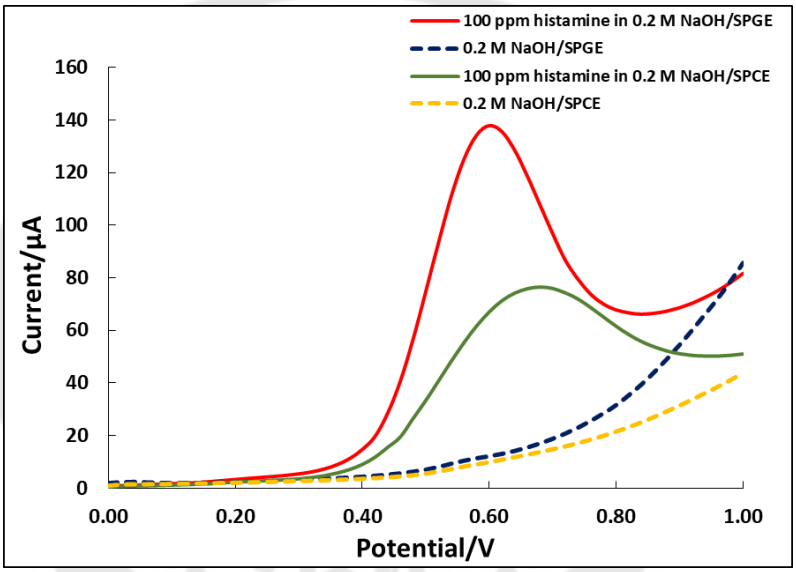

Figure 20 Square-wave voltammograms obtained from comparison study between using screen-printed carbon electrode (SPCE) and screen-printed graphene electrode (SPGE) at the best electrolyte condition (0.2 M NaOH).

2.2 Electrochemical characteristic of histamine at SPGE in 0.2 M NaOH solution and the influence of the scan rate

To study the efficiency of the proposed electrode for histamine determination, the electrochemical behavior of histamine at modifier-free SPGE was investigated using CV technique. The applied potential was operated in the range from -1.0 V to + 1.0 V (vs. Ag/AgCl) at a scan rate of 100 mV/s. The electrochemical measurement of histamine can be detected without any pretreatment of SPGE. The

obtained cyclic voltammograms in the absence and the presence of 100 ppm histamine in 0.2 M NaOH were shown in Figure 21. It can be noticed that a very well-defined oxidation peak of histamine was obtained at a low anodic peak potential (E_{pa}) of +0.58 V (vs. Ag/AgCl). This indicated that the electron transfer process easily occurred and the electrochemical process was an irreversible process. Due to the high surface area and high conductivity of graphene ink, the enhancement of signal towards the direct oxidation of histamine in 0.2 M NaOH solution was obtained as expected.

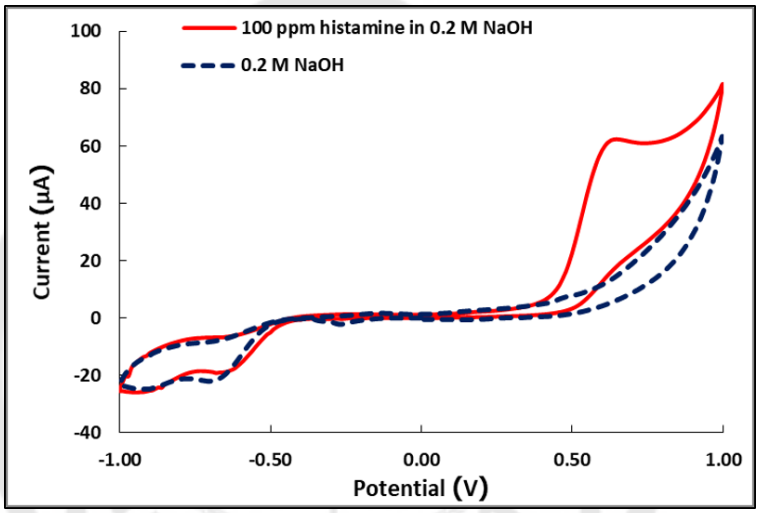

Figure 21 Cyclic voltammograms obtained from SPGE in 0.2 M NaOH (blue line) and 100 ppm histamine in 0.2 M NaOH (red line), respectively.

Besides, the effect of scan rate on the response of histamine at the SPGE was also examined by CV. The cyclic voltammograms of histamine at the SPGE using different scan rates were shown in Figure 22A. The results showed that when increasing the scan rate from 25 mV/s to 300 mV/s, the anodic peak current increases gradually while the oxidation peak potential shifts to more positive direction according to irreversible behavior. The anodic peak currents (I_{pa}) are proportional to the square root of the scan rate ($\upsilon^{1/2}$). The linear relationship between the anodic peak current and the square root of the scan rate was obtained with a linear equation: I_{pa} = 190.43X -1.6776 with R^2 = 0.9955 (Figure 22B). This result indicated that the nature of the mass transfer

process was controlled by diffusion-limited for the oxidation process of histamine at the unmodified SPGE. Moreover, the information obtained from the linear regression of the relationship between I_{pa} and $\upsilon^{1/2}$ can be used to calculate the diffusion coefficient (D_0) using Randles-Sevcík equation (Eq. 1) (Ferrari, Foster, Kelly, Brownson, & Banks, 2018). Where F is Faraday constant (96485 c mol^{-1}), A (electroactive surface area) = 0.342 cm^2, C_s (the concentration of histamine) = 9×10^{-7} mol cm^{-3}, R = 8.31447 J mol^{-1} K^{-1}, and T = 298 K. The D_0 of histamine was found to be 4.29 x 10^{-6} cm^2 s^{-1}.

$$I_{pa} = 0.496FAC_sD_0^{1/2}\left(\frac{F}{RT}\right)^{1/2}\upsilon^{1/2} \qquad \text{Eq. 1}$$

$$I_{pa} = (2.99\times10^5)n[(1-\alpha)n_\alpha]^{1/2}AC_sD_0^{1/2}\upsilon^{1/2} \qquad \text{Eq. 2}$$

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF}\ln\frac{RTk_s}{(1-\alpha)nF} + \frac{RT}{(1-\alpha)nF}\ln\upsilon \qquad \text{Eq. 3}$$

After calculating the value of D_0, the Eq. 2 was used to calculate the number of electron transfer (n) (Yomthiangthae, Kondo, Chailapakul, & Siangproh, 2020). To obtain the value of n, it is necessary to first determine the value of $(1-\alpha)n_\alpha$. For irreversible process, the calculation is based on Laviron's theory which is a linear relationship between the E_{pa} and ln υ (y = 0.0318x + 0.7182). The linear relationship revealed the slope value (0.0318). Then, the $(1-\alpha)n_\alpha$ value can be obtained from Eq. 3 (Laviron, 1979) and the parameter $(1-\alpha)n_\alpha$ was found to be 0.808. After that, the previously estimated D_0 of histamine from experiment and the value of $(1-\alpha)n_\alpha$ can be used to calculate the n value using Eq. 2, the factor n was found to be 1.11 ~ 1.00. It can be concluded that the oxidative pathway of histamine at the SPGE occurred with losing one electron.

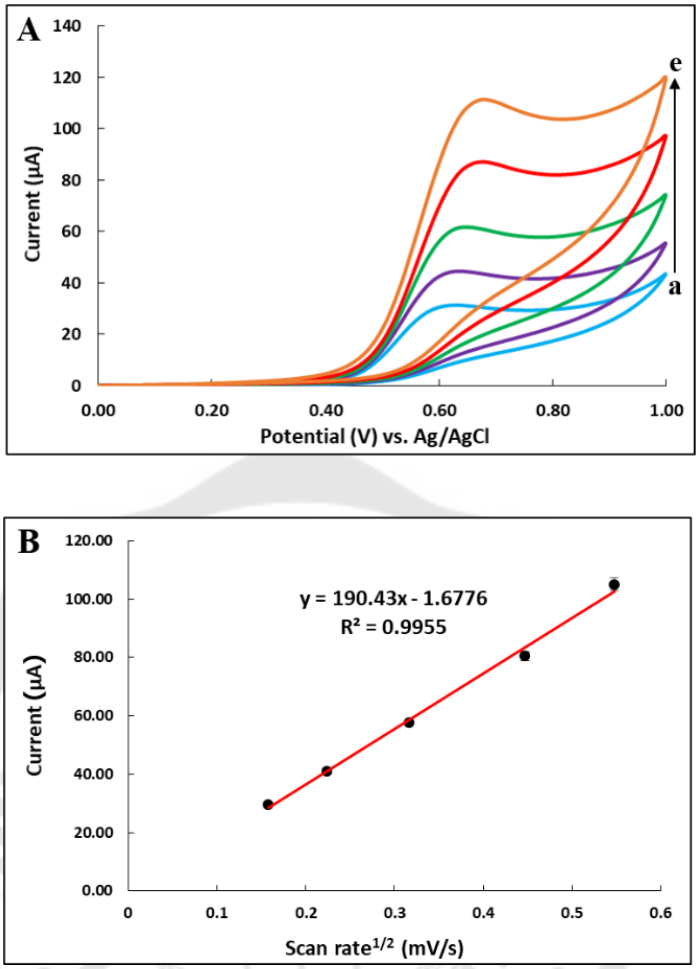

Figure 22(A) Cyclic voltammograms of histamine at the SPGE in 0.2 M NaOH using different scan rates (a-e: 25, 50, 100, 200, and 300 mV/s, respectively)
(B) Plot of the anodic peak currents versus the square root of the scan rate.

Furthermore, the electrochemical behavior of histamine at SPGE was also studied by SWV technique. The square wave voltammogram of 100 ppm histamine in 0.2 M NaOH was shown in Figure 18E. From this result, the SWV response displayed a higher oxidative current (~2 times) than CV response, suggesting that SWV offers a higher sensitivity than CV. Therefore, SWV was selected as a preferable technique for quantitative detection of histamine.

2.3 Optimization of the square-wave voltammetric parameters

The operating conditions and parameters of square-wave detection, including step potential, amplitude, and frequency were subsequently investigated. These parameters can affect the peak shape and peak current of the histamine response. To obtain the optimal value of these parameters, 100 ppm of histamine standard solution was used to record the square-wave voltammograms. The step potential in the range from 0.01 V to 0.03 V, the amplitude of 0.01 V to 0.1 V, and the frequency of 10 Hz to 30 Hz were monitored, respectively. From the results, the maximum currents were observed at a step potential of 0.025 V, amplitude of 0.075 V and frequency of 25 Hz. All results were shown subsequently in Figure 23A-C. Hence, the optimized step potential, amplitude, and frequency for the SWV were set at 0.025 V, 0.075 V, and 25 Hz, respectively. These parameters were used for all of the subsequent measurements.

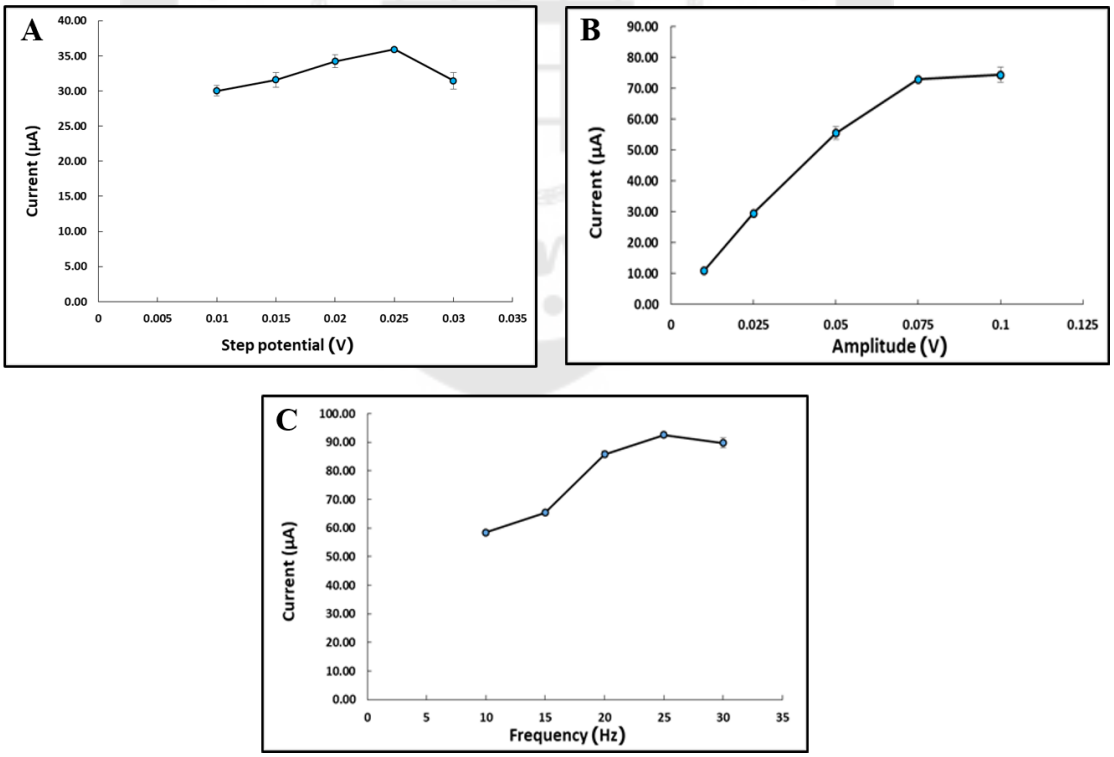

Figure 23 Optimization of square wave voltammetric parameters for histamine detection using SPGE: step potential (A), amplitude (B), and frequency (C)

2.4 Analytical performance

To evaluate the analytical performance of the proposed method, the quantitative analysis of histamine at the modifier-free SPGE was measured using SWV technique. Under the optimal conditions, the square wave voltammograms of the various concentrations of histamine were displayed in Figure 24. As can be seen that the anodic current increased with increasing the concentration of histamine up to 100 ppm. The calibration curve was constructed by plotting the anodic peak current versus the different concentrations of histamine (Figure 24 inset). The linearity was obtained in a wide concentration range of histamine from 5 ppm to 100 ppm with a correlation coefficient (R^2) = 0.9988. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the equation of 3SD/m and 10SD/m, respectively. Where SD and m were the standard deviation for ten replicates in blanks and the slope of the calibration curve, respectively. The obtained LOD and LOQ were found to be 0.62 ppm and 2.06 ppm of histamine, respectively.

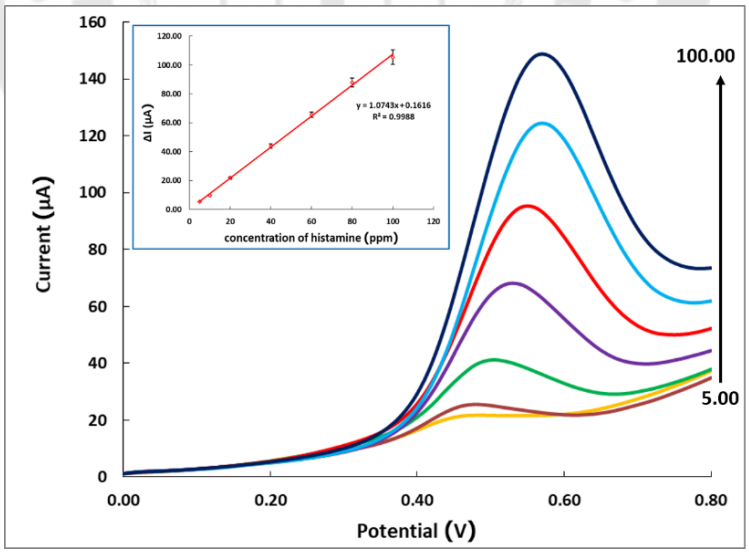

Figure 24 The square wave voltammograms of histamine in 0.2 M NaOH at various concentrations ranging from 5.00 ppm to 100.00 ppm.

Inset shows the plot of the anodic peak current vs. histamine concentrations.

The sensitivity of the proposed method was compared to the previous electrochemical methods and summarized in Table 2. Even though, the newly proposed methodology did not provide the lowest LOD. However, the proposed method exhibited excellent sensing performance enough for the determination of histamine in real-world analysis based on the criteria set by the EU and the FDA. Moreover, our proposed method offers the advantages of a simple, low-cost, portability and fast operation due to without any electrode pretreatment and electrode modification by enzyme or metal nanocatalysts.

Table 2 Comparison of the proposed method and other reported electrochemical methods for histamine detection

Working electrode	Peak potential (V)	Supporting electrolyte	Technique	Linear range (ppm)	LOD (ppm)
Boron-doped diamond electrode	+ 1.28	Phosphate buffer pH 7	Amperometry	0.05-10	0.05
AuNPs/GCE	+ 0.55	0.1 M NaOH	FIA-HPLC	0.2-44.46	0.06
Nickel-Film/GCE	+ 0.4	0.5 M NaOH	Chronoamperometry	0.5-110	0.11
MWCNTs/*p*-(AHNSA)/GCE	+ 1.0	Phosphate buffer pH 7	Differential pulse voltammetry	0.01-10	0.0076
DAO/nPt/graphene/CS/SPCE	+ 0.4	Phosphate buffer pH 7.4	Amperometry	0.01-30	0.0025
DAO/TiO_2/c-MWCNT/ RU/CS/SPCE	-0.30	0.05 M BR buffer pH 7	Chronoamperometry	0.99-110	0.69
MAO/TiO_2/c-MWCNT/ RU/CS/SPCE		0.05 M BR buffer pH 9		5.6-100	3.6
FrGO/GCE	+ 0.74	Phosphate buffer pH 8	Amperometry	0.02-8	0.0007
Cu@Pd/pencil graphite	+ 0.55	0.1 M KOH	Chronoamperometry	0.01-10	0.00032
Graphene nanoribbons/AgNPs/ EPPG	+0.94	Phosphate buffer pH 7.4	Square wave voltammetry	0.1-5, 6-50	0.0049
Modifier-free SPGE	**+ 0.58**	**0.2 M NaOH**	**Square wave voltammetry**	**5-100**	**0.62**

AuNPs: gold nanoparticles; GCE: glassy carbon electrode; MWCNTs: multi-walled carbon nanotubes; *p*-(AHNSA) : poly(4-amino-3-hydroxynaphthalene sulfonic acid); DAO: Diamine oxidase; nPt: platinum nanoparticles; CS: chitosan; SPCE: screen-printed carbon electrode; MAO: monoamine oxidase; TiO_2: titanium dioxide nanoparticles; c-MWCNT: carboxylated multiwalled carbon nanotubes; RU: hexaammineruthenium (III) chloride; FrGO: fluorine-doped reduced graphene oxide; AgNPs: silver nanoparticles; EPPG: edge plane of pyrolytic graphite; SPGE: screen-printed graphene electrode; BR; Britton–Robinson buffer.

2.5 Effect of the interference

To confirm the selectivity, the proposed electrode was used to investigate the current signals of histamine and other biogenic amines which probably found in fish samples such as putrescine, cadaverine, tyramine, and tryptamine. Using the optimal conditions of SWV experiments, 100 ppm individual standard solutions in 0.2 M NaOH solution were detected using the modifier-free SPGE as shown in Figure 25. Individually analyzing of each biogenic amine was compared to histamine oxidation (E_{pa} = + 0.58 V; I_{pa} = 104.54 μA). It was found that the oxidative peak of aliphatic amines, including putrescine and cadaverine, did not appear whereas the oxidative peak of aromatic amines, including tryptamine and tyramine, occurred at a less negative potential of +0.12 V and +0.22 V, respectively. From the results, tryptamine and tyramine may affect the oxidative current of histamine. However, the concentration of tryptamine and tyramine found in fish sample are very low levels (Yen & Hsieh, 1991) in comparison to histamine content. In addition, the standard addition curve was used for the quantitative calculation of histamine to get rid of any matrix influences. Therefore, these biogenic amines do not significantly affect to histamine detection using SPGE at the optimized conditions. Moreover, these results obtained suggested that only aromatic amines showed the oxidative peak current at the modifier-free SPGE, while aliphatic amines did not display this behavior. It can be concluded that the electrochemical conversion takes place at the imidazole ring of histamine rather than at the amine group. Therefore, the

developed modifier-free SPGE provided a high selectivity for determination of histamine in the absence of enzyme.

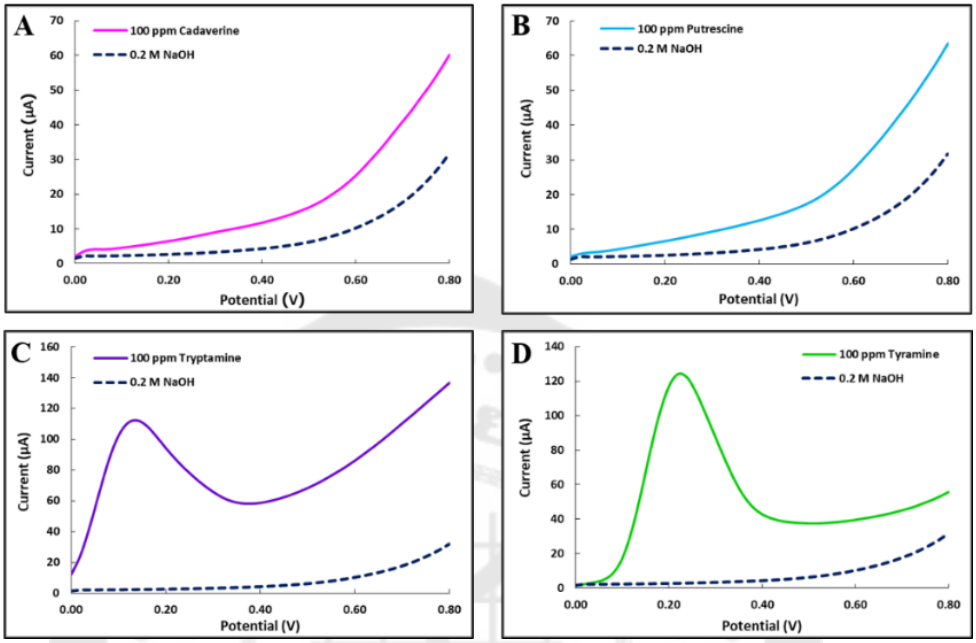

Figure 25 The square wave voltammograms of 100 ppm other biogenic amines using SPGE in 0.2 M NaOH: (A) cadaverine, (B) putrescine, (C) tryptamine, and (D) tyramine

2.6 Real sample analysis

Table 3 shows the recovery values for histamine detection in canned fish samples (tuna and mackerel) which were performed by using modifier-free SPGE as nonenzymatic sensing. The concentrations of sample were determined by using the standard addition method to get rid of any matrix influences as mentioned. This table shows the data for real sample analysis with and without histamine spiking. The obtained results showed that the average recoveries were in the range from 90.72 % to 101.21% . The values of recovery were very close to 100% with low % RSD values (< 5%), indicating that the developed electrode displayed a good accuracy and precision for histamine analysis.

Table 3 Comparison of the proposed method with HPLC method for the determination of histamine in canned fish samples (n=3)

Histamine spiked (ppm)	Histamine found (ppm), mean ± SD		Recovery (%) by this work	RSD (%)
	HPLC method	Proposed method		
T1	n.d.	n.d.	-	-
T1 + 10.00	9.95 ± 0.06	9.80 ± 0.07	98.01	0.73
T1 + 20.00	20.03 ± 0.52	19.97 ± 0.63	99.85	3.15
T1 + 40.00	39.56 ± 1.01	38.72 ± 1.03	96.79	2.65
T2	n.d.	n.d.	-	-
T2 + 10.00	9.65 ± 0.10	9.49 ± 0.14	94.87	1.47
T2 + 20.00	19.10 ± 0.50	18.28 ± 0.45	91.41	2.45
T2 + 40.00	37.72 ± 1.21	36.29 ± 1.22	90.72	3.36
M1	n.d.	n.d.	-	-
M1 + 10.00	9.95 ± 0.15	10.12 ± 0.27	101.21	2.70
M1 + 20.00	19.12 ± 0.45	18.90 ± 0.47	94.48	2.51
M1 + 40.00	38.72 ± 0.40	38.86 ± 0.47	97.15	1.21
M2	n.d.	n.d.	-	-
M2 + 10.00	9.85 ± 0.50	9.66 ± 0.42	96.62	4.37
M2 + 20.00	18.70 ± 0.60	18.74 ± 0.79	93.69	4.20
M2 + 40.00	37.42 ± 0.35	36.48 ± 0.40	91.19	1.11

T: canned tuna fish sample and M: canned mackerel fish sample

n.d.: not detected

Note that after histamine oxidation, it has lost one-electron to become a radical cation with a positive charge on imidazole nitrogen. Then, the radical stabilizes itself by dimerization with another leading to electropolymerization of histamine (Puthongkham, Lee, & Venton, 2019). This by-product can adsorb onto the electrode surface and this causes electrode fouling. From this critical found, our proposed disposable printed electrode is very useful for this application. In addition, the reproducibility between electrode and electrode was also investigated to confirm the performance of fabricated electrode. It was found that % RSDs between electrodes are less than 3%.

To verify the performance of the developed approach, the method was validated using the traditional HPLC method. The differences between both methods were verified by the paired Student's *t*-distribution test. At a confidence level of 95 %, the statistics revealed that $t_{calculated}$ (2.20) was below $t_{critical}$ (2.53), suggesting that there is no significant difference between the two methods. Therefore, it was summarized that the proposed method was reliable and appropriate to be applied for the determination of histamine in fish samples.

CHAPTER 5
CONCULSION

Herein, two simply alternative analytical methods were proposed for histamine detection, including colorimetric method and electrochemical method. The developed methods can be summarized into two parts as follows:

In the first part, a simple colorimetric method was developed using label-free silver nanoprisms (AgNPrs) as a chromogenic agent for rapid screening of histamine on paper-based analytical devices (PADs). PADs were fabricated using a wax printing method on filter paper substrate. The measurement is based on the oxidation of AgNPrs, which induces the etching of AgNPrs and leads to the changing of color. For colorimetric approach, the pink of AgNPrs at 400 ppm was selected as a suitable colorimetric agent for histamine detection in 0.1 M phosphate buffer solution (pH 5). Besides, the volume ratio between AgNPrs and histamine was 1:1 (10 µL: 10 µL). In the presence of histamine, the color change of the AgNPrs from pink to orange-yellow was rapidly visualized in 1 min. The semi-quantitative read-out by naked-eye over a broad histamine concentration range (20-100 ppm) can be obtained. The proposed method enables us to detect histamine by naked-eye with the detection limit of 20 ppm which is below the permissible limit by FDA (50 ppm). However, the proposed colorimetric sensor showed low selectivity because of the interference from other biogenic amines (cadaverine, putrescine, tyramine, and tryptamine).

Although the paper-based colorimetric sensor can provide a simple and rapid visual analysis method, however, its quantitative detection ability is low sensitivity and selectivity in comparison to those obtained from previous work. To overcome this problem, the electrochemical method was focused on a promising alternative choice to increase high selectivity and sensitivity.

Accordingly, in the second part, a novel non-enzymatic electrochemical assay using modifier-free screen-printed graphene electrode (SPGE) for the selective determination of histamine was first presented. SPGE is designed by screen-printed technology on transparency film. This electrode combined the benefits of graphene

nanomaterials and the disposable characteristic of screen-printed electrode. For electrochemical detection, the optimal conditions were investigated using square-wave voltammetry (SWV). From the results, 0.2 M NaOH was selected to use as a suitable supporting electrolyte for this work. Using a NaOH solution can reduce the oxidative potential of histamine from + 1.20 V to + 0.58 V (vs. Ag/AgCl). In addition, the current signal of histamine detection can also be enhanced from using conventional screen-printed carbon electrode. From this behavior, it can suggest that NaOH plays the significant role toward the oxidation of histamine at SPGE. Furthermore, the electrochemical behavior of histamine at modifier-free SPGE was investigated using cyclic voltammetry (CV). This result indicated that the nature of the mass transfer process was controlled by diffusion-limited for the oxidation process of histamine at the proposed SPGE. The mechanism of histamine oxidation at SPGE was also studied. It was found that the oxidative pathway of histamine at SPGE occurred with losing one electron. In addition, the optimal parameters of square-wave detection such as frequency, amplitude and step potential were subsequently studied. In this work, the optimized step potential, amplitude, and frequency for the SWV were set at 0.025 V, 0.075 V, and 25 Hz, respectively. Under the optimal conditions, the linearity was obtained in a wide concentration range of histamine from 5 ppm to 100 ppm with a correlation coefficient (R^2) = 0.9988. The limit of detection (LOD) and the limit of quantification (LOQ) were found to be 0.62 ppm and 2.06 ppm, respectively. The proposed method was also investigated the selectivity of modifier-free SPGE from other biogenic amines which are probably found in fish samples such as putrescine, cadaverine, tyramine, and tryptamine. It was observed that these biogenic amines do not significantly affect to histamine detection using SPGE by proposed method.

Finally, the proposed method was successfully applied to determine histamine in canned fish samples with satisfying accuracy (%recovery = 90.72 % - 101.21%) and precision (% RSD values < 5%). Moreover, the proposed electrochemical method was validated using the traditional HPLC method. The results suggested that there were no significant difference between the two methods. It can be concluded that the proposed

method was reliable and appropriate to be applied for the determination of histamine in canned fish samples.

The proposed method offers the advantages of a simple direct detection (enzyme-free sensor), low-cost, portability, and fast operation due to the unnecessary of electrode pretreatment. Therefore, the developed method offers a high potential of being an alternative option for freshness evaluation of fish to prevent toxic on human health and also provide a promising choice in routine sensing application for monitoring histamine in fish samples and may apply for the whole supply chain of food industry in the future.

Future works, the proposed colorimetric method in the first part of this dissertation is fascinating for the development of the test-kit device because it exhibited the rapid and simple assay for screening of histamine detection. To solve the selectivity of this method, thin-layer chromatography (TLC) is an interesting technique used in parallel for the separation of target analyte from interferences. Therefore, the colorimetric method combined with TLC will be developed for histamine detection in the future.

REFERENCES

Akyazi, T., Basabe-Desmonts, L., & Benito-Lopez, F. (2018). Review on microfluidic paper-based analytical devices towards commercialisation. *Analytica Chimica Acta*, 1-17.

Apetrei, I. M., & Apetrei, C. (2016). Amperometric biosensor based on diamine oxidase/platinum nanoparticles/graphene/chitosan modified screen-printed carbon electrode for histamine detection. *Sensors, 16*(4), 422-436.

Bard, A. J., & Faulkner, L. R. (2001). *Electrochemical methods fundamentals and applications* (second). New York: Wiley.

Carralero, V., González-Cortés, A., Yáñez-Sedeño, P., & Pingarrón, J. M. (2005). Pulsed amperometric detection of histamine at glassy carbon electrodes modified with gold nanoparticles. *Electroanalysis, 17*(4), 289-297.

Carrilho, E., Martinez, A. W., & Whitesides, G. M. (2009). Understanding wax printing: a simple micropatterning process for paper-based microfluidics. *Analytical Chemistry, 81*(16), 7091-7095.

Charoenkitamorn, K., Chaiyo, S., Chailapakul, O., & Siangproh, W. (2018). Low-cost and disposable sensors for the simultaneous determination of coenzyme Q10 and alpha-lipoic acid using manganese (IV) oxide-modified screen-printed graphene electrodes. *Analytica Chimica Acta, 1004*, 22-31.

Chena, Z., Lin, Y., Ma, X., Guo, L., Qiu, B., Chen, G., & Lin, Z. (2017). Multicolor biosensor for fish freshness assessment with the naked eye. *Sensors and Actuators B: Chemical, 252*, 201–208.

Comas-Baste, O., Latorre-Moratalla, M. L., Bernacchia, R., Veciana-Nogues, M. T., & Vidal-Carou, M. C. (2017). New approach for the diagnosis of histamine intolerance based on the determination of histamine and methylhistamine in urine. *Journal of Pharmaceutical and Biomedical Analysis, 145*, 379-385.

Dropsens. (May 20, 2019). Screen-printed electrodes. http://www.dropsens.com/en/screen_printed_electrodes_pag.html

FAO/WHO. (2013). Joint FAO/WHO expert meeting on the public health risks of histamine and other biogenic amines from fish and fishery products. *Meeting report, FAO/WHO; Rome, Italy*, 17.

Ferrari, A. G.-M., Foster, C. W., Kelly, P. J., Brownson, D. A. C., & Banks, C. E. (2018). Determination of the electrochemical area of screen-printed electrochemical sensing platforms. *Biosensors, 8*(2), 53-62.

Fiala, R. (May 18, 2019). Cycling Voltammetry: principle. https://physics.mff.cuni.cz/kfpp/povrchy/method/cv-principles

Fu, L. M., & Wang, Y. N. (2018). Detection methods and applications of microfluidic paper-based analytical devices. *Trends in Analytical Chemistry, 107*, 196-211.

Gajjala, R. K. R., & Palathedath, S. K. (2018). Cu@Pd core-shell nanostructures for highly sensitive and selective amperometric analysis of histamine. *Biosensors and Bioelectronics, 102*, 242-246.

Geto, A., Tessema, M., & Admassie, S. (2014). Determination of histamine in fish muscle at multi-walled carbonnanotubes coated conducting polymer modified glassy carbonelectrode. *Synthetic Metals, 191*, 135–140.

Halasz, A., Barath, A., Sarkadi, L. S., & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science & Technology, 5*(2), 42-49.

Hashemi, M., Nazari, Z., & Noshirvani, N. (2017). Synthesis of chitosan based magnetic molecularly imprinted polymers for selective separation and spectrophotometric determination of histamine in tuna fish. *Carbohydrate Polymers, 177*, 306-314.

Hassan, A. H. A., Sappia, L., Moura, S. L., Ali, F. H. M., Moselhy, W. A., Sotomayor, M., & Pividori, M. I. (2019). Biomimetic magnetic sensor for electrochemical determination of scombrotoxin in fish. *Talanta, 194*, 997-1004.

Heinze, J. (1984). Cyclic Voltammetry-"Electrochemical Spectroscopy". *Angewandte Chemie, 23*(11), 831-918.

Huang, C., Wang, S., Zhao, W., Zong, C., Liang, A., Zhang, Q., & Liu, X. (2017). Visual and photometric determination of histamine using unmodified gold nanoparticles.

Microchimica Acta, 184(7), 2249–2254.

Hwang, B. S., Wang, J. T., & Choong, Y. M. (2003). A rapid gas chromatographic method for the determination of histamine in fish and fish products. *Food Chemistry, 82*, 329–334.

Jiang, X. C., & Yu, A. B. (2008). Silver nanoplates: a highly sensitive material toward inorganic anions. *Langmuir, 24*(8), 4300-4309.

Keow, C. M., Bakar, F. A., Salleh, A. B., Heng, L. Y., Wagiran, R., & Siddiquee, S. (2012). Screen-printed histamine biosensors fabricated from the entrapment of diamine oxidase in a photocured poly(HEMA) film. *International Journal of Electrochemical Science, 7*(5), 4702-4715.

Kounaves, S. P. (1997). Voltammetric Techniques. *Handbook of Instrumental Techniques for Analytical Chemistry*, 709-725.

Kumar, N., & Goyal, R. N. (2018). Silver nanoparticles decorated graphene nanoribbon modifiedpyrolytic graphite sensor for determination of histamine. *Sensors and Actuators B: Chemical, 268*, 383–391.

Laviron, E. (1979). General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry, 101*, 19-28.

Leszczynska, J., Wiedlocha, M., & Pytasz, U. (2004). The histamine content in some samples of food products. *Czech Journal of Food Sciences, 22*, 81–86.

Lin, Y. T., Chen, C. H., & Lin, M. S. (2018). Enzyme-free amperometric method for rapid determination ofhistamine by using surface oxide regeneration behavior of copper electrode. *Sensors and Actuators B: Chemical, 255*, 2838–2843.

Luo, L., Yang, J. Y., Xiao, Z. L., Zeng, D. P., Li, Y. J., Shen, Y. D., . . . Xu, Z. L. (2015). A sensitivity-enhanced heterologous immunochromatographic assay based on a monoclonal antibody for the rapid detection of histamine in saury samples. . *Royal Society of Chemistry Advances, 5*(96), 78833–78840.

Martinez, A. W., Phillips, S. T., Butte, M. J., & Whitesides, G. M. (2007). Patterned paper as a platform for inexpensive, low volume, portable bioassays. *Angewandte Chemie*

International Edition, 46(8), 1318-1320.

Mattsson, L., Jungmann, C., Lieberzeit, P. A., & Preininger, C. (2016). Modified carbon black as label in a colorimetric on-chip immunoassay for histamine. *Sensors and Actuators B: Chemical, 246*, 1092–1099.

Nakthong, P., & Muangthai, P. (2015). Tyramine and histamine content in some Thai medicinal plants. *Proceedings of the Burapha University International Conference*, 370-376.

Nantaphol, S. (2016). *Electrochemical detection of organic and biological compounds with modified electrode* (Ph. D.). Chulalongkorn University, Bangkok. (Department of chemistry).

Niculescu, M., Frébort, I., Pe**č**, P., Galuszka, P., Mattiasson, B., & Csöregi, E. (2000). Amine oxidase based amperometric biosensors for histamine detection. *Electroanalysis, 12*(5), 369-375.

Panraksa, Y., Siangproh, W., Khampieng, T., Chailapakul, O., & Apilux, A. (2018). Paper-based amperometric sensor for determination of acetylcholinesterase using screen-printed graphene electrode. *Talanta, 178*, 1017-1023.

Parnklang, T., Lertvachirapaiboon, C., Pienpinijtham, P., Wongravee, K., Thammacharoen, C., & Ekgasit, S. (2013). H_2O_2-triggered shape transformation of silver nanospheres to nanoprisms with controllable longitudinal LSPR wavelengths. *The Royal Society of Chemistry Advances, 3*, 12886-12894.

Patange, S. B., Mukundan, M. K., & Kumar, K. A. (2005). A simple and rapid method for colorimetric determination of histamine in fish flesh. *Food Control, 16*, 465–472.

Puthongkham, P., Lee, S. T., & Venton, B. J. (2019). Mechanism of histamine oxidation and electropolymerization at carbon electrodes. *Analytical Chemistry, 91*(13), 8366-8373.

Rattanarat, P., Dungchai, W., Cate, D., Volckens, J., Chailapakul, O., & Henry, C. S. (2014). Multilayer paper-based device for colorimetric and electrochemical quantification of metals. *Analytical Chemistry, 86*(7), 3555-3562.

Saaid, M., Saad, B., Hashim, N. H., Mohamed Ali, A. S., & Saleh, M. I. (2009).

Determination of biogenic amines in selected Malaysian food. *Food Chemistry, 113*, 1356–1362.

Sarada, B. V., Rao, T. N., Tryk, D. A., & Fujishima, A. (2000). Electrochemical oxidation of histamine and serotonin at highly boron-doped diamond electrodes. *Analytical Chemistry, 72*(7), 1632-1638.

Schofield, C. L., Haines, A. H., Field, R. A., & Russell, D. A. (2006). Silver and gold glyconanoparticles for colorimetric bioassays. *Langmuir, 22*(15), 6707-6711.

Shahzad, F., Zaidi, S. A., & Koo, C. M. (2017). Synthesis of Multifunctional Electrically Tunable Fluorine-Doped Reduced Graphene Oxide at Low Temperatures. *ACS Applied Materials & Interfaces, 9*(28), 24179-24189.

Svarc-Gajic, J., & Stojanovic, Z. (2010). Electrocatalytic determination of histamine on a nickel-film glassy carbon electrode. *Electroanalysis, 22*(24), 2931–2939.

Taleat, Z., Khoshroo, A., & Mazloum-Ardakani, M. (2014). Screen-printed electrodes for biosensing: a review (2008–2013). *Microchimica Acta, 181*, 865–891.

Tao, Z., Sato, M., Han, Y., Tan, Z., Yamaguchi, T., & Nakano, T. (2011). A simple and rapid method for histamine analysis in fish and fishery products by TLC determination. *Food Control, 22*, 1154-1157.

Thangphatthanarungruang, J., Yakoh, A., Laocharoensuk, R., Chotsuwan, C., Chailapakul, O., & Siangproh, W. (2020). High-efficient of graphene nanocomposite: Application to rapidly simultaneous identification and quantitation of fat-soluble vitamins in different matric samples. *Journal of Electroanalytical Chemistry, 873*, 114361.

Thom, N. K., Lewis, G. G., Yeung, K., & Phillips, S. T. (2014). Quantitative fluorescence assays using a self-powered paper-based microfluidic device and a camera-equipped cellular phone. *The Royal Society of Chemistry Advances, 4*, 1334–1340.

Vilela, D., Gonzalez, M. C., & Escarpa, A. (2012). Sensing colorimetric approaches based on gold and silver nanoparticles aggregation: chemical creativity behind the assay. A review. *Analytica Chimica Acta, 751*, 24-43.

Wang, H., Li, Y. J., Wei, J. F., Xu, J. R., Wang, Y. H., & Zheng, G. X. (2014). Paper-based

three-dimensional microfluidic device for monitoring of heavy metals with a camera cell phone. *Analytical and Bioanalytical Chemistry, 406*(12), 2799-2807.

Wang, J. (2000). *Analytical Electrochemistry* (2nd). New York: Wiley.

Wifling, D. (2015). *Constitutive activity of the human histamine H_4 receptor: Molecular modelling, binding and functional studies on wild-type and mutant H_4R orthologs.* (Ph. D.). University Regensburg, Germany. (Faculty of Chemistry and Pharmacy).

Wu, L., Ma, C., Ge, L., Kong, Q., Yan, M., Ge, S., & Yu, J. (2015). Paper-based electrochemiluminescence origami cyto-device for multiple cancer cells detection using porous AuPd alloy as catalytically promoted nanolabels. *Biosensors and Bioelectronics, 63*, 450-457.

Xia, Y., Si, J., & Li, Z. (2016). Fabrication techniques for microfluidic paper-based analytical devices and their applications for biological testing: A review. *Biosensors and Bioelectronics, 77*, 774-789.

Yadav, S., Nair, S. S., Sai, V. V. R., & Satija, J. (2019). Nanomaterials based optical and electrochemical sensing of histamine: Progress and perspectives. *Food Research International, 119*, 99-109.

Yakoh, A., Rattanarat, P., Siangproh, W., & Chailapakul, O. (2018). Simple and selective paper-based colorimetric sensor for determination of chloride ion in environmental samples using label-free silver nanoprisms. *Talanta, 178*, 134-140.

Yamamoto, K., Takagi, K., Kano, K., & Ikeda, T. (2001). Bioelectrocatalytic detection of histamine using quinohemoprotein amine dehydrogenase and the native electron acceptor cytochrome c-550. *Electroanalysis, 13*(5), 375-379.

Yen, G.-C., & Hsieh, C.-L. (1991). Simultaneous analysis of biogenic amines in canned fish by HPLC. *Journal of Food Science, 56*(1), 158-160.

Yilmaz, U. T., & Inan, D. (2015). Quantification of histamine in various fish samples using square wave stripping voltammetric method. *Journal of Food Science and Technology, 52*(10), 6671-6678.

Yomthiangthae, P., Kondo, T., Chailapakul, O., & Siangproh, W. (2020). The effect of supporting electrolyte for the simultaneous determination of vitamin B_2, vitamin B_6,

and vitamin C using a modification-free screen-printed carbon electrode. *New Journal of Chemistry, 44*, 12603-12612.

Yu, J., Wang, S., Ge, L., & Ge, S. (2011). A novel chemiluminescence paper microfluidic biosensor based on enzymatic reaction for uric acid determination. *Biosensors and Bioelectronics, 26*(7), 3284-3289.

Zeng, K., Tachikawa, H., Zhu, Z., & Davidson, V. L. (2000). Amperometric detection of histamine with a methylamine dehydrogenase polypyrrole-based sensor. *Analytical Chemistry, 72*(10), 2211-2215.

Zhang, C., Hu, Z., & Deng, B. (2016). Silver nanoparticles in aquatic environments: Physiochemical behavior and antimicrobial mechanisms. *Water Research, 88*, 403-427.

Zhang, N., Wang, H., Zhang, Z. X., Deng, Y. H., & Zhang, H. S. (2008). Sensitive determination of biogenic amines by capillary electrophoresis with a new fluorogenic reagent 3-(4-fluorobenzoyl)-2-quinolinecarboxaldehyde. *Talanta, 76*, 791–797.

Zhang, W., Xiao, B., & Fang, T. (2018). Chemical transformation of silver nanoparticles in aquatic environments: Mechanism, morphology and toxicity. *Chemosphere, 191*, 324-334.

APPENDIX

Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d0ay01443e

Development of an unmodified screen-printed graphene electrode for nonenzymatic histamine detection†

Prangthip Nakthong,[a] Takeshi Kondo,[b] Orawon Chailapakul[c] and Weena Siangproh[*a]

Received 28th July 2020
Accepted 1st October 2020

DOI: 10.1039/d0ay01443e

rsc.li/methods

The high requirement for food quality control has inspired the creation of high-performance sensing, cost-effectiveness, and ease to use. Therefore, the aim of this work is to develop nonenzymatic electrochemical platforms for direct detection of histamine using unmodified screen-printed graphene electrodes (SPGEs) for their applications such as evaluation of fish freshness. In alkaline media (0.2 M NaOH), unmodified SPGEs showed a very low oxidation potential of histamine at +0.58 V (*vs.* Ag/AgCl) avoiding perturbations from other biogenic amines. The developed method offers an excellent selectivity, sensitivity (a limit of detection (at 3SD/slope) of 0.62 mg L^{-1}) and wide working linear range (5–100 mg L^{-1}) for histamine detection. In addition, the proposed method was successfully applied to detect histamine in canned fish samples with recovery values ranging from 90.72% to 101.21%. Therefore, this newly proposed method is promising as an alternative choice for the determination of histamine in fish samples and related food products.

1. Introduction

Histamine is an important biogenic amine found in many food products. It is derived from the bacterial decarboxylation of amino acid histidine by histidine decarboxylase.[1] Generally, histamine is produced during the microbial decomposition of scombroid fish such as tuna and mackerel because these fish species contain high levels of histidine.[2] The United States Food and Drug Administration (FDA) has established 50 ppm of histamine as the chemical index for fish spoilage.[3] Thus, the histamine content is used as a chemical freshness index of fish. Furthermore, consuming excess histamine can cause toxicity which is called histamine poisoning or scombroid poisoning. Several symptoms appear such as nausea, vomiting, diarrhea, and allergies.[4] To prevent the potential risk provoked by histamine in food, the European Union (EU) has assigned the amount of histamine in food at lower than 200 ppm. In addition, the FDA has also set a toxic level of histamine in fish at 500 ppm.[5] Hence, the detection of histamine is an important topic in food safety and clinical diagnosis.

To estimate histamine levels for fish freshness, various analytical methods have been reported including thin-layer chromatography (TLC),[6] colorimetric assay,[7] fluorometric assay and enzyme-linked immunosorbent assay (ELISA).[8] Among these mentioned, chromatographic techniques such as gas chromatography (GC),[9] high-performance liquid chromatography (HPLC)[10] and capillary electrophoresis (CE)[11] are mostly reported as a precise and reliable quantitative analysis for histamine. Although these techniques exhibit a high sensitivity and selectivity, they still have some limitations such as requirement of the derivatization step before detection and/or complex procedures of sample preparation. These lead to time consumption and expensive operating systems. Most importantly, they are not portable. To overcome these problems, it is a challenge to continuously develop a novel methodology for histamine detection to fulfill the role of field analysis in the food supply chain.

The quantification of histamine using the electrochemical method is a promising alternative choice due to high sensitivity, simplicity, rapid analysis, and low cost of instrumentation in comparison to chromatography and spectrophotometry. However, the main drawbacks for the detection of histamine by the electrochemical method are low sensitivity due to poor electrochemical properties (the high oxidation potential of histamine at 1.2–1.4 V (*vs.* SCE)[12]), electrode surface fouling, and the lack of selectivity. To solve these problems, enzyme-based biosensors using various enzymes such as amine

[a]*Electroanalytical and Imaging Sensor Research Group (EISRG), Department of Chemistry, Faculty of Science, Srinakharinwirot University, Sukhumvit 23, Wattana, Bangkok 10110, Thailand. E-mail: weena@g.swu.ac.th; weenasi@hotmail.com; Fax: +662 259 2097; Tel: +662 640 5000 ext 18208*

[b]*Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan*

[c]*Electrochemistry and Optical Spectroscopy Research Unit (EOSRU), Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Patumwan, Bangkok 10330, Thailand*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0ay01443e

oxidases,[13,14] diamine oxidase,[15,16] methylamine dehydrogenases,[17] and quinohemoprotein amine dehydrogenase[18] have been continuously reported in the past few decades. However, these biosensors still have drawbacks such as lifetime and cost of enzymes, complicated procedure, time consumption, and poor reproducibility. Furthermore, several reports published have described the outstanding performance of electrochemical electrodes for detection of histamine by modification of the working electrode surface using various nanoparticles such as metal nanoparticles[19–21] and carbon nanoparticles.[3,22] Nowadays, screen-printed electrodes (SPEs) have been extensively employed for development of various novel electrochemical sensing platforms for pharmaceutical analysis,[23] biosensors for agri-food safety,[24] and in flow systems.[25] SPEs became the candidate for use because of their economic production, miniaturized scale, capability of mass production, and suitability for on-site analysis.[26] Graphene is widely used for the fabrication of screen-printed electrodes because of its high electrical conductivity, high electron mobility, and large surface area, making it a very attractive material from an electrochemical viewpoint for increasing the sensitivity of detection.[27,28] Such benefits of SPGEs prompt us to develop a new electrode for analysis of histamine. And most importantly, as revealed by literature search there is no research reported on direct electrochemical oxidation of histamine using SPGEs. Therefore, this research is the first to develop a new electrode for direct quantitative analysis of histamine using SPGEs without any modifiers and enzymes.

To reach the ultimate goal, in this work, we presented a simple and cost-effective analytical assay as an alternative choice for histamine detection. To obtain high selectivity, the electrochemical measurements were performed using an unmodified SPGE in alkaline media (NaOH). These electrodes were designed by screen-printing technology on a transparent film substrate. The conditions of electrochemical measurements were studied and optimized using square wave voltammetry (SWV). Under the optimized conditions, the SPGE showed a very low oxidation potential of histamine at +0.58 V (*vs.* Ag/AgCl) avoiding perturbations from other biogenic amines. Finally, the proposed method was applied to determine histamine in canned fish samples with high accuracy and precision.

2. Experimental

2.1 Chemicals, reagents, and apparatus

Histamine dihydrochloride, cadaverine dihydrochloride, 1,4-diaminobutane dihydrochloride (putrescine), tyramine hydrochloride, and tryptamine hydrochloride were purchased from Sigma-Aldrich, USA. Disodium hydrogen orthophosphate ($Na_2HPO_4 \cdot 2H_2O$), sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$) and sodium hydroxide (NaOH) were purchased from Ajax Finechem, Australia. All chemicals were analytical reagent (AR) grade and used without any purification procedure. All solutions were prepared with deionized (DI) water from Millipore (R 18.2 MΩ.cm) throughout these experiments.

In this work, electrochemical experiments were carried out using a PalmSens handheld potentiostat (model Palm Instruments BV, Netherlands), and controlled with PSTrace 5.4 software. A three-electrode system consisting of a silver/silver chloride paste (Ag/AgCl, SSS-1913) from Sun chemical Ltd, UK served as the reference electrode, a graphene ink (ZP-1838) from Serve Science Co., Ltd, Thailand served as the auxiliary electrode and the working electrode was home-made printed. The screen-printed template block was fabricated by Chaiyaboon Co. Ltd (Bangkok, Thailand).

2.2 Fabrication of the screen-printed graphene electrode (SPGE)

The proposed SPGE was designed using Adobe Illustrator CS6 software. The SPGEs were fabricated using the home-made screen-printing method on a transparent sheet substrate (polypropylene, purchased from a local stationery store) according to previous work[29] with slight modification. First, graphene ink was printed onto a substrate to obtain the auxiliary and working electrodes, respectively. Then, these electrodes were placed in an oven at 55 °C for 30 min. After that, Ag/AgCl ink was printed onto the same substrate to obtain the reference electrode and conducting pads. Finally, these screened electrodes were placed in an oven at 55 °C for 30 min to complete the process of electrode fabrication. The obtained electrodes containing 20 devices per time were kept in a dry place. The image of the printed electrode is shown in Fig. S1(A).† The screen-printed carbon electrodes (SPCEs) were fabricated using the same screen-printed template block (geometrical area of electrode = 0.126 cm^2) as that used for screen-printed graphene electrodes by replacing graphene ink with carbon ink. All steps performed were the same as for SPGEs.

2.3 The electrochemical measurement of histamine using an unmodified SPGE

Voltammetric measurements were performed at room temperature using a PalmSens handheld potentiostat. The electrochemical behavior of the histamine was investigated with the SPGE using cyclic voltammetry (CV). The cyclic voltammograms were recorded from −1.0 V to +1.0 V at a scan rate of 100 mV s^{-1}. Square-wave voltammetry (SWV) was used for the direct determination of histamine. For the analytical procedure, 100 μL of histamine solution in 0.2 M NaOH was added to cover the electrochemical detection zone of the device. Then, a potential from 0.0 V to +0.8 V was applied to the system by using the setup as follows: step potential = 0.025 V; amplitude = 0.075 V; frequency = 25 Hz.

2.4 Analysis of histamine in canned fish samples

To demonstrate the benefit and capability of the proposed electrode and optimized conditions, the method was applied to detect histamine in canned fish samples. The canned fish samples were purchased from a local supermarket. The samples were prepared according to previous work.[30] Briefly, five grams of muscle tissue was homogenized with 40 mL of distilled water. This mixture was stirred for 5 min and then placed in an ultrasound bath and sonicated for 20 min. The resultant mixture was centrifuged for 10 min at 6000 rpm. Next, the

supernatant was filtered through a Whatman filter paper. Before measurement, the filtrate solution was diluted to 1000 μL with 0.2 M NaOH for electrochemical detection of histamine. For spiked samples, the standard histamine was added into the sample before performing the sample preparation, same as the previous step. The samples were spiked with different concentrations of standard histamine (10.00, 20.00 and 40.00 mg L^{-1}). The spiked samples were used to study the recovery to check the accuracy of the proposed method. To confirm the accuracy and reliability of the approach, the proposed method was validated using the traditional high performance liquid chromatography (HPLC) method.[31]

3. Results and discussion

3.1 Effect of different types of supporting electrolytes on the electrochemical behavior of histamine at the unmodified SPGE

A systematic study of the influence of the type of supporting electrolyte on the electrochemical behavior of histamine at the unmodified SPGE was first investigated. Preliminary experiments were carried out by SWV using 0.1 M phosphate buffer solution at different pH values (pH 3, 5, 7, and 9) as supporting electrolyte. These experiments revealed that the highest peak current and well-defined peak shape was obtained when using a 0.1 M phosphate buffer solution at pH 7 (Fig. 1C) as supporting electrolyte. However, the oxidation peak of histamine occurred at a high potential of +1.20 V (*vs.* Ag/AgCl). The results implied that the use of phosphate buffer solution at pH 7 as a supporting electrolyte still provided a low selectivity because another biogenic amine can be oxidized as well at high potential. Therefore, we tried to review the literature and we found that besides buffer solutions, NaOH solution was used as a supporting electrolyte for histamine detection at a thin film nickel electrode.[19] As the results demonstrated, the chronoamperometric response of histamine showed the oxidation process at a lower oxidation potential of +0.55 V (*vs.* Ag/AgCl). This means that histamine was easily oxidized in an alkaline medium. These findings also implied that the enhancement of selectivity by avoiding perturbations from interferences will be obtained. From this work, we were motivated to change the supporting electrolyte from phosphate buffer solution to NaOH solution to reduce the oxidation peak potential of histamine and to increase the selectivity of the proposed method. As shown in Fig. 1E, as expected, using a NaOH solution as the supporting electrolyte can reduce the oxidation potential of histamine from +1.20 V to +0.58 V (*vs.* Ag/AgCl) which agrees with previous work. In addition, the current signal of histamine detection can also be enhanced. From this behavior, we can suggest that NaOH plays a significant role toward the oxidation of histamine using the proposed SPGE. This may be due to the fact that histamine is a strong base with two N-groups of pK_a ~9.4 for the aliphatic amino group and pK_a ~5.8 for the imidazole ring. In a strong alkaline medium, we believed that the imidazole ring of histamine is deprotonated to form a negative charge which is easily oxidized at the SPGE. Moreover, we have preliminarily performed the experiment using other biogenic amines that have a similar chemical structure to histamine such

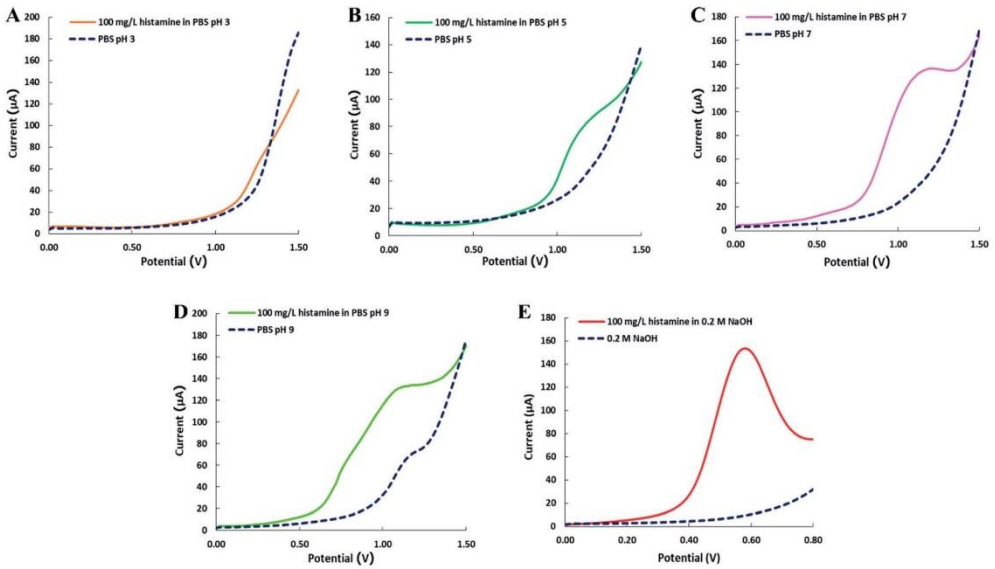

Fig. 1 Square-wave voltammograms of histamine in different types of supporting electrolytes obtained from using the SPGE: (A) 0.1 M phosphate buffer solution (PBS) pH = 3, (B) PBS pH = 5, (C) PBS pH = 7, (D) PBS pH = 9, and (E) 0.2 M NaOH solution.

as tryptamine and tyramine. It is clearly observed that there is no effect from these amines because the oxidation peak appeared at +0.12 V and +0.22 V for tryptamine and tyramine, respectively. Thus, NaOH solution was selected for use as a suitable supporting electrolyte for this work.

The results obtained from using a NaOH solution deeply prompted us to specifically study the effect of alkaline concentration on the oxidative peak current of histamine using the proposed SPGE. The experiment was conducted using various concentrations of NaOH solution in the range of 0.05–0.5 M, which have pH values from 12.7 to 13.7 corresponding to their concentrations. It can be observed that, the oxidative peak current was gradually increased until it reaches 0.2 M NaOH (pH 13.3). When the concentrations of NaOH solution were greater than 0.2 M, the oxidative current responses did not change significantly (Fig. S2†). It implied that this concentration of a supporting electrolyte is high enough for compensation of the IR drop in the electrochemical cell and pH of this solution is enough for complete conversion of histamine to the deprotonated form which it is easy to be oxidized at the SPGE surface. Therefore, 0.2 M NaOH solution was selected as the optimal concentration of the supporting electrolyte for this proposed method and used for the subsequent work.

To confirm that the SPGE is the most suitable electrode for the direct oxidation of histamine in alkaline media, a comparison was performed with the experiment set-up using screen-printed carbon electrodes (SPCEs), which have the same geometrical area of the SPGE, instead of the SPGE under the best electrolyte condition (0.2 M NaOH solution). It was clearly found that after their background subtraction, the net oxidative current of histamine obtained from the SPGE was 2 times higher than the net current obtained from the SPCE as shown in Fig. 2. This may be due to the fact that the SPGE provided a rough and porous surface with lots of graphene sheets as seen in the SEM image (Fig S1(B)†) leading to a larger electroactive surface area (0.342 cm^2 for the SPGE and 0.191 cm^2 for the SPCE) and higher electrical conductivity which agrees with the previous work reported by our group.[32] In addition, the use of the SPGE provided a lower oxidative peak potential than using the SPCE. Therefore, it is verified that the SPGE is a suitable electrode material for the determination of histamine using the proposed conditions.

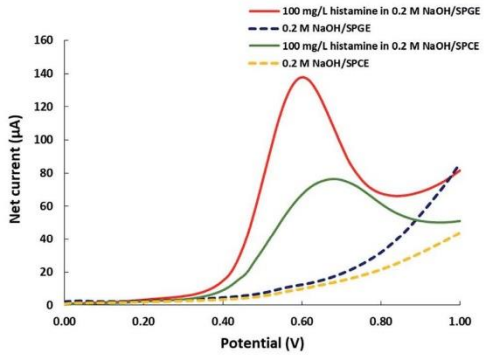

Fig. 2 Square-wave voltammograms obtained from comparison study between using the screen-printed carbon electrode (SPCE) and screen-printed graphene electrode (SPGE) under the best electrolyte condition (0.2 M NaOH). The SPCE and SPGE were fabricated using the same screen-printed template block (geometrical area of electrode = 0.126 cm^2).

3.2 Electrochemical characteristics of histamine at the unmodified SPGE in 0.2 M NaOH solution and the influence of the scan rate

To study the efficiency of the proposed electrode for histamine determination, the electrochemical behavior of histamine at the unmodified SPGE was investigated using the CV technique. The applied potential was in the range from −1.0 V to +1.0 V (*vs.* Ag/AgCl) at a scan rate of 100 mV s^{-1}. The electrochemical measurements of histamine can be detected without any pretreatment of the SPGE. The obtained cyclic voltammograms in the absence and the presence of 100 mg L^{-1} histamine in 0.2 M NaOH are shown in Fig. S3.† It can be noticed that a very well-defined oxidation peak of histamine was obtained at a low anodic peak potential (E_{pa}) of +0.58 V (*vs.* Ag/AgCl). This indicated that the electron transfer process easily occurred and the electrochemical process was an irreversible process. Due to the high surface area and high conductivity of graphene ink, the enhancement of signal towards the direct oxidation of histamine in the 0.2 M NaOH solution was obtained as expected.

Besides, the effect of the scan rate on the response of histamine at the SPGE was also examined by CV. The cyclic voltammograms of histamine at the unmodified SPGE using different scan rates are shown in Fig. S4A.† The results showed that when increasing the scan rate from 25 mV s^{-1} to 300 mV s^{-1}, the anodic peak current increases gradually while the oxidation peak potential shifts to a more positive direction according to irreversible behavior. The anodic peak currents (I_{pa}) are proportional to the square root of the scan rate ($\nu^{1/2}$). The linear relationship between the anodic peak current and the square root of the scan rate was obtained with a linear equation: $I_{pa} - 190.43X - 1.6776$ with $R^2 - 0.9955$ (Fig. S4B†). This result indicated that the nature of the mass transfer process was controlled by diffusion-limited processes for the oxidation process of histamine at the unmodified SPGE. Moreover, the information obtained from the linear regression of the relationship between I_{pa} and $\nu^{1/2}$ can be used to calculate the diffusion coefficient (D_0) using the Randles–Sevcik equation (eqn (1))[33] where F is the Faraday constant (96 485 C mol^{-1}), A (electroactive surface area) = 0.342 cm^2, C_s (the concentration of histamine) = 9×10^{-7} mol cm^{-3}, $R = 8.31447$ J mol^{-1} K^{-1}, and $T = 298$ K. The D_0 of histamine was found to be 4.29×10^{-6} cm^2 s^{-1}.

$$I_{pa} = 0.496FAC_sD_0^{1/2}\left(\frac{F}{RT}\right)^{1/2}\nu^{1/2} \quad (1)$$

$$I_{pa} = (2.99 \times 10^5)n[(1-\alpha)n_\alpha]^{1/2}AC_sD_0^{1/2}\nu^{1/2} \quad (2)$$

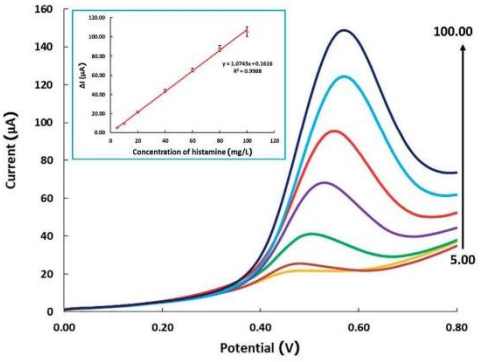

Fig. 3 The square wave voltammograms of histamine in 0.2 M NaOH at various concentrations ranging from 5.00 mg L^{-1} to 100.00 mg L^{-1}. Inset shows the plot of the anodic peak current *vs.* histamine concentrations.

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF}\ln\frac{RTk_s}{(1-\alpha)nF} + \frac{RT}{(1-\alpha)nF}\ln \nu \qquad (3)$$

After calculating the value of D_0, eqn (2) was used to calculate the number of electrons transferred (n).[34] To obtain the value of n, it is necessary to first determine the value of $(1-\alpha)n_\alpha$. For irreversible processes, the calculation is based on Laviron's theory which is a linear relationship between the E_{pa} and ln ν ($y = 0.0318x + 0.7182$). The linear relationship revealed the slope value (0.0318). Then, the $(1-\alpha)n_\alpha$ value can be obtained from eqn (3)[35] and the parameter $(1-\alpha)n_\alpha$ was found to be 0.808. After that, the previously estimated D_0 of histamine from the experiment and the value of $(1-\alpha)n_\alpha$ can be used to calculate the n value using eqn (2), the factor n was found to be 1.11 to 1.00. It can be concluded that the oxidative pathway of histamine at the SPGE occurred with losing one electron.

Furthermore, the electrochemical behavior of histamine at the SPGE was also studied by using the SWV technique. The square wave voltammogram of 100 mg L^{-1} histamine in 0.2 M NaOH is shown in Fig. 1E. From this result, the SWV response displayed a higher oxidative current (~2 times) than the CV response, suggesting that SWV offers a higher sensitivity than CV. Therefore, SWV was selected as a preferable technique for quantitative detection of histamine.

3.3 Optimization of the square-wave voltammetric parameters

The operating conditions and parameters of square-wave detection, including step potential, amplitude, and frequency were subsequently investigated. These parameters can affect the peak shape and peak current of the histamine response. To obtain the optimal value of these parameters, 100 mg L^{-1} of histamine standard solution was used to record the square-wave voltammograms. The step potential in the range from 0.01 V to 0.03 V, the amplitude of 0.01 V to 0.1 V, and the

Table 1 Comparison of the proposed method and other reported electrochemical methods for histamine detection[a]

Working electrode	Peak potential (V)	Supporting electrolyte	Technique	Linear range (mg L^{-1})	LOD (mg L^{-1})	Ref.
Boron-doped diamond electrode	+1.28	Phosphate buffer pH 7	Amperometry	0.05–10	0.05	12
AuNPs/GCE	+0.55	0.1 M NaOH	FIA-HPLC	0.2–44.46	0.06	20
Nickel-film/GCE	+0.4	0.5 M NaOH	Chronoamperometry	0.5–110	0.11	19
MWCNTs/p-(AIINSA)/GCE	+1.0	Phosphate buffer pH 7	Differential pulse voltammetry	0.01–10	0.0076	22
DAO/nPt/graphene/CS/SPCE	+0.4	Phosphate buffer pH 7.4	Amperometry	0.01–30	0.0025	16
DAO/TiO_2/c-MWCNT/RU/CS/SPCE	−0.30	0.05 M BR buffer pH 7	Chronoamperometry	0.99–110	0.69	14
MAO/TiO_2/c-MWCNT/RU/CS/SPCE		0.05 M BR buffer pH 9		5.6–100	3.6	
ErGO/GCE	+0.74	Phosphate buffer pH 8	Amperometry	0.02–8	0.0007	3
Cu@Pd/pencil graphite	+0.55	0.1 M KOH	Chronoamperometry	0.01–10	0.00032	21
Graphene nanoribbons/AgNPs/EPPG	+0.94	Phosphate buffer pH 7.4	Square wave voltammetry	0.1–5, 6–50	0.0049	1
Unmodified SPGE	+0.58	0.2 M NaOH	Square wave voltammetry	5–100	0.62	This work

[a] AuNPs: gold nanoparticles; GCE: glassy carbon electrode; MWCNTs: multi-walled carbon nanotubes; p-(AIINSA): poly(4-amino-3-hydroxynaphthalene sulfonic acid); DAO: diamine oxidase; nPt: platinum nanoparticles; CS: chitosan; SPCE: screen-printed carbon electrode; MAO: monoamine oxidase; TiO_2: titanium dioxide nanoparticles; c-MWCNTs: carboxylated multiwalled carbon nanotubes; RU: hexaammineruthenium (III) chloride; ErGO: fluorine-doped reduced graphene oxide; AgNPs: silver nanoparticles; EPPG: edge plane of pyrolytic graphite; SPGE: screen-printed graphene electrode; BR; Britton–Robinson buffer.

frequency of 10 Hz to 30 Hz were monitored, respectively. From the results, the maximum currents were observed at a step potential of 0.025 V, amplitude of 0.075 V and frequency of 25 Hz. All results are shown subsequently in Fig. S5A–C.† Hence, the optimized step potential, amplitude, and frequency for the SWV were set at 0.025 V, 0.075 V, and 25 Hz, respectively. These parameters were used for all of the subsequent measurements.

3.4 Analytical performance

To evaluate the analytical performance of the proposed method, the quantitative analysis of histamine at the unmodified SPGE was measured using the SWV technique. Under the optimal conditions, the square wave voltammograms of the various concentrations of histamine are displayed in Fig. 3. As can be seen, the anodic current increased with increasing the concentration of histamine up to 100 mg L^{-1}. The calibration curve was constructed by plotting the anodic peak current *versus* the different concentrations of histamine (Fig. 3 inset). The linearity was obtained in a wide concentration range of histamine from 5 mg L^{-1} to 100 mg L^{-1} with a correlation coefficient (R^2) = 0.9988. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the equation of 3SD/m and 10SD/m, respectively. Where SD and m were the standard deviation for ten replicates in blanks and the slope of the calibration curve, respectively. The obtained LOD and LOQ were found to be 0.62 mg L^{-1} and 2.06 mg L^{-1} of histamine, respectively. The analytical performance of the proposed method was compared to the previous electrochemical methods and summarized in Table 1, even though, the newly proposed methodology did not provide the lowest LOD. However, the proposed method exhibited excellent sensing performance, enough for the determination of histamine in real-world analysis based on the criteria set by the EU and the FDA. Moreover, the proposed method offers the advantages of a simple, low-cost, portable and fast operation method due to the fact that it does not require electrode pretreatment and electrode modification by enzyme or metal nanocatalysts.

3.5 Effect of the interferences

To confirm the selectivity, the proposed electrode was used to investigate the current signals of histamine and other biogenic amines which are probably found in fish samples such as putrescine, cadaverine, tyramine, and tryptamine. Using the optimal conditions of SWV experiments, 100 mg L^{-1} individual standard solutions in 0.2 M NaOH solution were detected using the unmodified SPGE as shown in Fig. 4. Individually analysis of each biogenic amine was compared to histamine oxidation (E_{pa} = + 0.58 V; I_{pa} = 104.54 μA). It was found that the oxidative peak of aliphatic amines, including putrescine and cadaverine, did not appear whereas the oxidative peak of aromatic amines, including tryptamine and tyramine, occurred at a less negative

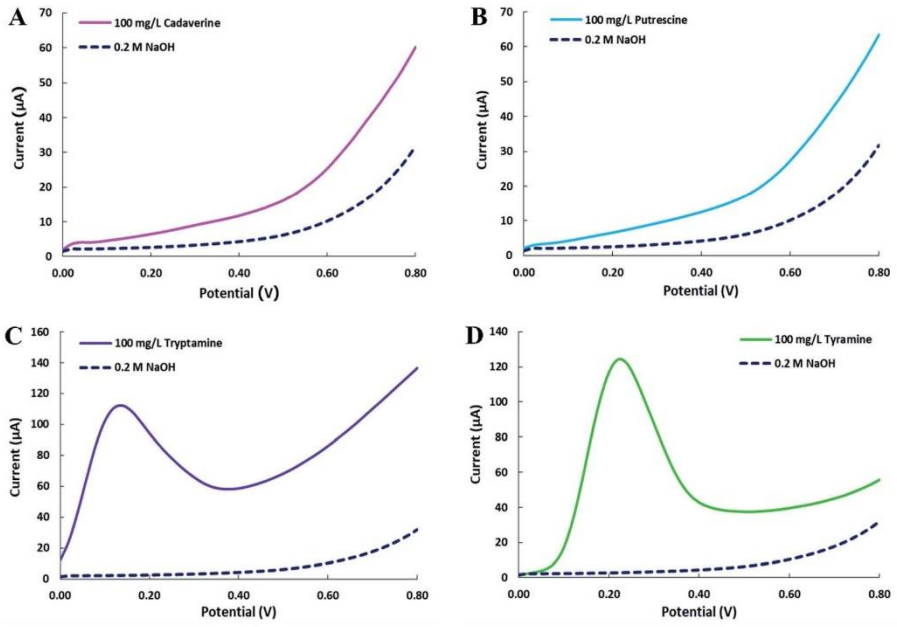

Fig. 4 The square wave voltammograms of 100 mg L^{-1} of other biogenic amines using the SPGE in 0.2 M NaOH: (A) cadaverine, (B) putrescine, (C) tryptamine, and (D) tyramine.

potential of +0.12 V and +0.22 V, respectively. From the results, tryptamine and tyramine may affect the oxidative current of histamine. However, the concentrations of tryptamine and tyramine found in fish samples are at very low levels[36] in comparison to the histamine content. In addition, we used the standard addition curve for the quantitative calculation of histamine to get rid of any matrix influences. Therefore, we are sure that these biogenic amines do not significantly affect histamine detection using the SPGE under the optimized conditions. Moreover, these results obtained suggested that only aromatic amines showed the oxidative peak current at the unmodified SPGE, while aliphatic amines did not display this behavior. It can be concluded that the electrochemical conversion takes place at the imidazole ring of histamine rather than at the amine group. Therefore, the developed unmodified SPGE provided a high selectivity for determination of histamine in the absence of enzymes.

3.6 Real sample analysis

Table 2 shows the recovery values for histamine detection in canned fish samples (tuna and mackerel) which was performed by using the unmodified SPGE as a nonenzymatic sensor. The concentrations of the sample were determined by using the standard addition method to get rid of any matrix influences as mentioned. This table shows the data for real sample analysis with and without histamine spiking. The obtained results showed that the average recoveries were in the range from 90.72% to 101.21%. The values of recovery were very close to 100% with low % RSD values (<5%), indicating that the developed electrode displayed a good accuracy and precision for histamine analysis.

Table 2 Comparison of the proposed method with the HPLC method for the determination of histamine in canned fish samples ($n = 3$)[a]

Histamine spiked (mg L^{-1})	Histamine found (mg L^{-1}), mean ± SD		Recovery (%) by this work	RSD (%)
	HPLC method	Proposed method		
T1	n.d.	n.d.	—	—
T1 + 10.00	9.95 ± 0.06	9.80 ± 0.07	98.01	0.73
T1 + 20.00	20.03 ± 0.52	19.97 ± 0.63	99.85	3.15
T1 + 40.00	39.56 ± 1.01	38.72 ± 1.03	96.79	2.65
T2	n.d.	n.d.	—	—
T2 + 10.00	9.65 ± 0.10	9.49 ± 0.14	94.87	1.47
T2 + 20.00	19.10 ± 0.50	18.28 ± 0.45	91.41	2.45
T2 + 40.00	37.72 ± 1.21	36.29 ± 1.22	90.72	3.36
M1	n.d.	n.d.	—	—
M1 + 10.00	9.95 ± 0.15	10.12 ± 0.27	101.21	2.70
M1 + 20.00	19.12 ± 0.45	18.90 ± 0.47	94.48	2.51
M1 + 40.00	38.72 ± 0.40	38.86 ± 0.47	97.15	1.21
M2	n.d.	n.d.	—	—
M2 + 10.00	9.85 ± 0.50	9.66 ± 0.42	96.62	4.37
M2 + 20.00	18.70 ± 0.60	18.74 ± 0.79	93.69	4.20
M2 + 40.00	37.42 ± 0.35	36.48 ± 0.40	91.19	1.11

[a] T: canned tuna fish sample and M: canned mackerel fish sample. n.d.: not detected.

Note that after histamine oxidation, it has lost one electron to become a radical cation with a positive charge on the imidazole nitrogen. Then, the radical stabilizes itself by dimerization with another leading to electropolymerization of histamine.[37] This by-product can adsorb onto the electrode surface and this causes electrode fouling. From this critical find, our proposed disposable printed electrode is very useful for this application. In addition, we also investigated the reproducibility between electrode and electrode to confirm the performance of the fabricated electrode. It was found that % RSDs between electrodes are less than 3%.

To verify the performance of the developed approach, the method was validated using the traditional HPLC method. The differences between both methods were verified by the paired Student's t-distribution test. At a confidence level of 95%, the statistics revealed that $t_{calculated}$ (2.20) was below $t_{critical}$ (2.53), suggesting that there is no significant difference between the two methods. Therefore, it was summarized that the proposed method was reliable and appropriate to be applied for the determination of histamine in fish samples.

4. Conclusion

In this report, we present for the first time a novel nonenzymatic electrochemical assay using an unmodified SPGE for the selective determination of histamine. This electrode combined the benefits of graphene and the disposable characteristics of screen-printed electrodes. In an alkaline medium, this electrode showed a good selectivity towards the oxidation of histamine without any interference from foreign compounds. The proposed method was successfully applied to determine histamine in canned fish samples with satisfactory accuracy and precision. Moreover, this approach provided a really simple method of direct detection (enzyme-free sensor) with rapidity, low cost, and portability. Therefore, the developed method has high potential as an alternative option for freshness evaluation of fish to prevent toxic effects on human health and also provides a promising choice in routine sensing application for monitoring histamine in fish samples and may be applicable for the whole supply chain of the food industry in the future.

Conflicts of interest

The authors are no conflicts to declare.

Acknowledgements

PN thankfully acknowledges the financial support from the Science Achievement Scholarship of Thailand (SAST). WS would like to acknowledge the partial financial support from the National Research Council of Thailand (NRCT, Grant no. NFS6400388) and Srinakharinwirot University.

References

1 N. Kumar and R. N. Goyal, *Sens. Actuators, B*, 2018, **268**, 383–391.

2 A. Halasz, A. Barath, L. S. Sarkadi and W. Holzapfel, *Trends Food Sci. Technol.*, 1994, **5**, 42–49.
3 F. Shahzad, S. A. Zaidi and C. M. Koo, *ACS Appl. Mater. Interfaces*, 2017, **9**, 24179–24189.
4 M. Hashemi, Z. Nazari and N. Noshirvani, *Carbohydr. Polym.*, 2017, **177**, 306–314.
5 Y. T. Lin, C. H. Chen and M. S. Lin, *Sens. Actuators, B*, 2018, **255**, 2838–2843.
6 Z. Tao, M. Sato, Y. Han, Z. Tan, T. Yamaguchi and T. Nakano, *Food Control*, 2011, **22**, 1154–1157.
7 S. B. Patange, M. K. Mukundan and K. A. Kumar, *Food Control*, 2005, **16**, 465–472.
8 J. Leszczynska, M. Wiedlocha and U. Pytasz, *Czech J. Food Sci.*, 2004, **22**, 81–86.
9 B. S. Hwang, J. T. Wang and Y. M. Choong, *Food Chem.*, 2003, **82**, 329–334.
10 M. Saaid, B. Saad, N. H. Hashim, A. S. Mohamed Ali and M. I. Saleh, *Food Chem.*, 2009, **113**, 1356–1362.
11 N. Zhang, H. Wang, Z. X. Zhang, Y. H. Deng and H. S. Zhang, *Talanta*, 2008, **76**, 791–797.
12 B. V. Sarada, T. N. Rao, D. A. Tryk and A. Fujishima, *Anal. Chem.*, 2000, **72**, 1632–1638.
13 M. Niculescu, I. Frébort, P. Peč, P. Galuszka, B. Mattiasson and E. Csöregi, *Electroanalysis*, 2000, **12**, 369–375.
14 İ. O. Koçoğlu, P. E. Erden and E. Kılıç, *Anal. Methods*, 2020, **12**, 3802–3812.
15 C. M. Keow, F. A. Bakar, A. B. Salleh, L. Y. Heng, R. Wagiran and S. Siddiquee, *Int. J. Electrochem. Sci.*, 2012, **7**, 4702–4715.
16 I. M. Apetrei and C. Apetrei, *Sensors*, 2016, **16**, 422–436.
17 K. Zeng, H. Tachikawa, Z. Zhu and V. L. Davidson, *Anal. Chem.*, 2000, **72**, 2211–2215.
18 K. Yamamoto, K. Takagi, K. Kano and T. Ikeda, *Electroanalysis*, 2001, **13**, 375–379.
19 J. Svarc-Gajic and Z. Stojanovic, *Electroanalysis*, 2010, **22**, 2931–2939.
20 V. Carralero, A. González-Cortés, P. Yáñez-Sedeño and J. M. Pingarrón, *Electroanalysis*, 2005, **17**, 289–297.
21 R. K. R. Gajjala and S. K. Palathedath, *Biosens. Bioelectron.*, 2018, **102**, 242–246.
22 A. Geto, M. Tessema and S. Admassie, *Synth. Met.*, 2014, **191**, 135–140.
23 H. M. Mohamed, *TrAC, Trends Anal. Chem.*, 2016, **82**, 1–11.
24 A. Smart, A. Crew, R. Pemberton, G. Hughes, O. Doran and J. P. Hart, *TrAC, Trends Anal. Chem.*, 2020, **127**, 115898.
25 A. L. Squissato, R. A. A. Munoz, C. E. Banks and E. M. Richter, *ChemElectroChem*, 2020, **7**, 2211–2221.
26 K. Charoenkitamorn, S. Chaiyo, O. Chailapakul and W. Siangproh, *Anal. Chim. Acta*, 2018, **1004**, 22–31.
27 Y. Panraksa, W. Siangproh, T. Khampieng, O. Chailapakul and A. Apilux, *Talanta*, 2018, **178**, 1017–1023.
28 E. P. Randviir, D. A. C. Brownson, J. P. Metters, R. O. Kadara and C. E. Banks, *Phys. Chem. Chem. Phys.*, 2014, **16**, 4598–4611.
29 W. Jesadabundit, S. Chaiyo, W. Siangproh and O. Chailapakul, *ChemElectroChem*, 2020, **7**, 155–162.
30 U. T. Yilmaz and D. Inan, *J. Food Sci. Technol.*, 2015, **52**, 6671–6678.
31 P. Nakthong and P. Muangthai, *Proceedings of the Burapha University International Conference*, 2015, pp. 370–376.
32 J. Thangphatthanarungruang, A. Yakoh, R. Laocharoensuk, C. Chotsuwan, O. Chailapakul and W. Siangproh, *J. Electroanal. Chem.*, 2020, **873**, 114361.
33 A. G.-M. Ferrari, C. W. Foster, P. J. Kelly, D. A. C. Brownson and C. E. Banks, *Biosensors*, 2018, **8**, 53–62.
34 P. Yomthiangthae, T. Kondo, O. Chailapakul and W. Siangproh, *New J. Chem.*, 2020, **44**, 12603–12612.
35 E. Laviron, *J. Electroanal. Chem. Interfacial Electrochem.*, 1979, **101**, 19–28.
36 G.-C. Yen and C.-L. Hsieh, *J. Food Sci.*, 1991, **56**, 158–160.
37 P. Puthongkham, S. T. Lee and B. J. Venton, *Anal. Chem.*, 2019, **91**, 8366–8373.

VITA

NAME	Miss Prangthip Nakthong
DATE OF BIRTH	30 Aug 1990
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Srinakharinwirot University
HOME ADDRESS	15 Soi Serithai 83 Yeak 7 Khannayao Khannayao Bangkok 10230
PUBLICATION	Nakthong, P., Kondo, T., Chailapakula, O., & Siangproh, W. (2020). Development of an unmodified screen-printed graphene electrode for nonenzymatic histamine detection. Analytical Methods, 12, 5407-5414.