FABRICATION OF A CARBON-BASED IMMUNO-BIOSENSOR FOR HEPATITIS B SURFACE ANTIGEN DETECTION

WILLY WONG XIU FA

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JULY 2014

ABSTRACT

Since the early diagnosis of HBV infection is crucial for the successful antiviral treatment, sensitive methods are urgently needed for measuring bio-diagnosis markers present at ultra-low levels during early stages of the infection. In this proposal, a graphene based biosensor is presented for performing highly sensitive pathogenic virus detection, particularly toward the detection of Hepatitis B virus surface antigen (HBsAg). A free-standing conductive graphene will be prepared using a modified Hummers method. Graphite will be oxidized to graphene oxide which will then be reduced to graphene by using hydrazine as the reducing agent. The product before and after hydrazine reduction was tested with UV-Vis spectrometer to obtain the peak at certain wavelength. Before reduction, the peak was shown around 230-240 nm while after reduction, the peak was around 270 nm. The peak shift from 230 nm to 270 nm shows that the synthesis of graphene was successful. The final product of the reduction was a black color powder. The immobilization of the antibody will be done using this graphene as substratum. The graphene is used to grow the Nafion-graphene nanosheet, and was then soak in Thionine solution. Following that, a solution of primary antibodies against hepatitis B surface antigen (anti-HBsAg IgG) was incubated on the biosensor. After the immobilization, the antigen was dropped onto the biosensor, follow by incubation of secondary antibodies conjugated to Horseradish Peroxidase (HRP). The color change to blue upon addition of Tetramethylbenzidine (TMB). Sulfuric acid stop solution was then added and colour changes from blue to yellow was observed. This colour change indicate that the immobilization of anti-HBsAg on the graphene surface was successful.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Anti-HBsAg	Antibodies against Hepatitis B virus surface antigen
FTIR	Fourier Transform Infrared Spectroscopy
GO	Graphene Oxide
rGO	reduced Graphene Oxide (graphene)
HBsAg	Hepatitis B virus surface antigen
HBV	Hepatitis B virus
SEM	Scanning Electron Microsope
TMB	Tetramethylbenzidine
UV-Vis	Ultraviolet-visible light

1 INTRODUCTION

1.1 Background of Study

Micro-organisms, such as bacteria and viruses, are found widely in the environment, in food, marine and estuarine waters, soil, and also in the body fluids of humans and animals. Many of these organisms have an essential function in nature, but certain potentially harmful micro-organisms can have profound negative effects on both animals and humans (Paul et al., 2002). One of the examples would be Hepatitis B virus that causes Hepatitis B.

Hepatitis B is a disease caused by HBV which infect the liver of hominoidae. As reported by Ganem and Varmus in 1987, chronic Hepatits B infections can cause a spectrum of different diseases ranging from inactive carrier state to the development of cirrhosis-related complication and hepatocellular carcinoma (liver cancel). Besides, Hepatits B virus is blood-borne, transfusion-transmitted human pathogen that has a major impact on blood safety and public health worldwide (Hsia et al., 2007). Hepatitis B virus (HBV) infection is a major global health problem and the tenth leading cause of death worldwide (Lavanchy, 2004). About a third of the world's population, an estimated 2 billion people have been infected with Hepatitis B virus and more than 360 million have chronic Hepatitis B. Additionally, at least 600,000 people die annually from acute or chronic consequences of Hepatitis B virus infections (Verma et al., 2011). Verma et al.(2011)'s research also shows that HBV is 50 to 100 times more infectious than Human Immunodeficiency Virus (HIV) that causes Acquired Immunodeficiency Syndrome (AIDS).

According to Sung et al. (2009)'s research, a high viral load in patients is the main cause of Hepatitis B progression, thus the ultimate goal for the treatment of Hepatitis B is to eliminate the virus before irreversible liver damage occurs. In other words, early diagnosis of HBV infections is crucial in making clinical decision for successful antiviral treatment. Therefore, sensitive methods are urgently needed for measuring the bio-diagnosis markers present at ultra-low levels during early stages of the infection. Sensitive and early detection of HBV may not only help monitor the viral dynamics associated with treatment but could also improve therapeutic decision making (Geng et

al., 2005). Conventional methods for detection of HBV have shown a negative outcome of being time consuming and expensive. As a matter of fact, the development of novel biosensor for highly sensitive, selective and rapid pathogen detection is of paramount importance for medical diagnostics, food safety screening and environment pollution monitoring.

As reported by Vashist et al. (2011), the electrochemical immunoassays and immuno-sensors are drawing more attention in a wide range of uses, due to their merits such as low cost, small size, short response time and the possibility of using in vivo. The immunoassay techniques, based on highly specific molecular recognition of antigens by antibodies, have become the main analytical tools in clinical and biochemical analyses and in other areas such as environmental control, food quality control, etc (Liu et al, 2006). The electrochemical methods in immuno-sensing have become very popular recently. Impedance technique, a type of electrochemical biosensors have been proven to be a promising method for pathogenic detection due to its portability, rapidity, sensitivity, and more importantly it could be used for on-the-spot detection.

Generally, the impedance detection techniques can be classified into two types depending on the presence or absence of specific bio-recognition elements. The first type works by measuring the impedance change caused by binding of targets to bio-receptors (antibodies and nucleic acids) immobilized onto the electrode surface, while the detection principle of the second type is based on metabolites produced by bacterial cells as a result of growth (Wang et al., 2012).

Owing to the emerging of nanotechnology, a variety of nanomaterial such as semiconductor quantum dots, metallic or semiconductor nanowires, carbon nanotubes (CNTs), and nanostructured conductive polymers has been incorporated into the optical and electrical transducers of biosensors. As Mahmoud and Luong (2003)'s stated, these incorporations lead to a significant improvement in the sensitivity and selectivity of the sensors. Among them, the two-dimensional carbon nanostructure, known as graphene, has stimulated research interest due to its remarkable electrical, mechanical and thermal properties (Haque et al., 2012). The additional findings of biocompatibility, facile surface modification with biomolecules, good water dispersibility, high surface-area-to-volume ratio, and unique optical properties endow graphene with high potential for bioelectronics and biosensing application (Liu et al., 2011). In conclusion, the

integration of nanomaterial (graphene) and biosensors has a promising future in detecting HBV.

1.2 Statement of Problem

A high viral load in patients is the main cause of Hepatitis B progression, thus the ultimate goal for the treatment of Hepatitis B is to eliminate the virus before irreversible liver damage occurs. In order to do so, diagnosis of HBV infections in the early stages is crucial for successful antiviral treatment. However, accurate detection of HBV using the conventional methods requires high cost. Moreover, most of the conventional methods for accurate detection are time consuming. Therefore, cheap, rapid and sensitive methods are needed to measure the bio-diagnosis markers present at ultra-low level during early stages of infections. As a result, the electrochemical immunoassays and immunosensors are drawing more attention in a wide range of uses such as in the analysis of trace substances in environmental science, pharmaceutical and food industries because of its low cost, small-sized, short response time and the possibility of using in vivo. Moreover, merging nanomaterial such as graphene oxide and graphene into biosensor enhance the performances of the detection for HBV. Both graphene oxide and graphene can be processed into a wide variety of novel materials with distinctly different morphological features, where the carbonaceous nanosheets can serve as either the sole component, as in papers and thin films, or as fillers in polymer and/or inorganic nanocomposites. Graphene is better than graphene oxide due to its biocompatibility, high surface area, facile surface modification with biomolecules, good water dispersibility, high conductivity and capacitance. Subsequently, incorporation of graphene with biosensor is believed to augment the detection of HBV in term of accuracy and time.

1.3 Objective of Research

The study aims to:

i) To reduce graphene oxide to graphene

ii) To immobilize Hepatitis B antibody (anti-HBsAg) onto graphene.

1.4 Scope of Proposed Research

Graphene, Hepatits B Virus and Hepatitis B antibodies (anti-HBsAg) were used in this research. Graphene oxide (GO) was synthesized from natural graphite based on the modified Hummer's methods. This GO was then being reduced to graphene. UV-Vis spectroscopy was used to confirm the peak shift. A peak shift from 230 nm to 270 nm indicates that the reduction was successful. The amount of graphene-Nafion solution to be dropped onto the 96-well microtiter plate was studied as well. The fabricated biosensor was tested with Hepatitis B virus surface antigen (HBsAg).

1.5 Expected Outcomes

This study would claim to produce a biosensor with high selectivity for rapid and simple detection of Hepatitis B virus surface antigen (HBsAg) in human blood serum, and it also studies the potential of the biosensor as a diagnostic tool for rapid and direct detection of viral antigens in clinical samples for preliminary pathogenic screenings.

1.6 Significance of Study

The biggest beneficiary will be the medical industry. This biosensor aims to detect the HBV in human blood serum. It appears to be a diagnostic tool for rapid and direct detection of HBV. Early diagnosis of Hepatitis B using this biosensor reduced the time consuming and cost for the detection of HBV. Thus improve the therapeutic decision making for the antiviral treatment.

1.7 Summary of Chapter

This chapter has explained on the background information of the research itself in term of Hepatitis B, type of detection for HBV and the promising benefits of graphene-based biosensor. Problem statement, research objectives and significance of research are discussed to explain the purpose and needs of this research. Lastly, scope of the research and expected outcomes are stated to ensure that the research objectives could be achieved.

2 LITERATURE REVIEW

2.1 Overview

This chapter will discuss in detail about the immuno-biosensors, characteristic of Hepatitis B virus, properties of graphene oxide and graphene.

2.2 Immuno-Biosensors (Immunosensors)

Biosensors are analytical devices which combine a biologically sensitive element with a physical or chemical transducer to selectively and quantitatively detect the presence of specific compounds in a given external environment (Nicolini et al., 1992). Immuno-biosensors or immunosensors will be an example of an easy-handling biosensor (Selvakumar & Thakur, 2012). As stated by Fu et al. in 2009, this type of biosensor have been extensively used for clinical diagnostics, environmental monitoring, and also for food safety. In fact, immunobiosensors are of great attention due to its potential utility as specific and direct detection tools and their simplicity compared to standard immunological test, which include Enzyme-Linked Immunosorbent Assays (ELISAs) (Sibbald, 1986). ELISAs are time consuming (Marquette, Coulet & Blum, 1999).

In addition, immunosensors, like other types of biosensors, uses a molecular recognition element that consists of a transduction system coupled to a receptor (Buch & Rechnitz, 1989; Thompson & Krull, 1991). The common recognition element in this immunosensors is achived by sensing the specific antigen-antibody binding reaction at the receptor (DeSelva et al., 1995). For instances, Tang et al. (2006) and Wang et al. (2005)'s research demonstrate an electrochemical immunobiosensor by immobilizing the antigens onto the surface of electrodes. When the antibodies bind to the immobilized antigens, electrical signals are generated. Next, the transduction system identifies and responds to changes in an optical, spectroscopic, chemical, electrochemical, radiochemical or electrical parameter of the receptor environment caused by the specific antigen-antibody binding (Tang et al., 2004). The study also highlights that the high selectivity and affinity of antibodies molecule to their corresponding antigens is the reason why this recognition element has become the most common element for immunobiosensor.

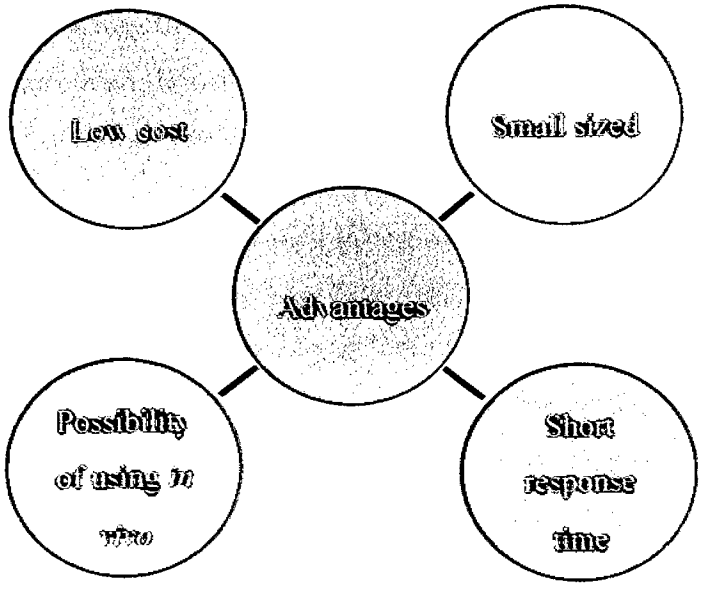

Figure 2.1: Advantages of Immuno-Biosensor

2.3 Hepatitis B Virus (HBV)

2.3.1 Virus Morphology

The HBV is an envelope virus belonging to the Hepadnaviridae family. It contains a 3.2-kb, partially double stranded open circular genome enclosed by a nucleocapsid core (HBcAg) and a viral encoded DNA polymerase (Aliyu et al., 2003). The HBV capsid is surrounded by a lipid bilayer envelope comprising three related surface glycoproteins known as L (large), M (middle), and S (short) surface antigen (HBsAg)(Ganem,1991). The viral capsid with an icosahedral structure is made up of 180 or 240 subunits of core antigen (HBcAg) (Crowther et al., 1994). Each HBcAg subunit consists of 183 or 185 amino acid residues (depending on virus subtypes) with a carboxy (C)-terminal region about 40 residues which are highly rich in positively charged residues (Tan et al., 2003).

2.3.2 Virus Detection Methods

Presently, enzyme-linked immunosorbent assays (ELISA) is the main method to clinically diagnose HBV (Moriya et al., 2002). However, information obtained from this method is indirect and the method itself has a low sensitivity. Moreover, ELISA is time consuming (Marquette & Blum, 1999).

The endogenous DNA amplification and dot blot methods can only detect 0.1pg HBV DNA, which corresponds to 3×104 virosomes (Liu et al., 1999). But, the sensitivity of clinical diagnosis has been enormously improved by the Polymerase Chain Reaction (PCR) method, which can sense 10–5pg HBV DNA (Desmet et al., 1994). In addition to the above methods, methods based on the use of fluorescence dye markers (Park et al., 2000; Stefanini et al., 1983), radioactive isotopes (Jilbert, 2000; Barlet et al., 1994), or chemiluminescence labels (Young et al., 2002) have also been developed for the detection of viral hepatitis. But, the radioactive isotope is difficult to handle and it is hazardous; while the fluorescence markers are prone to bleaching and the chemiluminescence labels may yield irreproducible results in some cases (Moriya et al., 2002). In conclusion, methods mentions above are complicated and the preparation of materials to perform such method is difficult. As a matter of facts, an alternative method characterized by simplicity, speed, and sensitivity is desired for the diagnosis of HBV in a typical clinical laboratory.

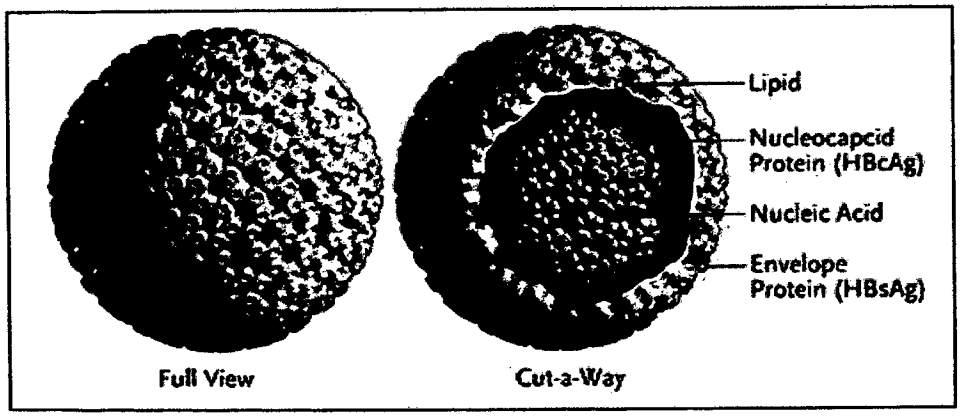

Figure 2.2: Model of Human Hepatitis B Virus (HBV) (Henderson, 2004)

2.4 Graphene Oxide (GO)

2.4.1 Morphology

The study of the GO structure is derived from the structural analysis of graphite oxide itself. Over the years, considerable effort has been directed toward understanding the structure of graphite oxide, both theoretically and experimentally. As a result, a few conflicting models have been continually proposed. Originally, Hofmann and Holst (as cited in Chen et al., 2010) proposed a simple model, in which graphite oxide was thought to consist of epoxy (1,2-ether) group modified planar carbon layers with a molecular formula of C2O. While Ruess (as cited in Chen et al., 2010) suggested that

the carbon layers were not in fact planar but puckered and that the oxygen-containing groups were hydroxyl and ether-like oxygen bridges between carbon atoms 1 and 3, randomly distributed on the carbon skeleton. In order to explain the acidic properties of graphite oxide, Claus et al. (as cited in Chen et al., 2010) further incorporated an enol- and keto-type structure into their model, which also contained hydroxyls and ether bridges at the 1 and 3 positions. However, Scholz and Boehm (as cited in Chen et al., 2010) proposed a new structure with corrugated carbon layers. Here the epoxide and ether groups were completely replaced by carbonyl and hydroxyl groups.

Meanwhile, Nakajima and Matsuo (1994) proposed a different model for graphite oxide. This model consisted of two carbon layers linked to each other by sp3 carbon–carbon bonds perpendicular to the layers and in which carbonyl and hydroxyl groups were present in relative amounts depending on the level of hydration. Based on expert NMR studies, Lerf et al. (as cited in Chen et al., 2010) proposed a structural model having a random distribution of flat aromatic regions with unoxidized benzene rings and wrinkled regions of alicyclic six-membered-rings bearing C=C, C–OH, and ether groups (reassigned to the 1 and 2 positions). In light of these previous models, Szabo et al. (2006) recently proposed a new structural model that involves a carbon network consisting of two kinds of regions: (i) trans-linked cyclohexane chairs and (ii) ribbons of flat hexagons with C=C double bonds as well as functional groups such as tertiary OH, 1,3-ether, ketone, quinone, and phenol (aromatic diol).

Even more recently, Dreyer et al. (as cited in Chen et al., 2010) reviewed the structural analogies and differences among the above structural models of graphite oxide. According to Chen et al. (2010)'s reviews, in GO, the carbon atoms that are covalently bonded with oxygen functional groups, such as hydroxyl, epoxy, and carboxy are sp3 hybridized. These can be viewed as oxidized regions, and they disrupt the extended sp2 conjugated network of the original honeycomb-lattice structured graphene sheet. The latter can be viewed as the unoxidized regions. These sp3 hybridized carbon clusters are uniformly but randomly displaced slightly either above or below the graphene plane.

2.4.2 Electrochemical Properties

Due to its specific 2D structure and the existence of various oxygenated functional groups, GO exhibits various excellent properties. These include electronic, optical, thermal, mechanical, and electrochemical properties, as well as chemical reactivity.

Recently, it has become popular to explore the electrochemical properties of GO at electrode surfaces. Due to its favorable electron mobility and unique surface properties, such as one-atom thickness and high specific surface area, GO can accommodate the active species and facilitate their electron transfer (ET) at electrode surfaces (Liu et al., 2009). For example, in 2010, Zuo et al. reported that GO supports the efficient electrical wiring of the redox centers of several heme-containing metalloproteins (cytochrome c, myoglobin, and horseradish peroxidase (HRP)) to the electrode. Next, GO possesses excellent electrocatalytic properties (Tang et al., 2009). In addition, Tang et al.'s research also demonstrated the electrocatalytic activity of GO toward oxygen reduction and certain biomolecules.

It has also been shown that GO exhibits high electrochemical capacitance with excellent cycle performance and hence has potential application in ultra-capacitors (Wang et al., 2009). Furthermore, Shao et al. (2010) reported that rGO shows much higher electrochemical capacitance and cycling durability than carbon nanotubes (CNTs). The specific capacitance was found to be ~165 and ~86 F/g for rGO and CNTs, respectively.

Due to the presence of a large number of oxygen-containing functional groups and structural defects, GO exhibits enhanced chemical activity compared with pristine graphene. It appears that one of the most important reactions of GO is its reduction. GO can be reduced to graphene by various approaches. In the past few years, there have been reports of reducing GO in the solution phase using various reducing agents, such as hydrazine (Park et al., 2011), sodium borohydride(Shin et al., 2009), or hydroquinone (Wang et al.,2008) and in the vapor phase using hydrazine/hydrogen or just by thermal annealing (Yang et al.,2009) or by electrochemical techniques. In this study, hydrazine will be used to reduce graphene oxide (GO) to grapheme.

2.5 Graphene

2.5.1 Morphology

Zhu et al. in 2010 reported that graphene honeycomb lattice is composed of two equivalent sub-lattices of carbon atoms bonded together with σ bonds. Each carbon atom in the lattice has a π orbital that contributes to a delocalized network of electrons. The microscopic corrugations were estimated to have a lateral dimension of about 8 to

10 nm and a height displacement of about 0.7 to 1 nm. Sub-nanometer fluctuations in height for graphene platelets deposited on a SiO2-on-Si substrate were studied by Scanning Tunneling Microscopy (STM).

2.5.2 Properties

Ever since its discovery in 2004, graphene has been making a profound impact in many areas of science and technology due to its remarkable physicochemical properties. These include a high specific surface area (theoretically 2630 m2/g for single-layer graphene) (Park & Ruoff, 2009), extraordinary electronic properties and electron transport capabilities (Novoselov et al., 2007), unprecedented pliability and impermeability (Bunch et al., 2008), strong mechanical strength (Lee, Wei, Kysar & Hone, 2008) and excellent thermal and electrical conductivities (Balandin et al., 2008; Bolotin et al., 2008).

These unique physicochemical properties suggest it has great potential for providing new approaches and critical improvements in the field of electrochemistry. For example, the high surface area of electrically conductive graphene sheets can give rise to high densities of attached analyte molecules. This in turn can facilitate high sensitivity and device miniaturization. Facile electron transfer between graphene and redox species opens up opportunities for sensing strategies based on direct electron transfer rather than mediation. Despite its short history, this 2D material has already revealed potential applications in electrochemistry, and remarkably rapid progress in this area has already been made.

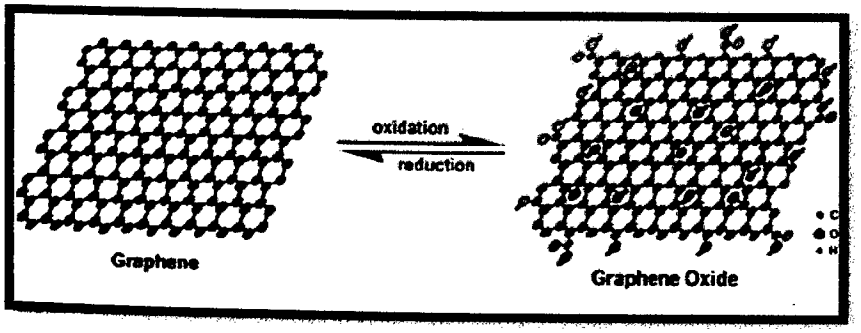

Figure 2.3: Scheme of structural model of graphene and graphene oxide (GO), showing that graphene consists of only trigonally bonded sp2 carbon atoms while GO consists of a partially broken sp2-carbon network with phenol, hydroxyl, and epoxide groups on the basal plane and carboxylic acid groups at the edges (Chen et al., 2010).

2.6 Summary of Chapter

This chapter discuss about the type of biosensors and the disadvantages of using conventional method for the detection of HBV. Besides, this chapter also talks about the morphology, virus markers and detection methods of HBV. Later, it discuss about the morphology and properties of graphene oxide (GO) and graphene. From the explanation given, it clearly define the difference between graphene oxide (GO) and graphene. It also explains the advantages and disadvantages for both nano-materials.

3 MATERIALS AND METHODS

3.1 Overview

This chapter will discuss on the methods used to perform the research in term of the production of graphene nanosheet, fabrication of biosensors, immobilization of Hepatitis B surface antigen antibodies (anti-HBsAg), electrochemical studies and lastly on the structural characterization of the graphene nanosheet. The overall process is as shown below.

3.2 Materials

Chemicals were obtained mostly from Sigma Aldriech (concentrated sulfuric acid, concentrated hydrochloric acid, citric acid, potassium persulfate, phophorus pentoxide, potassium permanganate, graphite powder, graphite rod, hydrazine hydrate, gold chloride hydrate, Nafion 117 solution and thionin acetate salt.). The Hepatitis B virus surface antigen antibodies (anti-HBsAg IgG) was purchased from Gene Tex. Some of the common chemicals such as acetone and hydrogen peroxides were obtained from UMP FKKSA chemical warehouse.

3.3 Instruments

UV- Vis Spectroscopy was used to identify GO and rGO. Ultrasonicator was used to exfoliate the GO produced before it goes through hydrazine reduction.

3.4 *Research Process/Procedures*

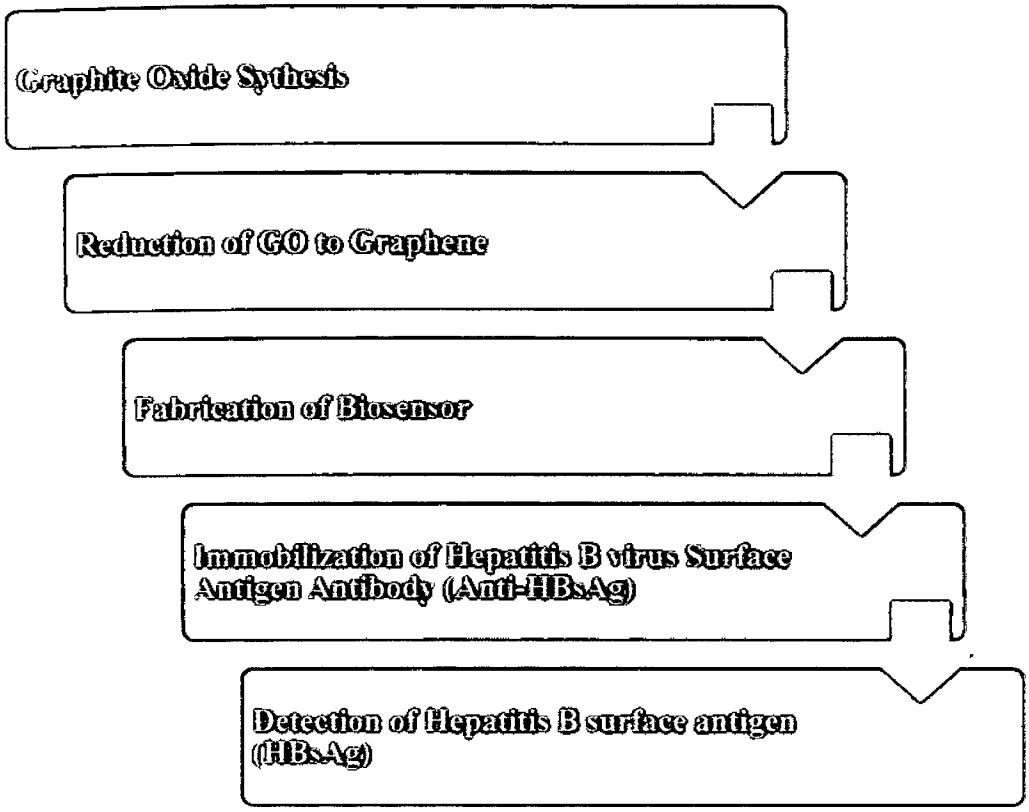

Figure 3.1: Process Flow Diagram of the Fabrication of Carbon-Based Immune-Biosensor for Hepatitis B Surface Antigen (HBsAg) Detection.

3.4.1 Synthesis of Graphite Oxide

A solution of concentrated sulfuric acid (H_2SO_4), potassium persulfate ($K_2S_2O_8$) and phosphorus pentoxide (P_2O_5) was prepared and heated up to 80°C. 20 grams of graphite powder was then be added into the solution and stirred for 30 minutes. A dark blue mixture was observed. The solution was then being cool to room temperature for 6 hours. Deionized (DI) water was added to filter and wash the filtrate until it becomes neutral pH. The filtrate was dried overnight at room temperature using a vacuum desiccator. By using a 2 liter conical flask, the 20 grams of dried graphite powder was poured into a solution of 0°C concentrated sulfuric acid (H_2SO_4). 60 grams of potassium permanganate (KMnO4) was added slowly into the solution with stirring and cooling. The temperature of the solution was maintained below 20°C. After that, the mixture was heated up to 35°C for 2 hours using oil bath. Effervescence and brownish grey paste appear during the process. Next, 920 mL of DI water was added into the mixture. The temperature was maintained at 98°C for 15 minutes. Another 2.8 liter of DI water and 30% hydrogen peroxide (H_2O_2) was added into the mixture. The color of

the mixture changes to bright yellow. The mixture was filtered with 5 liter of 1:10 concentrated hydrochloric acid (HCl). The filtrate was dried overnight at room temperature using a vacuum desiccator.

3.4.2 Reduction of Graphene Oxide (GO) to Graphene

The reduction was done by dispersing 0.1 grams of graphite oxide into a 50 mL DI water. It was then be ultrasonicated for 30 minute. After that, the solution was centrifuged to remove the unexfoliated materials. The supernatant, which is the top layer, is the graphene oxide (GO). The supernatant graphene oxide was poured into a round bottom flask and the pH of the solution was adjusted to 10 using 5M of potassium hydroxide (KOH). Later, 0.025 mL of hydrazine was added into the solution and reflux in an oil bath at 95°C with stirring for 24 hours. The solution was filtered using 0.45-micron PTFE filter paper and also washed with large amount of DI water. The filtrate was having a final wash using acetone instead of DI water. The filtrate was then being dried using a vacuum desiccator. The final products were the graphene, which will be used in the fabrication process.

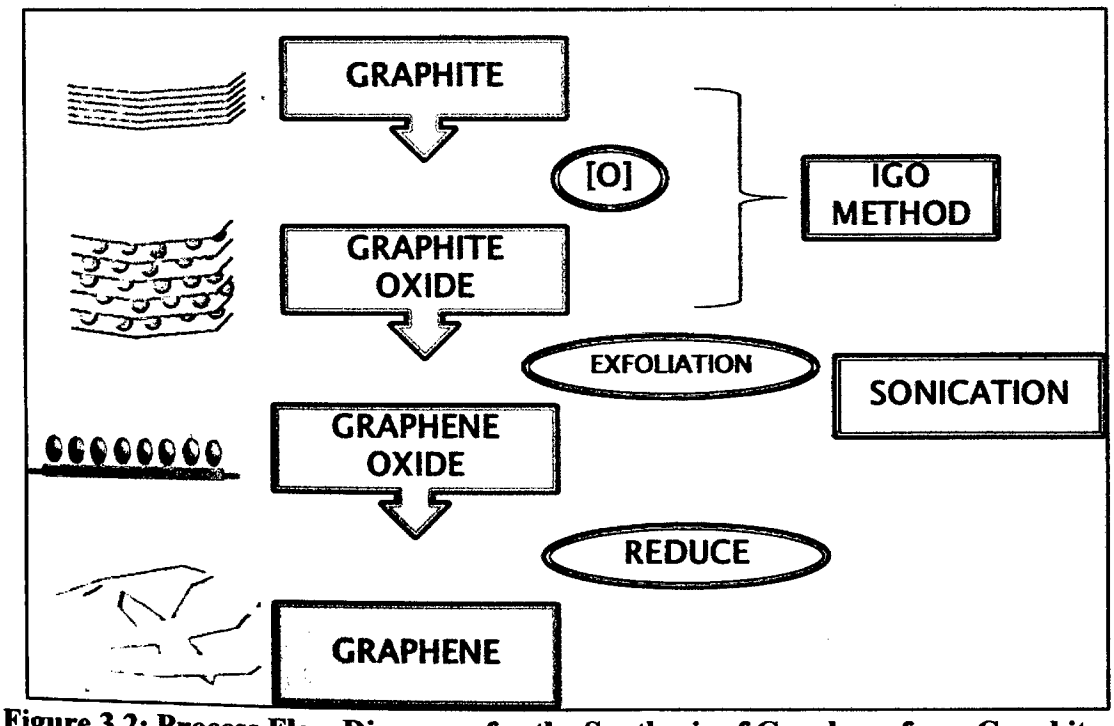

Figure 3.2: Process Flow Diagrams for the Synthesis of Graphene from Graphite

3.4.3 Fabrication of Biosensor

The graphene was used to grow the graphene-Nafion nanosheet by ultrasonicating the graphene in 0.25% Nafion-water. Subsequently, graphene-Nafion nanosheet was dropped onto the 96-well microtiter plate and allowed to dry. After the graphene-nafion layer dried, it was then being soaked in Thionine solution. The biosensor fabrication process is as described in Su et al. (2009). The amount of graphene-Nafion dropped onto the microtiter plate was examined to obtain the appropriate amount to coat the surface completely.

3.4.4 Immobilization of Hepatitis B Surface Antigen Antibody (Anti-HBsAg)

Immobilization of anti-HBsAg on the fabricated biosensor is based on carbodiimide-assisted amidation reaction. A solution of polyclonal antibodies against hepatitis B surface antigen (anti-HBsAg IgG) was incubated on the biosensor. After 12 hours, Bovine Serum Albumin (BSA) was applied to block the remaining active groups and eliminate non-specific binding sites; this was followed by washing step. The final biosensor can be stored at 4°C when not in use.

3.4.5 Identification of Graphene Oxide and Graphene

The graphene oxide and graphene produced was examined using UV-Vis spectroscopy. The peak shift was observed and results were compared with the literature.

3.5 Summary of Chapter

This chapter covers the materials and instrument used in the study. It explains in detail regarding to the procedure for the synthesis of graphene. It also discussed the process flow from sample preparation, fabrication of biosensor to immobilization of antibodies. Beside, this chapter also discuss on the methods in collecting data for the analysis.

4 RESULT AND DISCUSSION

4.1 Overview

This chapter will discuss on the result obtained. The result obtained focuses on the synthesis of graphene, the coating of graphene onto the microtiter plate, and lastly on the detection of HBsAg.

4.2 Synthesis of Graphene

There is one minor step in synthesizing graphite oxide known as the pre-oxidation step in which graphite powder was mixed with a solution of sulphuric acid, phosphorus pentoxide and potassium persulfate. The graphite supposed to change to dark blue mixture after the mixing. However, the colour does not seem to change much during the synthesis. Anyway, it was believed that this pre-oxidation step would not affect the quality of the graphite oxide as this step was done to speed up the whole experiment.

During solid-liquid separation process, several methods such as vacuum filtration and centrifugation using bench top centrifuge had been carried out to determine the best method in separating the graphite oxide from its solution. It was found that centrifugation was not a feasible method in separating the graphite oxide. The graphite oxide would not settle at the bottom of the centrifuge tube. The possible reason for this is that the centrifugal force applied was not sufficient. The maximum limit of rotation for bench top centrifuge is 10000 rpm.

The problem was then be solved by using vacuum filtration. It was clearly shown that the separation is more efficient using vacuum filtration. The filter cake was the product needed. The setup for vacuum filtration and its end product was shown in the figure below.

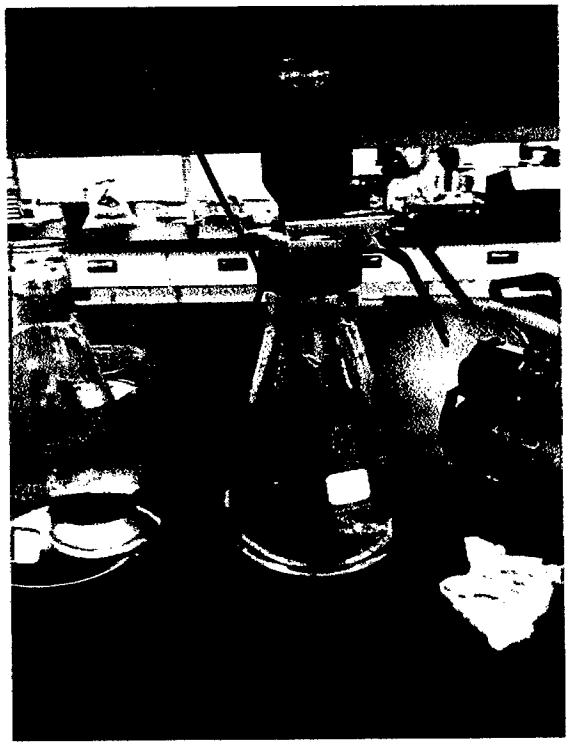

Figure 4.1: Setup for vaccum filtration.

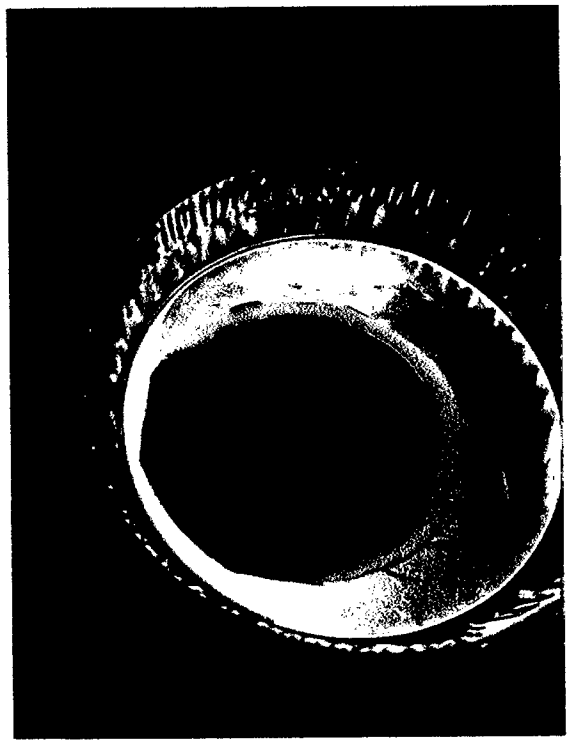

Figure 4.2: Graphite oxide cake after vacuum filtration