DEPOSITION OF NYLON MEMBRANE ON CARBON ELECTRODE FOR THE DEVELOPMENT OF ASCORBIC ACID ELECTROCHEMICAL BIOSENSOR

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by

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LIST OF SYMBOLS

A	Ampere
E_{we}	Potential
I	Current
mA	milliampere
υ	Scan rate
Ω	Ohm

LIST OF ABBREVIATIONS

AA	Ascorbic Acid
Ag	Silver
AgCl	Silver Chloride
CA	Citric Acid
CNPT	Carbon Nanoplate
CV	Cyclic Voltammetry
DA	Dopamine
EIS	Electrochemical Impedance Spectroscopy
Fe_2O_3	Iron(III) Oxide
FTIR	Fourier Transform Infrared
GCE	Glass Carbon Electrode
GCHs	Ground Cherry Husks
Glu	Glucose
HCl	Hydrochloric Acid
PANI	Polyaniline
SEM	Scanning Electron Microscopy
UV-VIS	Double Beam UV Visible Spectroscopy

PEMENDAPAN MEMBRAN NILON KE ATAS ELEKTROD KARBON UNTUK PEMBANGUNAN BIOSENSOR ELEKTROKIMIA ASID ASKORBIK

ABSTRAK

Biosensor elektrokimia adalah gabungan kaedah elektroanalitikal dengan kadar sensitif yang tinggi kerana kehadiran sistem pengesan sejenis biomolekul. Tujuan utama penyelidikan ini dilaksanakan adalah untuk mengkaji penyatuan antara asid askorbik (AA) dengan polyaniline (PANI) dan untuk menyiasat jenis - jenis respons elektrokimia yang terdiri daripada kitaran voltametri dan galangan elektrokimia spektroskopi. Dalam penyelidikan ini, penyatuan antara AA dan PANI dilakukan dengan mengsintesis PANI/Fe_2O_3 sebelum menggabungkannya dengan AA. Spektroskopi jenis UV-Vis digunakan untuk menyukat penyerapan untuk cecair berlainan iaitu PBS, 50 mM AA, PANI, PANI/Fe_2O_3 dan PANI/Fe_2O_3/AA dengan kepekatan 50 mM untuk memastikan penyatuan antara AA dan PANI/Fe_2O_3 berjaya. Seterusnya, jenis membrane-sensor disusun serta digabungkan iaitu, elektrod karbon-kaca, elektrod karbon-kaca bersama membrane serta elektrod karbon-kaca bersama membrane di mana, ascorbate oxidase dilekatkan di atas permukaan membrane tersebut. Respons kitaran voltametri dan galangan elektrokimia spektroskopi diukur untuk memerhati potensi pengoksidaan dan kekonduksian cecair tersebut

DEPOSITION OF NYLON MEMBRANE ON CARBON ELECTRODE FOR THE DEVELOPMENT OF ASCORBIC ACID ELECTROCHEMICAL BIOSENSOR

ABSTRACT

Electrochemical biosensor is the combination of electroanalytical methods of high sensitivity with a presence of biorecognition system that helps in identifying target species. The main objective of this work is to study the conjugation of ascorbic acid (AA) to polyaniline (PANI) and to explore the cyclic voltammetry and electrochemical impedance spectroscopy of the membrane-based sensor to detect AA. In this study, the conjugation of ascorbic AA was first done by the synthesis of PANI/Fe_2O_3, before the AA was interacted with PANI/Fe2O3. UV-Vis spectrometer absorbance for solutions PBS, AA of 50 mM, PANI, PANI/Fe2O3 and PANI/Fe2O3/AA of 50 mM were measured to prove the successful conjugation of AA. Next, membrane-sensor was assembled, bare glass carbon electrode (GCE), GCE-nylon membrane and GCE-nylon membrane immobilized with ascorbate oxidase were testing with AA. The cyclic voltammetry pattern and electrochemical impedance spectroscopy were recorded to study the redox potential and conductivity of the testing solutions.

CHAPTER ONE

INTRODUCTION

1.1. Research Background

From the range field of biomedical, biotechnology, medical and food technology, biosensor has been choosing eminently for either biological or, biochemical aspect for their respective quantification. For the past several years, with the advanced from the earliest stage in the application of sensor, biosensors had been widely used for the determination of species inside a sample.

Determination of ascorbic acid has been widely applied, especially in the food industry, for an instance, the determination of ascorbic acid in juice beverages as one of the highlights. It is to monitor the quantity of ascorbic acid to avoid the taste to be altered or worsen. The excessive level of ascorbic acid could also inhibit the oxidation process that contribute to the texture of the apple juice production. This is one of the factors on why the level amount of ascorbic acid needed to be considered in the food industry involvement (O'Connell, et al., 2001; Wawrzyniak, et al., 2005).

In recent study on medical diagnosis in biomedical field (Khan, et al., 2017), application of biosensor principle is applied to monitor the condition of patients that undergo eye surgeries. The study is focusing on the post-traumatic eye injuries and post-surgical where they were using multi-layered electrical-biosensor chip to help in detecting level of ascorbic acid on aqueous humour and point-of-care (POC) level on optical aspect. The usage of electrochemical immune-sensing technique is used to test a small, clinical sample taken from the patient.

1.2. Electrochemical Biosensor

Electrochemical techniques have been one of the selection for the low-cost and portable devices properties for a wide range of application in biosensors, especially in medical diagnosis and environmental monitoring. It behaves where it helps in transforming or converting the biological information from the recognition system to the electrical signal. In this technique, there are two important components involved which are, a biorecognition system and transducer. The biorecognition system is needed to recognize the desired specific species that is contained in an analyte. While the transducer serves as a purpose to transfer signal that was induced from the chemical information derived from the analyte sample which will be processed by selected equipment.

There are several definitions that are being suggested by Gennady Evtugyn in the biosensor field (Evtugyn, 2014) as shown in the table below:

Table 1.1: Definition in biosensor

Main component	Function
Analyte	Chemical compound or element that is to be determined in the study. The chemical compound to be detected in this research is ascorbic acid.
Transducer	It behaves as to transfer the response created from the biochemical reaction that occurred to electrical signal which could be analysed by an appropriated equipment.
Receptor	A complex molecule used for the recognition process and specific binding to the analyte. In the perspective of the study, the receptor being used is an ascorbate oxidase, which is a biomolecule compound to recognize the ascorbic acid
Biochemical recognition	This is to describe the capability of a biomolecule compound to bind specifically to the target species.
Biosensor signal (response)	This response is to describe the transformation based on the relation between the transducer and the biochemical recognition process. It usually contributes to the qualitative analysis that is shown, for instances, electrochemical impedance spectroscopy (EIS)

1.3. Research Problem Statement

The problem statement that we are focusing in this research is how the presence of polyaniline (PANI), will help for the performance of the electrochemical biosensor in detecting the ascorbic acid (AA). Secondly, how the different membrane-based biosensor will interact with different testing solution. Next, how the presence of ascorbate oxidase as the biomolecule compound on the surface of membrane, would help in detecting AA and what are the responses exhibit from performance of reduction and oxidation in the electrochemical biosensor when different testing solution is used. Next, how concentration of ascorbic acid, (AA) would affect the performance of the electrochemical biosensor.

1.4. Research Objective

There are several objectives involved in this research study. These are the following aim for this project:

1. To investigate the conjugation of ascorbic acid (AA) to composite PANI/Fe_2O_3. The result of the conjugation is observed through the absorbance of the testing solution prepared by UV-Vis spectrometer.
2. To assembly different type of membrane-based electrochemical biosensor. The different type of membrane-based biosensor is then, tested into different testing solution prepared prior being prepared.
3. To discover the cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) of the membrane sensor in detecting the ascorbic acid in the analyte solution. This is to investigate the redox potential and the electrical conductivity inside the electrochemical biosensor based on different testing solution.

3.1. Research Organization

For the organization in the research study, the chapter are separated into five section which consist of introduction, literature review, materials and methods, results and discussion and last but not least, conclusion and recommendation. In the introduction, the highlighted topic will be based on the current issue on the application of the electrochemical biosensor whether in the industry or in the research field.

Next is the literature review where we are going into detailed from the basic structure of the electrochemical biosensor which is the sole sensor, to the application of the electrochemical biosensor usage in between the involvement of biological element. Then, we are going to further discuss into the application of membrane in biosensor field. This will involve a discussion on previous work being done by researchers' prior that involved in these field.

This research will be proceeded with the research materials and methods where we will discuss the material used in this experiment and the methodology involved to carry out the experiment. The method chose in this experiment is discussed chronologically, step-by-step and the characterisation method is also mentioned.

The results and discussion are followed to elaborate further the outcome from experiment with several reference to synthesise the data and emphasised the data with details to the reference.

3.2. Research Scope of Project

In this research study, several scopes will be study for the development of the ascorbic acid electrochemical biosensor. The main part is to study the conjugation of AA to composite PANI/Fe_2O_3 to form PANI/Fe_2O_3/AA. The conjugation is based on the

absorbance of different testing solution which consist of phosphate buffer solution (PBS), pure AA in PBS, PANI solution, PANI/Fe_2O_3 solution and PANI/Fe_2O_3/AA solution.

Next, we are going to ventures into different assemble of membrane-based of sensor. Several sensors will be developed with the absence and presence of nylon membrane. The assembly sensors are consisting of bare carbon electrode (GCE), carbon electrode with deposition of nylon membrane on the surface (GCE-nylon membrane) and bare carbon electrode with deposition of nylon membrane with immobilization of ascorbate oxidase (AO) on the surface of the nylon membrane (GCE-nylon membrane-AO).

Next, we are going to look into the performance of redox potential in different testing solution. This is based on the cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) response. The testing solution that are involved for this part are buffer solution, buffer with presence of ascorbic acid, buffer solution with the presence of PANI and PANI/Fe_2O_3, and buffer solution with presence of PANI/Fe_2O_3/AA. This is to explore the interaction between the testing solution to the membrane, and also to the carbon electrode.

Moreover, we focused on investigate the detection performance on the membrane-based sensor with the presence of biological molecule recognition system, which in this study is ascorbate oxidase (AO). AO will be immobilized on the surface of the nylon membrane to observe the CV and EIS response in the testing solution that consist of AA.

CHAPTER TWO

LITERATURE REVIEW

2.1. Sensor

2.1.1. Definition

A chemical sensor is a tool of an application that help in interpret chemical information of a sample or an analyte taken, into an analytically useful signal. The sample taken could a specific sample component or total composition of various component for an analysis process (Thevenot, et al., 2001).

For biosensor is defined in which the system applied the concept of biochemical mechanism for the recognition of component in a provided analyte (Turner, et al., 1987) while Donald G. Buerk had mention that biosensor is any measuring devices that hold the presence of any biological component (Buerk, 1993). Biosensors are highly selective because of the presence of the immobilized, biological recognition system that would specifically bind and interact with the target species in the sample on the working electrode due to the specific binding affinity (Grieshaber, et al., 2008). The figure below shows the basic structure of a biosensor (Monosik, et al., 2012):

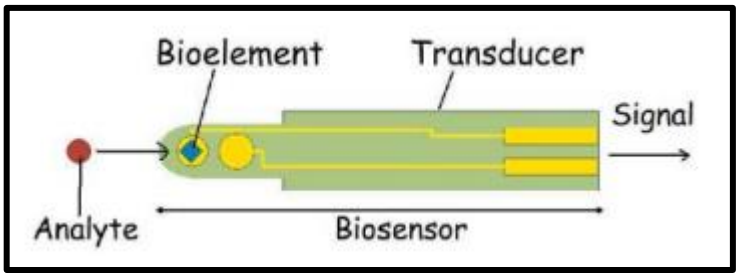


Figure 2.1: Basic Structure of Biosensor

2.1.2. Application of Chemical Sensor and Biosensor

Correlated with the chemical sensor, the usage of this devices is applied in the commercial aircraft to study the air quality inside the airplane which is studied by Gerhard Muller (Muller, et al., 2011). This is to ensure the passengers and crew to obtain the fresh air and water. The application of fuel cell is very useful as it is produced from kerosene as the fuel with the access of water and electricity in the flight.

For the features of biosensor, they already had their places in the past several decades. Leland C. Clark (Clark Jr. & Lyons, 1962) had presented the application with principle of the first enzyme electrode, by immobilizing the glucose oxidase which acts as the biological recognition receptor on the surface of the electrode. This is featured at the New York Academy of Sciences Symposium in 1962. This device system was used to detect the amount of glucose in the blood samples taken from diabetics' patients (Clark Jr. & Lyons, 1962; Setford & Newman, 2005). They are also frequently being targeted as they are easily fabricated, portable devices and their simplicity structure which contributes to the widely usage of this type of biosensor (Scampicchio, et al., 2010; Evtugyn, 2014).

In comparison, chemical sensors and biosensors are different based on their recognition system, where we can see that biosensors applied the usage of a biomolecule compound on the electrode to recognize the analyte. Chemical sensors could act as a transducer in the construction of the biosensor itself in order to enhance the processing information performance.

2.1.3. Classification of Biosensor

There are two main types of biosensors being suggested corresponding to their signal transduction principles which is shown in the table below (Evtugyn, 2014):

Table 2.1: Classification of biosensors according to transducer

Electrochemical Biosensors		**Optical Biosensors**	
Degree of reading	**Transducer**	**Degree of reading**	**Transducer**
Amperometric	Metal electrode Carbon material Chemically Modified Electrode	Colorimetric	Test Strips Fluorescent Reagent
Potentiometric	Ion-Selective Electrode Field-effect Transistors	Fluorometric	Chemiluminescence Bioluminescence
Conductometric	Conductometric Cell Impedimetric		Electroluminescence

2.2. Electrochemical Biosensor

Electrochemical sensors are where a reaction in which of oxidation and reduction, occur in a sample of an analyte, thus, forming an electrical signal that is proportional to the concentration of the provided sample. Electrochemical sensor typically, comprises of a sensing or working electrode (transducer) and a reference electrode separated by the sample or an analyte (Hammond, et al., 2016).

In respective of the electrochemical biosensor application, from the view of Daniel R. Thevenot (Thevenot, et al., 2001), it was being considered as a “self-contained integrated” tools of a receptor-transducer relation which are able to produce a particular

type of quantitative signal based on the implanted biological receptor when they come in contact with the electrochemical transducer. The closed contact on the mentioned of transducers and the biological component is a crucial feature to be considered in the construction of biosensors (Evtugyn, 2014).

2.2.1. Classification of Electrochemical Biosensor

Transducer is a part of where it transfer the signal received from the biorecognition system to the electrical receiver.There are several classifications of transducer being suggested in the electrochemical biosensor field where they usually being summarized based on the following table (Thevenot, et al., 2001; Pohanka & Skladal, 2008):

Table 2.2: Classification of electrochemical transducer

Measurement	**Transducer**
Potentiometric	Ion-selective electrode, glass electrode and gas electrode.
Amperometric	Metal or carbon electrode, chemically modified electrode (CME).
Conductometric	Metal electrode.
Ion charge or field effect	Ion-sensitive field effect transistor (ISFET)

2.2.2. Working Principle of Electrochemical BIosensor

In the perspective of electrochemical biosensor, a type of biological recognition system will help in transfer information from the biochemical sample which is an analyte, into an output signal with a type of sensitivity. The aim of the recognition process is to run the sensor in a shape of high selectivity state to measure the analyte (Mongra, et al., 2012). Biosensors are selective corresponding to an analyte. Design of certain biosensors

are based on a “class-specific” type as usually they are using specific biological compound, for example an enzyme (Thevenot, et al., 2001).

A typical electrochemical biosensor working principles are applied as follows where it shows as in the figure below (Putzbach & Ronkainen, 2013):

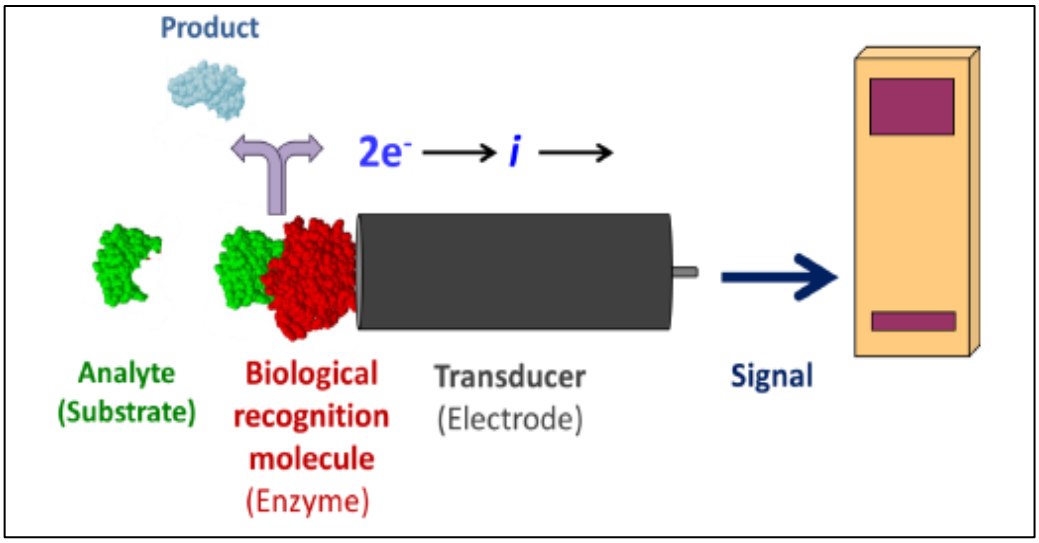


Figure 2.2: A typical design of electrochemical biosensor of enzyme-type

Firstly, the bioreceptors or biological molecule will specifically bind to the specific analyte which act as the substrate. Next, it will induce a specific biological response occurs and induces a signal or reading where the signal will be picked by a transducer. Then, the signal received will be transformed to an electronic signal where the electronic signal will be enlarged by a detector circuit using the setting reference provided. The signal will send to a software for processing where the provided computer software will help in transforming the signal into to a physical parameter or quantitative reading to help in elaborating the process that is being studied.

For example, the research that we are focusing on is the detection of ascorbic acid by an electrochemical biosensor. From the perspective that is studied by Aurelia Pisoschi

(Pisoschi, et al., 2008) and Gabor Csiffary (Csiffary, et al., 2016), the biorecognition system being used is ascorbate oxidase as the biomolecule compound, which is immobilized on the natural protein membrane on a glassy carbon electrode which act a transducer. The analyte is a various of juices and vitamin C effervescent tablet which contain the target substrate of ascorbic acid.

2.3. Electrochemical Detection Technique

2.3.1. Impedimetric Response

Impedimetric response is differentiated into two main response pattern which are, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

2.3.1. (a) Cyclic Voltammetry

Cyclic voltammetry, CV is one of the electro-analysis methods where it varies a certain potential and inducing current flow result. It is to study a redox potential of a provided analyte and electrochemical reaction that occurred. The measurement is indicated based on a scan of two value at fixed rate (Grieshaber, et al., 2008). Several studies are to be shown to investigate the redox potential of the biosensor applied. In the Figure 2.3.1. below, it shows a study on response based on different concentration of ascorbic acid (Pisoschi, et al., 2008):

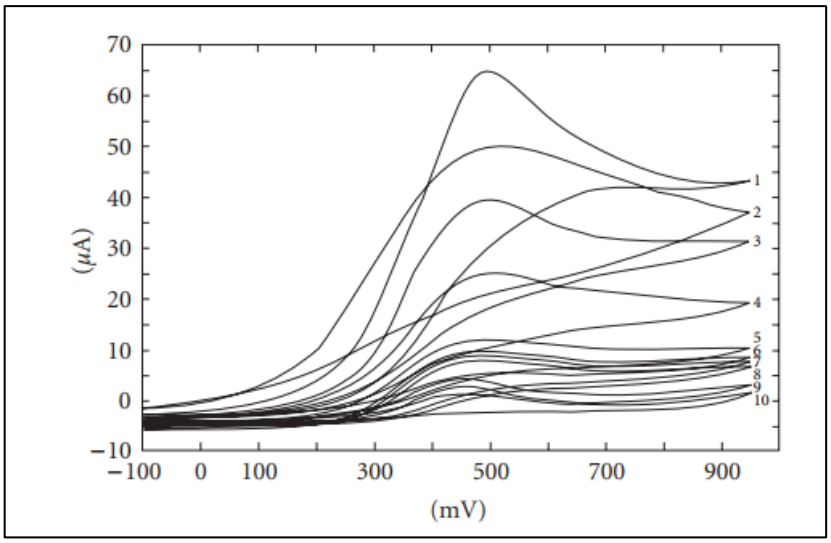


Figure 2.3: Cyclic voltammograms on different concentration of ascorbic acid

The figure above is based on the concentration of ascorbic acid between 0.1 mmol/L to 10 mmol/L where it is expressed as 0.1 (10), 0.5 (9), 0.75 (8), 1 (7), 1.5 (6), 2 (5), 4 (4), 6 (3), 8 (2), and 10 mmol/L (1) and the process is performed at two fixed value scan rate between -100 mV and 1000 mV. This shows that a higher concentration of ascorbic acid will increased the current flow.

While in this following study, cyclic voltammetry can be seen to be applied for the simultaneous determination of interference study between dopamine (DA), ascorbic acid (AA) and uric acid (UA). Figure following shows (Zheng, et al., 2009) the cyclic voltammetry on different based-carbon electrode:

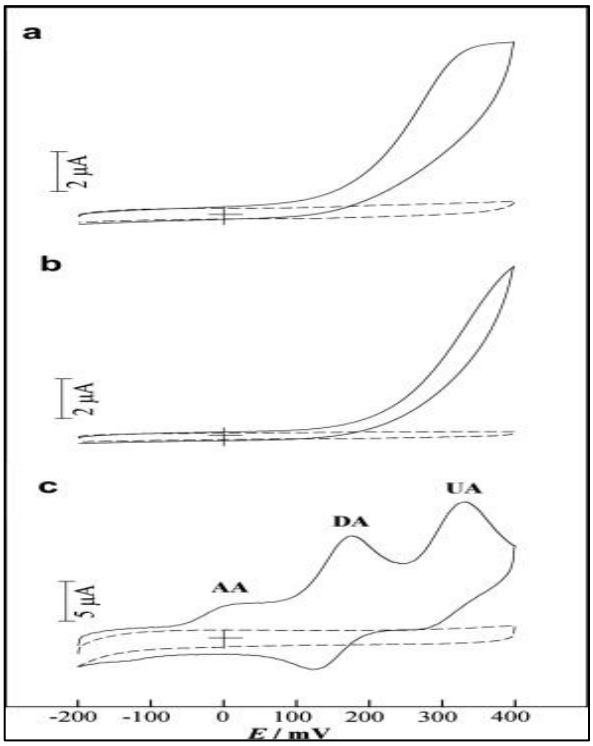


Figure 2.4: Cyclic voltammogram of (a) bare GCE, (b) Nafion/GCE and (c) OMC/Nafion film electrode

The figure above is to study the behaviour of AA, DA and UA on OMC/Nafion electrode. This is done as an interference study between different main species in the solution. This is to differentiate the pulse and peak current of a specific target in a noise solution. The scan rate is done between fixed value of -200 mV and 400 mV. It shows a different peak current of the redox potential of the respective analyte.

2.3.1. (b) Electrochemical Impedance Spectroscopy

This is the most common for the application among the impedimetric response where the impedance can be measured over a wide range of frequencies based on voltage potential, which usually between 100kHz to 1 mHz (Grieshaber, et al., 2008; Hammond, et al., 2016). This is helpful in the study of physical chemistry when the biorecognition system works in binding the receptor, which is the ascorbate oxidase immobilize on the membrane, to the desired molecule which is the ascorbic acid. Jules Hammond also

mentioned (Hammond, et al., 2016) that EIS is frequently chose for the characterization technique where it able to observe directly the specific binding affinity process between biomolecule compound and the analyte sample and also monitor the state of fabrication between the working electrode and the nylon membrane in which the ascorbate oxidase is immobilized.

The following graph is studied by Rui Zhang (Zhang, et al., 2009) to investigate the redox potential and the electron transfer resistance when different type of electrode is used. The performance of the Electrochemical Impedance Spectroscopy (EIS) is shown as in the figure below:

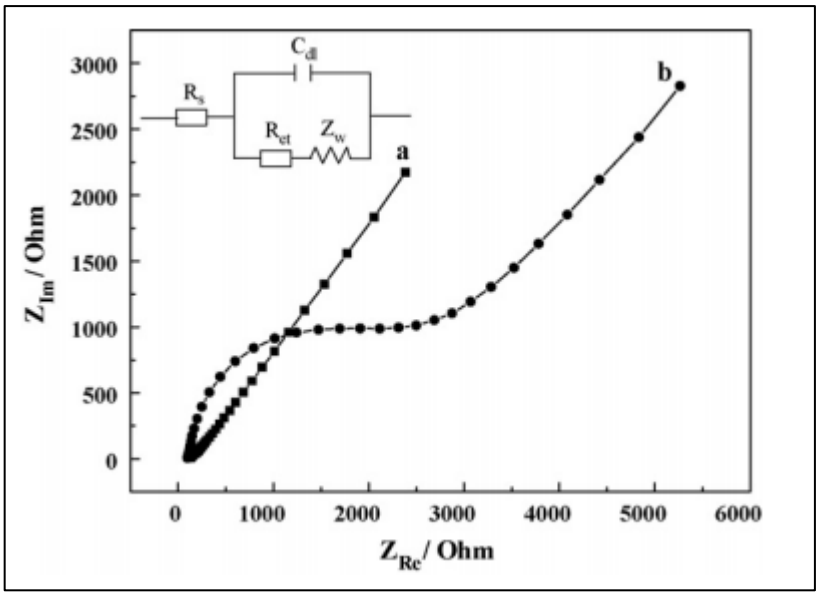


Figure 2.5: Nyquist plots in impedance measurements of electrodes: (a) a bare GCE and (b) the poly-ACBK film modified GCE

2.3.2. Amperometry Response

An amperometry response is based on the transfer of electron reaction during the oxidation and reduction of an electroactive species that is catalysed by the presence of enzyme (Pisoschi, et al., 2014). The system is usually performed at a constant potential on a working electrode in respect to the reference electrode and also, auxiliary electrode

(Pohanka & Skladal, 2008; Thevenot, et al., 2001). Amperometry biosensors are sensitive and suitable for quantifying the product from a sample compared to the corresponding potentiometric-type response. In the aspect of amperometry, current is measured at a constant potential and if a current is measured during controlled variations of the potential, this is referred as such to a voltammetry (Grieshaber, et al., 2008)

A study performed by Sanoe Chairam (Chairam, et al., 2011) to observe the interference study between AA, dopamine (DA) and glucose, (Glu) at constant potential of +0.25 V, as shown in figure below:

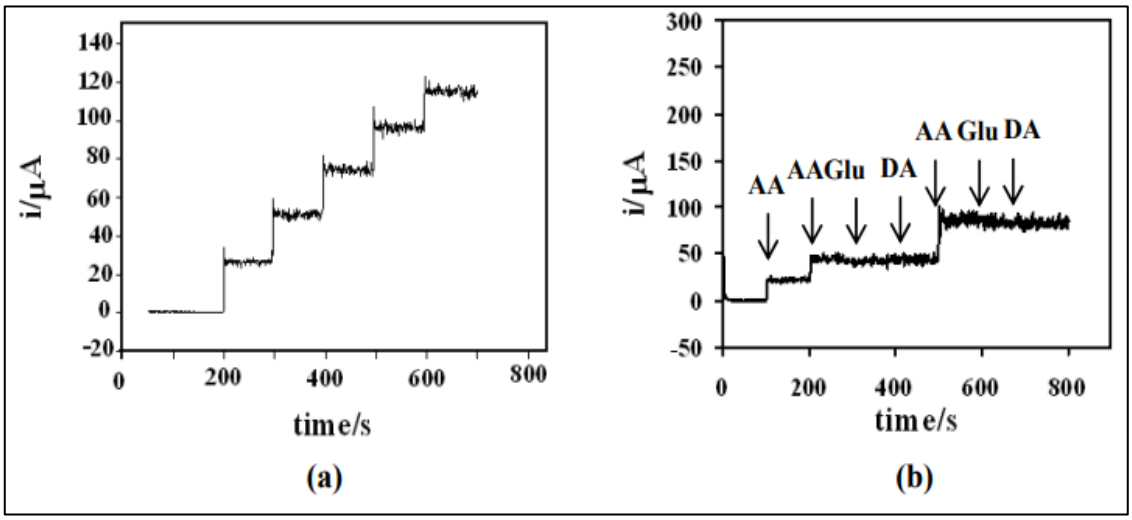


Figure 2.6: Amperometric curve of poly(Ani-co-m-FcAni)/GCE at 0.1 mM AA, (b) Amperometric response interference study with presence of 0.1 mM AA, 1.0 mM DA and 1.0 mM Glu

In addition, Xiuxiu Li has investigated the performance on the amperometric response based on different concentration of ascorbic acid, AA in different type of sample (Li, et al., 2017). The result from the response is shown as in below in the following figure:

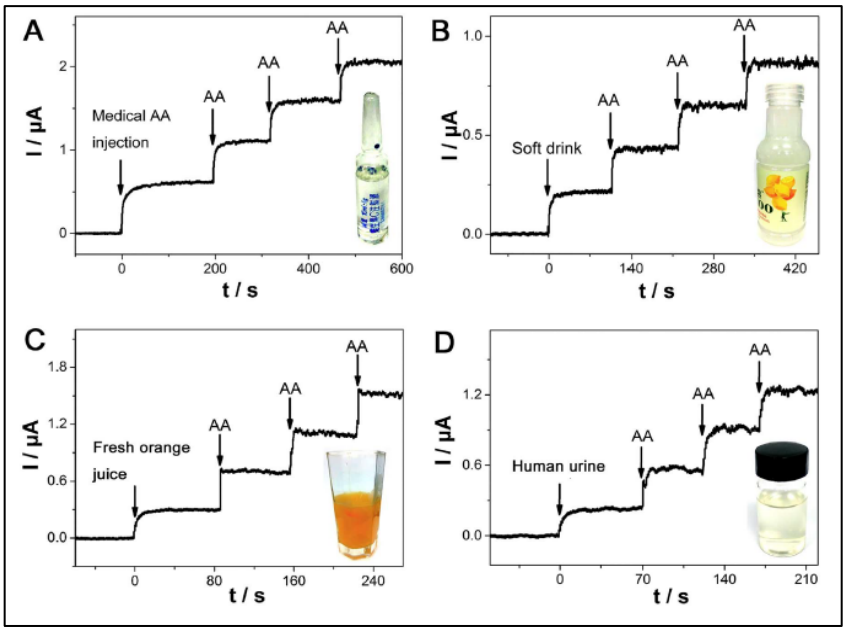


Figure 2.7: Amperometric responses at GCHs-CNPTs/GCE (A) medical AA, (B) soft drink sample, (C) fresh orange juice, (D) human urine

Ascorbic acid of 30μM in (A), 15μM in (B), 10μM in (C) and (D) in added and the applied of a constant potential at 0.026 V. This investigation is to evaluate which practical application is suitable for analyzation of AA by using the GCHs-CNPTs/GCE which is evaluated based on a data provided by a company which is shown below in:

Diluted Sample	Provided by company (μM)	Determined by GCHs-CNPTs/GE (μM)	Added (μM)	Found (μM)	RSD (%, n=3)	Mean recovery (%)
Medical AA injection	34.06	33.78	0	33.78	1.6	N/A
			30.0	63.82	1.7	100.13
			60.0	93.77	1.4	99.98
			90.0	123.50	2.1	99.69
Soft drink	10.85	10.76	0	10.76	2.1	N/A
			15.0	25.81	2.5	100.30
			30.0	39.98	1.7	99.98
			45.0	52.98	2.6	93.82
Fresh orange juice	N/A	18.15	0	18.15	2.0	N/A
			10.0	28.43	2.7	102.80
			20.0	38.09	1.9	99.75
			30.0	46.89	2.5	95.80
Human urine	N/A	19.34	0	19.34	2.4	N/A
			10.0	30.03	3.2	102.35
			20.0	38.86	2.1	98.78
			30.0	48.69	2.5	98.68

Figure 2.8 Comparison from Analytical Summary for sample (A) to (D)

2.3.3. Potentiometry Response

Potentiometric is about occurrence of potential difference between the sensing electrode and the reference electrode, or, a sensing electrode and two reference electrodes with the presence of perm-selective membrane as a barrier when there is no current flow in between. The potential differences between the corresponding electrode are directly proportional to the logarithmic ion-activity (Pohanka & Skladal, 2008) (Stefan, et al., 2001). Potentiometry helps in study the ion activity occurrence in the electrochemical reaction. The relationship between the concentration and the potential activity can be related through the application of the Nernst equation (Stefan, et al., 2001):

$$E_{eq} = E° + \frac{RT}{nF}\ln(\alpha) \qquad \text{Equation 2.1}$$

Where,

E_{eq} = equilibrium potential

E° = standard potential

α = activity of the ion

n = electron transfer in the reaction

F = Faradays constant, 96,500 C/mol

R = universal gas constant, 8.31 J/mol/K

T = standard temperature of 298 K

Potentiometric sensors are usually being selected because it is compatible in measuring a small sample with low concentration, as ideally in that state, they typically being taken as not bring any impact of a sample (Grieshaber, et al., 2008).

An example of a study based on potentiometric response is investigated by Milakin where Polyaniline-based biosensor is used to detect ascorbic acid as shown in the figure below (Milakin, et al., 2013):

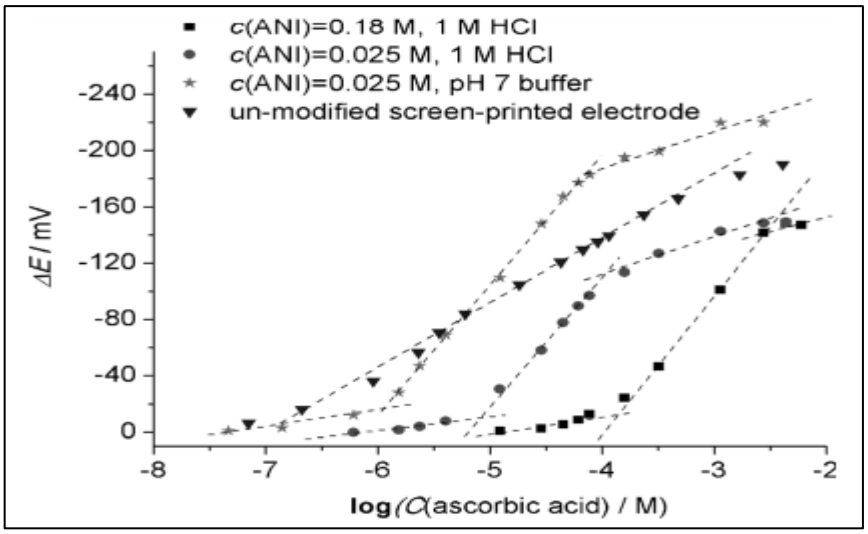


Figure 2.9 Effect of polymerization conditions on the sensor response

From the figure show, the increasing of ascorbic acid will lead to the increase in the potential inside the cell and further effect the concentration of PANI on the electrode surface. The presence of PANI in a solution would help in increasing the conductivity of the solution and it would help in transferring the electron on to the surface of the electrode and induce the current flow in the cell, with the condition of the PANI is in acidic condition (Tarver & Loo, 2013).

2.4. Nylon Membrane

2.4.1. Characteristic of Nylon Membrane

Nylon is a type of polyamide, hydrophilic membrane which is suitable to be used with aqueous and alcoholic solutions and solvents type. It is consisting of repeating units linked, called the peptide bonds and therefore, it's usually being referred as the polyamide. The characteristic of the nylon membrane is positive charge (cationic) and it will remain cationic over a wide range of pH in a sample. Nylon membrane also has a narrow pore size surface with good mechanical strength (Narang, et al., 2011).

Membrane structure that is made up from group of polymeric could help in enhance the interaction between biomolecule compound, that is ascorbate oxidase and target analyte which is ascorbic acid. This is because of their porous nature and desirable surface chemical compositions (Farahmand, et al., 2015). The figure below shows the schematic graphic of nylon nanofibrous membrane that usually involved in biosensing (Scampicchio, et al., 2010):

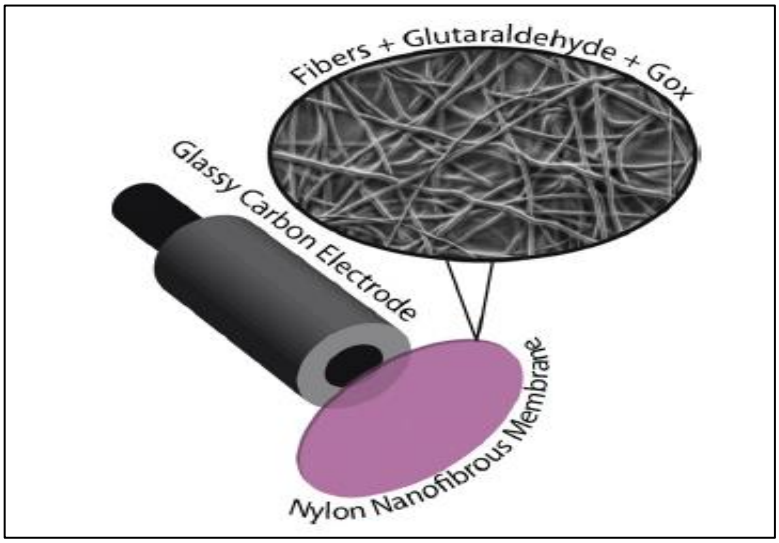


Figure 2.10: Schematic picture of nylon nanofibrous biosensor with GCE

2.4.2. Roles of Membrane in Biosensor

The usage of nylon membrane had been involved for years in the application involving biosensor due to their good mechanical properties (Gan, et al., 2000). Corresponding to the usage of membrane in the application of electrochemical biosensor, to determine the desired species in a sample, a biorecognition system is needed where a specific, biomolecule compound will be immobilized on the nylon membrane. Their behaviour that is stable could act as a support structure for the biomolecule compound which is the ascorbate oxidase (Hurk & Evoy, 2015). The membrane would help in binding the biomolecule to the corresponding species in the analyte where many

biochemical processes highly depend on the binding between the respective molecule (Lawley & Keener, 2017).

2.4.3. Function of Nylon Membrane

According to Daniel R. Thevenot (Thevenot, et al., 2001) the application of nylon membrane serves a purpose in the usage of the electrochemical biosensor. The function of the membrane are as follows:

It will act as a protection barrier. The membrane will have hindered certain molecule from permeate the membrane in order for the desires molecule to bind to the biomolecule compound. The selected membrane will also display perm-selectivity properties which will help in this study, for the detection of ascorbic acid. It will assist in reducing the possible interfering species or usually being called as noise, that present in the analyte sample to be detected by the transducer. It's also help in decreasing the potential of leakage of the targeted analyte, which in this case, ascorbic acid that should bind to the immobilized biomolecule compound.

Nylon membrane has a feature surfaces of biocompatibility and biostability. Typically, few modifications of biosensor will be applied in which an alteration will be made, either on the analyte sample or on the surface of the working electrode. Therefore, the selection for the membrane is crucial to ensure the stability of the response from the working electrode. If the changes applied as mentioned prior where the changes on biosensor doesn't affect the determination of the analyte and the changes applied in the sample doesn't affect the operation on the sensor, therefore, they are considered to be biocompatible.

Somehow, nylon films could contribute to insulation properties and this would indirectly affect their applicable usage in the bio-sensing field if it is proceeded without taking any precaution steps. Nylon membranes are very specific for biocompatibility criteria and they could also withstand any possible bacterial attack. Another advantage is the possibility of obtaining these membranes with different pore diameters (Portaccio, et al., 2002).

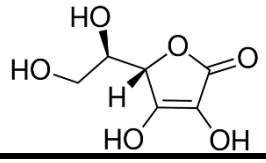
CHAPTER THREE

RESEARCH MATERIALS AND METHODS

3.1. MATERIALS

In this study, the ascorbic acid (AA) being used for the detection in the analyte solution which the analyte solution will comprises of polyaniline (PANI), iron(III) oxide (FE_2O_3) and phosphate buffer (PBS). The ascorbic acid is purchased from Sigma-Aldrich (M) Sdn. Bhd. Malaysia. The AA properties is shown in the following table:

Table 3.1: Properties of AA **(Sigma-Aldrich, Inc, 2018)**

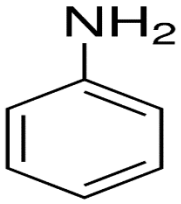
Properties	
Common name	Ascorbic Acid
IUPAC name	(2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one
Other name	L-ascorbic acid, Vitamin C, Ferrous Ascorbate
Molecular formula	$C_6H_8O_6$
Molecular weight	176.12 g/mol
CAS number	50-81-7
Chemical structure	

Ascorbic acid is conjugated to PANI and FE_2O_3 to form the analyte. Before the conjugation, the AA is weighed to set a different concentration of AA. The AA is then diluted in PBS solution.

Polyaniline is constructed from the basic of aniline with help of ammonium peroxodisulfate (APS), which helps in increase the conductivity of the solution by transfer

the electron from the ascorbic acid to the electrode. Aniline is purchased from Sigma-Aldrich (M) Sdn. Bhd. Malaysia. The properties of aniline are shown as in the following table:

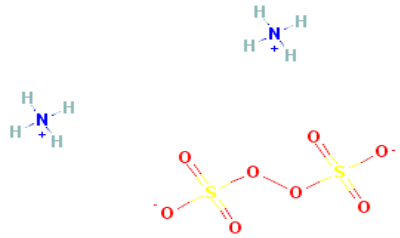
Table 3.2: Properties of Aniline **(Sigma-Aldrich, Inc, 2018)**

Properties	
Common name	Aniline
IUPAC name	Phenylamine
Other name	Benzenamine, Phenylamine
Molecular formula	$C_6H_5NH_2$
Molecular weight	93.13 g/mol
CAS number	62-53-3
Chemical structure	

Aniline is synthesized to form PANI before AA is conjugated to PANI. PANI is very useful to increase the transfer of electron in electrochemical cell, especially when it is in acidic condition where PANI is very conductive in lower pH (Ziadan & Saadon, 2012).

Ammonium peroxodisulfate (APS) is needed in synthesizing PANI. Ammonium peroxodisulfate, or ammonium persulfate is purchased from Merck Sdn. Bhd. Malaysia and the properties is shown in the following table:

Table 3.3: Properties of APS **(Merck Millipore , 2017)**

Properties	
Common name	Ammonium peroxodisulfate
IUPAC name	Diazanium sulfonatooxy sulfate
Other name	Ammonium persulfate, Ammonium peroxydisulfate
Molecular formula	$(NH_4)_2S_2O_8$
Molecular weight	228.19 g/mol
CAS number	7727-54-0
Chemical structure	

APS is diluted in distilled water before mixed into aniline to help in the synthetization of PANI.