

DEVELOPMENT OF A GLUCOSE SENSOR USING SCREEN-PRINTING AND  
ELECTROPHORETIC DEPOSITION

By

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Development of a Glucose Sensor Using Screen-Printing and Electrophoretic  
Deposition

**Abstract**  
by

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Diabetes is a worldwide health problem. This metabolic disorder and its related diseases are a leading cause of death in the world. These deaths can be reduced through personal control of blood glucose using a reliable and cost effective glucose biosensor.

In this research, thick-film microfabrication and electrophoretic deposition (EPD) technologies are used to produce practical glucose minibiosensors. These two technologies, in combination with Pt-Ir nano-metallic catalyst particles, were used to produce a glucose minibiosensor. The sensitivity of this minibiosensor was high and it required a lower applied potential than previous sensors.

The Pt-Ir/carbon nanoparticles showed excellent performance characteristics, such as good stability and high reactivity. The Pt-Ir/carbon nanoparticles showed good electrocatalytic activity for the oxidation of H<sub>2</sub>O<sub>2</sub>, at a relatively low applied potential.

Electrophoretic deposition (EPD) of the Pt-Ir/carbon nanoparticles, glucose oxidase, and Nafion binder was used to fabricate the glucose minibiosensors. The resulting sensors demonstrated very good stability and selectivity for glucose detection in both phosphate buffer saline (PBS) and bovine serum solution.

Electrophoretic deposition of both the nanoparticle catalyst and the enzyme was employed. The amount of deposited Pt-Ir/carbon nanoparticles and enzyme was controlled by controlling the applied potential and time of EPD.

Mixing the metallic electrocatalysts and the enzyme GOD with the carbon nanoparticles shortened the distance between the GOD and the working electrode. Therefore, the nanoparticles were in close contact with the carbon-based electrode. The H<sub>2</sub>O<sub>2</sub> generated from the enzyme transferred directly to the electrode. Mediator-free glucose detection based on direct electron transfer was achieved.

We used the EPD modified minibiosensor to study the kinetics of glucose oxidation in both PBS solution and bovine serum. The results showed a good fit to the Michaelis-Menten kinetic model. Furthermore, this biosensor showed good long term stability and shelf life at ambient temperature.

## **1. Introduction**

### **1.1 Brief history of biosensors**

Pioneering studies of an electrochemical sensor to measure the concentration of dissolved oxygen was reported in 1956 by Dr. L. Clark of the Children's Hospital in Cincinnati, Ohio.<sup>[1]</sup> His group has also reported an enzyme-based glucose sensor for continuous monitoring in cardiovascular surgery in 1962.<sup>[2]</sup> The concept of this pioneering biosensor research involved the immobilized glucose oxidase (GOD) (EC 1.1.3.4) enzyme on a Clark oxygen electrode for the measurement of glucose concentration in a test solution. This prototype has been the basis for the development of the first commercialized enzyme electrode and glucose analyzer.<sup>[3]</sup> Although the enzyme-based glucose biosensor has been commercialized, much continuing effort have been focused on finding the suitable membranes that can make the GOD-platinum electrode technique more reproducible and accurate.

The key research that led to the home-testing glucose sensors was carried out in the early 1980s.<sup>[4]</sup> The oxidized form of the mediator, such as ferrocene, reacts with the reduced GOD instead of oxygen and the reduced mediator was formed without generating hydrogen peroxide.<sup>[5,6]</sup> The redox mediator shuttles between GOD and electrode. This method eliminates the problem of variation of oxygen concentrations and partially decreases the interference caused by the vitamin C, uric acid, and other molecules in the sample.<sup>[6]</sup> The commercialized glucose biosensor system consists of a small, disposable, single-use, glucose-sensitive electrode and the corresponding handheld-sized portable meter

containing the IC electronics and LCD display.<sup>[5]</sup> Recently, immobilization techniques have been developed to bind the enzyme directly to an electrode, facilitating rapid electron transfer and high signal current densities.<sup>[7]</sup>

Many minimal and non-invasive glucose sensing methods measure, or attempt to estimate, glucose in the interstitial fluid instead of in the blood.<sup>[8]</sup> A so-called artificial pancreas that integrates a glucose biosensor, insulin delivery formulation, a pump and a control system has been reported.<sup>[9, 10]</sup> The problem is exacerbated by assays that consume glucose by the sensor itself such as GOD. Currently, an artificial pancreas using an optical fiber sensor has also been reported.<sup>[9]</sup> Commercialization of this artificial pancreas has been attempted by different groups. However, the key problems for in vivo glucose measurement include the degradation rate of the polyethylene glycol (PEG) matrix, the slow response time and relatively poor accuracy over time, and the possible effects of the relative motion between the sensing matrix and the surrounding tissues. The performance of this technology needs to be studied further. In conclusion, the requirements for an in vivo glucose monitoring are immense, making it difficult to develop a suitable sensor and artificial pancreas system.

## 1.2 General background on biosensors

### 1.2.1 Functions of biosensors

An individual person will have five types of sensing elements, i.e. nose, tongue, ears, eyes and fingers. These sensing elements represent the main types of sensors occurring in nature. The best known simple type of sensor is the litmus paper test for acids and bases. The litmus sensing element gives a qualitative

indication by changing color in the presence or absence of an acidic or basic aqueous solution. A more precise and automatic method of indicating the acidity is to use pH by a pH meter with a pH sensor. In such methods, the sensor that responds to the degree of acidity is either a dye litmus chemical or the glass membrane electrode in the pH meter. The chemical or electrical potential response then has to be converted or transduced into a signal that people can observe, usually with their eyes. The color change of litmus for acid-base titration arises from absorbance of visible light by the litmus material, which is detected by the eyes in a lighted room. The electrical potential change of the pH meter has to be transduced into an observable movement of the meter needle or the LCD digital display. The part of the device that converts of the physical or chemical change into light or an electronic signal is called a transducer. In general, the functions of the sensing elements of an individual person are fixed. On the other hand, the functions of electrochemical sensor, such as pH sensor, depend on the developed technologies of the sensor probes and the transducers.

### **1.2.2 Types of biosensors**

#### **1.2.2.1 Classifications of sensors**

There are three principal types of sensors: physical sensors, chemical sensors and biosensors. Classifications of sensors depend on the design, analytes, applications, and other criteria. Depending on the type of primary input energy, sensors can also be classified into thermal sensors, radiation sensors, mechanical sensors, magnetic sensors and chemical sensors (including biosensors).<sup>[11]</sup> In turns, chemical sensors can be classified into conductivity sensors, structured

semiconductor sensors, electrochemical sensors, solid electrolyte sensors, chemically sensitive field-effect transistors sensors (CHEMFET), and other designs such as biosensors and humidity sensors, etc.<sup>[12]</sup> The classification of biosensor will be described in more detail later in this study.

The main distinguishing feature of a biosensor is the type of recognition process for the analyte because this determines in the selectivity and sensitivity of the sensor itself. The design of a biosensor depends on the nature of the analyte, its concentration, and the presence of potential interferents. Accordingly, three types of biosensors are classified and briefly described.<sup>[13]</sup>

#### 1.2.2.2 Type 1 biosensors: Affinity binding biosensors

Antibody and antigen-binding elements are widely employed biomolecular recognition types used in biosensors for the corresponding analyte. For example, the sensitivity and selectivity of an immunosensor are determined by the specific affinity of the antigen-antibody interaction and consequently, by the signal to noise ratio of the system.

#### 1.2.2.3 Type 2 biosensors: Biocatalytic biosensors

This type of biosensors uses enzyme as the recognition and signal response element and is the most extensively studied and commercialized. The high selectivity and the rapid enzymatic reaction with the analyte give this type of biosensors very good selectivity and sensitivity. The enzyme catalyst is usually integrated into the materials comprising the transducer, such as the electrode, to ensure that the biocatalytic transformation is specifically transduced into an electrical signal.

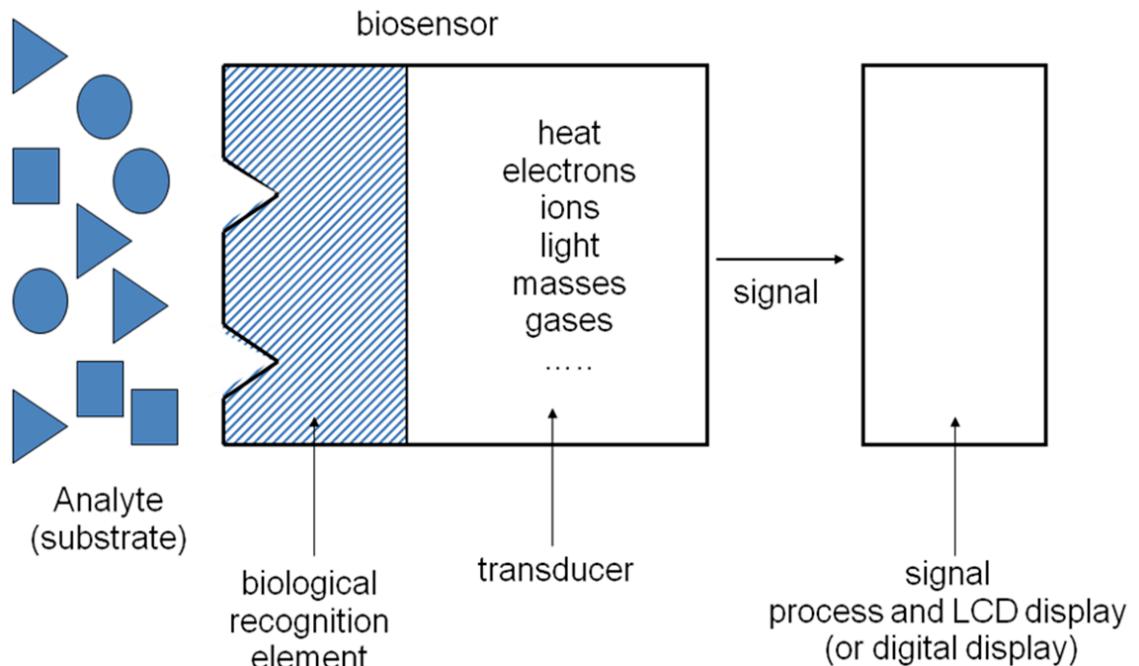
#### 1.2.2.4 Type 3 biosensors: Synthetic biomimetic biosensors

In order to improve the cost and durability of the natural biological recognition molecules used in biosensors, synthetic biomimetic materials are developed and used. Molecular imprinted polymers are artificial recognition elements that mimic natural molecular recognition. A variety of analytes, such as pesticides and others, have been used as template molecules to develop molecular imprinted polymer biosensors. However, the drawback of this approach is the difficulty associated with complete removal of the template molecule, the kinetics of the reaction, the selectivity, as well as the affinity of the biosensor, which still require further improvement.

The concept of using aptamers instead of antibody or antigen arises from the combination of a fraction of natural biomolecules and chemical synthesis. The aptamers are stable and produced by chemical synthesis. Recently, many aptasensors have been reported.<sup>[13]</sup>

### 1.2.3 Principle of biosensor operation

The biosensors of different types described above must be connected to a signal conversion device, i.e. transducer, so that a visibly observable response takes place. Biosensors are concerned with sensing and measuring a particular biological specie, which is defined as substrate or analyte. The general schematic layout of a biosensor is shown in Figure 1-1.



**Figure 1-1** Schematic layout of a biosensor

In Figure 1-1, the triangles schematically indicate the analyte which can fit into the triangular cavities of the biological recognition element. Both the circles and squares are potential interference compounds. The interference compounds cannot fit into the triangular cavities of the active sites of the biological recognition element. The biological recognition element includes an enzyme or antibody, or DNA, or whole cell, as well as the catalysts and immobilization matrix to bind and support this element.<sup>[11, 13]</sup> The transduction mechanism can be an electrochemical transducer, an optical transducer, thermal transducer, or a piezoelectric transducer depending on the change in temperature, electrons, ions, light, masses, and gases. In general, the transduction mechanism gives an electronic signal that can be transferred to a data processing system and then a LED or digital display of the signal output.<sup>[5, 13, 14]</sup>

#### **1.2.4 Immobilization of enzymes**

For the past three decades there has been an increasing interest in biosensor research due to their possible applications in clinical, food and environmental analysis.<sup>[15-28]</sup> One key requirement of these devices is the immobilization of an enzyme or other biological recognition molecules on the sensor surface with controlled spatial distribution in such a way to retain their biological recognition properties.<sup>[12]</sup>

Enzymes have been the research focus for the food, pharmaceutical and biosensor industries because of their unique ability to recognize target molecules quickly and accurately in a complex system. This molecular recognition ability has led investigators to improve the enzyme's functionality and reusability by using different immobilization matrices and techniques.<sup>[3]</sup> Enzymes have been used in a large number of practical applications through immobilization on a variety of supports.<sup>[16-18, 22-25]</sup> The immobilization techniques are a key factor in developing reliable biosensors. New immobilized schemes and novel materials that can improve the analytical capacities of biosensors are highly desirable. Glucose oxidase (GOD) (EC1.1.3.4) is a flavor protein that catalyzes the oxidation of β-D-glucose by molecular oxygen to gluconic acid and hydrogen peroxide for potential diabetic patient management applications.

The estimation of glucose in blood is an important parameter for the diagnosis and prevention of diabetes. Glucose biosensors using enzymatic method have been reported.<sup>[25]</sup> However, research in this field on new materials and approaches is continuing in order to further improve the sensitivity and the

stability of the biosensor. In general, GOD is immobilized on the electrode for sensing blood glucose. Long lifetime stability of the immobilized enzyme is an important factor. In order to enhance the properties such as reusability, stability, recovery and storage life, glucose oxidase has been immobilized on different supports using different immobilization techniques.<sup>[15]</sup> The development of low cost immobilization materials and methods with improved performance is also a key research issue.

Chemical sensors including biosensors with the ability of continuously sensing analytes have attracted considerable attention.<sup>[25]</sup> In these analytical methods, the reduction in testing cost is generally associated with the reduction of enzyme consumption. For example, it has been achieved by immobilization of the GOD in the glucose analytical system.

Screen-printed electrodes are frequently used in analytical applications because of their small size, low detection limit, fast response time, high reproducibility, mass production, and low cost.<sup>[18]</sup> These advantages have given to an improvement in many applications such as amperometric sensors and biosensors for the detection of glucose, and other biomarkers. The combination of screen-printed electrodes with immobilized enzymes and transition metal catalysts for glucose determination are attractive. The electrodes with these modified catalytic surfaces can be used for amperometric determination of analytes at a low potential if combined with oxidase – produced H<sub>2</sub>O<sub>2</sub>.<sup>[7,25]</sup>

The recent increasing research interest in the microbiosensor is due to the need for a miniature biosensor that can be mass produced. In order to explore

innovative ways to produce biosensors, new research approaches for the immobilization of enzyme bio-catalysts and the design and fabrication of cost effective mini size biosensors become important. Specially, biosensors that allow in vitro physiological monitoring, require minimum sample volume, are low cost with long shelf life and reusability are important.<sup>[21]</sup>

It is necessary that the immobilization process anchor the enzyme onto the surface of the electrode without adverse effect on the activity of the enzyme. The immobilization process is one of the key issues of an electrochemical glucose biosensor.<sup>[15-26,29]</sup> In general, there are several methods of immobilization including adsorption, microencapsulation, entrapment, cross-linking and covalent bonding, which are described below.<sup>[14]</sup>

#### 1.2.4.1 Adsorption:

Many substances such as cellulose can adsorb enzymes on their surfaces.<sup>[5,14,15]</sup> Adsorption can be divided into physical adsorption and chemical adsorption. Physical adsorption usually is weak and occurs by the formation of Van der Waals forces, hydrogen bonds and ion interactions. The adsorption can be described by many models such as the Langmuir adsorption isotherm.<sup>[14,30]</sup> Chemical adsorption is much stronger and it involves the formation of covalent bonds. The properties of the adsorbed enzymes or other biomolecules are usually changed with changes of temperature, pH, ionic strength and the substrate. Adsorption is the simplest method and involves minimal preparation. However, this method is only suitable for exploratory work over a short time-span, because the attachment of the enzyme or biological recognition molecules onto the

electrode surface often fails after a short period.

#### 1.2.4.2 Microencapsulation:

Microencapsulation is another immobilization method used in biosensors development.<sup>[2]</sup> It is a relatively stable method with respect to changes in temperature, pH, ionic strength and chemical composition. However, this system may become permeable to small molecules, which may affect the selectivity of the biosensor. Microencapsulation was used to develop the first glucose biosensor based on an oxygen electrode.<sup>[2, 13]</sup> The membranes used in this method include cellulose acetate, polytetrafluoroethylene (PTFE), etc.

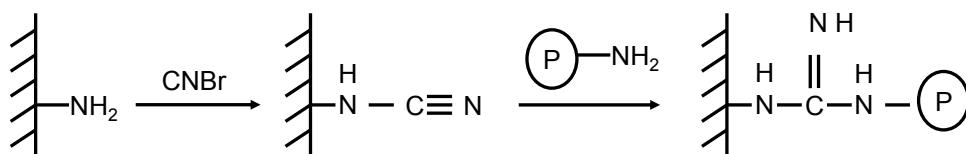
#### 1.2.4.3 Entrapment:

Entrapment usually is accomplished by mixing biomolecules with a monomer solution that is then polymerized in a polymeric matrix. This matrix can be in a gel or solid form.<sup>[15,16,23,25]</sup> Polyacrylamide, which is the copolymer of acrylamide with N, N'-methylenebisacrylamide, is a popular polymer used in this method.<sup>[14]</sup> Other materials such as polypyrrole, starch gel, and others are also used for entrapment. Unfortunately, this method can result in a diffusion barrier for the target analyte to the sensing electrode surface and a resulting loss of enzyme activity.

#### 1.2.4.4 Covalent bonding:

Covalent bonding usually involves a carefully designed bond between a functional group in the enzyme and its support matrix.<sup>[5, 11, 13, 14, 31]</sup> Various approaches are used to immobilize proteins onto solid supports.<sup>[31]</sup> The use of chemical cross-linkers is a popular method for the covalent immobilization of a

protein to a solid matrix. It involves the formation of a covalent bond between a functional group on the protein enzyme and a reactive group on the surface of the solid support. The methods of covalent bonding are as varied as organic chemistry itself. During the last three decades, thousands of proteins have been immobilized and hundreds of solid matrices have become available.<sup>[31,32]</sup> For example, amino group containing matrices can be activated with cyanogen bromide to which proteins will react forming guanidine linkages as shown in Figure 1-2.



**Figure 1-2** Coupling of proteins to cyanogen bromide activated amine matrices

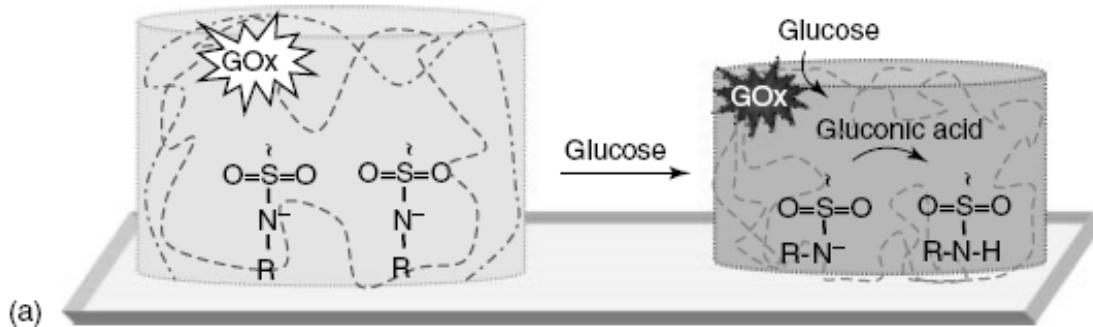
Almost any of the homobifunctional cross-linkers can be used to immobilize proteins. Depending on the matrix functional groups, different cross-linkers can be used. Cross-linking, an expansion of covalent bonding, is the technique in which the biomaterial is chemically bonded to a solid support. Sometimes, this approach can cause damage to the enzyme. However, this method has proven to be useful for stabilizing adsorbed enzymes. The cross-linking reaction must work under conditions of low temperature, low ionic strength, and neutral pH to ensure the enzyme activity will not decrease. In general, functional groups such as -NH<sub>2</sub>, -CO<sub>2</sub>H, -OH, -SH and imidazole etc., which are not related to the catalytic activity of an enzyme, can be covalently bonded to a transducer or the membrane of the biosensor. This method minimizes the leakage of the analyte

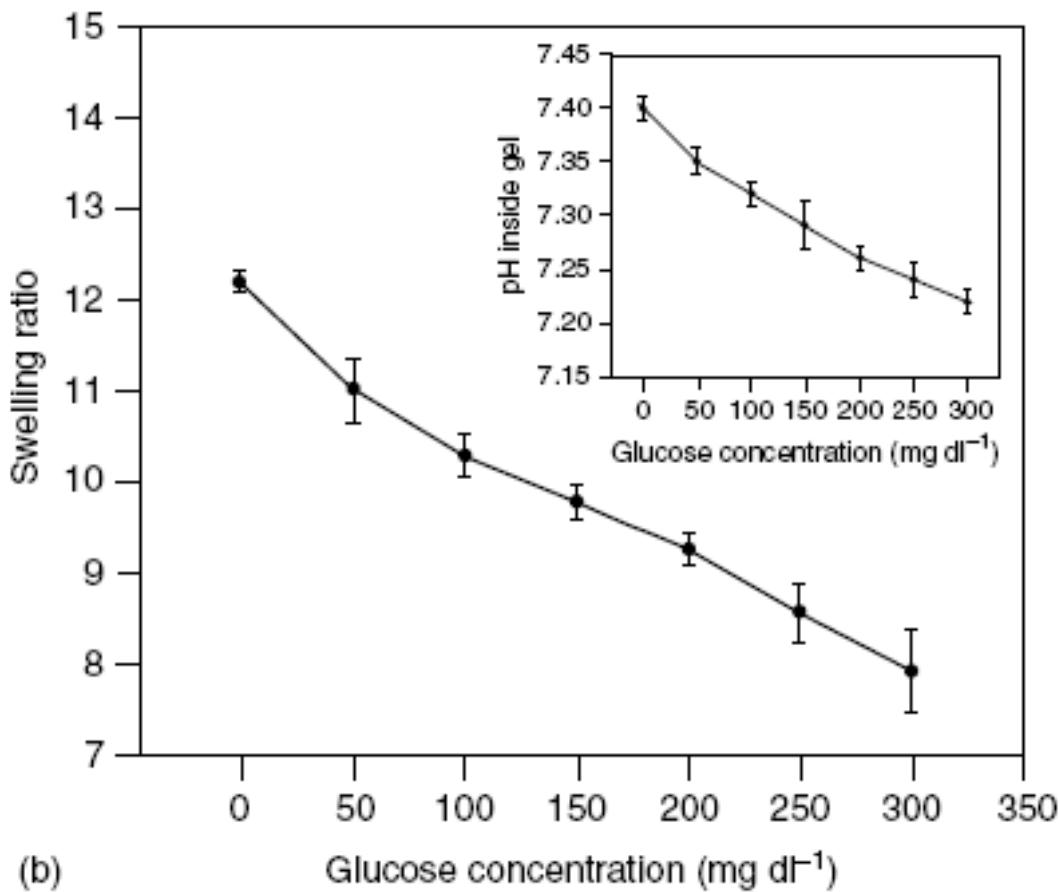
during sensing performance. Covalent-bonding may be the best enzyme immobilization method. However, from the manufacturing perspective, screen-printing with different immobilization methods may be better for commercialization. For example, glutaraldehyde is the most popular bifunctional cross-linker reagent used for the immobilization of proteins. The chemistry of cross-linking by glutaraldehyde is complex. The reaction is dependent on pH, temperature and ionic strength.<sup>[31]</sup> Glutaraldehyde can react with amino groups and other functional groups such as imidazole, thiol, and hydroxyl. Glutaraldehyde has been used to couple proteins to celluloses, to amino alkylsilytated glass, and in the preparation of GOD membranes for enzyme electrodes or biosensors use.<sup>[31]</sup>

The cross-linking method is not only used in the immobilization of enzyme, but also in the glucose biosensor design using smart hydrogel materials. Smart hydrogel materials are cross-linked three-dimensional polymer networks, which are composed of hydrophilic copolymers that are able to hold a large quantity of solvent inducing a volume change of this hydrogel.<sup>[31]</sup> The hydrogel materials can undergo substantial phase transitions in the presence of a specific biochemical agent. In this case, the hydrogel material is able to sense the analyte and translate this sensing event into a mechanical action. This ability makes the hydrogel materials useful in bioanalysis and the design of biosensors. One of the most important characteristics of smart hydrogels is their ability to sense a specific analyte, which is accomplished by the chemical recognition element integrated into the recognition element. The chemical recognition element binds

the target analyte, initiating a subsequent abrupt volume change in the bulk of the polymeric network of the smart hydrogel through the uptake of the solvent.

Several researchers developed a glucose-sensing hydrogel material based on a synthetic phenylboronate derivative.<sup>[13]</sup> The group of Guiseppi-Elie used a pH-sensitive monomer, i.e. dimethylaminoethyl methacrylate to prepare the smart hydrogel.<sup>[10]</sup> In the presence of glucose, the GOD catalyzes the conversion of glucose to gluconic acid and H<sub>2</sub>O<sub>2</sub>, decreasing the pH of the surrounding environment. When the pH decreases, the volume of the hydrogel increases owing to the change in the protonation state of the pH-sensitive monomer and the electrostatic repulsions arising in the hydrogel as shown in Figure 1-3. Upon the addition of glucose, the enzyme catalyzes the conversion of glucose to gluconic acid decreasing the pH in the bulk of the smart material. This results in the neutralization of the charge of the sulfadimethozine, minimizing electrostatic repulsions and shrinking the volume of the smart hydrogel material.





**Figure 1-3** (a) Principle of operation of a smart hydrogel material based on the pH-sensitive polymerizable sulfadimethozine ( $\text{SO}_2\text{-N}-$ ) and the enzyme glucose oxidase (GOx). (b) Dependence of the swelling ratio of the smart hydrogel in the presence of glucose in isotonic PBS solution at  $37^\circ\text{C}$ <sup>[31]</sup>

In the immobilization of proteins to a solid substrate, several factors must be considered in order to retain the optimal activity of the proteins. The proteins must be attached in such an orientation that their active sites or binding domains are accessible to the surrounding analytes and are not buried or blocked by the matrix or other components on the matrix surface. During the coupling reaction, the protein must be in its active state. Another factor that must be considered is

the possibility of chemically altering the protein in such a way that its reactivity is reduced. It is possible that the reactive groups associated with the active sites or binding sites of the proteins may be involved in the immobilization process. In addition, chemical cross-linking can take part intramolecularly causing damage to the protein, thereby reducing the attachment efficiency. Thus, a low cost automatic enzyme immobilization method that maintains the stability and activity of the enzyme is an important research topic.

A combination of microfabrication processing and a simple and effective enzyme immobilization method will make the manufacturing of electrochemical glucose mini-biosensors possible. The third generation electrophoretic deposition of both Pt-Ir/carbon nanoparticles and GOD, which is proposed by our research group, is a novel immobilization method to improve electrochemical glucose mini-biosensor manufacturing. This is the goal of this study.

### 1.3 Transducers

The analytical methods in analytical chemistry have mainly been based on photometric transducers such as spectroscopic and colorimetric methods. However, most chemical sensors or biosensors have been developed using electrochemical transducers because of their simplicity and low cost.

#### 1.3.1 Electrochemical transducers

In general, electrochemical transducers include five types. The first is voltammetric transducer.<sup>[30]</sup> It operates by increasing (or decreasing) the potential to the sensing system until oxidation(or reduction) of the analyte takes place, and there is a sharp rise(or fall) of the peak current. The height of the peak current is

directly proportional to the concentration of the electroactive analyte (or its redox mediator). Equation (1-1) shows the relationship between the current (also concentration of the analyte) and the overpotential (Butler-Volmer equation).<sup>[30]</sup>

$$i = i_0 \left[ e^{-\alpha F\eta / RT} - e^{(1-\alpha)F\eta / RT} \right]$$

(1-1)

$$i_0 = AFk^0 C \quad (1-2)$$

where  $i$  is the current produced in the electrochemical reaction at an applied potential and  $i_0$  is the exchange current.  $\alpha$  is the transfer coefficient,  $F$  is Faraday constant,  $\eta$  is the overpotential,  $R$  is the molar gas constant,  $T$  is absolute temperature,  $A$  is the surface area of the sensor,  $k^0$  is standard rate constant, and  $C$  is the concentration of analyte.

If the appropriate oxidation (or reduction) potential of Eq. (1-1) is known, the potential is fixed at that value and the current as function of time recorded. This mode is called an amperometric transducer, which is the second type of the electrochemical transducer<sup>[30]</sup>. For an amperometric transducer, the sensing current depends on the mass transfer of the electroactive analyte. The relationship between the limiting current and the concentration of analyte is expressed by the following equation:

$$i = \frac{KC}{\ell} \quad (1-3)$$

Where  $i$  is the limiting current,  $K$  is a constant,  $C$  is the concentration of analyte in the bulk solution, and  $\ell$  is the diffusion boundary layer at the electrode.

The third transducer type is the potentiometric transducer, which involves the measurement of the potential of the sensor cell at zero current.<sup>[29]</sup> Based on the Nernst equation, the potential is proportional to the logarithm of the concentration of the analyte as described in the equation (1-4).<sup>[30]</sup>

$$E = E^0 + \frac{RT}{nF} \ln \frac{a_{\text{ox}}}{a_{\text{red}}} \quad (1-4)$$

Where  $E$  and  $E^0$  are the electrode potential and the standard electrode potential respectively;  $a_{\text{ox}}$  and  $a_{\text{red}}$  are the activities of the reactants Ox and Red, respectively;  $R$  is the gas constant;  $T$  is the absolute operating temperature;  $n$  is the number of electrons transferred; and  $F$  is the Faraday constant.

The fourth electrochemical transducer is a conductometric transducer, which is based on the change in the composition of the solution or sensing material due to the chemical reactions taking place during sensing.<sup>[30]</sup> These will generally result in a change in the electrical conductivity that can be measured electrically at constant current or potential.

The fifth transducer type is the FET-based transducer, which is the miniaturization one of the above types of the electrochemical transducers on a silicon-chip-based field-effect transistor.<sup>[11,13]</sup> The FET transducer has mainly been used with potentiometric, voltammetric or conductometric sensors.

There are other types of transducers in addition to electrochemical transducers, such as optical, piezo-electrical or thermal sensors and brief

discussions of each of these transducers is given in the following sections.

### 1.3.2 Optical transducers

Fiber optic sensors are a flexible and miniaturized transducer.<sup>[11,13]</sup> This transducer uses absorption spectroscopy, fluorescence spectroscopy, luminescence spectroscopy, light scattering, and other detection techniques. For example, fluorescence emission that is indicative of selective reactions can be detected and converted into an analytical signal. Fluorescence intensity is defined by an extension of the Beer-Lambert law:<sup>[32]</sup>

$$F = \phi K I_0 (1 - 10^{abC}) \quad (1-5)$$

where  $F$  is fluorescence absorption,  $\phi$  is the quantum yield,  $K$  is the sensor response coefficient,  $I_0$  is the intensity of the excitation radiation,  $a$  is the molar absorptivity of the fluorophore,  $b$  is the path length, and  $C$  is the concentration of fluorescent species.

### 1.3.3 Piezo-electric transducers

These transducers are based on the generation of electric currents from a vibrating crystal. The sensing principle or transduction function is the change in frequency of vibration caused by the mass of material adsorbed, often on a quartz crystal.<sup>[11,13]</sup>

### 1.3.4 Thermal transducers

This type of transducers involves a chemical or biochemical reaction of the analyte that generates heat. The resulting temperature change is measured by a thermocouple or thermistor. The magnitude of the change is proportional to the analyte concentration.<sup>[11,13]</sup>

## **1.4 Applications of biosensors**

Biosensors have been applied in many fields such as health care, environmental monitoring, food analysis, defense and military, agriculture, and the medical and biochemical industries. Electrochemical biosensors are the most important and popular, because they can be applied in many fields such as medical and food applications.<sup>[5,11-13,30,32]</sup> Applications of biosensors in food, environmental, and medical applications<sup>[5,11-13,30,32]</sup> are briefly described below.

Food safety is an important concern of consumers and to food producers and processors. Increasing illnesses from foodborne pathogens require rapid analysis of food products for contaminants.<sup>[11-13,32]</sup> In recent years, global political instability has raised serious concerns about potential food bioterrorism. Government and industrial agencies recognize the need for sensitive and low-cost detection tools. Biosensors are an important solution for the food safety and control of food bioterrorism.

One promising application of biosensors is the monitoring of toxic chemicals, complex microorganisms and bacterial pathogens. The important idea is to fabricate a chemical or biosensor that will operate as an alarm system that is able to provide an early warning signal about the presence and toxicity of an analyte using minimal reagents and sample preparation. Also, this alarm system should be portable, robust and easy to use. A major problem of toxicity monitoring is the large number of samples and analytes that must be tested. Traditional methods need many sample preparation steps, which are expensive

and time-consuming. Biosensors are able to provide rapid information about the toxicity of an analyte.

Affinity-based biosensors are devices incorporating a molecular recognition element such as antibody, receptor protein, nucleic acid, molecular imprinting polymer, or aptamer.<sup>[5,11-13,32]</sup> The use of affinity-based sensors has increased significantly over the past decades. Many types of affinity biosensors have been developed and used to detect the biomolecules such as immunoglobulin E ( $I_gE$ ) for diagnosis of allergies.<sup>[32]</sup> The applications of biosensors for the detection of viruses have been reported.<sup>[32]</sup>

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## **2. Literature Review and Background of Glucose Biosensors**

### **2.1 Diabetes mellitus**

Diabetes mellitus is a chronic but treatable disease affecting millions of people around the world. The total number of individuals with diabetes mellitus worldwide is 171 million in 2000, and is estimated to increase to 366 million in 2030.<sup>[1]</sup> These metabolic diseases, characterized by unstable blood glucose levels, result from defects in insulin secretion or action. A healthy individual is able to regulate the amount of glucose in the blood to less than 6.11 mM (110 mg/dL) with the hormone insulin. On the other hand, people who are suffering from diabetes mellitus are not able to control their blood glucose level, which changes in the range between 1 to 30 mM (18 to 540 mg/dL).<sup>[1,2]</sup> Both a high level of glucose (hyperglycemia) and a low level of glucose (hypoglycemia) are dangerous. It is one of the leading causes of death and disability around the world. Diabetes mellitus, especially due to the duration and severity of hyperglycemia, causes long-term tissue complications, called microangiopathy, which leads to eye, kidney, and nerve damage.<sup>[2]</sup>

There are two types of diabetes; type 1 and type 2. Type 1 diabetes is usually referred to as juvenile diabetes or insulin dependent diabetes mellitus and strikes children and young adults. The insulin producing islet cells in the pancreas of type 1 diabetes patient fail due to damage by the patient's own immune system. The type 1 diabetes patients, in general, lose all insulin-producing capabilities and must inject themselves with insulin before each meal to allow their bodies to consume glucose from the food and generate energy for their bodies. Type 2

diabetes, referred to as non-insulin dependent diabetes mellitus, is very complex and, in general, strikes older people. In type 2 diabetes patient, either the body does not produce enough insulin, or the body fails to properly use insulin.<sup>[3]</sup>

Most people who are diagnosed with diabetes have type 2 diabetes. Type 2 diabetes patients can improve their glucose regulation by losing weight, exercising and using drugs that assist the body to metabolize glucose. Exact and rapid determination of glucose in human blood is essential for the diagnosis and management of diabetes. Thus, glucose biosensors are necessary for monitoring glucose of blood glucose levels.<sup>[1,3]</sup>

## 2.2 Importance of glucose biosensors

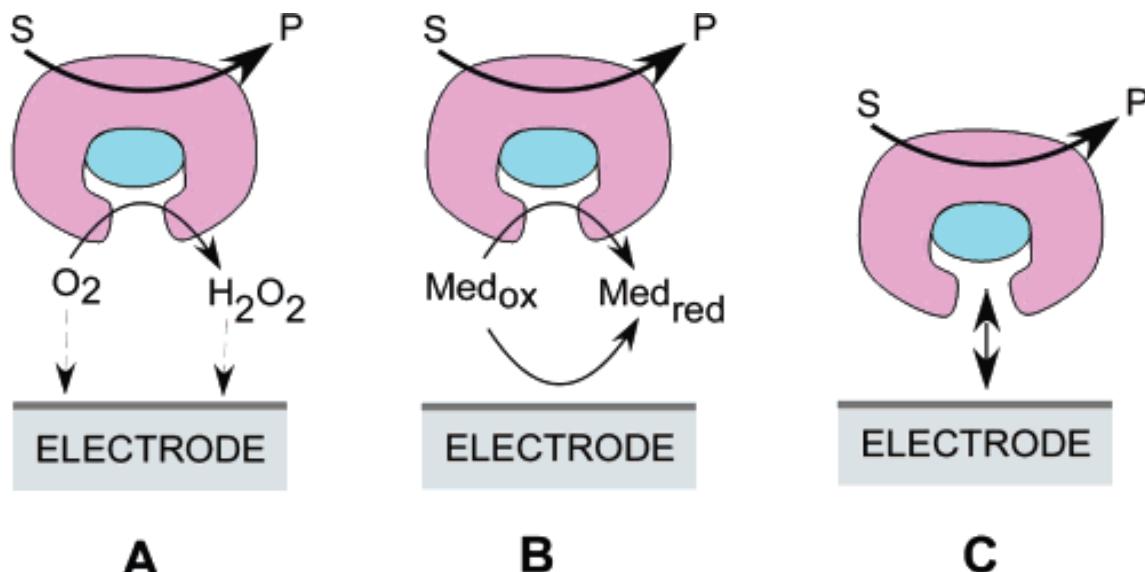
Millions of diabetic patients test their blood glucose levels daily; glucose tests are the most common bio-assay. Glucose biosensors are estimated to be about 85% of the entire biosensor market.<sup>[3]</sup> Such a large market size makes diabetes important for developing new biosensing concepts and technologies.

Novel research and innovative sensing strategies have been proposed, and numerous studies published and patents filed in recent years.<sup>[3]</sup> Based on glucose oxidase, amperometric enzyme electrode biosensors have played a leading role in the development of a simple, easy-to-use blood glucose testing method and it is expected to play a similar role in the future in the advancement of continuous glucose monitoring and the development of the artificial pancreas.

## 2.3 Technology improvement of electrochemical glucose biosensors

Based on the technology improvement of electrochemical glucose biosensors, three generations of glucose biosensors have been reported.<sup>[3]</sup> Figure

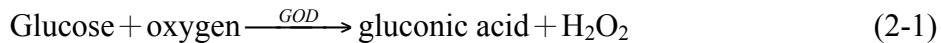
2-1 shows the schemes of these glucose biosensors. The first glucose biosensor was an electrochemical sensor that measured the concentration of dissolved oxygen; it was the beginning of the first generation of glucose biosensors.<sup>[4]</sup> Guilbault and Lubrano in 1973 using an enzyme electrode for detecting blood glucose based on the amperometric monitoring of the liberated hydrogen peroxide from the reaction of glucose with dissolved oxygen catalyzed by the enzyme, glucose oxidase, GOD.<sup>[5]</sup> The second generation glucose biosensors are mediator-based glucose biosensors reported in 1984.<sup>[6]</sup> The third generation of glucose biosensors are mediator-free and low applied potential glucose biosensors, which are related directly to own study.<sup>[7]</sup>



**Figure 2-1** Three generations of amperometric enzyme electrodes for glucose based on the use of natural oxygen cofactor (A), artificial redox mediators (B), direct electron transfer between GOD and the electrode (C)<sup>[3]</sup>; S: substrate; P: product

### 2.3.1 First generation glucose biosensors

The first glucose enzyme electrode had on a thin layer of GOD entrapped over an oxygen electrode by a semipermeable dialysis membrane.<sup>[3]</sup> Oxygen was measured based on the enzyme-catalyzed reaction as shown in equation (2-1):



A negative potential was applied to the Pt working electrode for a reductive detection of the oxygen consumption as shown in the following reaction



However, the background oxygen in the test sample resulted in poor accuracy for this type of glucose sensor. The effect of background oxygen and interference from other species could be corrected by using two oxygen sensing electrodes, one of which was covered with the GOD and the another one without. The difference of the current outputs of these two oxygen sensors was then used to quantify the glucose concentration.<sup>[4]</sup> This approach was complicated and difficult to use.

An enzyme electrode for the measurement of blood glucose based on amperometric monitoring of the anodic oxidation of the hydrogen peroxide product was also reported, as shown in equation (2-3).<sup>[5]</sup>



This type of measurement gave good accuracy and precision with a 100 μL blood sample. Anodic detection of enzymatic produced hydrogen peroxide became the main technique for blood glucose biosensors. In this generation of glucose sensor development, the biocatalytic reduction of the flavin group (FAD)

in the enzyme by reaction with glucose produced the reduced form of the FADH<sub>2</sub>.<sup>[3]</sup> Direct measurement of hydrogen peroxide for glucose detection had many advantages, especially for further device miniaturization. In general, the measurement of glucose was performed on a Pt electrode at a moderate anodic potential, about +0.6V vs. Ag/AgCl, at which ascorbic and uric acids would, unfortunately, also be oxidized. There were methods to minimize the interference from these coexisting electroactive compounds. The first method to decrease electroactive interferences was use of a permselective coating that would minimize the access of these compounds to the electrode surface.

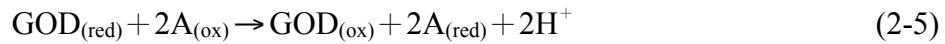
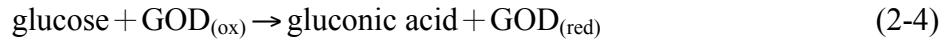
Electropolymerized films were shown to be useful to improve the selectivity.<sup>[8-10]</sup> The second method was to use electrocatalytic detection of the generated H<sub>2</sub>O<sub>2</sub> by lowering the anodic detection potential.<sup>[11,12]</sup> The third method was to use metallized carbons, such as rhodium or ruthenium on carbon, to decrease the anodic potential and increase the selectivity of glucose biosensors.<sup>[13,14]</sup> The catalytic oxidation of the H<sub>2</sub>O<sub>2</sub> product depended on the presence of a metal oxide film. The hydrogen peroxide would reduce the metal oxide film to the metal, which was then reoxidized electrochemically, generating the anodic current signal. The metal oxide catalysts actually played the roles of both catalyst and redox coupling. Electrochemical co-deposition of ruthenium and glucose oxidase on carbon fiber microelectrodes for the development of miniaturized or disposable glucose microsensors has been reported.<sup>[14]</sup> Similarly, dispersing of metal microparticles or metallized carbon particles within a screen-printable ink for the preparation of the microsensors by thick film screen printing was also

reported.<sup>[13,15]</sup> Furthermore, it was shown that combining the dispersed rhodium particles within a Nafion film improved both the sensitivity and the selectivity of the glucose biosensors.<sup>[16]</sup> The reduction in the applied overvoltage for H<sub>2</sub>O<sub>2</sub> at carbon-nanotube-modified electrodes resulted in high selectivity and low applied potential.<sup>[17,18]</sup> The coupling of carbon nanotubes to Pt nanoparticles has been shown to be useful for enhancing the sensitivity and decreasing response time of the GOD based biosensors.<sup>[19]</sup> Nanotechnology has led to applications of nanomaterials in bioanalytical chemistry. Carbon nanotubes can be coupled to enzymes to provide a favorable surface orientation and served as an electrical connector between their redox center and the electrode surface. Owing to the similar dimensions of the nanoparticles and the redox proteins such as GOD, such nanomaterials can be used for effective electrical wiring of redox enzymes.<sup>[20-22]</sup> The nanocarbon particles served as molecular wires between the electrode and a redox enzyme. At present, the activation of the bioelectrocatalytic functions of GOD by nanoparticles or nanocarbontubes requires electrical overpotentials. Improving the contact between the nanoparticles and the electrode might decrease this overpotential. In our study, the use of electrophoretic deposition which generates compact Pt-Ir/nanocarbon and GOD layers may solve part of these problems.<sup>[23]</sup>

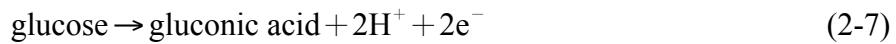
### 2.3.2 Second generation glucose biosensors

Glucose oxidase does not directly transfer electrons to conventional electrodes because of a thick protein layer surrounding its flavin adenine dinucleotide (FAD) redox center generating a barrier to direct electron transfer.

In order to overcome this problem, artificial mediators that shuttle or carry electrons between the FAD center and the electrode surface are used<sup>[3]</sup>



The overall reaction is then



where A(ox) and A(red) are the oxidized and reduced forms of the mediator, respectively. As shown in equation (2-6), the reduced form is reoxidized at the electrode while generating the oxidized form of the mediator. Equation (2-7) shows that current produced is proportional to the glucose concentration. In most cases, oxidation of the reduced GOD by oxygen can occur even in the presence of mediator. This will lead to an effect by the oxygen present. However, the oxygen competition can be minimized if the rate of electron transfer by the mediator is higher than the rate of the enzyme reaction with oxygen. On the other hand, even at a relatively low applied potential, the oxidation of other compounds such as ascorbic acid and uric acid in blood cannot be totally eliminated. In-vivo devices are usually mediator-free due to leaching and the toxicity of the mediator.

### 2.3.3 Third generation glucose biosensors

This generation of the glucose biosensor tries to eliminate the mediator and develop a mediator-free glucose biosensor which operates at a low potential. The electrons are transferred directly from glucose to the electrode by the active

sites of the enzyme. The absence of mediator and the low overpotential are the main advantages of this approach. The challenge is to overcome any difficulty of this direct electron transfer route due to the spatial separation of the electron donor-acceptor pair. There are reports of such mediator-free electron transfer between GOD and the electrode.<sup>[3]</sup> However, proof for such mediator-free sensing is not yet in evidence. The optimally designed electrode configuration has to ensure that the electron-transfer distance between the immobilized enzyme and the sensing electrode surface is as short as possible.

## 2.4 Home testing of blood glucose

The blood glucose home testing devices are used daily to diagnose millions of patients who suffer potentially life-threatening events due to diabetes. The blood glucose sensor must be reliable, simple to use, and be cost effective. The personal blood glucose sensors, in general, are single use disposable enzyme test strips.<sup>[24,25]</sup> The simple-use strips can be mass produced by rapid, simple and low cost thick film (screen printing) microfabrication.<sup>[15]</sup> In the long run, a desirable glucose sensor will provide a reliable real-time continuous monitoring of blood glucose with high selectivity and sensitivity over an extended period of time for potential use in an artificial pancreas system.<sup>[3]</sup>

## 2.5 Microfabrication of minibiosensors

### 2.5.1 Thick film printing

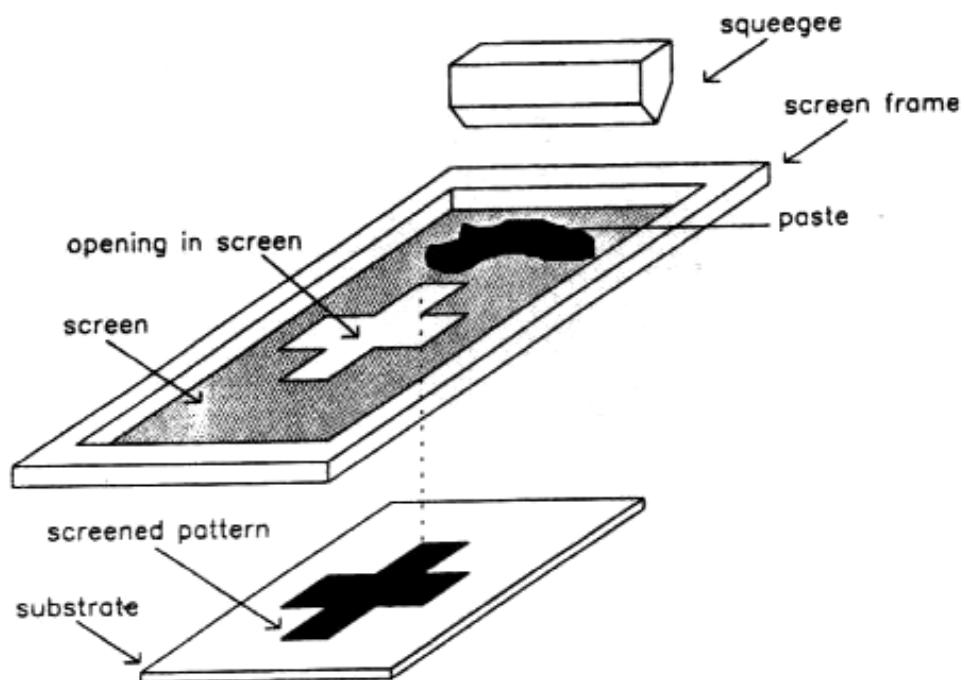
Thick film printing includes screen printing techniques. Screen printing technology involves the printing of a designed pattern of conductors and insulators onto the surface of planar solid (usually, plastic or ceramic) substrates.

The corresponding printing inks are forced through a patterned screen mask. In the development of an electrochemically based sensor, each sensor contains working, counter and reference electrodes. The working electrode is usually coated with the necessary reagents such as enzyme (mediator), catalyst, stabilizer, surfactant, cross-linking, binding agents and membrane. A more detailed example description is given as follows: The thick-film process uses a squeegee to spread the ink onto a substrate or screen that is masked with a designed pattern produced by a photolithographic technique. Figure 2-2 shows the scheme and the components of the thick film printing process.<sup>[26]</sup> Different inks can be screen-printed on the substrate in sequence, based on their properties and the needs. Typically, a thick film ink contains the active material, a binder and a solvent. After the squeegeeing process, the substrates are placed in an oven at 70~90°C in order to remove the solvent. The printed substrates are then place in a high temperature furnace or oven to solidify the printed film. The heating temperature and the heating profile are often provided by the manufacturer.

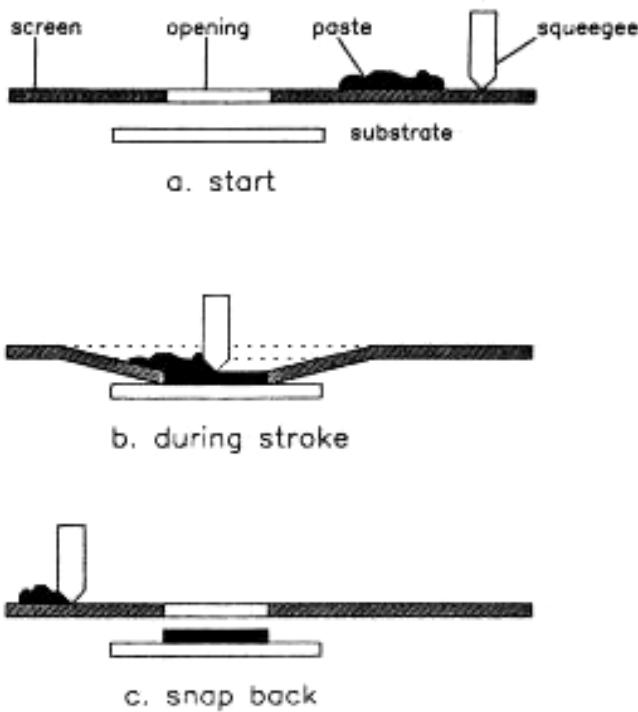
A typical thick film printing sequence can be described as follows:

1. The screen and the substrate are placed and aligned appropriately into the printing machine.
2. The ink is placed onto the screen (Fig. 2-3-a)
3. The squeegee is then controlled mechanically moving and pushing the ink through the screen making a direct contact with the substrate. (Fig. 2-3-b)
4. The screen then snapping back into its original position, after the printing step above. (Fig. 2-3-c)

5. The printed substrate is then placed in a low temperature oven to remove the solvent from the printed film.
6. The printing of each metallic and insulation layer is carried out in a sequence based on the curing temperatures of the films. The film that requires the highest solidified temperature will be printed first.



**Figure 2-2** Schematic of thick-film process <sup>[26]</sup>



**Figure 2-3** Steps of thick film printing process<sup>[26]</sup>

### 2.5.2 Thin film fabrication

Thin film fabrication is a microfabrication process that can be used for the production of highly uniform and reproducible micro-structures. Thin film processing has been used in the construction of biosensor electrodes. Two thin film processing steps, the lift-off technique and the chemical etching technique, are often used in the fabrication of biosensor prototypes.<sup>[26-28]</sup> In our study, thin film fabrication processing was not used, for this processing is not cost effective for the development of single use, disposable biosensor. However, it is appropriate to briefly describe the lift-off and the chemical etching techniques here in order to provide a complete description of biosensor development.

The general procedure of the lift-off technique can be described as follows.

A photo-resist layer is first deposited onto the substrate, usually by a spin-coating method. The photo-mask with the designed pattern is then aligned on the photo-resist layer and an ultra-violet light source is applied over the photo-mask, polymerizing the photo-resist materials. Depending on the types of the photo-resist material chosen, positive or negative, and the polarity of the photo-mask design, light field or dark field, an appropriate solvent will then be used to remove the non-polymerized photoresist, providing the patterned structure. A thin metal layer of the material of interest will then be deposited over the photoresist layer on the substrate by either chemical or physical vapor deposition. The whole substrate is then immersed into an appropriate solvent to lift off the remaining photo-resist, leaving the metallic structure of the desired design.

In the chemical etching process, the sequence of the deposition of the metal film and the photo-resist layer is reversed compared to those in the lift-off process. In the chemical etching process, the metal layer is first deposited following by deposition of the photo-resist layer. The chemical etching of the photo-resist and the metal layer are carried out in a similar manner to that described above. This procedure yields the metallic structure based on the designed pattern.

As mentioned, thin film metallization is not used in this study. However, it is necessary to recognize that it can be used in micro-size biosensor development.

### **2.5.3 Ink-jet printing**

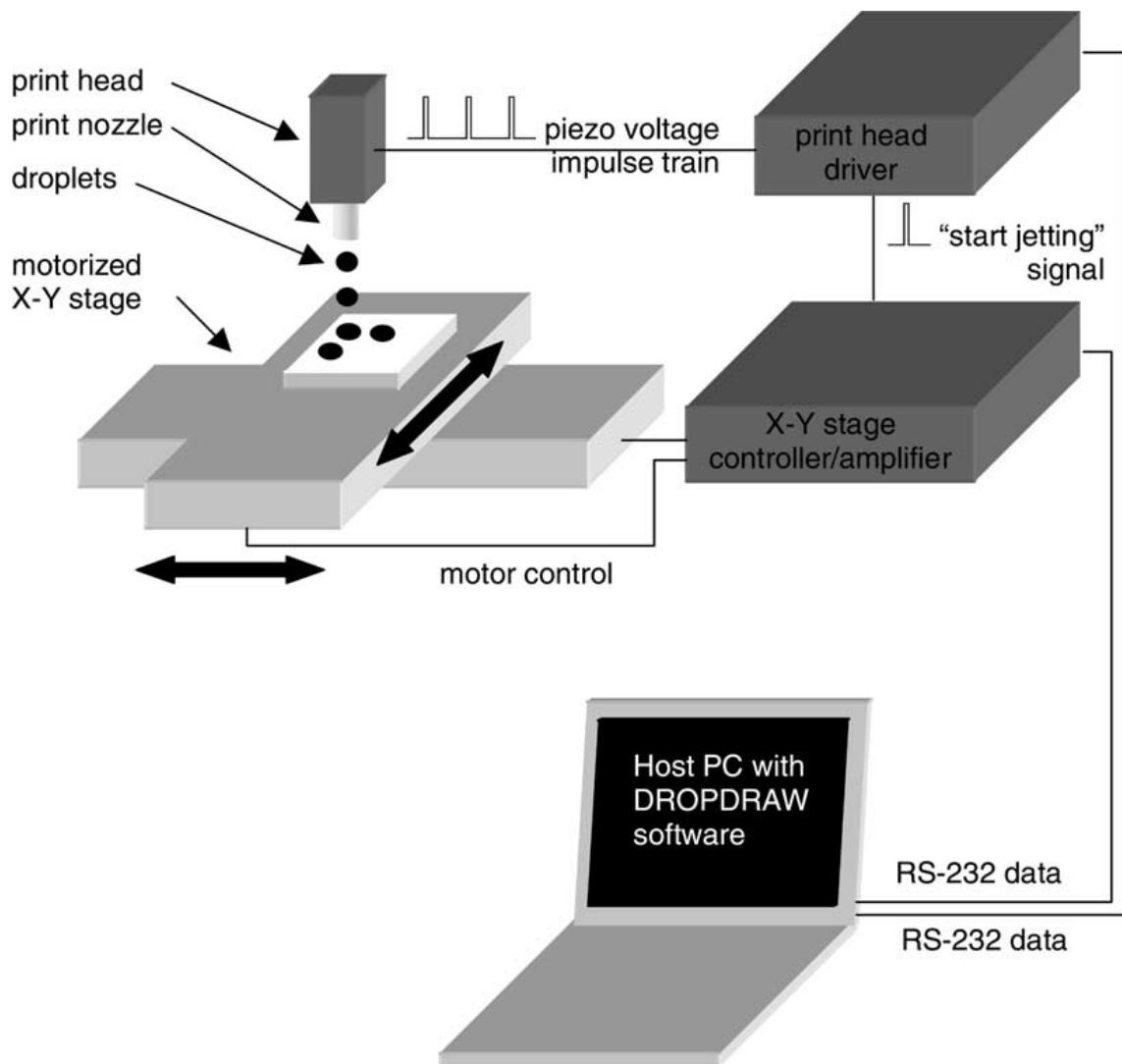
Ink-jet printing is another microfabrication technique that can be used to

produce a pattern on a substrate for manufacturing of biosensors.<sup>[26, 29, 30]</sup> The ink-jet printing is also commonly used in tissue engineering.<sup>[29]</sup> The schematic of the elements of the custom-built ink-jet printer is shown in Figure 2-4.<sup>[29]</sup>

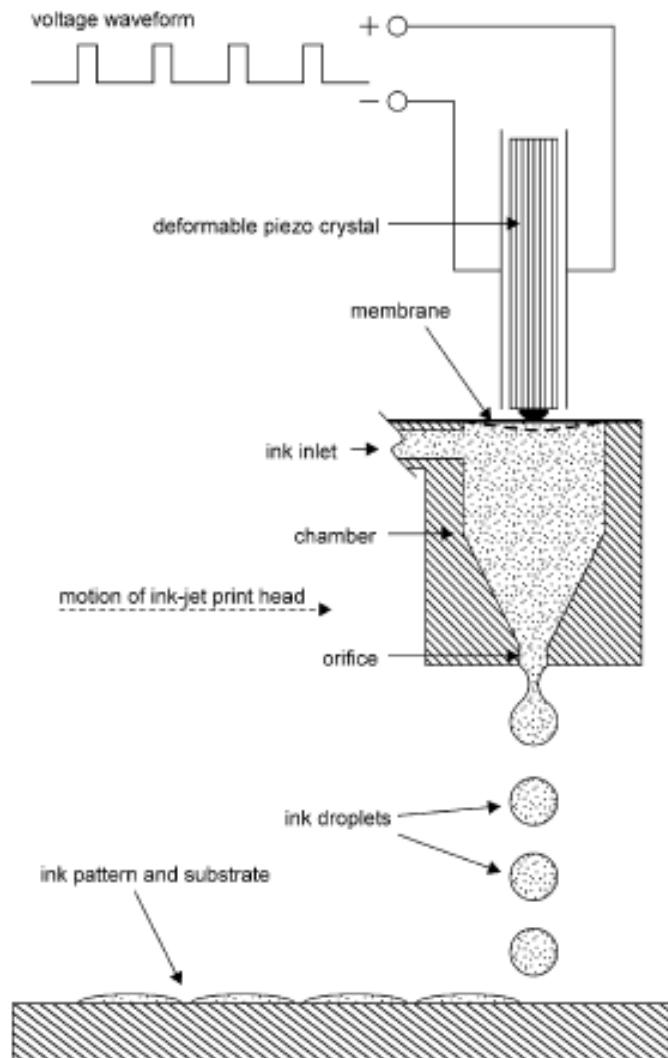
In general, the ink-jet nozzle is connected to an ink reservoir above the computer controlled X-Y stage where the substrate for the printing is placed directly underneath.

As shown in Figure 2-4, during operation, when a desired position is reached, the stage controller sends a signal to the print head driver to generate a particular waveform specified by serial commands from the PC. This signal causes one or more droplets to be ejected from the nozzle orifice at the desired position.<sup>[33]</sup> In most biosensor applications, the ink contains the metal nanoparticles of interest. Also, after printing, it is necessary to evaporate the solvent in the ink first in a relatively low temperature oven, i.e. 80-100°C.

The size of the pattern can be printed using different sizes of nozzles to form the printed pattern. A commonly used piezo electric ink-jet printer consists of a thin membrane with an attached piezoelectric crystal on the top cover of the ink reservoir as shown in Figure 2-5.<sup>[33]</sup>



**Figure 2-4** Schematic of the elements of the custom-built ink-jet printer. A PC with a custom-written software environment sends data through a serial port to command an X-Y stage servomotor controller to move according to a drawing stored in the software.<sup>[29]</sup>



**Figure 2-5** Diagram of the operation of a piezo-electric drop-on-demand ink-jet print head. Microscopic ink droplets are ejected individually through an orifice by means of a pressure impulse delivered by a piezo-electric crystal. Each droplet,  $10\text{--}100\mu\text{m}$  in diameter, takes a ballistic trajectory a small distance (1 mm) to the glass substrate. The print head is moved robotically in two-dimensions above the substrate as droplets are ejected, leaving a pattern of round dots on the substrate.<sup>[29]</sup>

The drop size from the nozzle can be as small as a few picoliters.

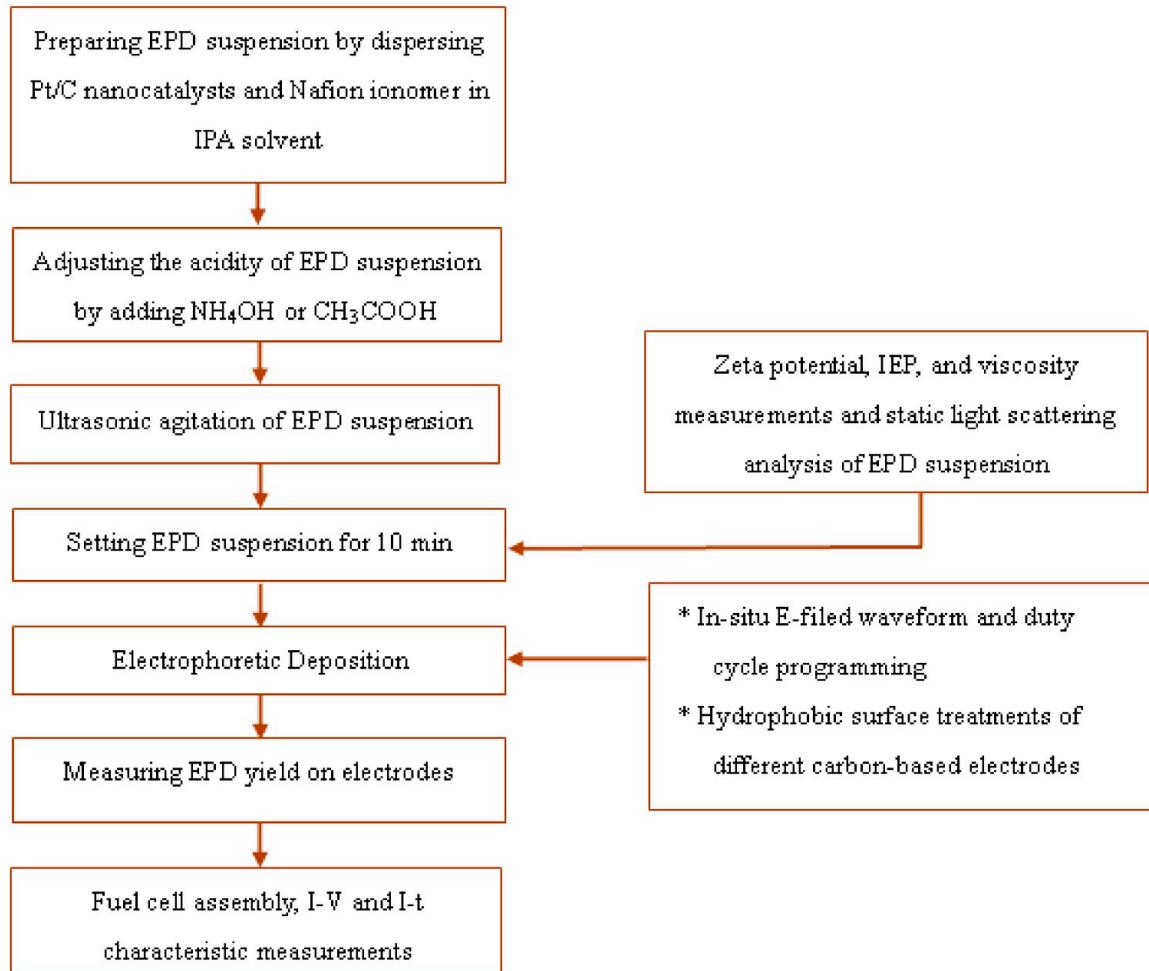
Another common type of ink-jet printing uses a thermal printing approach

employing a bubble jet in which a superheated vapor explosion vaporizes a controlled quantity of ink forming an expanding bubble that ejects a drop of ink onto the substrate from the ink cartridge.<sup>[26]</sup> This type of ink-jet printing can produce small and thin metal layers on the substrate with high reproducibility, but the cost is higher than the standard thick film screen printing. In our study, ink-jet printing was not used for production of biosensor prototypes. However, we anticipate in future manufacturing of single use, disposable enzymatic based biosensors, and deposition of the appropriate enzyme solution will be accomplished using ink-jet printing.

## **2.6 Electrophoretic deposition of coupled catalysts to modify glucose minibiosensors**

### **2.6.1 Electrophoretic deposition of catalyst layers from non-aqueous suspensions**

Recently, electrophoretic deposition (EPD) has been studied as a novel method to produce electrodes for membrane type fuel cells.<sup>[31-37]</sup> The electrophoretic deposition (immobilization) is usually carried out from a non-aqueous suspension solution at high-voltage conditions.<sup>[38]</sup> Figure 2-6 shows a process flow chart for preparation of an electrode catalyst Pt/C layer from a non-aqueous suspension by the electrophoretic technique. The procedures are relatively complicated.

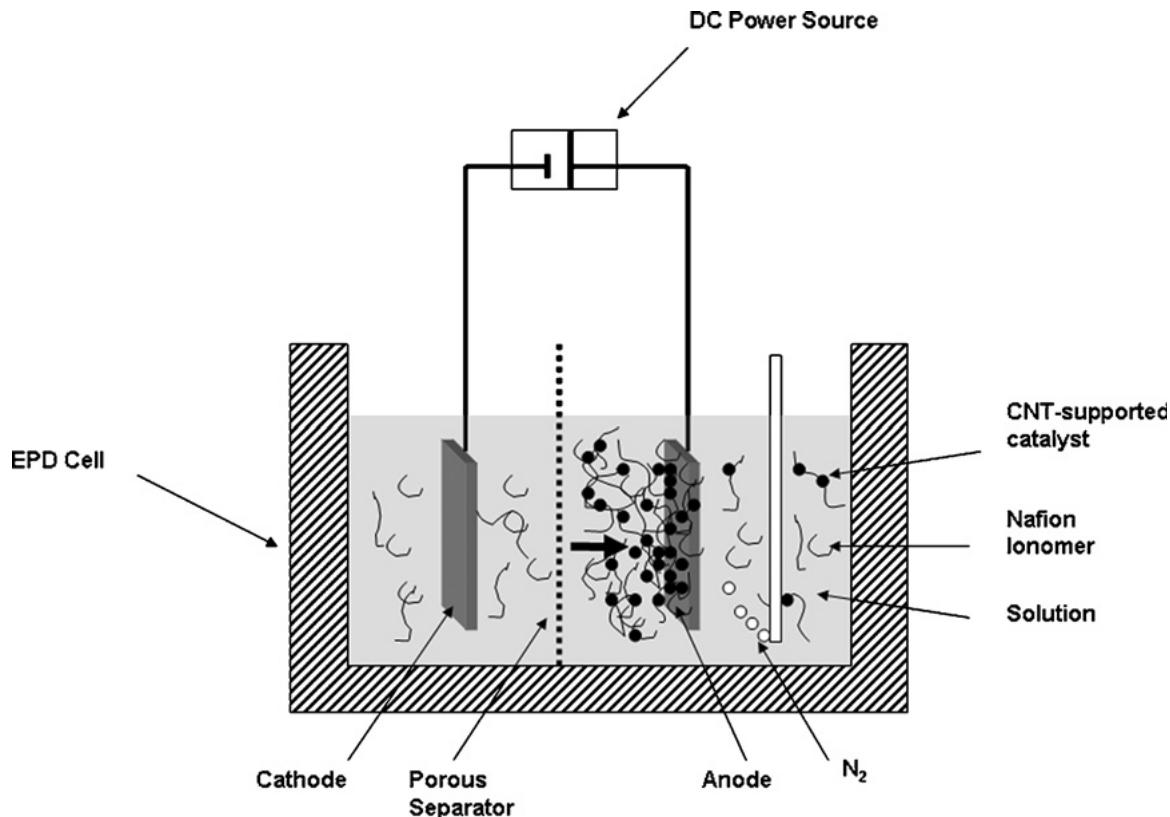


**Figure 2-6** Process flow of electrophoretic deposition of Pt/C nanocatalysts and Nafion® solution on carbon-based electrodes <sup>[38]</sup> IPA: isopropyl alcohol

## 2.6.2 Electrophoretic deposition of catalyst layer in aqueous suspension

Electrophoretic deposition can produce an even and compact layer of catalyst and provide the desired catalyst composition and morphology at the electrode. Furthermore, electrophoretic deposition of suspension catalyst particles can be accomplished from aqueous suspensions solutions.<sup>[39,40]</sup> This can be achieved by applying a low voltage, which gives a safer and more environmentally friendly process. The fabrication of a direct methanol fuel cell

electrode catalyst layer, incorporated with carbon nanotube-supported Pt-Ru catalyst in an undivided cell has been reported.<sup>[23]</sup> The Pt-Ru/CNT catalyst layer is mainly a composite structure with Nafion® ionomers and a catalyst binding. The experimental arrangement is shown in Figure 2-7.<sup>[23]</sup>

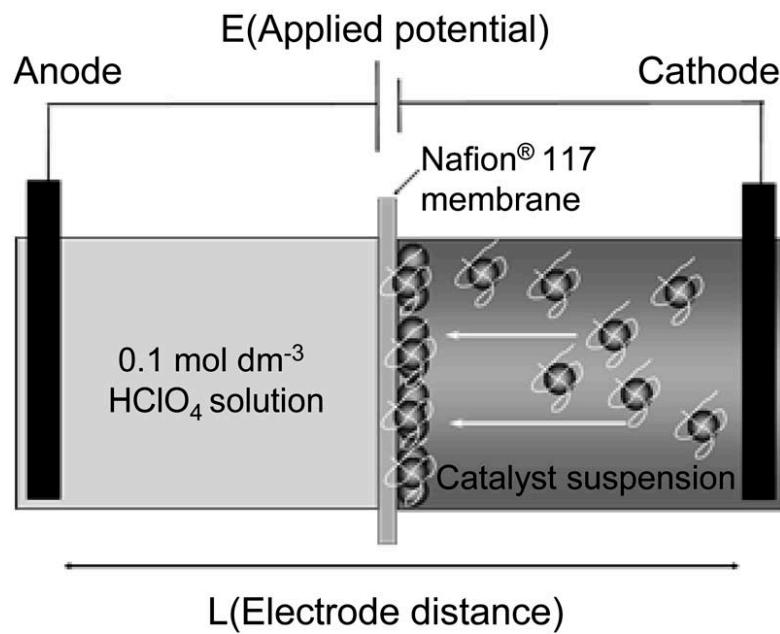


**Figure 2-7** Schematic illustration of the experimental setup for low-voltage EPD operation<sup>[23]</sup>

### 2.6.3 Electrophoretic deposition of charged particles on a membrane

A process to produce a coating on a substrate by using electrophoretic deposition of suspended charged particles from a dilute suspension has been reported.<sup>[37]</sup> The particles in the suspension are deposited and migrated onto a target substrate using to a D.C. electric field as shown in Figure 2-8. The catalyst layer on a Nafion 117 membrane is successfully prepared by electrophoretic

deposition as shown and the catalyst layer is attached well to the membrane without mechanical pressing and heating. If a microelectrode is used and an electric field is locally generated in a suspension, micropatterns of deposits can be fabricated.<sup>[41]</sup>



$$\text{Electric field intensity} = \frac{E}{L}$$

**Figure 2-8** Schematic illustration of the EPD cell<sup>[37]</sup>

Synthesis of Pt-Sn nanoparticles and in situ deposition on a graphite felt support using electrophoretic technique have been reported.<sup>[35]</sup> The electrophoretic current and the field, which lead to the migration and adsorption of the stabilizer reductant on the graphite felt surface, were described. The reductant reduces the  $\text{PtCl}_2\text{-SnCl}_2$  metal salt mixture forming Pt-Sn alloy nanoparticles.<sup>[35]</sup>

Based on the above literature review and discussion, many advantages can

be obtained by using electrophoretic deposition to modify glucose mini electrodes.

The methods given above concern the preparation of fuel cell electrodes.

There are no reports for the preparation or immobilization of catalyst layers on the electrodes of a biosensor. It is of interest to immobilize the enzyme and the metal (or the metal oxide) catalyst nanoparticles on carbon nanoparticles of the biosensor electrodes by EPD. Furthermore, the compact layer stabilizes the conformation of enzyme molecules resulting in a modified biocomposite electrode that exhibits excellent sensitivity and stability.<sup>[61]</sup>

Therefore, modification of glucose minibiosensor was carried out by simultaneous electrophoretic deposition of the Pt-Ir alloy bimetallic catalyst, carbon particle catalyst, and enzyme.

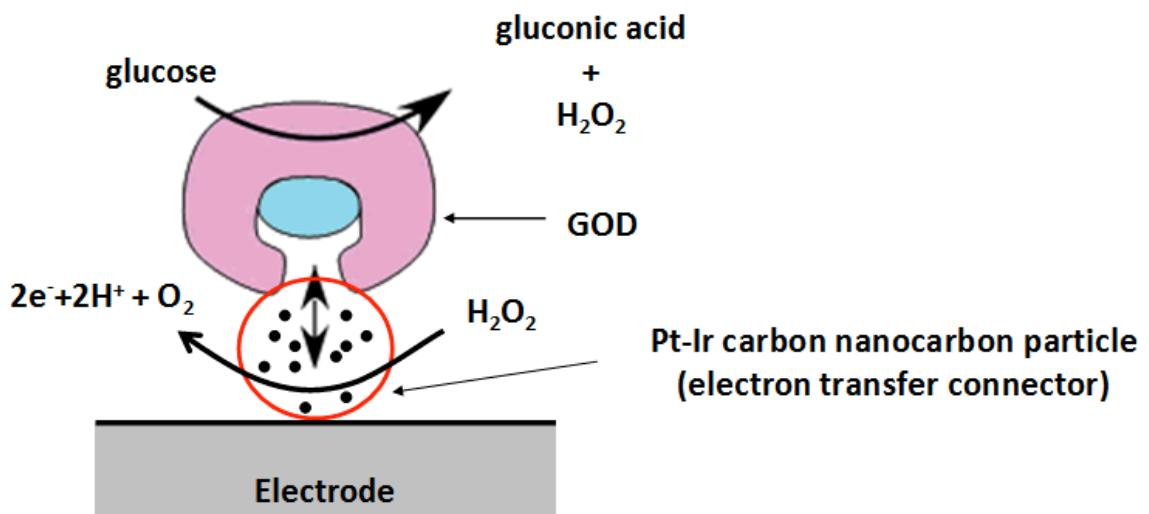
#### **2.6.4 Methods for electrophoretic deposition of Pt-Ir and GOD coupled catalysts**

Based on the above literature review of electrophoretic deposition, electrophoretic deposition methods can create a compact layer of electrophoretic reagents or suspended nanoparticles. It is desirable that the enzyme, GOD, be immobilized in a compact layer directly on the electrode surface, for this will enhance the stability of the enzyme. In order to minimize potential interference from other biological components, such as ascorbic acid, a relatively low oxidation potential for the measurement of the H<sub>2</sub>O<sub>2</sub> produced from the enzymatic reaction of glucose is also desirable. Nano catalyst particles, such as Pt-Ir will be useful to lower the activation energy and the oxidation potential. Thus, simultaneous electrophoretic deposition of GOD and the Pt-Ir nano catalyst is carried out in this study.

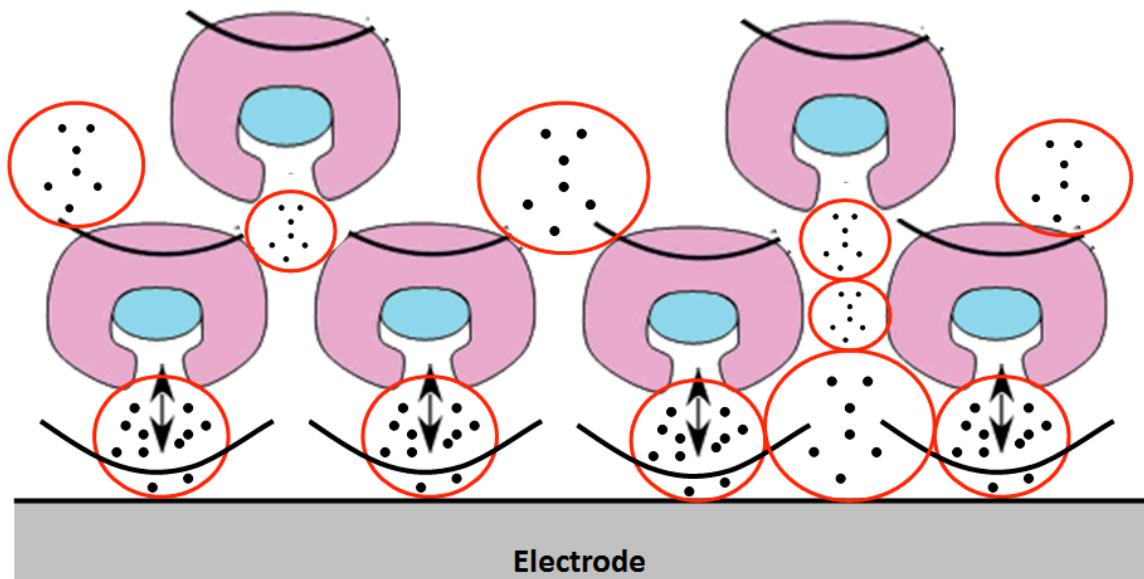
## 2.7 Third generation glucose biosensors

The distances between the GOD, the Pt-Ir, and the carbon particles should be relatively short and the catalyst and the carbon particles (the electron carriers) closely assembled. Figure 2-9 shows the scheme of this third generation glucose minibiosensor with the GOD and the Pt-Ir nano catalyst particles co-deposited on the carbon particles of the working electrode surface. The compact modified layer containing the Pr-Ir/carbon nanoparticles, GOD and Nafion binder on the screen printed carbon working surface is shown in Figure 2-10. The Pr-Ir/carbon nanoparticle is the direct connector between GOD and the carbon electrode for electrons are that generated by equation (3). The H<sub>2</sub>O<sub>2</sub> produced from the enzymatic reaction of glucose is then oxidized electrochemically at a relatively low potential in the presence of the Pt-Ir nano catalyst particles. This unique approach results in a mediator-free glucose mini biosensor. The operation of the biosensor at a relative low potential is accomplished using the Pt-Ir bi-metallic nano catalyst particles. The EPD deposition of the enzyme allows the enzyme catalyst to be immobilized closely to the electrode surface. In order to provide a compact layer of the catalyst involved, GOD and Pt-Ir catalyst, Nafion is used as a binder strengthening the integrity of the EPD deposited layer.

Based on the above literature review and discussion, many advantages can be seen by using electrophoretic technique to modify the glucose mini electrode. The proof of this concept and the experimental evaluation of the prepared sensor prototypes are carried out.



**Figure 2-9** Scheme of the third generation electrochemical glucose biosensor combining Pt-Ir/carbon nanoparticle, GOD, and mini electrode



**Figure 2-10** Scheme of the EPD compact modified layer containing Pt-Ir/carbon nanoparticle, GOD, and the binding Nafion

## 2.8 Bibliography

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### **3. Experimental program**

#### **3.1 Scope**

The scope of this research is to apply thick-film microfabrication and electrophoretic deposition (EPD) technologies to produce single use, disposable and cost-effective glucose biosensors. The techniques developed in this research contribute well to the advancement of biosensors.

#### **3.2 Overview of the experimental program**

This chapter describes the microfabrication of the minibiosensor, materials used, and the experimental arrangement. The preparation of the platinum-Iridium Pt-Ir/ carbon nanoparticles catalyst, and the cell assembly for the electrophoretic deposition and the H<sub>2</sub>O<sub>2</sub> test procedure are also given. The electrophoretic deposition of Pt-Ir and enzyme, GOD, coupled catalysts on the minibiosensor and the evaluation procedure of Pt-Ir modified minibiosensor for H<sub>2</sub>O<sub>2</sub> and glucose detection are also described. Details are described in the following sections.

### **3.3 Microfabrication of the basic structure of the minibiosensors**

#### **3.3.1 Thick film screen printing**

The minibiosensor was fabricated using thick film screen techniques. Typical thick film printing steps were described in section 2.5.1.<sup>[1]</sup>

The minibiosensor prototype consisted of a carbon modified working electrode, a Ag / AgCl reference electrode, and a carbon modified counter electrode. The surface area of the working electrode was approximately 7.85×10<sup>-3</sup> cm<sup>2</sup>. These electrodes were printed on a polyester substrate.

The procedures for the fabrication of this three-electrode configuration

enzyme minibiosensor were described in related publications.<sup>[2,3]</sup> Firstly, the thick film ink-based solution was prepared. The ink-based solution was prepared by mixing phosphate buffer, enzyme immobilizing agent and thickening polymer. Typically, 10 ml pH 7.0 phosphate buffer was used to with 1.36 ml polyethylenimine, and 0.34 g 2-hydroxyethyl cellulose to form the ink-based solution for the printing of the working and the counter electrodes. Mixing was completed when a clear homogeneous solution was obtained.

Secondly, the carbon ink was prepared. The ink-based solution described above was mixed with the carbon particles, amorphous 5~7nm in diameter, forming the carbon based ink. Typically 0.9 g of carbon was added into 5 ml of the ink-based solution described in the previous step. Mixing and homogenization (for approximately 5 min) were than carried out resulting in a carbon based ink for the printing of the working and the counter electrodes.

Thirdly, the working and the counter electrodes were fabricated. This three-electrode configuration sensor was prepared by screen-printing on a polyester substrate. The electrical contacts were silver that were printed first using a commercial silver ink. Both the working and the counter electrodes were screen-printed using the carbon ink over the silver-based current connector.

Finally, the preparation of the Ag/AgCl reference electrode was carried out. A commercial AgCl thick film ink was used and printed over the silver-based current collector, to form the Ag/AgCl reference electrode.

### **3.4 Materials**

#### **3.4.1 Materials for thick film printing**

The mini biosensor prototypes were fabricated on a polyester substrate (Melinex 329, Du Pont Co.). Active carbon particles, amorphous 5~7nm in diameter, were purchased from E-TEK<sup>SM</sup>. Polyethylenimine and 2-hydroxyethyl cellulose were purchased from Sigma–Aldrich. All other chemical used were of analytical grade and were used as received. All solutions were prepared using de-ionized water.

#### **3.4.2 Materials for Pt-Ir/carbon nanoparticles and test procedure**

The materials for the preparation of Pt-Ir/carbon nanoparticles included: H<sub>2</sub>PtCl<sub>6</sub>.6H<sub>2</sub>O (Acros 363590010), Na<sub>2</sub>CO<sub>3</sub> (Acros 123670010), NaHCO<sub>3</sub> (Acros 217120010), IrCl<sub>2</sub>.3H<sub>2</sub>O(Acro 195500010), NaHSO<sub>3</sub> (Acros 223075000) Carbon XC-72R (Cabot), NaOH (Acros 383052500), NH<sub>4</sub>OH (Acros 423305000), H<sub>2</sub>O<sub>2</sub> (Acros 202460250), NaBH<sub>4</sub> (Acros 189301000), H<sub>3</sub>BO<sub>3</sub> (Acros 217085000). All other chemicals were of analytical grade and used as-received. All solutions used were prepared using de-ionized water.

#### **3.4.3 Materials used for electrophoretic deposition**

The materials for the electrophoretic deposition included the prepared Pt-Ir/carbon nanoparticles, Nafion (Aldrich 292567), Glucose Oxidase (Sigma 7141), CH<sub>3</sub>COOH (Acros 124040010). All solutions were prepared using de-ionized water.

### **3.4.4 Materials used for the glucose sensing tests**

The materials used for the glucose sensing tests included D-glucose (Sigma G, 136,100U/g), bovine serum (Invitrogen 16170), phosphate buffer saline (PBS) solution(Sigma P5493), ascorbic acid (Fluka 59209), Uric acid (Sigma U-0881). All other chemicals were analytical grade and used as-received.

All solutions used were prepared using de-ionized water.

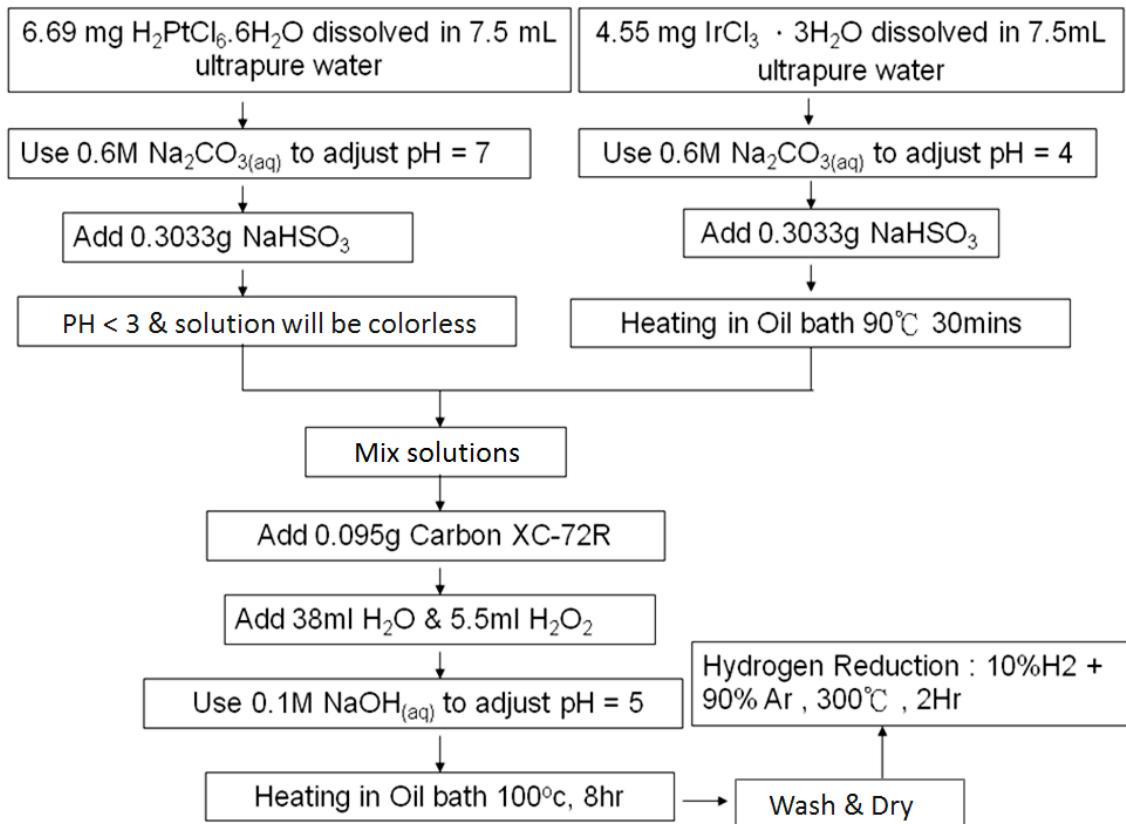
### **3.5 Instrumentation**

The instruments used for the thick film screen printing were described in section 2.5.1 and 2.5.3.

The electrophoretic deposition of the combined GOD and nano metallic particle layer, and the glucose testing were carried out using a CHI 660A Electrochemical workstation (CH instruments, Inc., Austin, TX)

### **3.6 Preparation of the Platinum – Iridium/carbon nanoparticles**

The preparation of the Pt-Ir/carbon nanoparticles were based on the procedures reported in the literature.<sup>[4]</sup> The experimental procedure for preparing the Pt-Ir/carbon nanoparticles is shown in Figure 3-1.



**Figure 3-1** Experimental procedure for preparing Pt-Ir/carbon nanoparticles

In a typical experiment, the XC-72R (Cabot) active carbon particles were used as the support for the Pt-Ir electrocatalyst. The carbon particles were first pretreated with nitric acid to oxidize the surface to generate  $-COO^-$  and  $-OH$  functional groups. The functional groups, especially  $-COO^-$ , repel each other and enhance the dispersion of the carbon particles in the aqueous solution.

The pretreatment procedure of the carbon nanoparticles by nitric acid is described as follows:

- Placed 5 g of the XC-72R carbon particles in a glass reactor, add 50mL of 65 wt% nitric acid and mix.

2. A reflux condenser was connected to the glass reactor and the mixture of the carbon particles and the nitric acid was boiled at 100°C for 8 hours.
3. The mixture was then cooled to room temperature and washed several times with de-ionized water to remove any remaining nitric acid.
4. The pH of the carbon particles in the de-ionized water was then adjusted to pH 7.0 by using 35 wt% NH<sub>3</sub> aqueous solution. The slurry was then filtered and washed again using de-ionized water. The pretreated carbon particles were then baked in an oven at 80°C.

The modified Watanabe method for the synthesis Pt-Ir/carbon particles catalyst is shown in Figure 3-1 and is briefly described as follows:

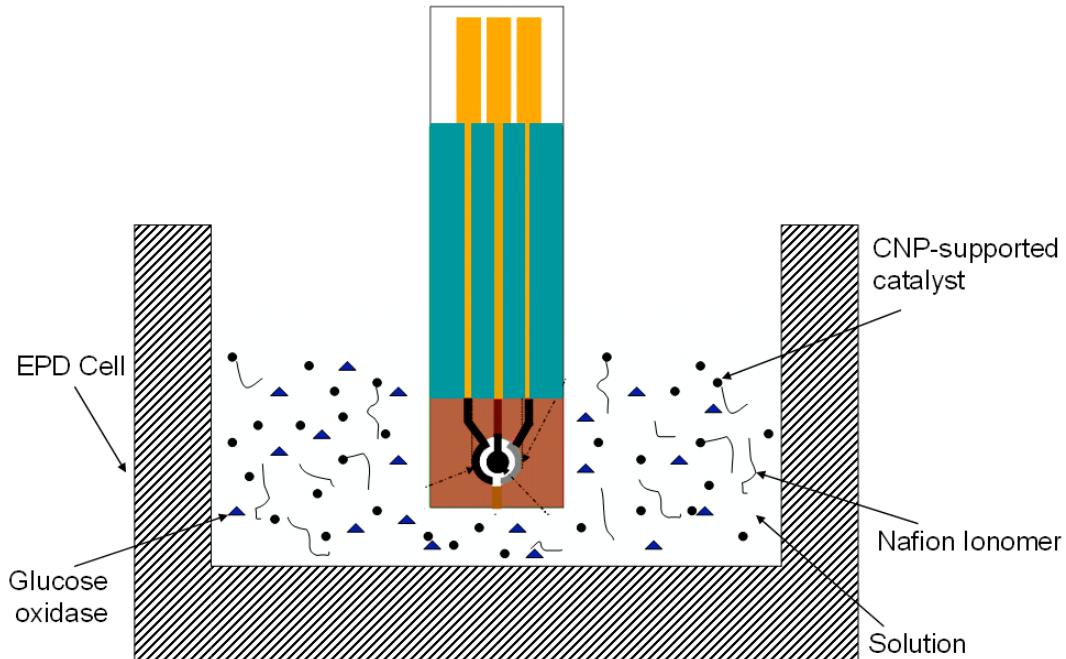
1. Prepare a H<sub>2</sub>PtCl<sub>6</sub> solution and a IrCl<sub>3</sub> solution by dissolved 6.69mg H<sub>2</sub>PtCl<sub>6</sub> · 6H<sub>2</sub>O and 4.45mg IrCl<sub>3</sub> · 3H<sub>2</sub>O, respectively, in 7.5 mL de-ionized water, each.
2. Adjust the IrCl<sub>3</sub> solution to pH 4 by adding 0.6M Na<sub>2</sub>CO<sub>3</sub> solution.
3. Adjust the H<sub>2</sub>PtCl<sub>6</sub> solution to pH 7 by adding 0.6M Na<sub>2</sub>CO<sub>3</sub> solution.
4. Ensure that the IrCl<sub>3</sub> and H<sub>2</sub>PtCl<sub>6</sub> were completely dissolved by adding 0.3033g NaHSO<sub>3</sub> solution, respectively.
5. Heat the IrCl<sub>3</sub> solution in a test tube in an oil bath at 90°C for 30 min.
6. The prepared H<sub>2</sub>PtCl<sub>6</sub> solution, IrCl<sub>3</sub> solution and 0.095 g of the pretreated XC-72R carbon particles are mixed in a flask and placed in an ultrasonic bath for mixing for 30 minutes.
7. Add 38 mL of 35 wt% H<sub>2</sub>O<sub>2</sub> into the flask and adjust the solution to pH 5 by adding 1M NaOH solution.

8. The solution is then boiled at 100°C in a reflux reactor for 8 hours.
9. The mixture is then cooled to room temperature, and washed with 95 wt% alcohol and de-ionized water, sequentially. The mixed particles are then baked in an oven at 80°C.
10. The mixed particles are then exposed to hydrogen at 300°C in a 10% H<sub>2</sub> in Ar stream for 2 hours in an oven.
11. This final product was defined as Pt-Ir/C (5%) or Pt-Ir/carbon particles in this study.

### **3.7 Electrophoretic deposition of Pt-Ir/carbon nanoparticles and GOD coupled catalysts**

#### **3.7.1 Electrodes and cell assembly**

The minibiosensor produced by the thick film screen-printing is shown in Figure 2-4. The mini biosensors were fabricated in bulk (150/sheet, i.e. 25/row × 6rows per sheet) on a polyester substrate (Melinex 329, DuPont Co.) by Conductive Technologies, York, PA.<sup>[2]</sup> The diameter of the carbon working electrode was about 1mm (0.4in.). A schematic illustration of the experimental arrangement for the EPD operation is shown in Figure 3-2. In this figure CNP-supported catalyst refers to the Pt-Ir/carbon particles and EPD to the electrophoretic deposition. The minibiosensor was placed, fixed and operated in the EPD cell as shown in Figure 3-3. Detailed procedures will be described in the section 3.7.2.



**Figure 3-2** Schematic illustration of the experimental setup for EPD operation, where CNP is carbon nanoparticles and EPD is electrophoretic deposition.

### 3.7.2 Electrophoretic deposition procedures

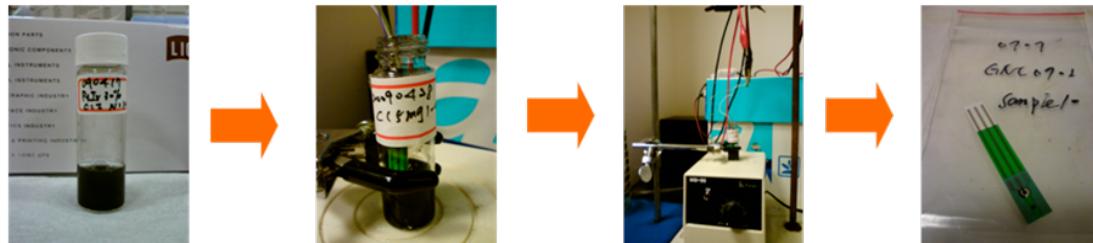
The operating conditions of the applied potential and the composition of the Pt-Ir/carbon nanoparticles, Nafion binders, as well as GOD were important factors in the fabrication of the minibiosensors. In general, these factors affected the performance of the EPD combined minibiosensor. Figure 3-3 shows the EPD process for the fabrication of the minibiosensor.

The EPD process for the deposition of both GOD and Pt-Ir / carbon nanoparticles is described as follows:

1. Add a suitable quantity of Pt-Ir/carbon nanoparticles (1mg/mL) and Nafion binder (5 $\mu$ L of 5% Nafion solution L/mL) into 0.01M PBS solution (pH = 7.4) in a bottle and place it in a supersonic bath for mixing.

2. Add a desired amount of GOD (136,100U/g, 10mg/mL) into the prepared solution above and mix it again. The prepared solution is then placed in a refrigerator at 4°C.
3. Place the minibiosensor into the prepared solution in a bottle at room temperature for EPD. Apply a potential +1V vs. printed Ag / AgCl electrode for 30 minutes.
4. After the EPD process, wash the treated biosensor with deionized water.
5. The modified minibiosensor is stored in a bag at room temperature for the sensing test.

Figure 3-3 shows the sequence of this preparation procedure.



**Figure 3-3** The sensor and cell assembly for making electrophoretic deposition of both GOD, Nafion and Pt-Ir/carbon nanoparticles

### 3.8 Experimental procedures of H<sub>2</sub>O<sub>2</sub> testing and glucose sensing

#### 3.8.1 Procedures of H<sub>2</sub>O<sub>2</sub> testing

The following procedures were carried out in the evaluation of the sensor performance in the presence of H<sub>2</sub>O<sub>2</sub>

1. Place five 10mL-beakers on the table and add 5mL phosphate buffer saline (PBS) (Sigma P5493) solution (pH7.4) to each beaker.

2. The different H<sub>2</sub>O<sub>2</sub> concentration tests were carried out by adding 1.0, 2.0, 3.0, 4.0, to 5.0 mM hydrogen peroxide to each of the five beakers using a pipette. For example, 0.5µL of the prepared 10M H<sub>2</sub>O<sub>2</sub> solution (stored in 4 °C refrigerator) was added to the first beaker forming a 1.0mM H<sub>2</sub>O<sub>2</sub> concentration in the PBS solution. Similarly, other H<sub>2</sub>O<sub>2</sub> concentrations of the test solutions were prepared.
3. Fix and immerse the minibiosensor in the center of the testing beaker as shown in Figure 3-4.
4. Adjust the scanning potential window in the range from -0.4 to +0.3V vs. the printed Ag/AgCl reference electrode, and set the cyclic voltammetry (C.V.) scanning rate at 50mV/sec. Start from 0V towards +0.3V vs. the Ag/AgCl reference electrode.
5. After about 11 cycles of the C.V. performance, the C.V. peak reached a steady state.
6. Record the oxidation peak current at +0.2V vs. the printed Ag/AgCl reference electrode as a function of H<sub>2</sub>O<sub>2</sub> concentration.
7. The above steps 3 to 6 are then repeated in other H<sub>2</sub>O<sub>2</sub> concentration PBS solutions.

### **3.8.2 Procedures of glucose testing**

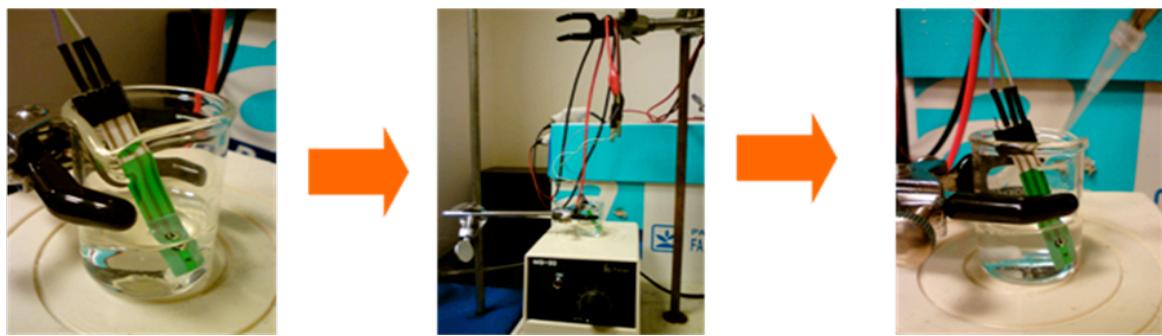
Two types of testing solutions were used. One was phosphate buffer saline (PBS) (Sigma P5493) solution (pH7.4); the other was bovine serum (Invitrogen 16170). All chemicals used were of analytical grade.

(I) Typical operating steps of glucose sensing in PBS solution were

1. A 1.0M glucose solution was first prepared.
2. Place ten 10ml-beakers and add 5ml PBS solution into each beaker.
3. For sensing glucose concentration in PBS solution, add, 1.0 to 9.0 mM prepared glucose solution separately to each beaker using a pipette. For example, 5 $\mu$ L of the prepared 1.0M glucose solution was added to a beaker forming a 1.0 mM glucose concentration of the PBS solution. Similarly, other glucose concentration solutions were prepared.
4. Fix and immerse the minibiosensor in the center of the testing beaker.
5. Adjust the scanning potential window in the range from -0.4 to +0.3V vs. the printed Ag/AgCl reference electrode, and set the cyclic voltammetry (C.V.) scanning rate at 50mV/sec. The performance of the C.V. was started from 0.0 to +0.3V vs. the Ag/AgCl reference electrode and reduced to -0.4V vs. the Ag/AgCl reference electrode.
6. After approximately 11 cycles of the C.V. performance, the C.V. peaks reached a steady state.
7. The peak current at +0.2V vs. the Ag/AgCl reference electrode was then recorded.
8. The above steps were repeated in other glucose concentration PBS solutions.

(II) Typical operating steps of glucose sensing in bovine serum are

The procedure for testing the glucose concentration in bovine serum was similar to that described above for the PBS test medium. The only difference was that bovine serum was used as the test medium instead of PBS solution.



**Figure 3-4** Measuring process by using minibiosensor

### 3.9 Bibliography

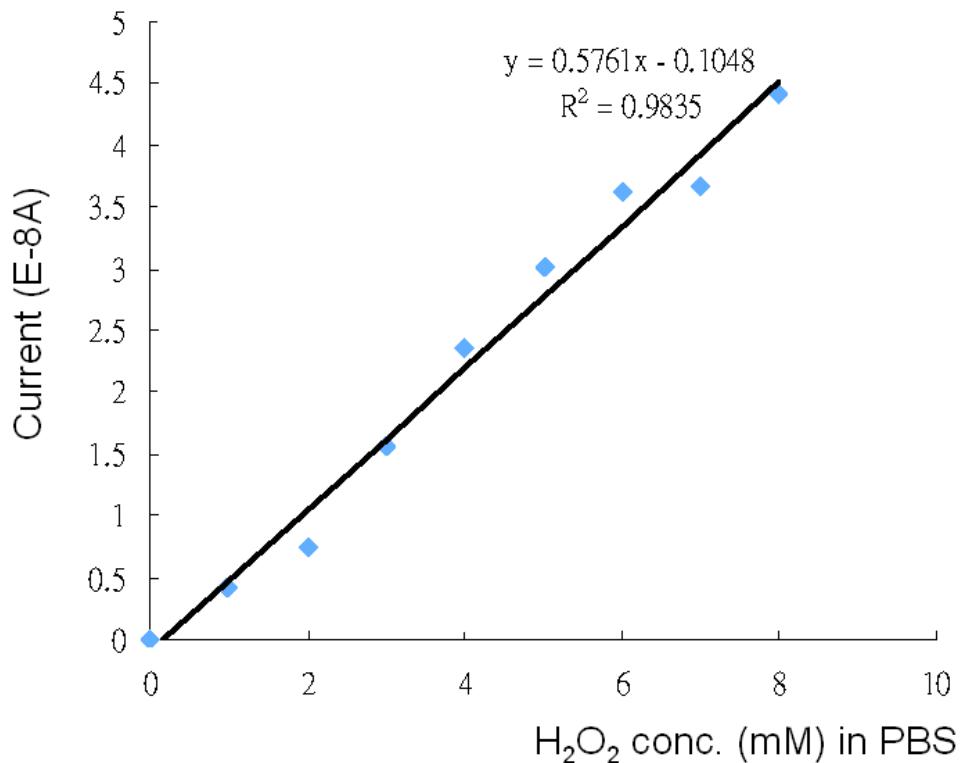
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#### **4. Results and Discussion**

Based on the discussion in Chapter 3, EPD minibiosensors were prepared and reported for the first time in this study. Successful detection of enzymatic generated H<sub>2</sub>O<sub>2</sub> and consequently the glucose in PBS solution using the EPD minibiosensors are described. Detection of glucose in bovine serum by the EPD minibiosensors is also reported. Methods to immobilize the GOD, as well as the Pt-Ir / carbon nanoparticles, on the screen-printed mini electrodes by electrophoretic deposition (EPD) are also described in this chapter. In addition, the reaction mechanism and kinetics of the detection of glucose by this minibiosensor are discussed.

##### **4.1 Detecting hydrogen peroxide in PBS solution by repeated use of the same electrophoretic deposition (EPD) minibiosensor**

The EPD minibiosensor was used to detect and measure the concentration of H<sub>2</sub>O<sub>2</sub> in PBS (100mM, pH7.4, containing 1mM KCl as supporting electrolyte). The experimental results are shown in Figure 4-1.



**Figure 4-1** Current output as a function of H<sub>2</sub>O<sub>2</sub> concentrations using the EPD minibiosensor for H<sub>2</sub>O<sub>2</sub> detection

Figure 4-1 shows the current of the EPD minibiosensor versus concentration of H<sub>2</sub>O<sub>2</sub> using the amperometric technique at an oxidation potential at +0.2V vs. the Ag/AgCl reference electrode. The oxidation potential was obtained in a cyclic voltammetric measurement over the voltage window of -0.4 to +0.3V with a scanning rate of 50mV/sec. Figure 4-1 shows an excellent linear relationship between the oxidation current and the H<sub>2</sub>O<sub>2</sub> concentration. The results show that the EPD minibiosensor is able to quantify of hydrogen peroxide in PBS solution.

## **4.2 Development of electrophoretic deposited (EPD) glucose minibiosensors**

### **4.2.1 Different designs of EPD minibiosensors**

In order to understand the role of GOD, Nafion binder, and Pt-Ir/carbon nanoparticles catalyst, four types of the minibiosensors were designed and assessed. These electrophoretic deposition (EPD) minibiosensors included (1) EPD GOD minibiosensor, (2) EPD of both GOD and Nafion binder (GOD + Nafion) minibiosensor, (3) EPD deposition of both GOD and Pt-Ir/carbon nanoparticle catalyst (GOD + Pt-Ir catalyst) minibiosensor, and (4) EPD of all three components including GOD, Nafion binder and Pt-Ir/carbon nanoparticle catalyst (GOD + Nafion + catalyst) or EPD minibiosensor. The preparation and the results from these four types of electrophoretic deposited minibiosensors are described as follows:

#### **4.2.1.1 The EPD GOD minibiosensor**

The enzyme, GOD, was electrophoretically deposited on the working electrode of the screen-printed minisensor containing a carbon working electrode, a carbon counter electrode, as well as a printed Ag / AgCl reference electrode. The schematic illustration of the experimental setup for this EPD operation was similar to the setup shown in Figure 3-2. In this particular preparation, the solution contained only the enzyme, GOD, for electrophoretic deposition (EPD). The glucose oxidase (**EC 1.1.3.4**) (G7141 Sigma) from *Aspergillus niger* (136,100 unit/g) was purchased from the Sigma Company. The type used in this research was Type X-S, lyophilized powder 100,000~250,000 unit/g solid (without added oxygen). In a typical run, the electrophoretic deposition solution

contained 7.5 mg GOD (or 102 U/mL) in 10mL of 0.01M PBS solution (pH=7.4).

A potential of +1V vs. Ag/AgCl was applied for 30 minutes. As described in section 3.7.2, this EPD GOD minibiosensor was taken out and washed with de-ionized water. The modified mini biosensor was stored in a sealed plastic bag at room temperature until testing. The activity of this EPD GOD minibiosensor was tested for glucose sensing in PBS solution and the results are given in section 4.2.2.

#### 4.2.1.2 The EPD GOD + Nafion minibiosensor:

For the preparation of the EPD GOD + Nafion minibiosensor, both the GOD and Nafion binder were electrophoretically deposited simultaneously on the screen-printed working electrode surface. The experimental setup was similar to that shown in Figure 3-2. Nafion binder is a polyelectrolyte (cation exchange polymer) that contains  $\text{SO}_3^-$  functional group as shown in Figure 2-12. In the preparation of this mini biosensor, a 0.05mL(5%) Nafion solution and 7.5mg GOD were added in a 10mL of 0.01 M PBS solution, which was well dispersed by placing the solution in a beaker in an ultra-sonic machine (DELTA Ultrasonic Cleaner DC400H) for 5 minutes. The experimental setup and the operating procedure were similar to that described in section 4.2.1, except that the electrophoretic deposition solution contained both GOD and Nafion binder. The modified EPD GOD +Nafion minibiosensor was also stored in a sealed plastic bag at room temperature until used for testing.

#### 4.2.1.3 The EPD GOD + catalyst minibiosensor

For the preparation of the EPD GOD + catalyst minibiosensor, the GOD and Pt-Ir/carbon nanoparticles were electrophoretically deposited simultaneously on the screen-printed working electrode of the minisensor . The schematic illustration of the experimental setup for EPD operation was similar to that as shown in Figure 3-2. The Pt-Ir/carbon nanoparticles for the electrode catalyst of H<sub>2</sub>O<sub>2</sub> anodic oxidation was synthesized by using the synthesis method reported in the literature<sup>[1]</sup> as modified in section 3.6. The Pt and Ir atomic ratio on the carbon particle was 1 to 0.95, which was determined Inductively Coupled Plasma-Atomic Emission Spectrometry ICP-AES (JY 2000-2). Typically, 5 mg of Pt-Ir/ carbon particle catalyst and 7.5 mg GOD were added to a 10mL of 0.01M PBS solution, which was well dispersed by placing the solution in a beaker placed in an ultra-sonic machine (DELTA Ultrasonic Cleaner DC400H) for 5 minutes. The experimental setup and operating procedure were similar to those described in section 4.2.1.1 and section 4.2.3.1. However, the electrophoretic deposition solution contained both GOD and Pt-Ir/carbon nanoparticles catalyst in this case. In general, the Pt-Ir/carbon nanoparticles did not contain any charged functional groups so there was almost no ionic migration when a potential was applied.

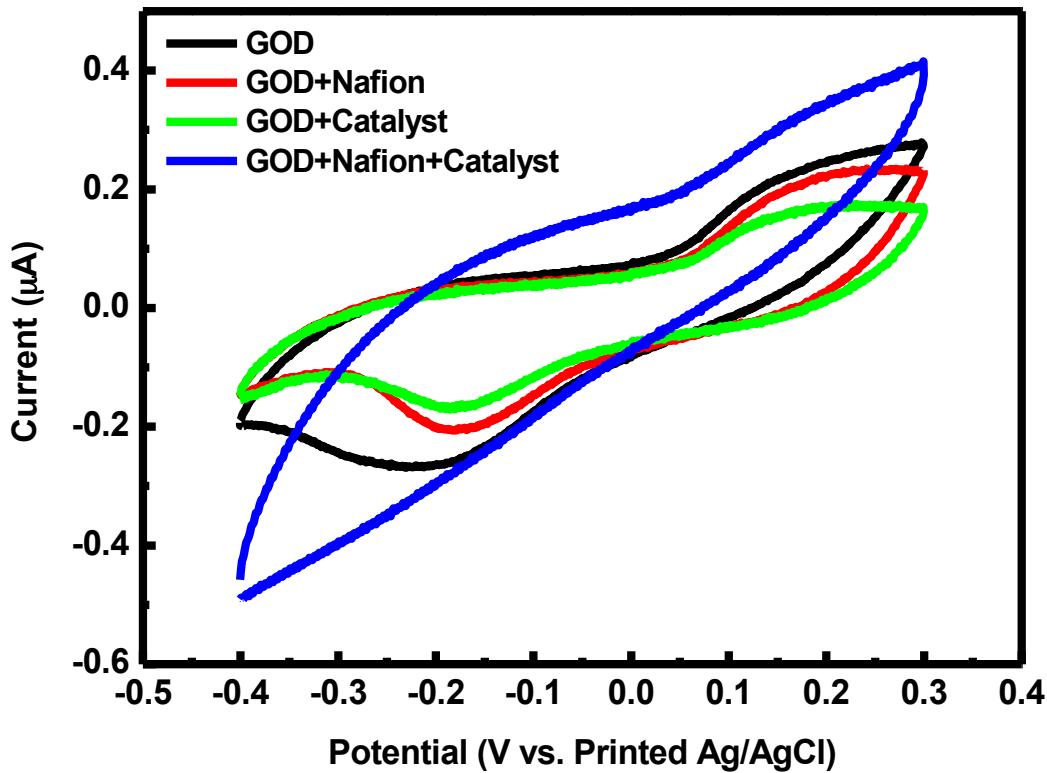
#### 4.2.1.4 The EPD GOD + Nafion + catalyst minibiosensor

For the preparation of the EPD GOD + Nafion + catalyst, combined minibiosensor, the GOD, Nafion binder and the Pt-Ir/carbon nanoparticles were electrophoretically deposited simultaneously on the screen-printed working electrode. The experimental setup for EPD operation was similar to that described

previously. Typically, 5 mg of Pt-Ir/ carbon nanoparticle catalyst, 0.05 mL Nafion binder solution, and 7.5mg GOD were added to a 10mL of 0.01 M PBS solution that was well dispersed by placing the solution in a beaker which was placed in an ultra-sonic machine (DELTA Ultrasonic Cleaner DC400H) for 5 minutes. The experimental setup and operating procedures are similar to those described previously. However, the electrophoretic deposition solution contained GOD, Nafion binder and Pt-Ir/carbon nanoparticles catalyst. After the solution was well dispersed, the electrophoretic deposition of the EPD deposited GOD, Nafion, and Pt/Ir combined particles was carried out by application of a +1.0V vs. Ag/AgCl potential for 30 minutes. Subsequently, this modified mini biosensor was taken out and washed with de-ionized water. The combined mini-biosensor was stored in a sealed plastic bag at room temperature until testing.

#### **4.2.2 Comparison of the performance of the EPD glucose minibiosensors**

Cyclic voltammetry using the four types of EPD glucose minibiosensors was carried out and the results are shown in Figure 4-2. The scan potential range of the cyclic voltammetry was from -0.4 to +0.3 volts vs. the Ag/AgCl reference electrode; the scan rate was kept at 50mV/sec in a 100mM PBS solution with 4mM glucose. The cyclic voltammograms shown in Figure 4-2 were obtained after 13 cycles. The highest anodic oxidation current was obtained at +0.2V with the type(4) prototype containing GOD, Nafion binder and Pt-Ir/ carbon nanoparticles. The order of anodic oxidation currents were EPD GOD minibiosensor > EPD GOD + Nafion minibiosensor > EPD GOD + Pt-Ir/carbon nanoparticles catalyst minibiosensor.



**Figure 4-2** Cyclic voltammetry results from the four different glucose minibiosensors using 4mM glucose in PBS solution.

Table 4-1 summarizes the results of the anodic currents at +0.2V vs. the Ag/AgCl reference electrode using the four different glucose minibiosensors, operating in a 100mM PBS solution with 4mM glucose.

**Table 4-1** Anodic oxidation currents at +0.2V vs. Ag / AgCl using the four EPD glucose minibiosensors

Operation condition: scan Rate: 50mV/sec, 13 cycles. , 4mM Glucose in 100mM PBS at 0.2 V vs. printed Ag /AgCl

Sample	Current ( $\mu$ A)
<b>GOD</b>	0.245
<b>GOD + Nafion</b>	0.222
<b>GOD + Catalyst</b>	0.171
<b>GOD + Nafion + Catalyst</b>	0.343

As shown in Table 4-1, the highest current at +0.2 V vs. Ag/AgCl was 0.343  $\mu$ A. This highest response current was the result of the contribution of both GOD and Pt-Ir bimetallic catalysts that were electrophoretic deposited on the working electrode. The Nafion binder containing anion functional groups enhanced the migration of GOD and Pt-Ir/carbon nanoparticles catalyst that were deposited on the surface of the mini-electrode. The current outputs at +0.2V versus the Ag/AgCl reference electrode for EPD deposited GOD and GOD+ Nafion prototypes were +0.245 $\mu$ A and +0.222 $\mu$ A respectively. These two values should be considered approximately the same. This observation was reasonable, since both prototypes had identical quantities of the enzyme catalyst, GOD. The addition of Nafion may affect the available active sites of the GOD slightly. Consequently, a minute decrease of the oxidation current occurred. The lowest

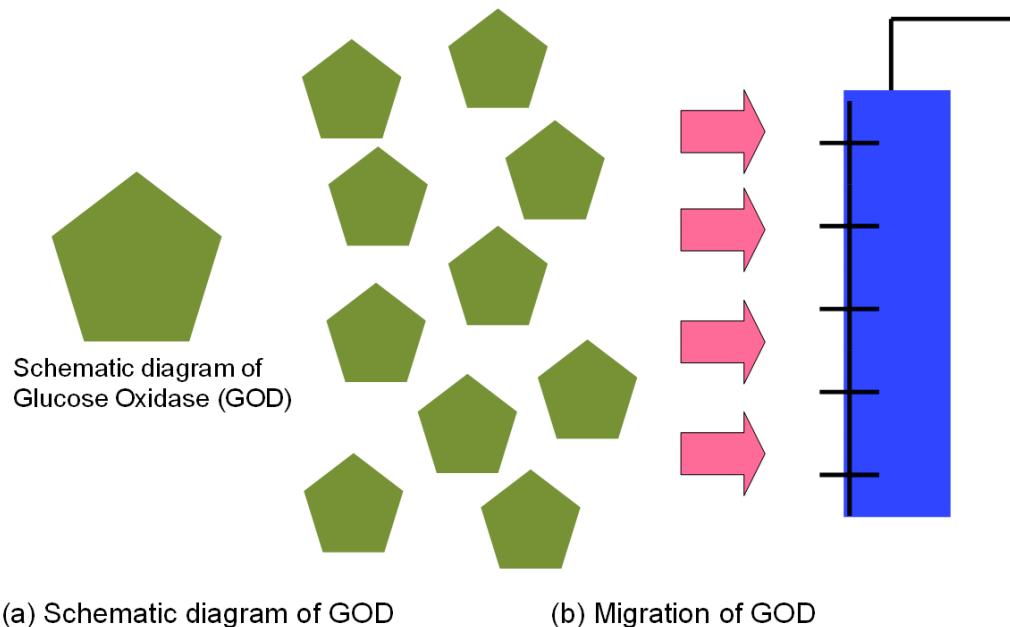
oxidation current among the four prototypes was +0.171 $\mu$ A for the GOD+ catalyst prototype. In this case, without the Nafion as a binding reagent, the adhesion of both the GOD and the bimetallic catalyst were poor. This would result in the loss of both catalysts leading to lower available catalyst sites and lower oxidation current. One also recognizes that the actual size of the bimetallic catalyst was larger compared to that of the GOD. Thus, when the EPD process deposited the bimetallic catalyst and GOD simultaneously, a larger surface area of the working electrode would be occupied by the bimetallic catalyst, minimizing the quantity of the GOD deposited on the surface. Consequently, the available active sites of the GOD would be less than when only GOD was used in prototype (1). The combination of the loss of both bimetallic catalyst and GOD due to poor adhesion without the Nafion binder and fewer available active sites of the GOD to start with, resulted in the low oxidation current obtained in this prototype.

#### **4.2.3 Discussion of possible mechanisms of electrophoretic deposition**

##### **4.2.3.1 Possible mechanism of the electrophoretic deposition of glucose oxidase**

The isoelectric point (PI) of GOD is 4.2.<sup>[2]</sup> Thus, the molecular charge of GOD is positive when it is dissolved in an aqueous solution with pH<4.2. GOD molecular charge is negative when it is dissolved in a pH>4.2 aqueous solution. Thus, the GOD molecules would have negative charge or would have –COO<sup>-</sup> terminals or anion functional groups in the PBS solution (pH7.4). Figure 4-3(a) shows the schematic diagram of the glucose oxidase. In electrophoretic deposition as described in Figure 3-2, the large negative charged glucose oxidase molecule migrate to the anode and adsorb on the surface of the anode when a potential

(+1V vs. Ag/AgCl) was applied for 30 minutes as shown in Figure 4-3(b). The GOD molecules were packed on the surface of the working electrode, which was active carbon. Based on the experimental results obtained shown in Figure 4-2 and Table 4-1, as well as shown in the reactions of Equations (2-1) and (2-3), without a binder, the quantity of GOD deposited by EPD may be relatively low. Consequently this reaction produced a lesser amount of  $\text{H}_2\text{O}_2$ , and the oxidation current of this sensor was  $+0.245\mu\text{A}$  at  $+0.2\text{V}$  vs. Ag/AgCl as shown in Table 4-1. The direct adhesion between the GOD and the carbon working electrode without a binder was relatively poor. Consequently, this would contribute to the relatively low  $\text{H}_2\text{O}_2$  oxidation current.

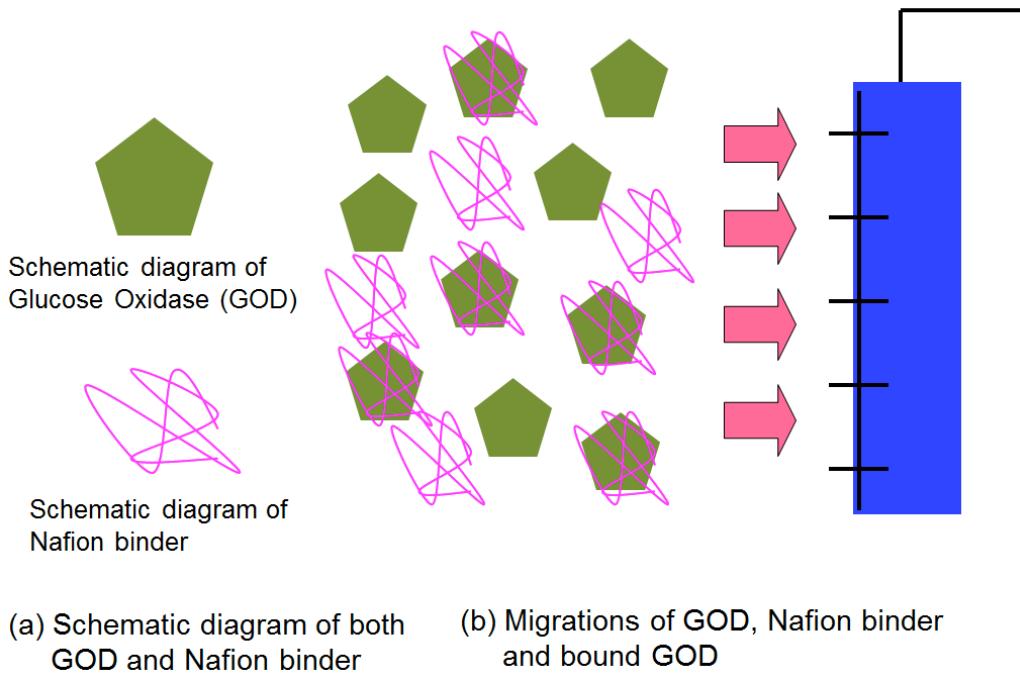


**Figure 4-3** Electrophoretic deposition of glucose oxidase (GOD) in a 10mL of 0.01M PBS solution (a) GOD is dissolved in PBS solution (b) negative charged GOD migrate to the anode and deposit on the surface of electrode under an applied potential +1V for 30 minutes.

#### 4.2.3.2 Possible mechanism of simultaneous electrophoretic deposition of both GOD and Nafion binder

Nafion binder is a polyelectrolyte molecule that contains  $-SO_3^-$  functional groups. A schematic diagram of the Nafion binder is shown in Figure 4-4(a). The large negative charged GOD protein molecules and the cation exchange Nafion binder migrated simultaneously to the anode and adsorbed on the surface of the active carbon anode when a potential (+1V vs. Ag/AgCl) was applied for 30 minutes as shown in Figure 4-4(b). The material of the working electrode (anode) was active carbon. The GOD, Nafion binder, and bound GOD composite were packed on the surface of the carbon working electrode. In this case, the Nafion binder enhanced the adhesion of the GOD to the surface of the working electrode. However, the Nafion binder occupied some of the surface area of the working electrode. This would lead to a smaller quantity of GOD on the working electrode surface, compared to the quantity of GOD deposited in the absence of the Nafion. Consequently, this may affect the total enzymatic oxidation of the  $H_2O_2$  resulting in relatively low oxidation current. The oxidation current obtained in this case was approximately  $+0.222\mu A$  at an oxidation potential of +0.2V versus the Ag/AgCl reference electrode. In the case of EPD deposited GOD, the oxidation current was approximately  $+0.245\mu A$ . at the same oxidation potential. Under the experimental conditions and the potential experimental accuracy, these two oxidation currents should be viewed as approximately equal. In the absence of the Nafion binder, the adhesion of GOD to the electrode surface was relatively weak, and this would result in the loss of GOD on the electrode surface. In the presence

of the Nafion binder the adhesion of the GOD to the electrode surface improved, but the Nafion binder would occupy some of the surface area of the working electrode. In both cases, the small quantity of GOD on the electrode surface resulted in a lower enzymatic reaction rate, and therefore lower oxidation current.

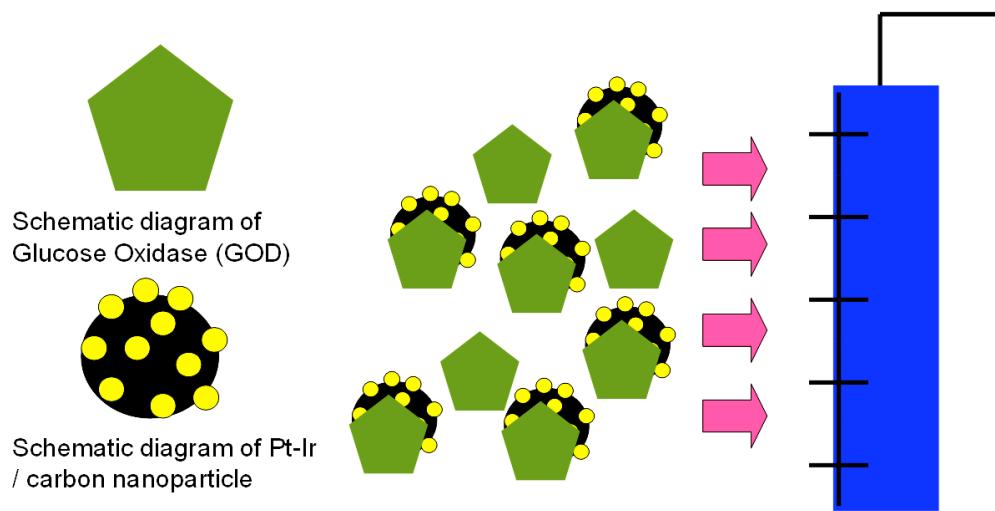


**Figure 4-4** Electrophoretic deposition of glucose oxidase (GOD) and Nafion binder in a 10mL of 0.01M PBS solution (a) GOD is dissolved in PBS solution (b) negative charged GOD and Nafion binder migrate to the anode and deposit on the surface of electrode under an applied potential +1V for 30 minutes

#### 4.2.3.3 Possible mechanism of simultaneous electrophoretic deposition of GOD and Pt-Ir/carbon nanoparticles

The structure of the Pt-Ir/carbon particle is shown in Figure 4-5(a). In this case, the electrophoretic deposition solution contained both GOD and Pt-Ir/carbon nanoparticles. The ionic migration of GOD as well as GOD and Pt-Ir/carbon

composite nanoparticles to the anode took place when a potential (+1V vs. Ag/AgCl) was applied, see in Figure 4-5(b). In this case, both the GOD molecules and the Pt-Ir/carbon nanoparticles migrated towards the anode. There was a substantial size difference between the GOD molecules and the Pt-Ir/carbon nanoparticles. Without a binding agent, Nafion in this case, the adhesion between the GOD and the Pt-Ir/carbon nanoparticles was relatively poor. Consequently, both the available GOD and the Pt-Ir/carbon nanoparticle catalyst on the working electrode surface would be relatively limited. Thus, it would be reasonable to observe a low oxidation current of  $+0.171\mu\text{A}$  in this case.



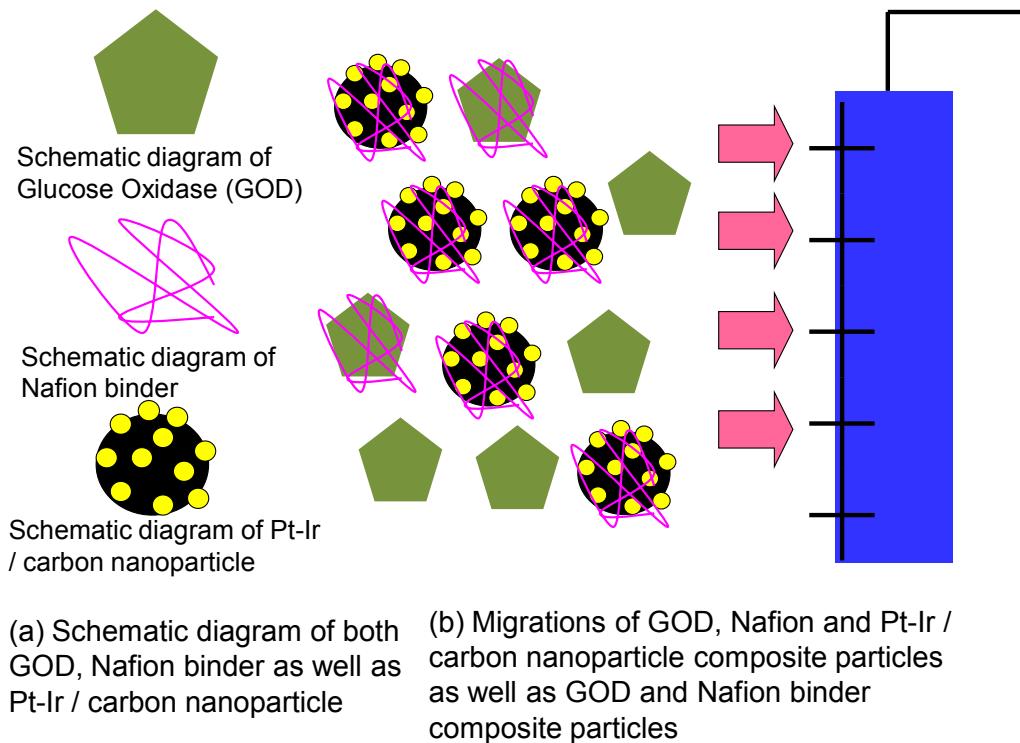
(a) Schematic diagram of both GOD and Pt-Ir / carbon nanoparticle

(b) Migrations of GOD and Pt-Ir / carbon nanoparticle composite particles

**Figure 4-5** Electrophoretic deposition of GOD and Pt-Ir/carbon nanoparticle composite nanoparticles

#### 4.2.3.4 Possible mechanism of electrophoretic deposition of GOD, Nafion binder, and Pt-Ir/carbon nanoparticles composite

In this case, EPD was carried out in the presence of the enzyme catalyst, GOD, Pt-Ir/carbon nanoparticles and the Nafion binder simultaneously. As shown in Figure 4-6, these three components migrated towards the anode upon applying +1V versus the Ag/AgCl reference electrode. In this case, the Nafion binder served its function by binding effectively both the enzymatic catalyst, GOD, and the nano-bimetallic catalyst, Pt-Ir/carbon, onto the working electrode surface simultaneously. Consequently, the nano-bimetallic catalyst lowered the overpotential of the oxidation reaction of H<sub>2</sub>O<sub>2</sub>, and gave a relatively complete enzymatic conversion of glucose to H<sub>2</sub>O<sub>2</sub>. Thus, it is reasonable to anticipate that a biosensor with EPD GOD+ Nafion binder + Pt-Ir/carbon nanoparticles combined would give the best results of the four different types of biosensor prototypes.



**Figure 4-6** Electrophoretic deposition of GOD, Nafion and Pt-Ir/carbon particle composite particles as well as GOD and Nafion binder composite particles

#### 4.2.4 Comparison of the typical immobilization of GOD and electrophoretic deposition immobilization developed in this study

The immobilization of GOD has been reported in the literatures<sup>[3,4]</sup> and is briefly described here. These immobilization methods will be compared with the electrophoretic deposition immobilization developed in this study.

##### 4.2.4.1 Conventional GOD immobilization

The procedure for typical glucose oxidase immobilization has been reported.<sup>[3,5]</sup> For example, glucose oxidase was immobilized on the surface of an iridium containing carbon working electrode using glutaraldehyde as the covalent linking agent between the enzyme and the chemical polyethylenimine

incorporated in the carbon working electrode.<sup>[4]</sup> As reported, a glutaraldehyde solution (2%, 6 $\mu$ L) was first applied to the iridium-containing carbon working electrode surface and then the biosensor was dried overnight at room temperature in air.<sup>[5]</sup> After 24 hours of drying, a quantity of 6 $\mu$ L of an enzyme solution (15 mg GOD in 100 $\mu$ L PBS solution) was deposited onto the iridium-contained carbon working electrode surface. The biosensor prototype was stored in a refrigerator at 4°C for drying overnight. Prior to the testing of the biosensor, it was carefully rinsed using de-ionized water to remove any loosely attached enzyme. Several layers were formed. The first layer (top surface layer) was enzyme. The second layer was the iridium catalyst. The third layer was the silver metal (current collector) and the fourth layer was the substrate. It was based on this immobilization procedure that the enzymatic catalyst (GOD) and the metallic catalyst (iridium) layer were separated. Thus, the H<sub>2</sub>O<sub>2</sub> generated by the enzymatic reaction might diffuse outward to the bulk solution resulting in an incomplete anodic oxidation on the iridium catalytic sites. Consequently, an error would occur in this biosensor prototype system.

#### 4.2.4.2 Electrophoretic deposition and GOD immobilization

Our new method is a one step GOD and bimetallic catalyst electrophoretic deposition (EPD) and immobilization in which the catalyst Pt-Ir/carbon nanoparticles were readily available. The one-step process packs the GOD, Nafion binder, and Pt-Ir/carbon nanoparticles tightly and uniformly on the surface of the carbon working electrode as shown in Figure 4-6. In general, the potential gradient near the surface of the carbon anode (double layer of the anode) was

about  $10^6 \sim 10^7$  V/cm at +1V vs. Ag/AgCl (assuming the double layer was about 10Å to 100Å, and the potential difference was 1 V. As mentioned in section 4.2.3.4, the electrophoretic deposition solution was comprised of GOD, Nafion binder and Pt-Ir/carbon nanoparticles. In the solution, GOD, Pt-Ir/carbon nanoparticles and the Nafion binder formed composite particles. Based on the potential gradient, these composite mixtures as well as the individual components would migrate to and pack tightly on the surface of the carbon anode. The GOD and the metallic catalyst, Pt-Ir/ carbon was not formed in a layer by layer structure, and it was uniformly and homogeneously mixed as shown in Figure 2-10. In the structure formed by EPD immobilization, the H<sub>2</sub>O<sub>2</sub> generated enzymatically would be oxidized on the Pt-Ir/carbon nanoparticles where the electrons generated were directly transferred through the carbon particles to the carbon electrode as shown in Figure 2-9 and Figure 2-10. Mediator-free electron transfer and a lower overpotential to obtain the response current were achieved. A current of 0.343μA was obtained by using this EPD GOD + Nafion binder + Pt-Ir/carbon nanoparticles minibiosensor. See Table 4-1 and Figure 4-2.

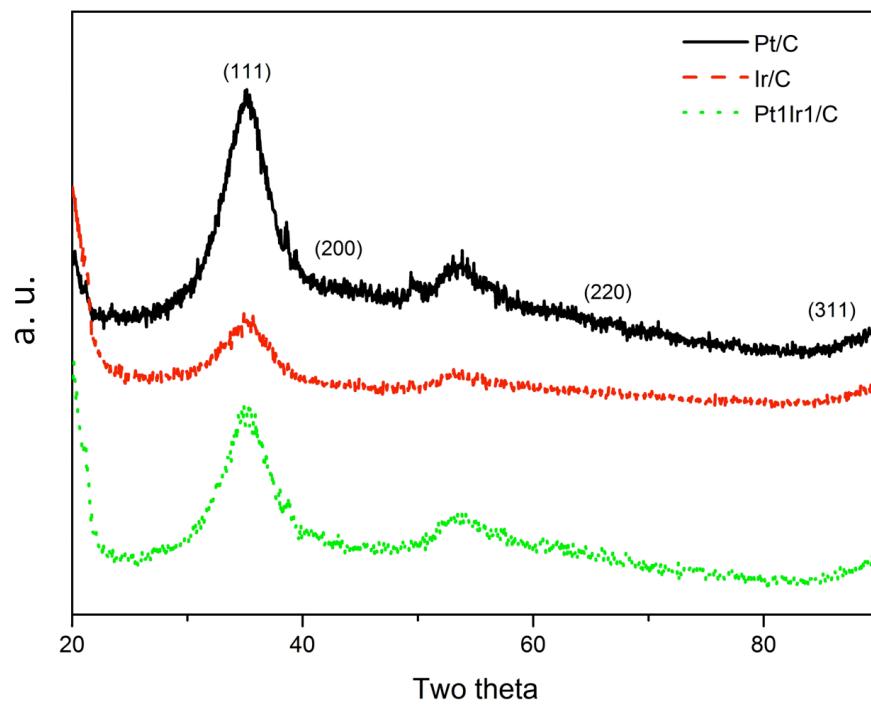
The electrophoretic deposition of GOD and Pt-Ir/carbon nanoparticles simultaneously formed a uniform compact dual catalytic (GOD and bimetallic catalyst) immobilization layer. This new approach provided advantages such as the stabilization of the conformation of glucose oxidase, which preventing it from being denatured. This resulted in a good minibiosensor without the need of storing it at 4°C in a refrigerator. Furthermore, this glucose minibiosensor prototype could be repeatedly used. The GOD stabilized by EPD was confirmed

by reports in the literature.<sup>[6,7]</sup> Glucose oxidase was stabilized by EPD due to the polymer network which stabilized the conformation of GOD. The EPD of GOD, Nafion binder and Pt/Ir bimetallic catalyst on carbon nanoparticles developed in this study gave a similar stabilization of the glucose oxidase. The bimetallic catalysts decreased the applied potential for the oxidation of H<sub>2</sub>O<sub>2</sub> as discussed in Chapter 2.<sup>[8]</sup> The use of carbon nanotubes or carbon nanoparticles to direct electron transfer between the glucose oxidase protein and the electrode were also reported elsewhere.<sup>[9,10]</sup> These literature reports confirmed and supported the feasibility and advantages of our approach of electrophoretic deposition to fabricate the minibiosensors.

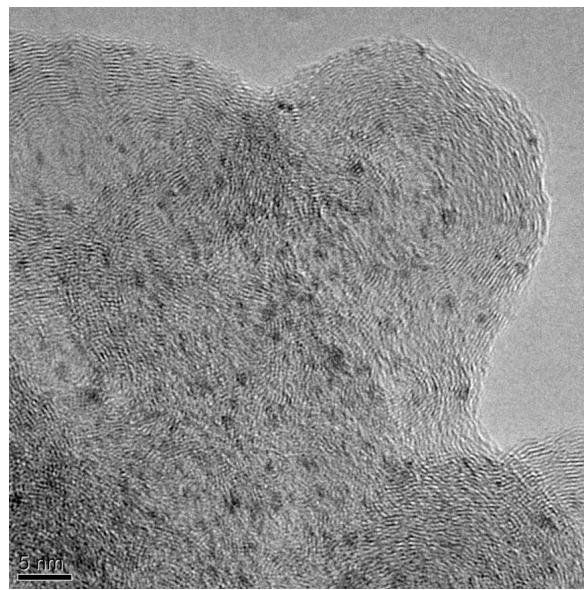
#### 4.3 The characteristics of Pt-Ir/carbon nanoparticles

The Pt-Ir/carbon nanoparticles were prepared by putting 0.475g carbon nanoparticles (XC-72R, Cabot) 22mg PtCl<sub>4</sub> and 23mg IrCl<sub>2</sub>•3H<sub>2</sub>O in 50mL deionized water adjusted to pH8~9; 0.125g NH<sub>4</sub>F and 0.625g H<sub>3</sub>BO<sub>3</sub> were than added. A 0.1 M NaBH<sub>4</sub> solution was slowly added dropwise into the solution. The produced particles were washed and dried. Based on analytical data obtained by Inductively Coupled Emission Spectrometry, the Pt to Ir atomic ratio on the carbon nanoparticles was 1:1. Figure 4-7 shows the X-ray Diffraction (XRD) of Pt/carbon nanoparticles, Ir/ carbon nanoparticles, and Pt-Ir/carbon nanoparticles. (Pt<sub>1</sub>Ir<sub>1</sub>/c indicates Pt-Ir/carbon nanoparticles with Pt/Ir atomic ratio of 1:1). The XRD of Pt/carbon nanoparticle, Ir/carbon nanoparticles, and Pt-Ir/carbon nanoparticles were put together for comparing but measured independently. At 2θ= 39.4°, a characteristical (111) peak was found for all three types of metal/

carbon nanoparticles. The atomic numbers of Pt and Ir are 78 and 77 respectively, and the atomic weights of Pt and Ir are  $195.084 \text{ g.mol}^{-1}$  and  $192.217 \text{ gmol}^{-1}$ . The size of atom or atomic radius of Pt and Ir was 139 pm and 136pm, respectively. The properties of these two elements were very similar. The crystal structures of Pt and Ir are both FCC and we found  $2\theta=39.4^\circ$  using XRD (Rigaku Dmax-B, Japan). The peaks corresponding to the (220) plane of Pt, Ir and Pt-Ir on the carbon nanoparticles were very weak. It was possible that the quantity of the metal on the carbon nanoparticles was too small to provide a more definitive analysis. Figure 4-8 shows the TEM image of the Pt-Ir/carbon nanoparticles by high resolution TEM. The size of the black grain particles (Pt-Ir grains) on the carbon nanoparticles was approximately 1~2nm, and the distribution of the Pt-Ir nano grains was uniform. Based on the literature review of Chapter 2, the most active Pt-Ir alloy bimetallic catalyst was the Pt-Ir alloy with Pt to Ir ratio 1:1. This type of Pt-Ir/carbon nanoparticle was prepared and used in this study.



**Figure 4-7** XRD pattern of homemade catalysts of Pt/ carbon nanoparticles, Ir/carbon nanoparticles and Pt1Ir1/carbon nanoparticles with a Cu K $\alpha$  radiation source operated at 40 KV and 100mA. The diffraction pattern was obtained at a scan rate of 0.05deg s-1 for  $2\theta$  between  $20^\circ$  and  $90^\circ$

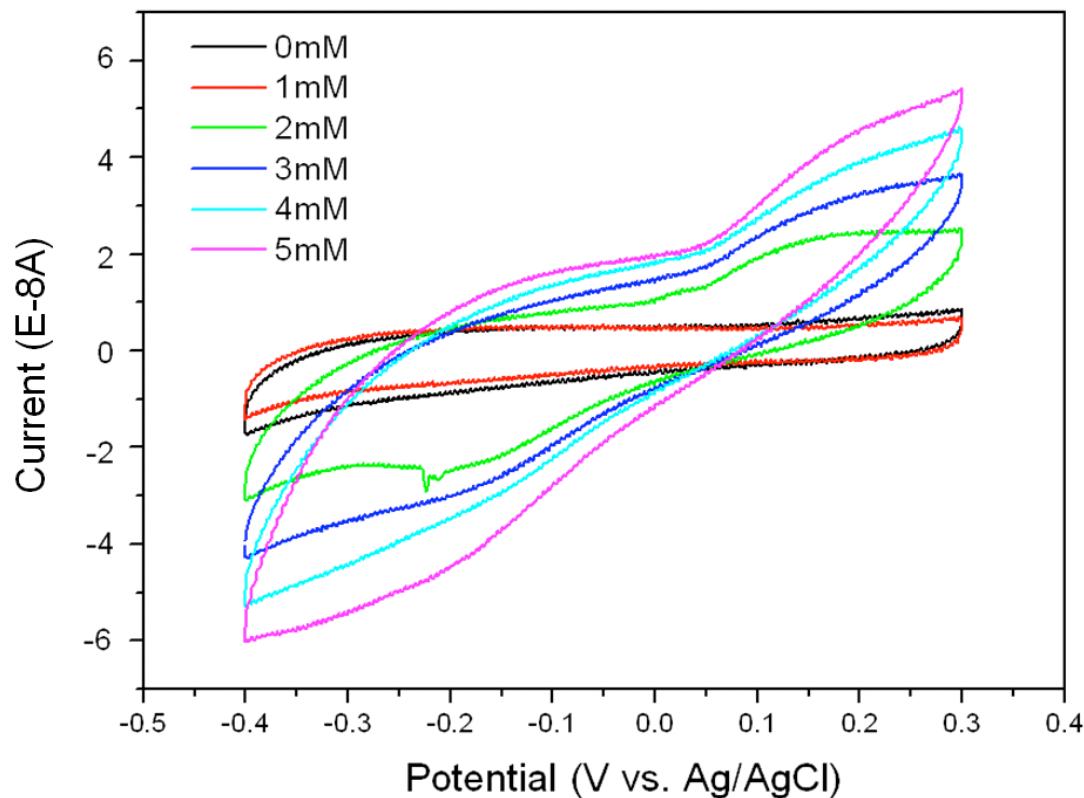


**Figure 4-8** TEM image of homemade Pt-Ir / carbon nanoparticles catalyst

## 4.4 Repeated detection of glucose in PBS solution using the same EPD minibiosensor

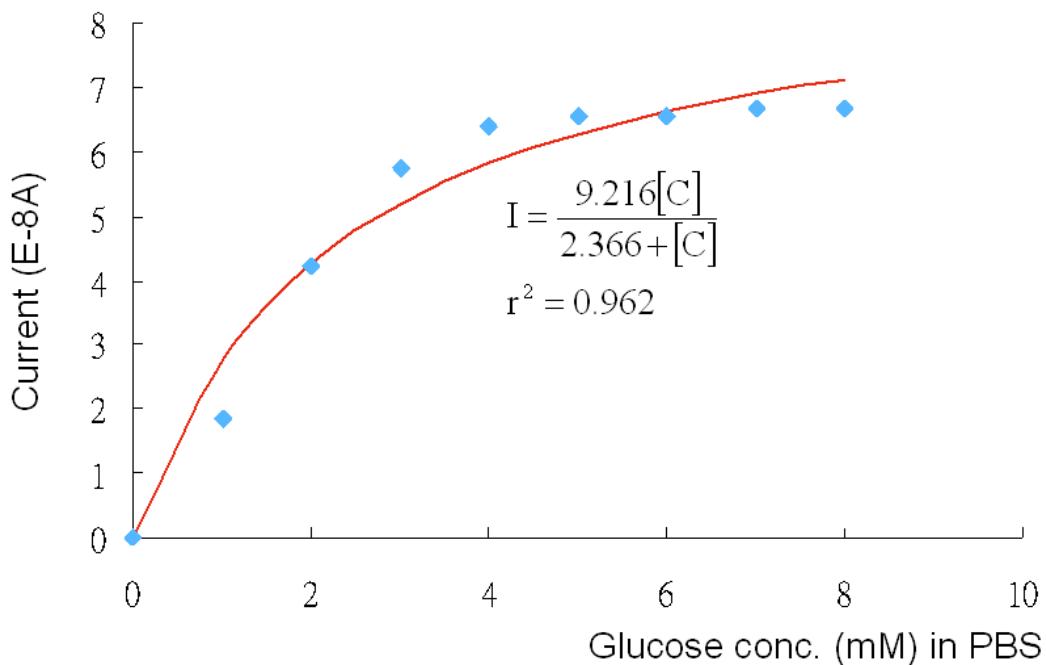
### 4.4.1 Detecting glucose by cyclic voltammetry

The use of the EPD technique to deposit GOD, Nafion binder and Pt-Ir/carbon nanoparticles onto the working electrode of the screen-printed mini-biosensor was undertaken in this study. We refer to this sensor prototype as the EPD minibiosensor. We investigated the feasibility of detecting glucose concentration in PBS solution using this minibiosensor. The glucose solutions were prepared using a commercially available phosphate buffer solution (100mM, pH7.4, containing 1mM KCl from Sigma P5493).



**Figure 4-9** CV diagram of current vs. potential (-0.4~+0.3V) from glucose in PBS solution (100mM, pH7.4, containing 1mM KCl) using EPD minibiosensor scanning rate 50mV/sec.

Figure 4-9 shows typical CV diagrams using this EPD minibiosensor for the detection of glucose in PBS solution. Figure 4-9 shows that the current outputs of this biosensor can be used to quantify the glucose concentration based on the oxidation of H<sub>2</sub>O<sub>2</sub> produced by the enzymatic reaction of glucose by GOD. The observation was consistent with the measurements of H<sub>2</sub>O<sub>2</sub> by the Pt-Ir/carbon electrode biosensor as shown in Figure 4-1. When glucose contacted GOD, it reacted with GOD to produce hydrogen peroxide. Furthermore, based on the CV results shown in Figure 4-9, an oxidation potential of +0.2V versus the printed Ag/AgCl reference electrode was observed. The current at this oxidation potential can then be used to quantify the glucose concentrations as shown in Figure 4-10.

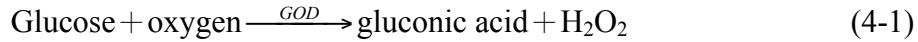


**Figure 4-10** The relationship between the current output of the biosensor versus the glucose concentration in PBS solution at +0.2V vs. screen-printed Ag / AgCl reference electrode. Note! I=current; [C]=concentration;  $I_{\max} = 9.216$ ;  $K_M = 2.366$

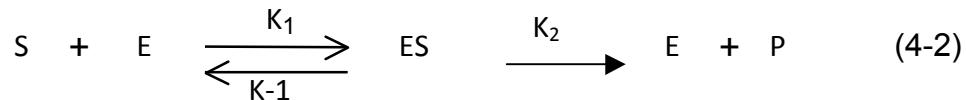
Figure 4-10 shows that the current output of the EPD minibiosensor does not have a simple linear relationship with the glucose concentration in PBS. Rather, the experimental results appear to be more closely follow the Michaelis-Menten expression. This is a reasonable assessment for the enzymatic oxidation of glucose by GOD. Thus, the reaction mechanism of this GOD reaction could be better evaluated using the Michaelis–Menten mechanism.

#### 4.4.2 Kinetics of electrochemical glucose biosensor in PBS solution

The oxidation of glucose in the present of oxygen and enzyme-GOD can be express follows:



The equation (4-1) can be further expressed as shown in equation (4-2)



where S is glucose, E is the enzyme GOD, ES is the GOD-glucose complex, and P is the product. The oxidation of glucose catalyzed by GOD can be expressed as shown in Equation (4-3) :



The Michaelis-Menten equation states that<sup>[10]</sup>

$$r = \frac{d[P]}{dt} = -\frac{d[S]}{dt} = k_2[ES] = \frac{K_2[E_0][S]}{K_M + [S]} \quad (4-4)$$

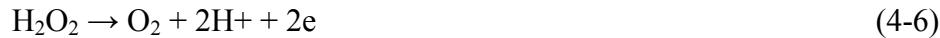
where r is the overall rate of the reaction (4-3) (rate of formation of product; gluconic acid or H<sub>2</sub>O<sub>2</sub>); K<sub>2</sub> is rate of breakdown of the complex; [ES] and K<sub>M</sub> is the Michaelis-Menten constant which is equal to (K<sub>-1</sub>+K<sub>2</sub>)/K<sub>1</sub>; [S] is glucose concentration; [E<sub>0</sub>] is the initial GOD concentration; V<sub>Max</sub> is the maximum reaction rate of glucose oxidation by [E<sub>0</sub>]; when [S]>>K<sub>M</sub>,

$$r = V_{\text{max}} = K_2[E_0]$$

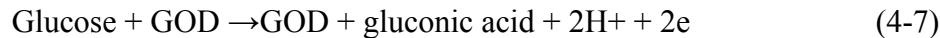
Equation (4-4) can be arranged as

$$\frac{1}{r} = \frac{K_M}{K_2[E_0][S]} + \frac{1}{V_{\text{Max}}} \quad (4-5)$$

The anodic oxidation of H<sub>2</sub>O<sub>2</sub> can be expressed as :



The combination of equations (4-3) and (4-6) obtains



The rate of Michaelis-Menten rate equation can then be expressed in term of the oxidation current, since

$$I = nFr \quad (4-8)$$

where  $n=2$ , which is the number of electrons transferred per mole of product in this reaction and F is Faraday's constant, 96,500 coulomb/mole; r is the overall rate of equation (4-7) mole/sec; I is the current in ampere(coulomb/sec).

Substituting equation (4-8) into equation (4-4), one obtains,

$$I = \frac{I_{\max}[C]}{K_M + [C]} \quad (4-9)$$

where  $I_{\max} = nFK_2 [E_0]$  and it is the maximum current,  $K_M$  is a constant, and [C] is the concentration of glucose.

Equation (4-9) can also be expressed as following:

$$\frac{1}{I} = \frac{1}{I_{\max}} + \frac{K_M}{I_{\max}} \cdot \frac{1}{[C]} \quad (4-10)$$

The use of metal or metal oxide electrocatalysts to oxidize hydrogen peroxide have been reported.<sup>[11-17]</sup> In general, the metal oxide is reduced by the hydrogen peroxide.

The iridium used in this bimetallic catalyst is actually IrO<sub>2</sub>. Iridium oxide can be reduced by H<sub>2</sub>O<sub>2</sub> and then re-oxidized as shown in eq. (4-11) and (4-12):<sup>[12]</sup>



For a metal such as Pt, the reaction mechanism is



And the overall reaction of equations (4-11) and (4-12) yields



Thus,  $\text{Pt}(\text{OH})_2$  and  $\text{IrO}_2$  electrocatalysts will oxidize  $\text{H}_2\text{O}_2$

electrochemically as shown in equations (4-14) and (4-11) respectively. The overall oxidation reaction, however, remains as shown in Eq.(4-15). Therefore the Michaelis-Menten equation can be used for the bimetallic catalysts used in this study for the mechanisms of  $\text{H}_2\text{O}_2$  detection.

For a bimetallic alloy electrocatalyst, the oxidation reaction mechanism can be complicated. The reaction rate would depend on the composition of the Pt-Ir bimetallic catalyst. The Pt and  $\text{IrO}_2$  active sites will also affect the catalytic effect. However, as expressed in eq.(4-15), the oxidation of  $\text{H}_2\text{O}_2$  with Pt-Ir bimetallic electrode can still be expressed in the Michaelis-Meten equation as,

$$I = \frac{I_{\max} [C]}{K_M^{\text{app}} + [C]} \quad (4-16)$$

$K_M^{\text{app}}$  is an apparent constant of  $K_M$ .

The experimental results of the cyclic voltammetric study are shown in Figure 4-9. The results were obtained in PBS solution using the EPD minibiosensor at +0.2 V versus the Ag/AgCl reference electrode. Table 4-2

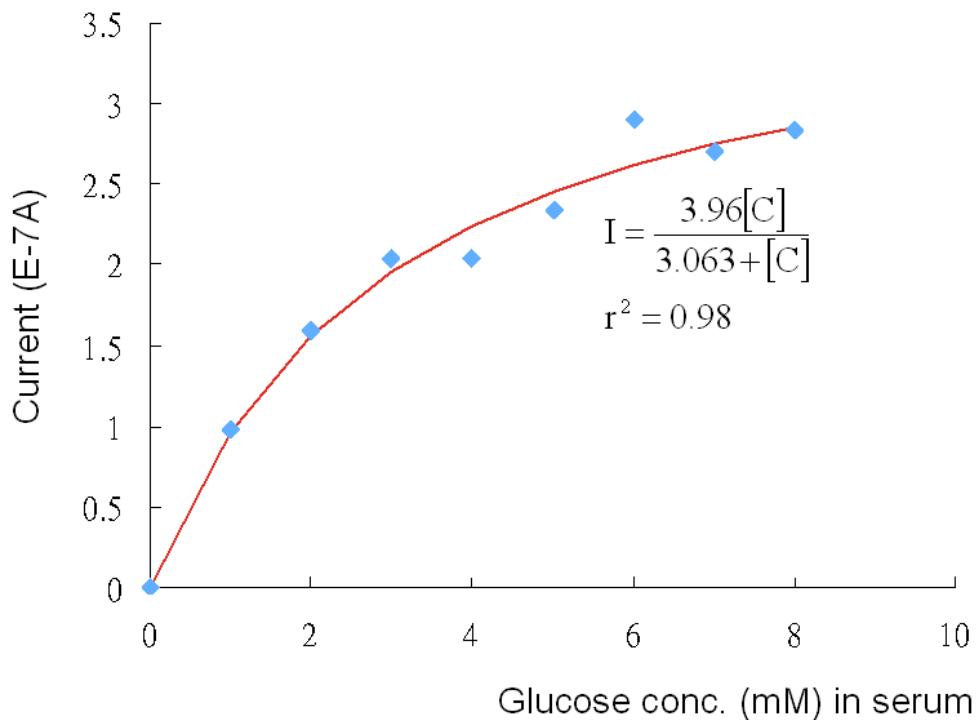
summarizes the experimental results calculated. Based on the experimental results, the values of  $I_{max}$  and  $K_M^{app}$  in the Michaelis-Menten equation (Equation 4-16) were  $9.22 \times 10^{-8} \text{ A}$  and  $2.37 \text{ mM}$  respectively.

**Table 4-2** Effect of glucose concentrations on the response currents using the EPD minibiosensor in 100mM PBS solution at +0.2V vs. printed Ag/AgCl reference electrode

Glucose concentration (mM)	Current $\times 10^8$ (A)
0.0	0.0
2.0	4.10
3.0	5.98
4.0	6.50
5.0	6.80
6.0	6.70
7.0	6.80
8.0	6.80

#### 4.5 Detecting glucose in bovine serum using the EPD minibiosensors

A critical assessment of this bi-metallic catalyst based EPD minibiosensor would be based on its performance in a physiological fluid medium, such as bovine serum. Figure 4-11 shows the current outputs of this biosensor as a function of the glucose concentrations in bovine serum.



**Figure 4-11** The current vs. glucose concentration in bovine serum solution at +0.2V vs. Ag/AgCl

A good relationship following the Michaelis-Menten mechanism between the current output of the biosensor and the glucose concentration in the bovine serum was found. Table 4-3 summarizes the relationship between the current outputs of the biosensor and the glucose concentration in bovine serum. The  $I_{max}$  value is  $0.4\mu\text{A}$  and  $K_M^{\text{app}}$  is  $3.06\text{mM}$ .

**Table 4-3** Glucose concentrations and response currents using the EPD minibiosensor in bovine serum solution

Glucose concentration (mM)	Current $\times 10^7$ (A)
0.0	0.00
1.0	1.00
2.0	1.70
3.0	2.00
4.0	1.98
5.0	2.40
6.0	2.90
7.0	2.60
8.0	2.80

The oxidation potential was +0.2V vs. the printed Ag/AgCl reference electrode. The calibration curve of glucose concentration in serum was with the calibration curve of glucose concentration in PBS solution following the Michaelis-Meten mechanism profile.

In this study, the EPD minibiosensor was used repeatedly with only standard rinsing with de-ionized water between usages. The biosensor performed well with no observable deterioration, which had been a concern for conventional immobilized GOD biosensors used in serum.

#### **4.6 Comparison of the glucose detection in PBS and bovine serum solutions using EPD minibiosensors**

The parameters in the Michaelis-Menten equation were obtained as described previously. Table 4-4 summarizes the calculated values of  $I_{max}$  and  $K_M^{app}$  in PBS and bovine serum solutions. Many reports [2, 10] of glucose biosensors used PBS solutions to simulate the whole blood or serum solution. Our studies suggest that the evaluation of glucose concentration using PBS and bovine serum fluid media showed similar Michaelis-Menten profile, but the values of  $I_{max}$  and  $K_M^{app}$  were substantially different. The  $I_{max}$  obtained in the bovine serum was significantly larger than that in PBS. This suggested that other biological species in the bovine serum may contribute to the background current at the applied potential of +0.2V versus the Ag/AgCl reference electrode. Fortunately, this background current did not interfere with the current output from the glucose concentration. Hence, this EPD minibiosensor can be used effectively for the measurement of glucose concentrations in bovine serum.

**Table 4-4** Parameters of the Michaelis-Menten Equation in PBS and bovine serum solutions

$I_{max}$ ( $\mu\text{A}$ )		$K_M^{app}$ (mM)	
PBS	bovine serum	PBS	bovine serum
0.092	0.4	2.37	3.066

#### **4.7 Long-term stability tests of the EPD minibiosensors**

One of the important characteristics of the EPD minibiosensors developed in this study are their long term stability and long shelf life.

The EPD minibiosensors were stored in a plastic bag sealed at room temperature, and ambient atmosphere for both 14 days and 160 days. The sensors were tested at different glucose concentrations in PBS solutions; the results are given in Table 4-5. The results indicate that the EPD minibiosensors performed quite well compared to the conventional GOD immobilization screen-printed mini sensors,<sup>[2, 18]</sup> which had to be stored at 4°C after they were prepared. In the latter sensors, the GOD activity would decrease substantially if they were not stored at 4°C. However, the EPD combined biosensor could be stored at 25°C and ambient atmosphere. Table 4-5 showed that the EPD minibiosensors maintained relatively high enzyme catalytic activities after 160 days shelf storage time. For instance, the current output of this biosensor decreased from +0.063 µA at day 14 in the PBS solution of 5mM glucose concentration to +0.041µA 146 days later. This showed a total current output decrease of 0.016 µA, representing a 25% decrease in enzymatic activity over 146 days in shelf-life or 0.17% per day of enzyme activity lost over the 146 days in room temperature, 25°C.

**Table 4-5** The effect of shelf storage time on long-term stability of glucose detection in PBS solution.

The current difference for detecting glucose concentration in PBS solution at 0.2V vs. printed Ag/AgCl reference electrode (at room temperature, 25°C and ambient atmosphere)

Glucose concentration (mM)	Current outputs			Current difference ( $\mu\text{A}$ )	
	Shelf storage		Shelf storage time 160 days		
	time 14 days				
2.0	0.042		0.026	0.016	
3.0	0.052		0.033	0.019	
4.0	0.058		0.040	0.018	
5.0	0.063		0.047	0.016	

## 4.8 Bibliography

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## 5. Summary

The goal of this research is to apply both thick-film microfabrication and electrophoretic deposition (EPD) technologies to produce clinically important glucose biosensors. The use of these two technologies, as well as Pt-Ir nano-metallic catalyst particles, in an electrochemical glucose minibiosensor is a major objective of this study.

The carbon nanoparticles were pretreated with nitric acid to generate –COOH and –OH functional groups, which were used to attach to the Pt-Ir alloy and the GOD enzyme. The –COOH on the surface of carbon nanoparticles could dissociate forming -COO<sup>-</sup> and H<sup>+</sup> in a buffer solution (pH7.4) The –COO<sup>-</sup> was useful for electrophoretic deposition of the particles on the microelectrode.

The Pt-Ir/carbon nanoparticles showed good electrocatalytic activity for the detection of H<sub>2</sub>O<sub>2</sub>, either by oxidation or reduction, at a relatively low applied potential as described above.

The sensitivity of the glucose minibiosensors using a Pt-Ir/carbon nanoparticle catalyst on a thick-film screen-printed active carbon based electrode was good. The H<sub>2</sub>O<sub>2</sub> oxidation current increased for the Pt-Ir/carbon nanoparticle microelectrode compared to the conventional active carbon electrode. A lower applied potential, i.e., +0.2V vs. Ag / AgCl, was required for the Pt-Ir/carbon nanoparticle glucose minibiosensor.

The Pt-Ir/carbon nanoparticles showed excellent stability, fast and reversible reactivity, less dissolution, and good biocompatibility. The carbon nanoparticles provided –COOH and –OH functional groups, which aided

electrophoretic deposition and bonding to the glucose oxidase enzyme. Thus, the Pt-Ir alloy/carbon nanoparticles were chosen as the electrocatalyst in this glucose minibiosensor.

Electrophoretic deposition of both the Pt-Ir/carbon nanoparticles and glucose oxidase in the presence of Nafion binder was used for the fabrication of the glucose minibiosensors. The resulting sensors showed good stability and selectivity for glucose detection in both PBS and serum solutions.

The electrophoretic deposition of both Pt-Ir/carbon nanoparticles and glucose oxidase (GOD) on the printed microelectrode formed the glucose minibiosensor. This new technique was relatively simple, easy, and cost-effective. The amount of Pt-Ir/carbon nanoparticles and the glucose oxidase was controlled by controlling the applied potential and the deposition time.

Preparation of the immobilized catalysts (Pt-Ir alloy carbon nanoparticles and the glucose oxidase) on the working electrode has not been reported previously. This study is the first to report the combined advantages of this approach. The electrophoretic deposition produced an even and well-mixed nanocatalyst and enzyme layer, providing the desired composition and morphology on the electrode surface. This approach led to a glucose biosensor with high stability, high response current, and high selectivity. The EPD glucose minibiosensor had a long shelf life (more than 6 months) at room temperature. This result was different from conventional biosensors that usually are stored in a refrigerator at 4C. Furthermore, the EPD technique was simple and could employ aqueous suspensions. This process is achieved with low voltages, high quality control, and

is environmentally friendly.

In general, the conventional glucose immobilized minibiosensor is used as a one-time, disposable biosensor. In contrast, the new sensors can be stored and used again. We studied the kinetics of this glucose minibiosensor in both PBS solution and serum. The results followed the Michaelis-Menten mechanism.

This third generation glucose sensor was accomplished by binding both the metallic electrocatalysts and GOD on conductive carbon nanoparticles. The Pt-Ir/carbon nanoparticles were in direct contact with the GOD and the carbon-based electrode. In this approach, a mediator was not required; the resulting metallic catalyst and enzyme were in a well-mixed configuration.

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