DEVELOPMENT OF GLUCOSE BIOSENSOR USING FERROCENE CONTAINING CARBON NANOTUBES MODIFIED GLASSY CARBON ELECTRODE

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

MAJOR IN CHEMISTRY

FACULTY OF SCIENCE

UBON RATCHATHANI UNIVERSITY

ACADEMIC YEAR 2014

UBON RATCHATHANI UNIVERSITY

THESIS APPROVAL

MASTER OF SCIENCE

MAJOR IN CHEMISTRY FACULTY OF SCIENCE

TITLE DEVELOPMENT OF GLUCOSE BIOSENSOR USING FERROCENE CONTAINING CARBON NANOTUBES MODIFIED GLASSY CARBON ELECTRODE

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ACKNOWLEDGEMENT

I would like to thank my advisor Asst. Prof Dr. Maliwan Amatatongchai for her constant advice, guidance, insight, and for sharing her extensive chemistry knowledge. I wish to thank Dr. Purim Jarujumrus and Asst. Prof Dr. Sanoe Chairum for comments and suggestions.

I also would like to thank Dr. Monpichar Srisa-art for her advice and criticism as the external examiner.

I would like to thank the Center of Excellence for innovation in Chemistry (PERCH-CIC) and Ubon Ratchathani University for financial support. My gratitude is extended to Deparment of Chemistry, Faculty of Science, Ubon Ratchathani University for providing all laboratory facilities. Above all, my deepest gratitude is given to my beloved parents for their eternal love and care throughout my life.

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ชื่อเรื่อง : การพัฒนากลูโคสไบโอเซนเซอร์โดยใช้คาร์บอนนาโนทิวบ์ที่มีองค์ประกอบของเฟอร์โรซีนมาดัดแปรขั้วไฟฟ้ากลาสซีคาร์บอน

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ศัพท์สำคัญ : กลูโคสไบโอเซนเซอร์ กลูโคสออกซิเดส โฟลอินเจคชันอะนาลิซิส สารประกอบมัลติวอลล์คาร์บอนนาโนทิวบ์เฟอร์โรซีน

งานวิจัยนี้นำเสนอวิธีการแก้ปัญหา เฟอร์โรซีนซึ่งใช้เป็นสารนำส่งอิเล็กตรอนในกลูโคสไบโอเซนเซอร์หลุดออกจากไบโอเซนเซอร์ทำให้ประสิทธิภาพของไบโอเซนเซอร์ไม่ดีจึงได้นำเสนอวิธีการตรึงเฟอร์โรซีนลงในไบโอเซนเซอร์ดังนี้ 1) ตรึงเฟอร์โรซีนในท่อนาโนคาร์บอนแบบผนังหลายชั้นที่มีหมู่อะมิโนและ 2) ตรึงเฟอร์โรซีนในไคโตซาน การสร้างกลูโคสไบโอเซนเซอร์ในงานวิจัยนี้แบ่งเป็นสองวิธี วิธีแรกสร้างโดยอาศัยการตรึงกลูโคสออกซิเดสลงบนวัสดุเชิงประกอบที่เกิดจากการสร้างพันธะโคเวเลนต์ของเฟอร์โรซีนกับท่อนาโนคาร์บอนแบบผนังหลายชั้นที่มีหมู่อะมิโน และเฟอร์โรซีนสร้างพันธะกับไคโตซาน เพื่อช่วยป้องกันการหลุดออกของเฟอร์โรซีนจากเมทริกซ์ และรักษาสมบัติของสารนำส่งอิเล็กตรอน ก่อนนำไปเคลือบบนขั้วไฟฟ้ากลาสซีคาร์บอน กลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้น (GC/CNTs-NH_2-Fc/-CS-Fc/GOx) นำมาประยุกต์ใช้เป็นตัวตรวจวัดกลูโคสแบบแอมเพอร์โรเมทรีในระบบโฟลอินเจคชันที่ใช้สารละลายฟอตเฟตบัฟเฟอร์ พีเอช 7 เป็นสารละลายตัวพา และให้ศักย์ไฟฟ้า 0.3 โวลต์ ผลการศึกษาพบว่าขั้วกลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้นให้กราฟมาตรฐานที่มีการตอบสนองแบบเส้นตรงอยู่ในช่วง 2-80 มิลลิโมลาร์ ค่าความชันเท่ากับ 5.0 นาโนแอมแปร์ต่อมิลลิโมลาร์ และค่าสัมประสิทธิ์สหสัมพันธ์ (r^2) เท่ากับ 0.997 ขีดจำกัดต่ำสุดในการตรวจวัดเท่ากับ 1.86 มิลลิโมลาร์ (S/N = 3) ค่ากระแสที่ได้จากการวัดมีความเที่ยงของการวิเคราะห์กลูโคสเข้มข้น 10 มิลลิโมลาร์ซ้ำ 10 ครั้ง (% RSD) เท่ากับร้อยละ 3.0 และสามารถวิเคราะห์ได้รวดเร็วถึง 42 ตัวอย่างต่อชั่วโมง วิธีที่สองเตรียมกลูโคสไบโอเซนเซอร์โดยอาศัยการสร้างพันธะโคเวเลนต์ของเฟอร์โรซีนกับท่อนาโนคาร์บอนแบบผนังหลายชั้นที่มีหมู่อะมิโนเพื่อช่วยป้องกันการหลุดออกของเฟอร์โรซีนออกจากเมท

ริกซ์และรักษาสมบัติของสารนำส่งอิเล็กตรอน จากนั้นเอนไซม์กลูโคสออกซิเดสจะถูกตรึงโดยอาศัยการเชื่อมไขว้ด้วยกลูตารัลดีไฮด์ในวัสดุเชิงประกอบท่อนาโนคาร์บอนแบบผนังหลายชั้นที่มีหมู่อะมิโน-เฟอร์โรซีน-โบวีนซีรัมอัลบูมิน แล้วนำไปเคลือบบนขั้วไฟฟ้ากลาสซีคาร์บอน กลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้น (GC/CNTs-NH_2-Fc-BSA-GOx) นำมาประยุกต์ใช้เป็นตัวตรวจวัดกลูโคสแบบแอมเพอร์เมทรีในระบบโฟลอินเจคชันที่ใช้สารละลายฟอตเฟตบัฟเฟอร์พีเอช 7 เป็นสารละลายตัวพา และให้ศักย์ไฟฟ้า 0.6 โวลต์ ผลการศึกษาพบว่าขั้วกลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้นให้กราฟมาตรฐานที่มีการตอบสนองแบบเส้นตรงอยู่ในช่วง 2-16 มิลลิโมลาร์ ค่าความชันเท่ากับ 323 นาโนแอมแปร์ต่อ มิลลิโมลาร์ ค่าสัมประสิทธิ์สหสัมพันธ์ (r^2) เท่ากับ 0.999 ขีดจำกัดต่ำสุดในการตรวจวัดเท่ากับ 0.65 มิลลิโมลาร์ (S/N = 3) ค่ากระแสที่ได้จากการวัดมีความเที่ยงของการวิเคราะห์กลูโคสเข้มข้น 2 มิลลิโมลาร์ ซ้ำ 7 ครั้ง (% RSD) เท่ากับร้อยละ 1.9 และสามารถวิเคราะห์ได้รวดเร็วถึง 72 ตัวอย่างต่อชั่วโมง การสร้างแอมเพอร์โรเมทรีกลูโคสไบโอเซนเซอร์ทั้งสองวิธีมีสภาพไว มีความจำเพาะเจาะจงสามารถทำซ้ำได้ตอบสนองได้รวดเร็วและง่ายต่อการเตรียมและราคาถูก

ABSTRACT

TITLE : DEVELOPMENT OF GLUCOSE BIOSENSOR USING FERROCENE CONTAINING CARBON NANOTUBES MODIFIED GLASSY CARBON ELECTROD

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DEGREE : MASTER OF SCIENCE

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KEYWORDS : GLUCOSE BIOSENSOR / GLUCOSE OXIDASE / FLOW INJECTION ANALYSIS / CARBON NANOTUBES / FERROCENE

The works presented solve of leakage of ferrocene (Fc) which is a mediator in glucose biosensor from the biosensor, making the performance of the biosensor poorly. This work presented two immobilization methods of ferrocene onto the biosensor 1) ferrocene immobilized onto the NH_2-functionalized multiwall carbon nanotubes and 2) ferrocene immobilized onto chitosan. This work consists of two methods for the construction of novel amperometric glucose biosensors. The first method is based onglucose oxidase (GOx) entrapped on composite of ferrocene covalently bound onto the NH_2-functionalized multiwall carbon nanotubes (CNTs-NH_2-Fc) and ferrocene bound chitosan (CS-Fc) to prevent ferrocene leakage from the matrix and retain its activity as an electron mediator before being coated on a glassy carbon (GC) electrode. The developed glucose biosensor (GC/CNTs-NH_2-Fc/CS-Fc/GOx) was applied in a flow injection analysis system for amperometric detection of glucose using a solution of 0.1 M phosphate buffer (pH 7.0) as a carrier and applying a potential of 0.3 V. The proposed glucose biosensor exhibits linear calibration over the range of 2-80 mM of glucose with a slope of 5.0 nA /mM and a correlation coefficient of 0.997. The limit of detection, based on a signal-to-noise ratio (S/N = 3) of three, was 1.86 mM. The developed biosensor also provides good precision (% RSD = 3.6) for glucose signal (10 mM, n = 10) with rapid sample

throughput (42 samples/h). The second method, the biosensor was developed based on ferrocene (Fc) covalently bound onto the NH_2-functionalized multiwall carbon nanotubes (CNTs-NH_2) to prevent ferrocene leakage from the matrix and retain its activity as an electron mediator. Glucose oxidase (GOx) was immobilized to ferrocene-modified NH_2-functionalized multiwalled carbon nanotubes and bovine serum albumin (CNTs-NH_2-Fc -BSA) composite film by using glutaraldehyde (Glu) before being coated on a glassy carbon (GC) electrode. The developed glucose biosensor (GC/CNTs-NH_2-Fc-BSA-GOx) was applied in a flow injection analysis system for amperometric detection of glucose using solution of 0.1 M phosphate buffer (pH 7.0) as a carrier and applying a potential of 0.6 V. The proposed glucose biosensor exhibits linear calibration over the range of 2 - 16 mM of glucose with a slope of 323 nA /mM and a correlation coefficient of 0.999. The limit of detection, based on a signal-to-noise ratio (S/N = 3) of three, was 0.65 mM. The developed biosensor also provides good precision (% RSD = 1.9) for glucose signal (2 mM, n = 7) with rapid sample throughput (72 samples/h). Both fabrication methods of amperometric glucose biosensors exhibited good reproducibility, sensitivity, selectivity, stability, fast response time and ease of preparation and low cost.

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LIST OF ABBREVIATION

CNTs-NH_2	NH_2-functionalized multiwall carbon nanotubes
CNTs-NH_2-Fc	Ferrocene covalently bound onto the NH_2-functionalized multiwall carbon nanotubes
GOx	Glucose oxidase
Glu	Glutaraldehyde
Fc	Ferrocene
BSA	Bovine serum albumin
CS	Chitosan
CS-Fc	Ferrocene bound chitosan
CV	Cyclic voltammetry
FIA	Flow Injection Analysis
GCE	Glassy carbon electrode
H	Hour
S	Second
Min	Minute
Mg	Milligram
mL	Milliter
mM	Millimolar
SD	Standard Daviation
μA	Microampare
$i_{p.a}$	Anodic peak current

CHAPTER 1

INTRODUCTION

1.1 Importancein research and development

The Development of a reliable, rapid, simple, accurate and highly sensitive biosensors for glucose detection has been attracted much attention due to a worldwide public health problem because of the increasing incidence of diabetes. This metabolic disorders result from insulin deficiency and hyperglycemia which is reflected by higher glucose concentrations in blood than the normal range of 80-120 mg/dL (4.4-6.6 mM) [1-5]. In addition, measuring the amount of glucose in body fluids including urinary and blood is necessary for diagnosis of diabetes disorder. Glucose monitoring in fermentation of food industry is also necessary as the amount of glucose in the fermentation greatly influences the quality of the food products [4].

Glucose oxidase (GOx) have been widely used in the construction of glucose biosensor due to its high selectivity to glucose and high activity over a broad range of pH values [6]. To improve the performance of GOx based biosensors, effective immobilization of GOx in a biocompatible environment and inclusion of components that intensify electron transfer between electrode and GOx are strongly required [4, 7]. Materials commonly used as a matrix improving the performance of the glucose biosensor could possessability i) to promote electron transfer, such as metal nanoparticles, carbon nanotubes (CNTs) and ferrocene (Fc) and ii) enzyme compatibility such as bovine serum albumin (BSA) and chitosan (CS) [7-12].

CNTs have attracted much attention due to their high chemical stability, high surface area, unique electronic properties, excellent electricity, and biocompatibility. These unique properties make CNTs suitable materials for the promotion of electron transfer between GOx and the electrodes surface [3-4, 8-9, 11]. CNTs can be modified to the desired properties by surface functionalization with -OH, -COOH or $-NH_2$ to improve the immobilized ability with biological substances, including enzymes, proteins or DNA.

BSA as the most abundant globular protein in plasma is naturally biodegradable/biocompatible, non-toxic and enhancement the activity of enzyme. It has been extensively used for the fabrication of biosensors [13-16].

CS contains large groups of -NH_2 and -OH and has been widely used as a modifying reagent to prepare the modified electrode due to its non-toxicity, high mechanical strength and biocompatibility. These properties make it a promising matrix for enzyme immobilization [17-21]. It is preferable to maintain the high biological activity of immobilized biomolecules and enhance the sensitivity of biosensor.

Fc and its derivatives, which are well-known as mediators, have been widely used in the development of biosensor due to their various highly desirable properties, e.g. relatively low molecular mass, reversibility, rapid responses to many electro-active substances, pH independent stability in both oxidized and reduced forms at low potentials and fast electron transfer [16, 22-23]. Nevertheless, the Fc and its derivatives modified electrodes are unstable and difficult to be controlled because of their weak adsorption on electrode surface. Fc can easily diffuse away from the electrode surface into the bulk solution when the biosensor is used continuously, which would lead to significant signal loss and greatly affect the performance and lifetime of the biosensor [3, 12].

In this work, high sensitive and rapid measurements of glucose biosensors were developed for amperometric detection in a flow injection system. This work consists of two main parts. In the first part, a glucose biosensor was developed based on immobilization of glucose oxidase (GOx) on chitosan-ferrocene (CS-Fc) hybrid and ferrocene modified NH_2- functionalized multiwalled carbon nanotubes (CNTs-NH_2-Fc) coated on a glassy carbon (GC) electrode. The developed glucose biosensor was GC/CNTs-NH_2-Fc/CS-Fc/GOx.

In the second part, a glucose biosensor was developed based on ferrocene (Fc) covalently bound onto the NH_2 functionalized multiwall carbon nanotubes (CNTs-NH_2) to prevent ferrocene leakage from the matrix and retain its activity as an electron mediator. Glucose oxidase (GOx) was immobilized to ferrocene-modified NH_2-functionalized multiwalled carbon nanotubes and bovine serum albumin (CNTs-NH_2-Fc-BSA) composite film by using glutaraldehyde (Glu) before being coated on a glassy carbon (GC) electrode. The developed glucose biosensor was GC/CNTs-NH_2-Fc-BSA-GOx.

1.2 Objectives

1.2.1 To investigate the possibility of using a developed glucose biosensor for evaluation of glucose oxidation by using cyclic voltammetry.

1.2.2 To optimize parameters effecting the sensitivity of glucose biosensor for evaluation oxidation of glucose by using cyclic voltammetry and amperometry.

1.2.3 To implement the amperometric detection on the glucose biosensor in a FIA system for evaluation determination of glucose.

1.3 Scope of Research

1.3.1 Part I: Development of glucose biosensor based on CNTs-NH_2-Fc/CS-Fc/GOx for evaluation of glucose.

1.3.1.1 The possibility of improving stability of a glucose biosensor using ferrocene modified chitosan (CS-Fc) is investigated.

1.3.1.2 Parameters that affect the sensitivity of the glucose biosensor for evaluation of glucose oxidation using cyclic voltammetry and amperometry are evaluated.

1) Optimization of pH of 0.1 M phosphate buffer solution used as a supporting electrolyte.

2) Optimization of the CNTs-NH_2-Fc composite loading.

3) Optimization of the CS-Fc hybrid concentration.

4) Optimization of the glucose enzyme concentration

5) Optimization potential for amperometric detection

1.3.1.3 Amperometric detection of glucose using the developed glucose biosensor in a FIA system will be studied.

1) Characteristics of the amperometric response assessed in terms of linear concentration range, sensitivity and limit of detection will be explored.

2) Study of interferences

3) Stability of biosensor

4) Characterization of the composites by FI-IR spectroscopy

1.3.2 Part II: Development of glucose biosensor based on CNTs-NH_2-Fc-BSA composites for evaluation of glucose.

1.3.2.1 Development of glucose biosensor were carried out by immobilizing GOx to ferrocene-modified NH_2-functionalized multiwalled carbon nanotubes and bovine serum albumin (CNTs-NH_2-Fc-BSA) composites for evaluation of the oxidation of glucose.

1.3.2.2 Parameters that affect the sensitivity of the glucose biosensor for evaluation of glucose oxidation using cyclic voltammetry and amperometry will be studied.

1) Optimization of pH of 0.1 M phosphate buffer solution used as a supporting electrolyte.

2) Optimization of the CNTs- NH_2- Fc composite loading.

3) Optimization of the glucose oxidase enzyme concentration

4) Optimization of the BSA concentration.

1.3.2.3 Studies of amperometric detection of the developed glucose biosensor in a FIA system for evaluation of glucose

1) Optimization of the potential for amperometric detection

2) Optimization of flow rate for amperometric detection of glucose in the FIA system.

3) Characteristics of the amperometric response assessed in terms of concentration range, sensitivity and limit of detection were explored

4) Study of interferences

5) Performance on real sample applications

6) Stability of biosensor.

7) Characterization of the nanocomposites by FI-IR spectroscopy

CHAPTER 2

LITERATURE REVIEWS

2.1 Biosensor

A biosensor can be defined as a compact analytical device or unit incorporating a biological or biologically derived sensitive recognition element integrated or associated with a physio-chemical transducer [24]. A biosensor is a device composed of two intimately associated elements (Figure 2.1):

(1) A bioreceptor, that is an immobilized sensitive biological element (e.g. enzyme, DNA probe, antibody) recognizing the analyte (e.g. enzyme substrate, complementary DNA, antigen). Although antibodies and oligonucleotides are widely employed, enzymes are by far the most commonly used biosensing elements in biosensors.

(2) A transducer, that is used to convert the (bio)chemical signal resulting from the interaction of the analyte with the bioreceptor into an electronic one. The intensity of generated signal is directly or inversely proportional to the analyte concentration. Electrochemical transducers are often used to develop biosensors. These systems offer some advantages such as low cost, simple design or small dimensions. Biosensors can also be based on gravimetric, calorimetric or optical detection [25].

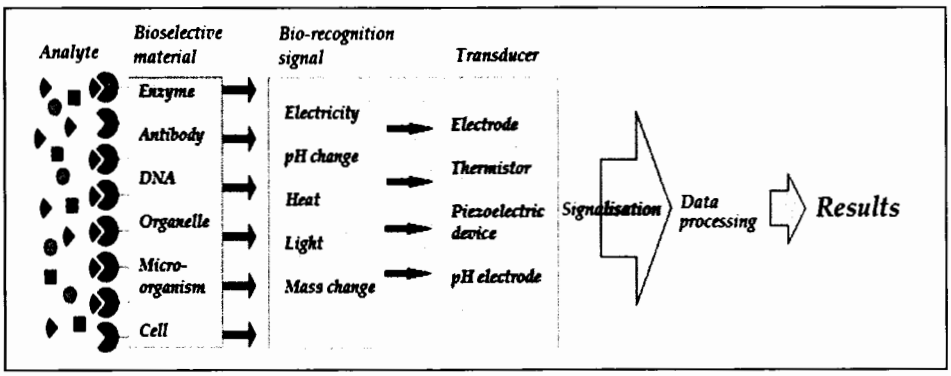


Figure 2.1 Schematic of biosensor components (bioreceptor and transducer) [26].

Biosensors are categorized according to the basic principles of signal transduction and biorecognition elements. According to the transducer elements, biosensors can be classified as electrochemical, optical, piezoelectric, and thermal sensors. Electrochemical biosensors are also classified as potentiometric, amperometric and conductometric sensors [24, 27]. The application of biosensor areas are clinic, diagnostic, medical applications, process control, bioreactors, quality control, agriculture, veterinary medicine, bacterial and viral diagnostic, drug production, control of industrial waste water and mining, military defense industry, etc [28-31]. Examples of biosensors advantages are listed below:

(1) They can measure non polar molecules that do not respond to most measurement devices.

(2) Biosensors are specific due to the immobilized system used in them.

(3) Rapid and continuous control is possible with biosensors.

(4) Response time is short (typically less than a minute).

(5) Practical use in real samples.

There are also some disadvantages of biosensors [32]:

(1) Heat sterilization is not possible because of denaturalization of biological material,

(2) Stability of biological materials (such as enzyme, cell, antibody, tissue, etc.), depends on the natural properties of the molecule that can be denaturalized under environmental conditions (pH, temperature or ions)

(3) Cells used in biosensors can become intoxicated by other molecules that are capable of diffusing through the membrane.

2.2 Enzyme immobilization

The most important step in the development of an enzyme biosensor is the stable attachment of an enzyme onto the surface of the working electrode. This process is governed by various interactions between an enzyme and an electrode material, and strongly affects the performance of the biosensor in term of sensitivity, stability, response time and reproducibility [33]. Various immobilization strategies can be envisioned: adsorption, covalence, entrapment, cross-linking or affinity (Figure 2.2) [25].

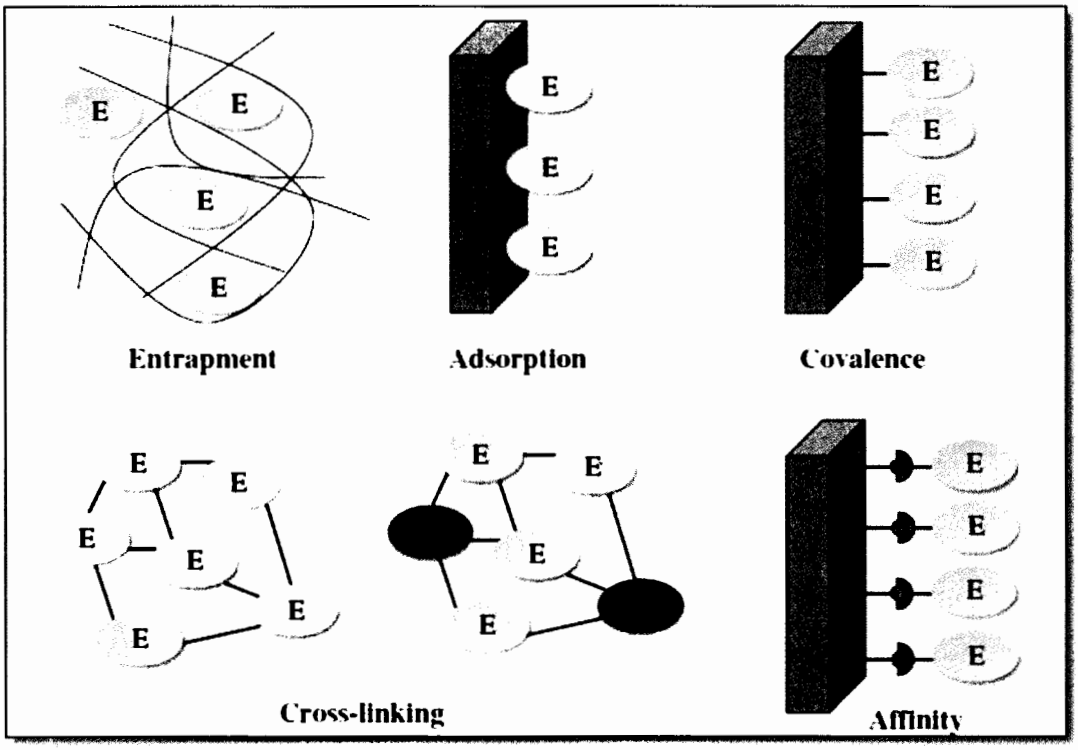


Figure 2.2 Schematic representation of the main different methods of enzyme immobilization, E:enzyme, P: inert protein [25].

2.2.1 Entrapment

For an entrapment method, generally a solution of polymeric materials is prepared containing biologic material that was entrapped onto the working electrode. The solution is coated on the electrode with various coating methods. This immobilization method is easy to perform. Enzyme, mediators and additives can be simultaneously deposited in the same sensing layer. There is no modification of the biological element so that the activity of the enzyme is preserved during the immobilization process. Biosensors based on physically entrapped enzymes are often characterized by increased operational and storage stability. However, limitations such as leaching of biocomponent and possible diffusion barriers can restrict the performances of the systems [25, 34].

2.2.2 Adsorption

The easiest and least denaturing method is physical adsorption. The procedure consists of simple deposition of enzyme onto an electrode material and attachment of the enzyme is weak bonds such as Van der Waal's forces and electrostatic and/ or hydrophobic interactions. This technique does not involve any functionalization of electrode materials or covalent links and is generally non-destructive for enzyme activity. Although this immobilization method causes little or no enzyme inactivation, this technique presents drawbacks: enzymes are loosely bound to the support and desorption of the enzyme resulting from changes in temperature, pH and ionic strength, appears to be the main problem. Thus, biosensors based on adsorbed enzyme suffer from poor operational and storage stability. Another drawback is the non-specific adsorption of other proteins or substances [25, 34-35].

2.2.3 Covalent Coupling

The most popular chemical immobilization for enzyme-based biosensors is covalent bonding of residues react with amino acid residues of the enzymes to form a monolayer of enzyme on the solid surface. Similarly, solid surfaces can be functionalised to carboxylic acid with subsequent attachment with enzymes. The carboxylic acid residues are converted to an active ester using carbodimide reagents, to acyl halides using thionyl chloride, or to a mixed anhydride by the reaction with an anhydride prior to the coupling of the enzymes on the solid support as displayed in Figure 2.3 a. Likewise, solid surfaces can be functionalised to amino groups with subsequent attachment with enzymes. The enzyme is coupled with the amino residues surface by glutaraldehydeas shown in Figure 2.3 b [35]. Covalent immobilization can be performed directly onto the transducer surface or onto a thin membrane fixed onto the transducer.

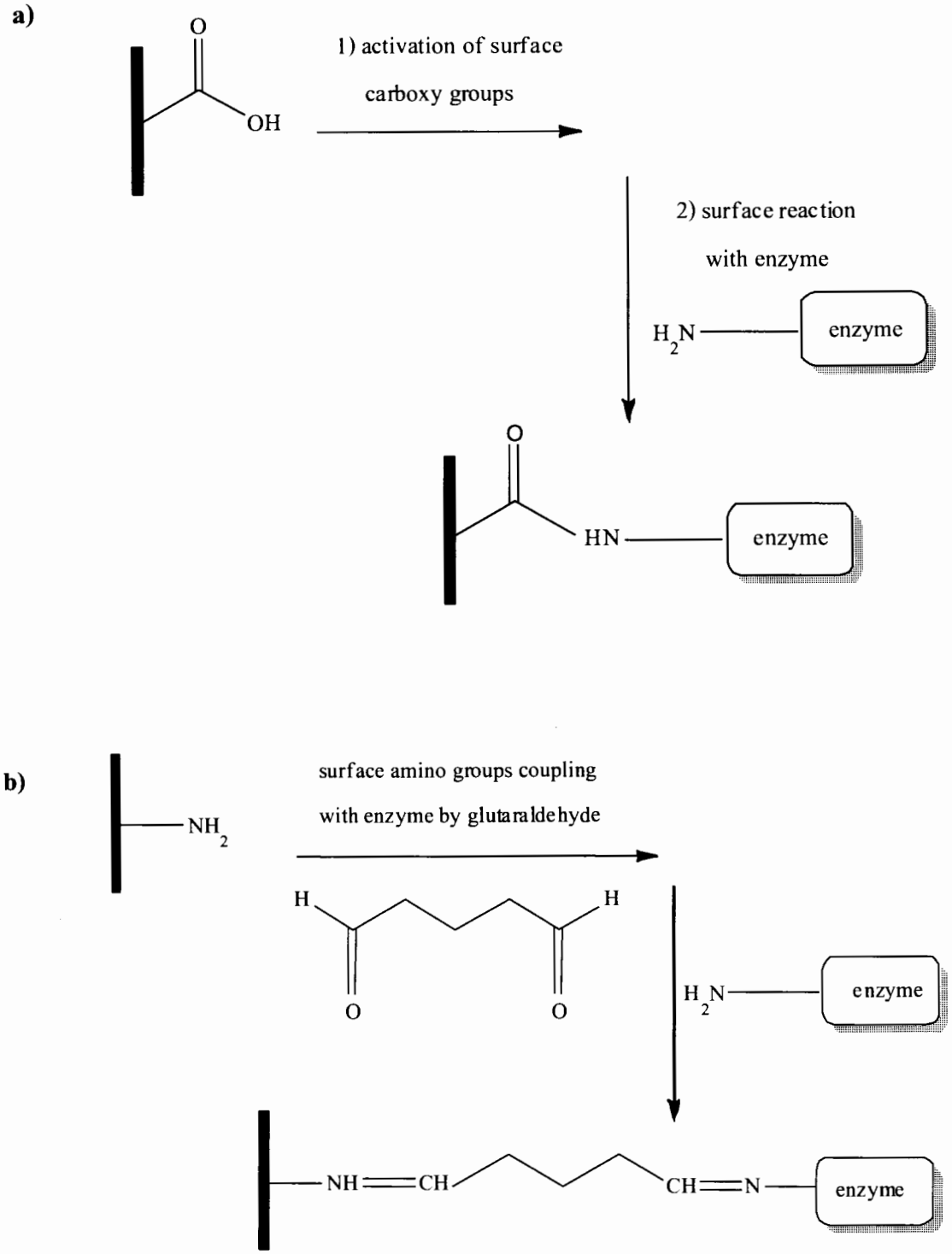


Figure 2.3 Examples of covalent linkage of enzyme layers to solid surfaces by coupling of a) carboxyand b) amino functionalities [35].

2.2.4 Cross-linking

In many cases the immobilization of enzymes has been achieved by cross-linking enzyme molecules to each other or to some functional groups on a carrier matrix. The result is a coupling one enzyme molecule to another, thus forming large matrices of enzyme molecules. The cross-linking is accomplished with bifunctional reagents, which may either contain two identical functional groups or two different functional groups. Of these reagents, glutaraldehyde is by far the most widely used. Glutaraldehyde is used to cross-link enzymes or link them to supports. It is particularly useful for producing immobilized enzyme membranes for use in biosensors by cross-linking the enzyme plus a non-catalytic diluents protein within a porous sheet [35].

The enzyme can be either cross-linked with each other or in the presence of a functionally inert protein such as bovine serum albumin. This method is attractive due to its simplicity and the strong chemical binding achieved between biomolecules. The main drawback is the possibility of activity losses due to the distortion of the active enzyme conformation and the chemical alterations of the active site during cross-linking [25].

2.2.5 Affinity

Efforts have been achieved in order to develop biosensors based on oriented and site-specific immobilization of enzymes. A strategy is to create (bio)affinity bonds between an activated support (e.g. with lectin, avidin, metal chelates) and a specific group (a tag) of the protein sequence (e.g. carbohydrate residue, biotin, histidine). This method allows to control the biomolecule orientation in order to avoid enzyme deactivation and/ or active site blocking. Several affinity methods have been described to immobilize enzymes through (strept)avidin-biotin, lectin-carbohydrate and metal cation-chelator interactions. An enzyme can contain affinity tags in its sequence (e.g. a sugar moiety) but, in some cases, the affinity tag (e.g. biotin) needs to be attached to the protein sequence by genetic engineering methods such as site-directed mutagenesis, protein fusion technology and post-transcriptional modification. Table 2.1 presents the main advantages and drawbacks of each immobilization method [25, 34].

Table 2.1 Advantages and drawbacks of the six basic immobilization methods.

	Binding nature	Advantages	Drawbacks
Adsorption	Weak bonds	- Simple and easy - Limited loss of enzyme activity	- Desorption - Non-specific adsorption
Covalent coupling	Chemical binding between functional groups of the enzyme and those on the support	- No diffusion barrier - Stable - Short response time - High enzyme activity loss	- Matrix not regenerable - Coupling with toxic product
Entrapment	Incorporation of the enzyme within a gel or a polymer	- No chemical reaction between the monomer and the enzyme that could affect the activity - Several types of enzymes can be immobilized within the same polymer	- Diffusion barrier - Enzyme leakage - High concentrations of monomer and enzyme needed for electro polymerization
Cross-link	Bond between enzyme/cross-linker (e.g. glutaraldehyde)/inert molecule (e.g. BSA)	- Simple	- High enzyme activity loss
Affinity	Affinity bonds between a functional group on a support and affinity tag on a protein sequence	- Controlled and oriented immobilization	- Need of the presence of specific groups on enzyme (e.g. His, biotin)

2.3 Glucose oxidase

One of the most important sensing materials widely used in glucose biosensors is glucose oxidase (GOx). Most of the electrochemical glucose biosensors are based on the GOx enzyme, which catalyzes the oxidation of glucose to gluconolactone which was hydrolyzed to gluconic acid in water as seen in Figure 2.4 [36].

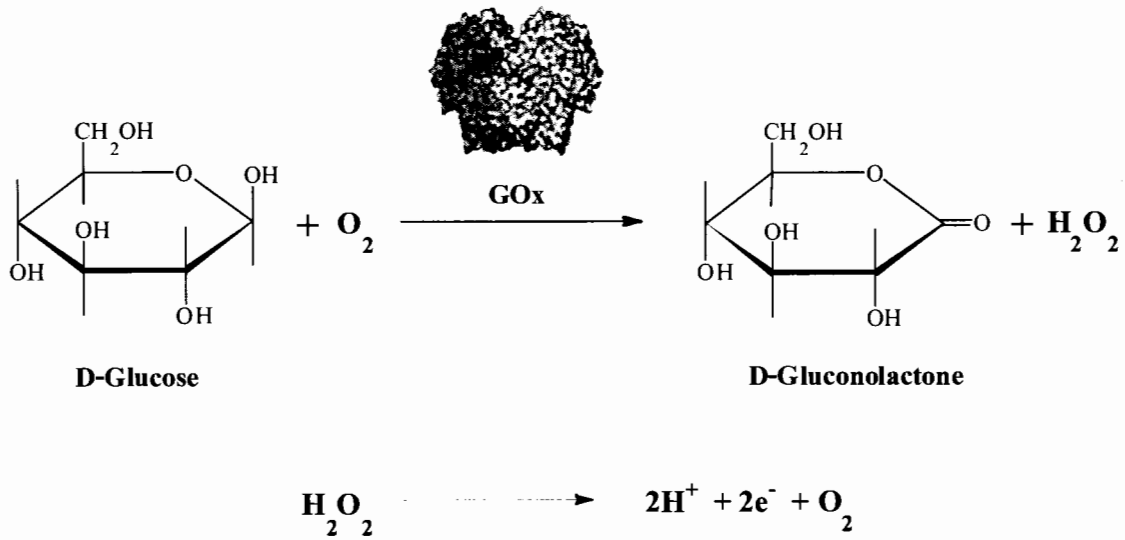


Figure 2.4 Schematic diagram of glucose oxidase (GOx) enzymatic reaction [36].

In general, GOx is selected as a model enzyme due to its being inexpensive, stable, and of practical use. The detection of glucose by electrochemical biosensors is based on the electrochemical oxidation of hydrogen peroxide generated by enzyme-catalyzed oxidation of glucose at anodic potentials (> 0.6 V vs. Ag/AgCl) [9]. Sensitivity and stability of a glucose biosensor are key features for its quantitative analysis applications. To improve the features of the biosensors, many attempts have been made, including making use of novel immobilization techniques and new enzyme immobilization materials [4].

2.4 Carbon nanotubes

Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure. Nanotubes have been constructed with length-to-diameter ratio of up to 132,000,000:1, significantly larger than for any other material [37]. The lamellar planes of sp^2 carbon in graphite sheets are organized in hexagons with a tremendously high degree delocalization of π-electron. Thus, CNTs can display metallic, semiconducting and superconducting electron transport properties [38-39]. An important advantage displayed by CNTs is their high chemical stability, high surface area, unique electronic properties, excellent electricity, metal and semiconductors, especially biocompatibility, have become suitable candidates for the promotion of heterogeneous electron transfer [3, 11]. CNTs are categorized as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). The structure of SWCNTs can be conceptualized by wrapping a one-atom-thick layer of graphite called graphene into a seamless cylinder. The way that the graphene sheet wrapped is represented by a pair of indices (n,m) called the chiral vector. The integers n and m denote the number of unit vectors along two directions in the honeycomb crystal lattice of graphene. If $m = 0$, the nanotubes are called zigzag nanotubes, and if $n = m$, the nanotubes are called armchair nanotubes. Otherwise, they are called chiral in Figure2.5 [40].

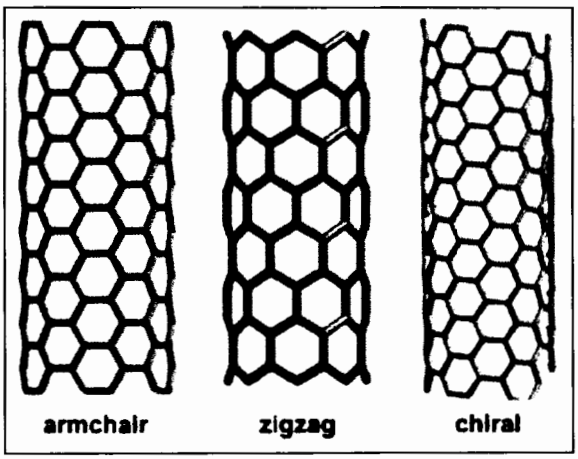


Figure 2.5 SWCNTs with different chiralities. The difference in structure is easily shown at the open end of the tubes (armchair, zigzag s and chiral structure).

Multi-walled carbon nanotubes (MWCNTs) consist of multiple rolled layers (concentric tubes) of graphite as shown in Figure 2.6. The interlayer distance in multi-walled carbon nanotubes is close to the distance between graphene layers in graphite, approximately 3.4 Å [41]. MWCNTs have found enormous number of applications in many areas including electrochemical devices, composite-polymer materials, gas storage, and in the area of sensors and biosensors.

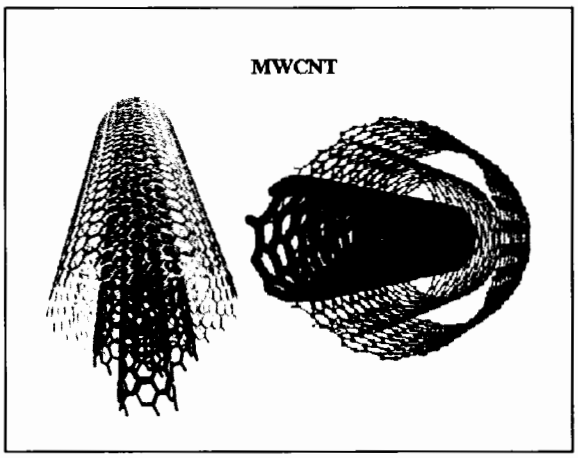


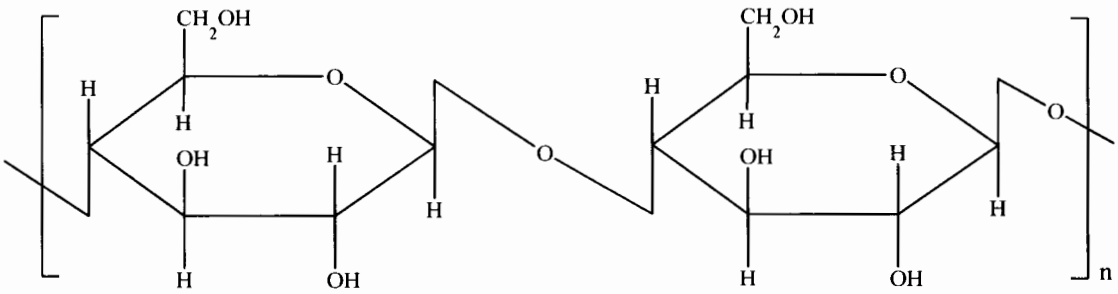
Figure 2.6 Structure of multi-walled carbon nanotubes (MWCNTs).

2.5 Chitosan

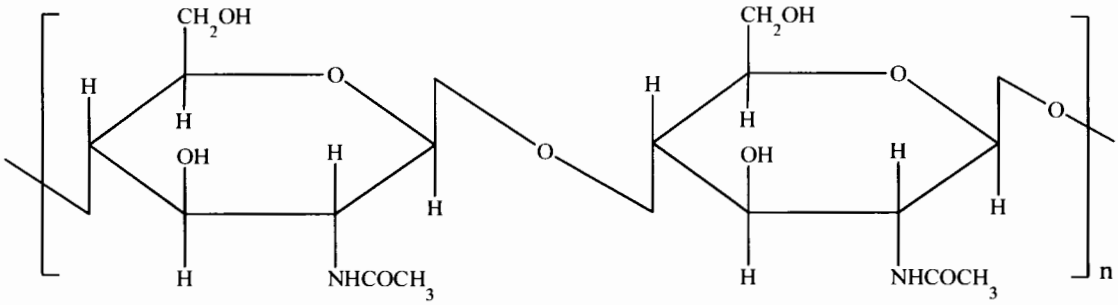
Chitosan is a polysaccharide extracted from the shells of crustaceans, such as shrimp, crab and other sea crustaceans, including pandalus borealis and cell walls of fungi. Chemical name is 2-amino-2-deoxy-b-D-glucopyranose.molecular formula is $(C_6H_{11}O_4N)_n$.

Chitosan is also known as soluble chitin. Chitin consists mainly of unbranched chains of beta-(1⟶4)-2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine). It is similar to cellulose, in which the C-2 hydroxyl groups are replaced by acetamido residue. Chitin is practically insoluble in water, dilute acids, and alcohol, with variation depending on product origin. Chitosan, the partially deacetylated polymer of *N*-acetyl-D-glucosamine, is water-soluble. Structure of the chitin molecule (Figure 2.7), showing two of the *N* –acetyl glucosamine units that repeat to form long chains in beta-1, 4 linkage. Structure of the chitosan shown in Figure 2.7

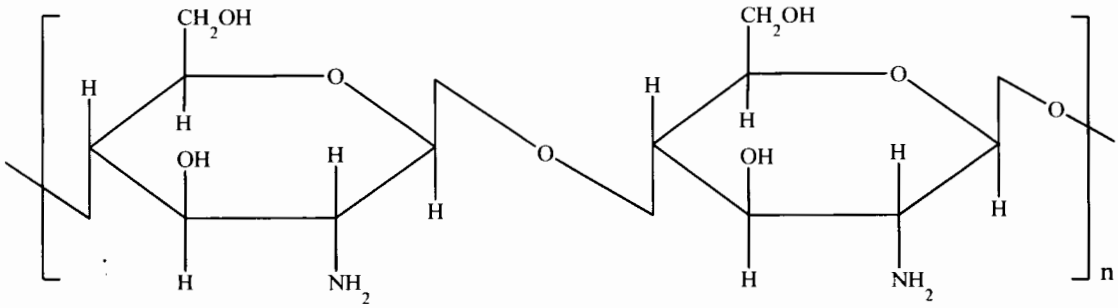
composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit) [42-43].



Cellulose



Chitin



Chitosan

Figure 2.7 Structures of cellulose, chitin and chitosan.

2.5.1 Properties of chitosan

Most of the naturally occurring polysaccharides e.g. cellulose, dextran, pectin, alginic acid, agar, agarose and carragenans, are neutral or acidic in nature, whereas chitin and chitosan are examples of highly basic polysaccharides. Their unique properties include polyoxysalt formation, ability to form films, chelate metal ions and optical structural characteristics. Chitosan, the deacetylated shells obtained as a food industry waste is product of chitin, the amino group in chitosan has a pKa value of ~ 6.5, which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the % DA-value. Chitosan is soluble in dilute acids such economically feasible, especially if it includes as acetic acid, formic acid, etc [43].

Chitosan was selected as a matrix for immobilization of an enzyme because of the unusual combination of its properties, which includes an excellent membrane-forming ability, high permeability toward water, good adhesion, biocompatibility, non-toxicity, high mechanical strength and biocompatibility, making it a promising matrix for enzyme immobilization [21, 44-45].

2.6 Bovine serum albumin

Bovine serum albumin (BSA) is a highly abundant protein in blood of all mammals [46]. BSA has several known benefits, including good stability, low cost, non-toxicity and enhancement the activity of enzyme. It is often used as a blocking agent of immunoblots during western blotting to reduce non-specific binding, and as model protein applied in enzyme-linked immunosorbent assay and protein micro-array technology. Therefore, the assembly of BSA and other proteins on carriers or electrodes becomes increasingly important for the fabrication of biosensors anddevelopment of protein-based devices.

Despite the fact that BSA has many advantages, the two main problems still exist in the fields of protein electrochemistry. (1) Strong adsorption of BSA and its oxidized products on the electrode surfaces leads to an unpredictable and irreproducible depression of electrochemical signals. (2) Electron transfer reaction between BSA and electrode is relatively poor due to lack of redox-active centers [47].

2.7 Mediator

Mediators are artificial electron transferring agents that can readily participate in the redox reaction with a biological component and thus help in the rapid electron transfer. There is a low molecular weight redox couple, which shuttles electrons from the redox center of an enzyme to the surface of an indicator electrode. During the catalytic reaction, the mediator first reacts with the reduced enzyme and then diffuses to the electrode surface to undergo rapid electron transfer. For example:

$$\text{Glucose} + \text{FAD} + H_2O \longrightarrow \text{Gluconic acid} + FADH_2 \quad (2.1)$$

$$FADH_2 + M_{ox} \longrightarrow FAD + M_{red} + 2H^+ \quad (2.2)$$

$$\text{At electrode: } M_{red} \longrightarrow M_{ox} \quad (2.3)$$

The rate of production of the reduced mediator (M_{red}) is measured amperometrically by oxidation at the electrode.

A mediator is expected to be stable under required working conditions and should not participate in the side reactions during electron transfer. The mediator should be used in such a way that it has a lower redox potential than other electrochemically active interferences in samples. The redox potential of a suitable mediator should provide an appropriate potential gradient for electron transfer between enzyme's active site and electrode. The redox potential of the mediator (compared to the redox potential of enzyme active site) should be more positive for oxidative biocatalysis or more negative for reductive biocatalysis.

Characteristics of an ideal mediator [23]

(1) It should be able to react rapidly with the reduced enzyme.

(2) It should exhibit reversible heterogeneous kinetics.

(3) The over potential for the regeneration of the oxidized mediator should be low and pH independent.

(4) It should have stable oxidized and reduced forms.

(5) The reduced form should not react with oxygen.

Advantages of using mediators [23]

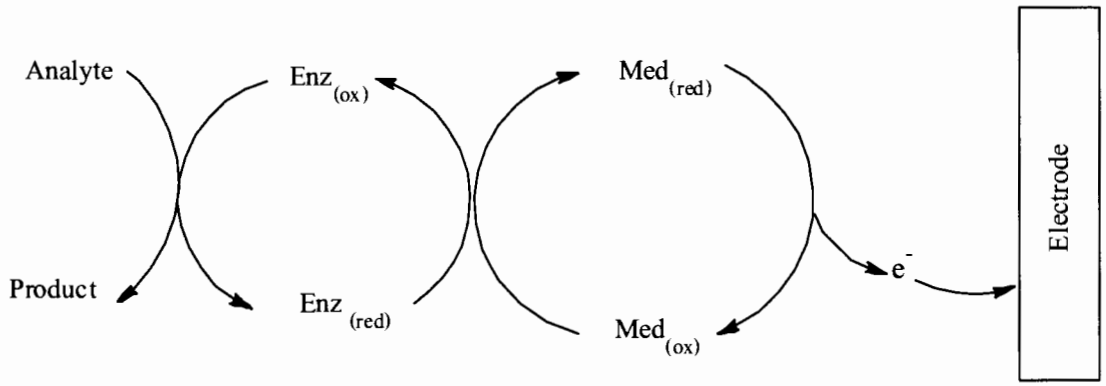
(1) Measurements are less dependent on oxygen concentration.

(2) The working potential of the enzyme electrode is determined by the oxidation potential of the mediator.

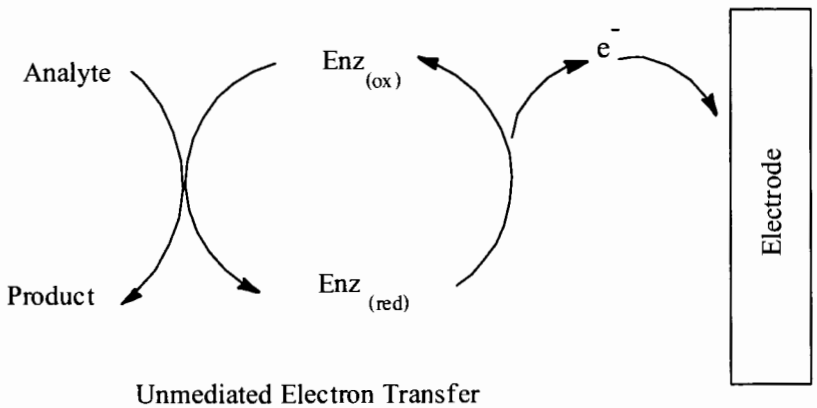
(3) With the use of mediators at low oxidation potentials, interferences can be avoided.

(4) If oxidation of reduced mediator does not involve protons, it can make the enzyme electrode relatively pH insensitive.

Electron transfer in electrochemical biosensors is based on mediated or unmediated electrochemistry as shown in Figure 2.8.



Mediated Electron Transfer



Unmediated Electron Transfer

Figure 2.8 The scheme of mediated and unmediated electron transfer in electrochemical biosensor [23].

Ferrocene (Fc) and its derivatives, ferricyanide, methylene blue, benzoquinone and *N*-methyl phenazine etc. are attractive organometallic compounds due to their sandwich structure resulting in interesting physical and chemical properties [48]. Fc and its derivatives have been used as mediators in the development of enzyme-based biosensor, due to their properties of relatively low molecular mass, rapid responses to many electroactive substances, being pH-independent, stability in both oxidized and reduced forms at low potential and having fast electron transfer [22-23, 49]. The structure of ferrocene is shown in Figure 2.9.

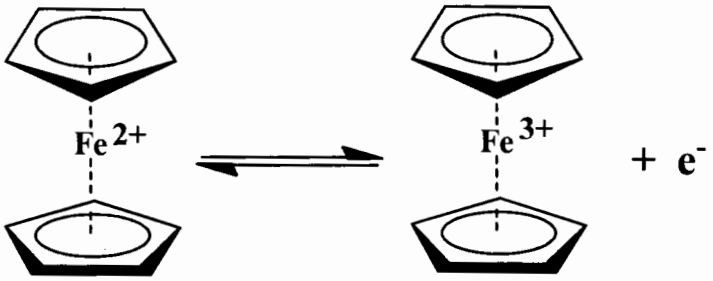


Figure 2.9 Structure of ferrocene.

2.8 Electrochemical biosensors

Electrochemical biosensors are the most commonly used class of biosensors. These are based on the fact that during a bio-interaction process, electrochemical species such as electrons are consumed or generated producing an electrochemical signal which can in turn be measured by an electrochemical detector. Electrochemical biosensors have been widely accepted in biosensing devices. These biosensors can be operated in turbid media, have comparable instrumental sensitivity and are more amenable to miniaturization [23].

2.8.1 Amperometry

Amperometry is operated at a given applied potential between the working electrode and the reference electrode, and the generated signal is correlated with the concentration of target compounds. In the amperometric detection, the current signal is generated as a function of the reduction or oxidation of an electro-active product on the surface of a working electrode [23].

Amperometric detectors, which are the most commonly used, are of the three-electrode type. The potential of working electrode is set relatively to a reference electrode, the iR drop between the working and counter electrodes is compensated for using the potentiostat and current flowing through the working electrode is the measured signal. Current, which is in the pA to μA range, is amplified and recorded as a function of the time flow of the mobile phase. This gives the concentration-time profile or chromatogram of the analyte in the effluent [50]. The principle of amperometric detection is outlined in Figure 2.10.

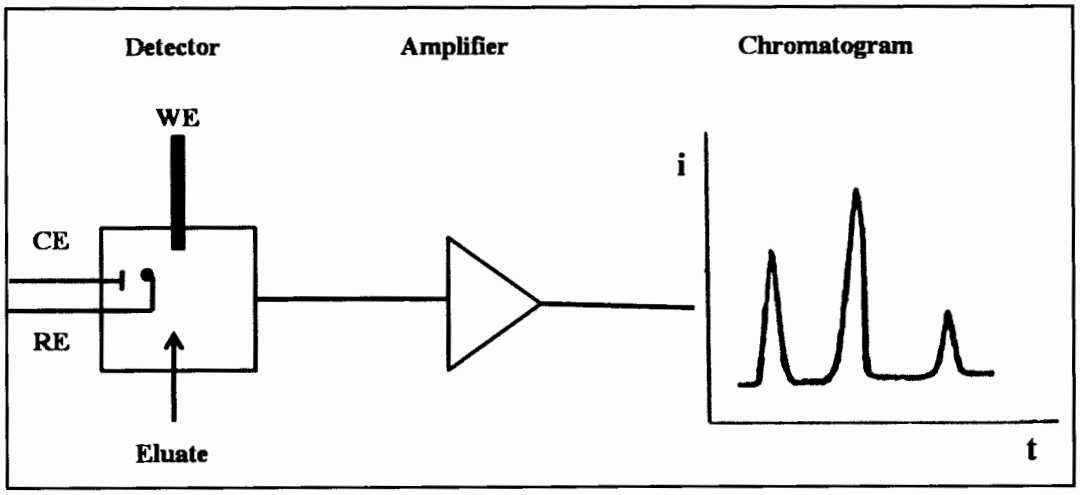


Figure 2.10 Outline of amperometric detection. WE - working electrode; CE - counter electrode; RE - reference electrode. Adopted from [50].

2.8.2 Voltammetry

Voltammetry is the most versatile technique in electrochemical analysis. Both current and potential are measured. The position of peak current is related to the specific chemical and the peak current density is proportional to the concentration of the corresponding species. A remarkable advantage of voltammetry is the low noise which can endow the biosensor with higher sensitivity. In addition, voltammetry is able to detect multiple compounds, which have different peak potentials, in a single electrochemical experiment (or scan), thus offering the simultaneous detection of multiple analytes. The voltammetric technique is one of the most sensitive electro-analytical methods [23].

2.8.2.1 Cyclic voltammetry

Cyclic voltammetry is one of the most widely used forms and it is useful to obtain information about the redox potential and electrochemical reaction rates (*e.g.* the chemical rate constant) of analyte solutions. In this case, the voltage is swept between two values at a fixed rate, however, when the voltage reaches V_2 the scan is reversed and the voltage is swept back to V_1, as is illustrated in Figure 11 a. The scan rate, $(V_2 - V_1)/(t_2 - t_1)$, is a critical factor, since the duration of a scan must provide sufficient time to allow for a meaningful chemical reaction to occur. Varying the scan rate, therefore, yields correspondingly varied results.

The voltage is measured between the reference electrode and the working electrode, while the current is measured between the working electrode and the counter electrode. The obtained measurements are plotted as current vs. voltage, also known as a voltammogram, as is illustrated in Figure 11 b. As the voltage is increased toward the electrochemical reduction potential of the analyte, the current will also increase. With increasing voltage toward V_2 past this reduction potential, the current decreases, having formed a peak as the analyte concentration near the electrode surface diminishes, since the oxidation potential has been exceeded. As the voltage is reversed to complete the scan toward V_1, the reaction will begin to reoxidize the product from the initial reaction. This produces an increase in current of opposite polarity as compared to the forward scan, but again decreases having formed a second peak as the voltage scan continues toward V_1. The reverse scan also provides information about the reversibility of a reaction at a given scan rate [51]. The cyclic voltammetry system consist of three-electrodes including, the potential is applied to the working electrode (WE) with respect to a reference electrode (RE), and counter electrode (CE) is used to complete the electrical circuit, as is illustrated in Figure 11 c. A working electrode is typically made of noble metals (platinum or gold) or carbon (i.e., glassy carbon). The counter electrode usually uses a Pt wire. Two commonly used reference electrodes are Ag/AgCl electrode (E_o = 0.20 V vs. SHE) and saturated calomel electrode (SCE, E_o = 0.25 V vs. SHE) [52].

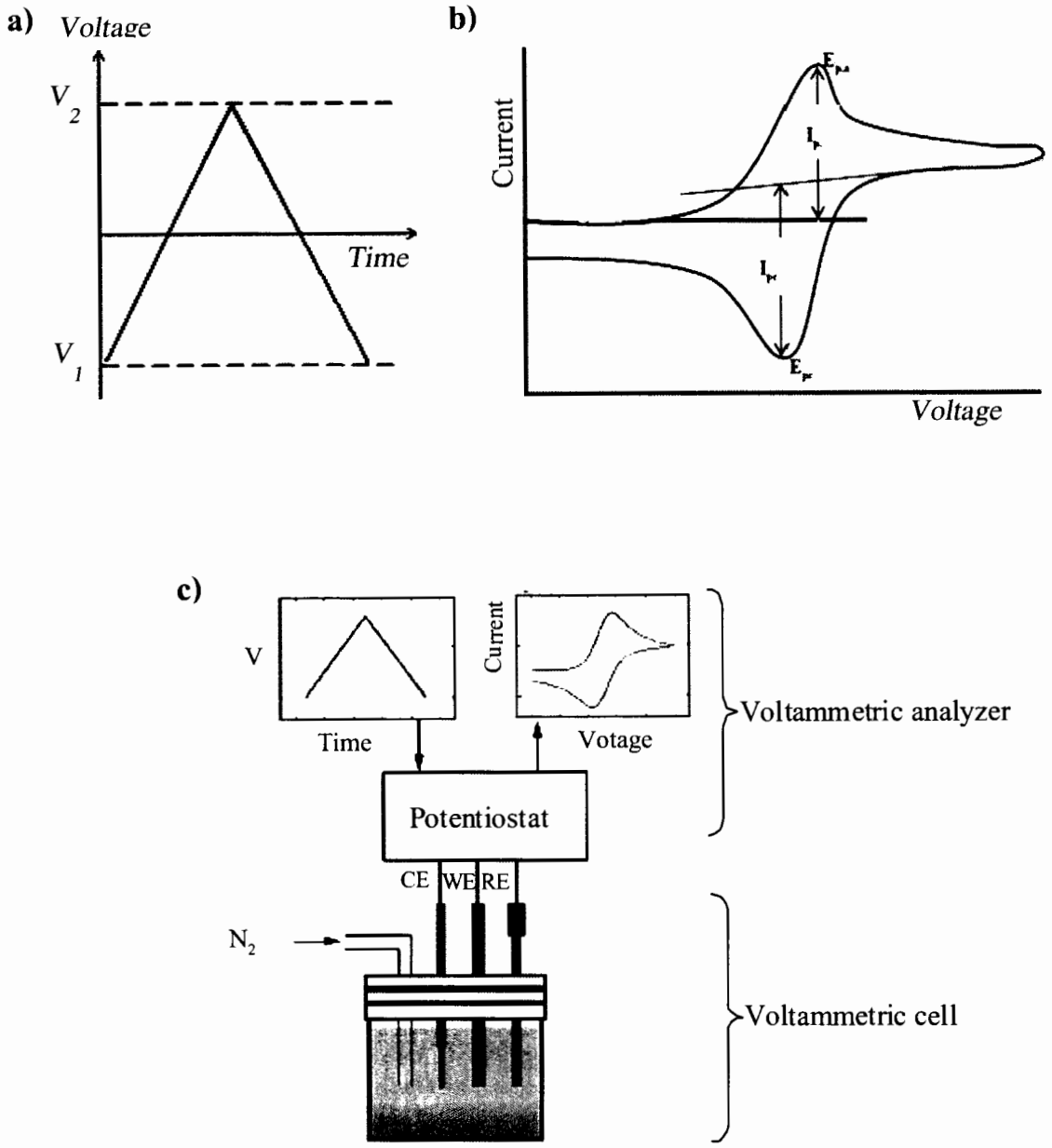


Figure 2.11 Typical of a) cyclic voltammogram wave from: variation of the potential applied to the working electrode with time, b) cyclic votammogram: resulting current-potential curves, c) voltammetric or components of cyclic voltammetry systems which composed of voltammetric analyzer and voltammetric cell adopted from [53].

2.9 Flow injection analysis

Flow injection analysis (FIA) is based on the injection of a liquid sample into a moving, nonsegmented continuous carrier stream of a suitable liquid. FIA is a simple, rapid and versatile technique that is now firmly established, with widespread application in quantitative chemical analysis. The designation of FIA was proposed in 1975 by Ruzicka and Hansen [54]. The inclusion of the term injection in the name of this technique occurred because the technique originally entailed using a syringe to inject a sample through a septum into a reagent flow.

FIA may be defined as the sequential insertion of discrete sample solution into an unsegmented continuously flowing stream with subsequent detection of the analyte [55]. Even this definition, however, is frequently made obsolete by new developments. The first definition, given by Ruzicka and Hansen [54] was "A method based on injection of a liquid sample into a moving unsegmented continuous stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the absorbance, electrode potential, or other physical parameter as it continuously changes due to the passage of the sample material through the flow cell [56]. This definition, however, was soon considered obsolete and was revised to describe a technique for information gathering from a concentration gradient formed from an injected, well-defined zone of a fluid, dispersed into a continuous unsegmented stream of a carrier in order to accommodate new developments in stopped-flow FIA, merging zones, zone sampling and other gradient techniques. This new definition was soon challenged by FI systems which were segmented in one way or another, or which dealt with samples eluted from columns without well-defined boundaries. Furthermore, Fang [57] defines FIA as A flow analysis technique performed by reproducibly manipulating sample and reagent zones in a flow stream under thermodynamically non-equilibrated conditions.

The simplest flow injection analyzer (Figure 2.12 (a)) consists of a pump, which is used to propel the carrier stream through a narrow tube; an injection port, through which a well-defined volume of a sample solution S is injected into the carrier stream in a reproducible manner; and a microreactor in which the sample zone disperses and reacts with the components of the carrier stream, forming a species which is sensed by a flow through detector and recorded. A bypass loop allows passage of carrier when the injection valve is in the load position. A typical recorder output has the form of a peak (Figure 2.12 (b)) the height H, width W, or area A of which

is related to the concentration of the analyte. The time span between the sample injection S and the peak maximum, which yields the analytical readout as peak height H, is the residence time t during which the chemical reaction takes place. A well-designed FIA system has an extremely rapid response, because T is in the range 5 - 20 s. Therefore, a sample cycle is less than 30 s (roughly $T + t_b$) and thus, typically, two samples can be analyzed per minute. The injected sample volumes may be between 1 and 200 mL (typically 25 - 50 mL), which in turn requires no more than 0.5 mL of reagent per sampling cycle. This makes FIA a simple, automated microchemical technique, capable of a high sampling rate and minimum sample and reagent consumption.

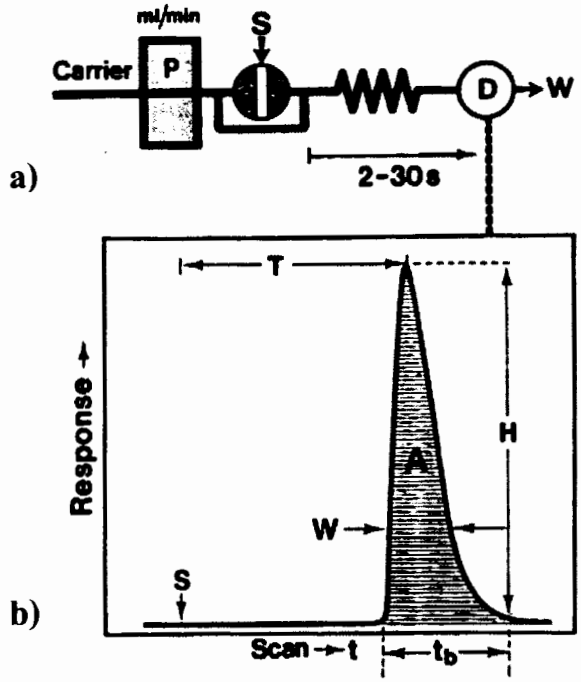


Figure 2.12 a) The simplest single line FIA manifold utilizing a carrier stream of reagent; S is the injection port, D is the flow cell, and W is the waste. b). The analog output has the form of a peak, the recording starting at S (time of injection to). H is the peak height, W is the peak width at a selected level, and A is the peak area. T is the residence time corresponding to the peak height measurement, and t_b is the peak width at the baseline [56].

It follows from the preceding discussion that FIA based on a combination of three factors: sample injection, controllable dispersion of the injected sample zone and reproducible timing of the injected sample through the flow system [56]. Except for detector warm-up, the system is ready for instant operation as soon as the sample is introduced.

FIA offers several advantages in term of considerable decrease in sample (normally using 10 to 50 μL) and reagent consumption, high sample throughput (50 to 300 samples per hour) reduced residence times (reading time is about 3 to 40 s), shorter reaction times (3 to 60 s), easy switching from one analysis to another (manifolds are easily assembled and/or exchanged), reproducibility (usually less than 2% RSD), reliability, low carry over, high degree of flexibility, and ease of automation. Perhaps the most compelling advantage of the FIA technique is the great reproducibility in the results obtained by this technique that can be setup without excessive difficulties and at very low cost of investment and maintenance [55]. These advantages have led to an extraordinary development of FIA, unprecedented in comparison to any other technique.

2.10 Related Research

In 2003, Wang, S. G. et al. [4] presented the bio-electrochemical characteristics of a novel multi-walled carbon nanotube (MWCNTs)-based biosensor for glucose detection. The MWCNTs-based biosensor was studied and compared with those of glassy carbon (GC)-based biosensor. The MWCNTs based biosensor exhibits a strong glucose response at applied potentials of 0.65 and 0.45 V vs Ag/AgCl, respectively, while a GC-based biosensor shows a weak glucose response at 0.65 V and no response at 0.45 V. Besides, the MWCNTs-based biosensor shows a high stability of 86.7% of the initial activity to glucose after four-month storage. This response current was about 37.2% higher than the corresponding value obtained from a GC-based biosensor. MWCNTs based enzyme electrodes were prepared in the following steps. After MWCNTs was grown on Si substrate, a thin gold film was evaporated on the top surface of the MWCNTs. Si substrate was subsequently etched away in a mixture of nitric acid (HNO_3) and hydrofluoric acid (HF) solution at a volumetric ratio of 1:3. After the Si substrate was removed, the retained MWCNTs vertically adhered to the Au film with good adhesion. The Au/ MWCNTs electrode was immersed in a 0.1 M phosphate buffer solution containing glucose oxidase (GOx) to form a structure of Au/MWCNTs-GOx.

In 2004, Luo, X.-L. et al. [58] developed an amperometric biosensor for quantitative measurement of glucose. The biosensor is based on a bio-composite which is homogeneous and easily prepared. This bio-composite is made of chitosan hydrogel, GOx and gold nanoparticles (AuNPs) by a direct and facile electrochemical deposition method under enzyme-friendly conditions. The resulting biocomposite provided a shelter for the enzyme to retain its bioactivity at considerably extreme conditions, and the decorated AuNPs in the biocomposite offer excellent affinity to enzyme.

In 2005, Liu, Y. et al. [11] presented fabrication of glucose biosensors based on GOx entrapped in the composite of CNTs/CS and direct electron transfer reaction between GOx and electrode. The preparation of GOx/CNTs/CS/GC electrode was as following, 2 mg of CNTs and 1 mL of 1% CS solution were firstly mixed with ultrasonic agitation over 15 min. After that, the viscous and black suspension was mixed thoroughly with appropriate amount of GOx solubilized in pH 7.5 PBS. Next, 5 μL of the GOx/CNTs/CS mixture was spread evenly onto the GC electrode surface with syringe. Finally, to get more uniform films, the modified electrode was covered with a small bottle and allowed to dry for over 24 h at 4 °C. Then an adherent and robust film electrode containing GOx/CNTs/CS was obtained. Electrochemical experiments were performed by using a CHI 660B Model electrochemical analyzer with three electrodes system. The modified electrodes were the working electrode, coiled platinum wire as a counter electrode and Ag/AgCl (saturated KCl) as a reference electrode. The electrochemical detection based on cyclic voltammetry and chronamperometry measurements were performed in an electrochemical cell containing 0.1M PBS pH 7.5, 5 mM glucose and 0.5 mM FMCA under nitrogen purge to remove oxygen. Results from electrochemical behavior of GOx/CNTs/CS/GC electrode demonstrate good bioactivity of GOx. Therefore, the developed electrode can be used as an amperometric biosensor for glucose detection using 0.5 mM FMCA added to supporting electrolyte as the mediator. These developed electrodes displayed satisfy sensitivity and better stability.

In 2006, Tripathi, V. S. et al. [16] proposed method for prevent of mediator leakage from the matrix. This paper demonstrated a novel approach for both encapsulation of the mediator in ormosil and improvement of the conductivity using both ferrocene monocarboxylic acid-bovine serum albumin (FMC–BSA) conjugate and multiwall carbon nanotubes (MWCNTs). FMC-BSA

conjugate was prepared using a carbodiimide coupling reaction using a molar ratio of 1:30 (BSA: FMC). The FMC–BSA/MWCNTs/ormosil composite was prepared by mixing 125.0 μL double distilled water with 25.0 μL 4% PEG, 17.0 μL sol–gel precursors APTES, 9.0 μL Epoxy, 4.0 μL 25 mg/mL MWCNTs suspension, 10.0 μL FMC–BSA conjugate (2.64 mg protein/mL) and 2.0 μL of 0.1 M HCl solution sequentially. 3.0 μL of the resulting homogeneous solution was cast on well-polished glassy carbon electrode surface, which was kept at room temperature (28 °C) for 4 h and 4 °C for 20 h for the formation of FMC BSA/MWCNTs/ormosil composite film. The formed ormosil film was washed with PBS and stored at 4 °C till using. Results from electrochemical behavior of FMC BSA/MWCNTs/ormosil composite are highly useful for the bioelectrochemical/ biosensing applications. This composite possesses excellent conductive, entrapment and biocompatible properties and the doped ferrocene group can act as electron transfer mediator for preparation of reagent less biosensors.

In 2009, Qiu, J.-D. et al. [12] reported a simple electrochemical approach to controllable fabrication of a biosensor with a homogeneous CS-Fc/Au NPs/GOx nano-composite film. The preparation of CS-Fc/AuNPs/GOx modified electrode is as follows: firstly, Fc covalently bound CS was prepared by dissolving CS (75.0 mg) in 0.1 M acetic acid solution (15 mL). FcCHO (20 mg) was dissolved in methanol (15 mL) and added to the CS solution. After the mixture was stirred at room temperature for 2 h, $NaCNBH_3$ (100 mg) was added and the reaction mixture was further stirred for another 24 h. The reaction was quenched by precipitation with 5% NaOH and the yellow product was exhaustively washed with ethanol and water. The product was dried in air and finally dispersed in 0.2 M acetate buffer (pH 5.0) using sonication. Secondly, polished GC was immersed in the deposition solution containing 2.0 mL CS-Fc (0.5 mg/mL), Au NPs (0.06 mg/mL) and GOx (4.0 mg/mL), and a constant potential of + 1.5 V was applied for 120 s. When the pH was higher than 6.3, CS became insoluble, and CS hydrogel incorporated Au NPs and enzyme was electrodeposited at the electrode surface. Finally, the modified electrode was removed from the solution and rinsed with water, then dried in air at room temperature for about 3 h. The results showed that the redox polymer, the leakage of both enzyme and mediator was effectively prevented by using CS-Fc. Moreover, its activity can be retained by controllable electro-deposition method.

In 2009, Qiu, J.-D. et al. [3] presented an amperometric biosensor for glucose was developed by entrapping GOx in a new composites of MWCNTs-Fc and CS. Ferrocenecarboxaldehyde (FcCHO) was covalently bound onto the MWCNTs with ethylenediamine (EDA) as cross-linker to achieve Fc-MWCNTs conjugate. The prepared MWCNTs-Fc/CS composites possessed high surface area, good mechanical stability, and good conductivity, which provided a compatible microenvironment for maintaining the activity of the immobilized enzyme and mediator, increased the mediator loading, and more importantly prevented the leakage of mediator. Preparation of MWCNTs-Fc nanocomposite by the amount of 15 mg of MWCNTs was prepared by dispersing in 5 mL doubly distilled water with ultrasonicating for 10 min, and then added to the FcCHO aqueous solution (10 mL, 7 mg/mL) with stirring at room temperature. After 1 h, $NaCNBH_3$ (200 mg) was added in the mixture, and left stirring for 24 h. Then the MWCNTs-Fc nanocomposites were centrifuged, and washed with methanol and water several times to remove the dissociative FcCHO or any physically adsorbed FcCHO from the surface of MWCNTs. Finally, the MWCNTs-Fc nanocomposites were dispersed in doubly distilled water with ultrasonicating. The authors have demonstrated that the MWCNTs-Fc nanocomposites can be effectively employed for fabrication of the reagent less glucose biosensor. This composite not only provides a very suitable environment for enzyme entrapment but also establishes efficient electronic communication between GOx and the electrodes.

In 2010, Zhilei, W. et al. [7] presented an ingenious approach for the fabrication of a promising glucose sensor, GOx/C_{60}Fc-CS-IL, that exploits the synergistic beneficial characteristics of fullerene (C_{60}), ferrocene (Fc), chitosan (CS) and ionic liquid (IL) for glucose oxidase (GOx). The biosensor prepared by polishing glassy carbon (GC) electrode (3 mm in diameter) with 0.3 and 0.05 μm alumina powder, and sonicating in a 6.0 M HNO_3/H_2O and $EtOH/H_2O$ for 20 min, respectively. Then, 40 μL of the C_{60} solution was coated onto the surface of the pretreated GC using a micro syringe and dried in a stream of hot air (50 °C). Next, 0.1 mL of the Fc solution was well mixed with0.1 mL of the IL solution, and 2 μL of the mixture solution was then coated on the surface of C_{60}-GC. After that, 50 μL the IL was well dispersed in 0.2 mL of the CS solutions. After the mixture was sonicated for 30 min, 5 μL of the mixture was coated on the surface of the C_{60}-Fc-GC. GOx stock solution was prepared by dissolving 10 mg of GOx in 1.0 mL of pH 7.0 PBS. 8 μL of the enzyme solution was dropped onto the surface of C_{60}-Fc-CS-

IL-GC to fabricate the glucose biosensor. The modified electrode exhibits stable and reversible electrochemistry, it can be used as electron transfer mediator to shuttle electrons between GOx and the modified electrode.

In 2011, Gomathi, P. et al. [8] presented the new method for fabrication of glucose biosensor based on multiwalled carbon nanotubes (MWCNTs) grafted chitosan (CS) nanowire (NW) was prepared by phase separation method. GOx was sequentially immobilized into MWCNTs-CS-NW to obtain MWCNTs-CS-NW/GOx biosensor. The MWCNTs-CS-NW/GOx biosensor shows an excellent performance for glucose at + 0.34 V with a high sensitivity (5.03 A/mM) and lower response time (3 s) in a wide concentration range of 1-10 mM (correlation coefficient of 0.9988). In addition, MWCNTs-CS-NW/GOx biosensor possesses good reproducibility, storage stability and there is negligible interference from other electroactive components.

In 2012, Huang, C. et al. [59] reported a novel flow injection analysis (FIA) system suitable for measurements of glucose in blood serum. In this proposed FIA system of glucose sensor based on immobilized GOx into the inter space of poly (1,3 phenylenediamine/resorcinol) molecules during electropolymerization followed by modification of Nafion perfluorinated ion exchange resin (5 wt.% in solution in lower aliphatic alcohols/ H_2O mix containing 45% water) film. A wide linear range of 0.1-50 mM for glucose detection was reported in virtue of the new configuration of the sensor and the developed FIA system. The reproducibility of signals was quite good with relative standard deviation (%RSD) values for n = 4 injections (typically 5.7 %). Good analytical recovery of glucose spiked into serum samples, with recoveries in the range of 96.7-105.0%, was obtained.

In 2012, Yılmaz, Ö. et al. [49] proposed the advantages of covalently linked chitosan-ferrocene (CS-Fc) hybrid. Ferrocene bound chitosan (CS-Fc) was synthesized by dissolving CS (75.0 mg, 0.39 mmol NH_2) in 15 mL of 0.1 M acetic acid. FcCHO (39.0 mg, 0.18 mmol) was dissolved in 15 mL of methanol and added to the CS solution. After stirring the reaction mixture at room temperature for 1 h, $NaCNBH_3$ (23.0 mg, 0.36 mmol) was added to the mixture and stirred for 24 h. Then 3 mL of 5.0% NaOH was added, the orange precipitates were formed. Subsequently, the orange products were separated by filtration and washed with distilled water and methanol. Finally, the products were first dried in air for a few hours and then dried under

vacuum. 1.0 wt.% CS-Fc solution was prepared by dissolving 2.5 mg of CS-Fc in 250 μL of 1.0 wt.% acetic acid with stirring for 1 h at room temperature. The biosensor was prepared by spreading 17 μL of 1% CS -Fc solution onto the electrode surface. Subsequently, 5.0 μL of 1.0% Glu (in 50 mM sodium phosphate buffer, pH 7.5) was dropped on GC/CS-Fc electrode surface for cross linking. Then, 5.0 μL of GOx (in 50 mM sodium acetate buffer pH 5.5) was placed on the GC/CS-Fc/Glu electrode and dried at room temperature for 90 min. The results form electrochemical behaviors demonstrated this immobilization technique was effective in preventing the leakage of enzyme and mediator. Furthermore, the Fc group improved the electron transfer efficiency of the biopolymer film, which provides a biocompatible microenvironment around the enzyme. The biosensors exhibited good analytical characteristics form including wide linear ranges, fast response times, sufficient stability and reproducibility towards the quantification of glucose.

CHAPTER 3

EXPERIMENTAL

3.1 Instrumentation

Table 3.1 Instrumentation used for cyclic voltammetric measurements.

Instrument	Model	Company
Potentiostat	EA161	eDAQ, Australia
Data System	e-Corder210	eDAQ, Australia
Working electrode	Glassy carbon electrode (3 mm)	CH Instruments, USA
Reference electrode	Ag/AgCl electrode (3 M KCl)	CH Instruments, USA
Counter electrode	Pt wire	CH Instruments, USA

Table 3.2 Instrumentation used for FIA experiments.

Instrument	Model	Company
Injector	20 µL injection loop	Rheodyne, USA
Pump	LC-10AD	Shimadzu
Detector	EA161	eDAQ, Australia
Data System	e-Corder210	eDAQ, Australia
Thin layer flow cell	CHI 130	CH Instruments, USA
Working electrode	Glassy carbon electrode (3 mm)	CH Instruments, USA
Reference electrode	Ag/AgCl electrode (3 M KCl)	CH Instruments, USA
Counter electrode	Stainless steel tube	CH Instruments, USA

3.2 Reagents and Chemicals

Table 3.3 List of reagents, grade and their suppliers.

Chemical and reagent	Grade	Supplier
Glucose oxidase, from Aspergillusniger (50 KU/g)	AR	Sigma-Aldrich
Bovine serum albumin (BSA) (20 mg/mL)	AR	Sigma-Aldrich
Ferrocenecarboxaldehyde (FcCHO)	98% pure	Sigma-Aldrich
Sodium cyanoborohydride ($NaCNBH_3$)	95% pure	Acros-Organics
Glutaraldehyde (Glu) (50%)	Potographic	Kemika
Muti-walled carbon nanotubes	95% pure	Nanolabinc. (MA, USA)
Chitosan (CS)	Medium molecular weight, 78-85% deacetylated	Sigma-Aldrich
di- Sodium hydrogen phosphate anhydrous (Na_2HPO_4)	AR	Carlo Erba
Sodium phosphate monobasic ($NaH_2PO_4\ H_2O$)	AR	Carlo Erba
D(+)glucose ($C_6H_{12}O_6$)	AR	Sigma-Aldrich
Sucrose ($C_{12}H_{22}O_{11}$)	AR	Sigma-Aldrich
Fructose ($C_6H_{12}O_6$)	AR	Sigma-Aldrich
Sodium chloride (NaCl)	AR	Sigma-Aldrich
Ascorbic acid ($C_6H_8O_6$)	AR	Sigma-Aldrich
Dopamine ($C_6H_{11}NO_2$)	AR	Sigma-Aldrich

3.3. Part I: Development of glucose biosensor based on GC/CNTs-NH_2-Fc/CS-Fc/GOx

3.3.1 Chemical preparation

Preparation of Ferrocene bound chitosan (CS-Fc).

Ferrocene bound chitosan (CS-Fc) was synthesized according to the method described by Yilmaz et al. [49]. Briefly, CS (75 mg) was dissolved in 15 mL of 0.1 M acetic acid solution. FcCHO (39 mg) was dissolved in 15 mL of methanol and a then added to the CS solution. After stirring the reaction mixture at room temperature for 1 h, $NaCNBH_3$ (23 mg) was added into the mixture and stirred for 24 h. Then 3 mL of NaOH 5.0% was added, the orange precipitates were formed. Subsequently, the orange products were separated by filtration and washed with distilled water and methanol. Finally, the products were first dried in air for a few hours and then dried under vacuum. A schematic diagram of CS-Fc synthesis is illustrated in Figure 3.1.

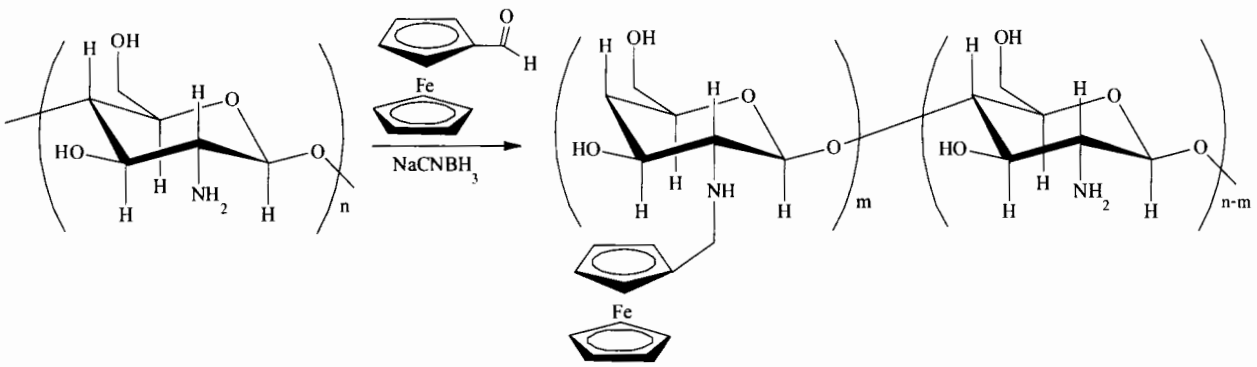


Figure 3.1 Synthesis of ferrocene bound chitosan (CS-Fc).

Preparation of CNTs-NH_2-Fc composites.

Preparation of CNTs-NH_2-Fc composites according to Qiu et al. [3] was adopt. Briefly, CNTs-NH_2 (30 mg) was dispersed in 10 mL of deionized water with ultrasonicating for 30 min, and then FcCHO aqueous solution (20 mL, 7 mg/mL) was added into the solution and continuously stirring for 1 h at room temperature. After that, 20 mg of $NaCNBH_3$ was added in the mixture, and then the mixture was stirred for 24 h. After that the CNTs-NH_2-Fc composites were centrifuged and washed with methanol and water to remove dissociative FcCHO or any physically adsorbed FcCHO from the surface of CNTs-NH_2 and dried at room temperature.

0.5% CS-Fc solution

0.5% CS-Fc solution was prepared by dissolving 25 mg of CS-Fc in 5 mL of 2% acetic acid with stirring for 1 h at room temperature.

7.5 mg/mL of CNTs-NH_2 –Fc solution

7.5 mg/mL of CNTs-NH_2-Fc solution was prepared by dispersing 7.5 mg of CNTs-NH_2-Fc in 1 mL of 0.1 M phosphate buffer pH 7.0 with ultrasonicating for about 1 h.

7.5 mg/mL of GOx solution

7.5 mg/mL of GOx solution was prepared by dissolving 7.5 mg of GOx in 1 mL of 0.1 M phosphate buffer pH 7.0. The solution was shaken using vertex mixer, until homogenous dispersion of GOx was achieved.

0.1 M Na_2HPO_4 solution

14.196 g of di-Sodium hydrogen phosphate (Na_2HPO_4) was dissolved and diluted with deionized water to 1.00 L in a volumetric flask.

0.1 M NaH_2PO_4 solution

13.799 g of sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$) was dissolved and diluted with deionized water to 1.00 L in a volumetric flask.

0.1 M Phosphate buffer pH 7.0

0.1 M phosphate buffer pH 7.0 was prepared by mixing 59 mL of 0.1 M Na_2HPO_4 and 41 mL of 0.1 M NaH_2PO_4 then the mixture was adjusted to pH 7.0 by adding small amount of 3 M NaOH or 3 M HCl.

3.3.2 Electrode preparation

Glassy carbon (GC) electrodes (diameter of 3 mm, from CH Instrument inc.) were polished using 1.0, 0.3 and 0.05 μm alumina slurry, sequentially. The electrodes were rinsed with distilled water and then sonicated in deionized water for 5 min to remove any residual abrasive particles.

GC/CNTs-NH_2-Fc electrode

A GC/CNTs-NH_2-Fc electrode was prepared by dropping 40 μL of 7.5 mg/mL CNTs-NH_2-Fc solution on the surface of well polished glassy carbon (GC) electrode, and then dried at room temperature. A schematic diagram for preparation of GC/CNTs-NH_2-Fc electrode is shown in Figure 3.2 a.

GC/CS electrode

A GC/CS electrode was prepared by coating 20 μL of 0.5% CS solution on the surface of GC electrode. Then electrode was dried at room temperature. A schematic diagram for preparation of GC/CS electrode is shown in Figure 3.2 b.

GC/CS-Fc electrode

A GC/CS-Fc electrode was prepared by dropping 20 μL of 0.5% CS-Fc solution on the surface of GC electrode. Then electrode was dried at room temperature. A schematic diagram for preparation of GC/CS-Fc electrode is shown in Figure 3.2 c.

GC/GOx electrode

A GC/GOx electrode was prepared by dropping 30 μL of 7.5 mg/mL GOx solution on the surface of GC electrode. Then electrode was dried at room temperature. A schematic diagram for preparation of GC/CS electrode is shown in Figure 3.2 d.

GC/CNTs-NH_2-Fc/CS-Fc electrode

A GC/CNTs-NH_2-Fc/CS-Fc electrode was prepared by coating 40 μL of CNTs-NH_2-Fc (7.5 mg/mL) on the well-polished GC electrode surface. The electrode was dried at room temperature. Then, 20 μL of 0.5% CS-Fc solution was dropped on the GC/CNTs-NH_2-Fc electrode. After that, electrode was dried at room temperature. A schematic diagram for preparation of GC/CNTs-NH_2-Fc/CS-Fc electrode is shown in Figure 3.2 e.

GC/CNTs-NH_2-Fc/GOx electrode

A GC/CNTs-NH_2-Fc/GOx electrode was prepared by coating 40 μL of CNTs-NH_2-Fc (7.5 mg/mL) on the surface of GC electrode. The electrode was dried at room temperature. Then, 30 μL of GOx (7.5 mg/mL) was dropped on the GC/CNTs-NH_2-Fc electrode, After then, electrode was dried at room temperature. A schematic diagram for preparation of GC/CNTs-NH_2-Fc/GOx electrode is shown in Figure 3.2 f.

GC/CS-Fc/GOx electrode

A GC/CS-Fc/GOx electrode was prepared by 20 μL of 0.5% CS-Fc solution dropped on the surface of GC electrode. The electrode was dried at room temperature. Then, 30 μL of GOx (7.5 mg/mL) was dropped on the GC/CS-Fc electrode, and then dried at room temperature. A schematic diagram for preparation of GC/CS-Fc/GOx electrode is shown in Figure 3.2 g.

GC/CNTs-NH_2-Fc/CS-Fc/GOx electrode

A CNTs-NH_2-Fc/CS-Fc/GOx electrode was prepared by 40 µL of CNTs-NH_2-Fc (7.5 mg/mL) was dropped on the surface of GC electrode and the electrode was left to dry at room temperature. Then, 20 µL of 0.5% CS-Fc solution was coated on the GC/CNTs-NH_2-Fc electrode, and then dried. After that 30 µL of GOx (7.5 mg/mL) was dropped on GC/CNTs-NH_2-Fc/CS-Fc, and then dried. Figure 3.2 h shows a diagram for preparation of the GC/CNTs-NH_2-Fc/CS-Fc/GOx biosensor.

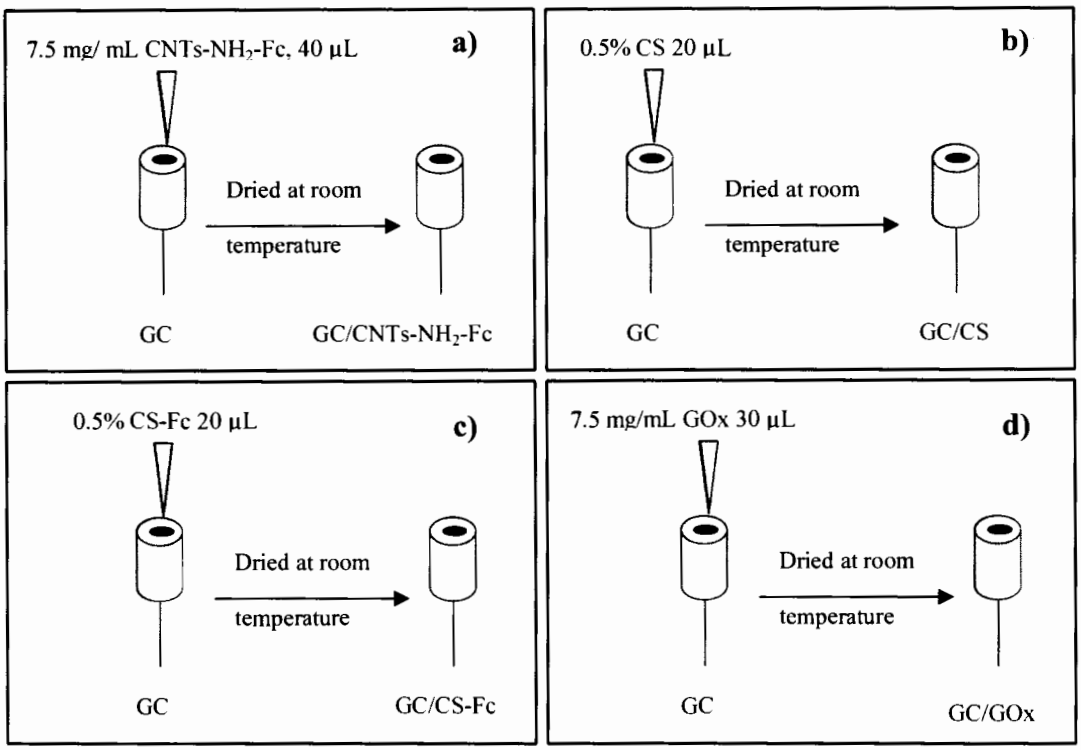


Figure 3.2 Preparation procedure of a) GC/CNTs-NH_2-Fc, b) GC/CS, c) GC/CS-Fc, d) GC/GOx, e) GC/CNTs-NH_2-Fc/CS-Fc, f) GC/CNTs-NH_2-Fc/GOx, g) GC/CS-Fc/GOx and h) GC/CNTs-NH_2-Fc/CS-Fc/GOx electrodes.

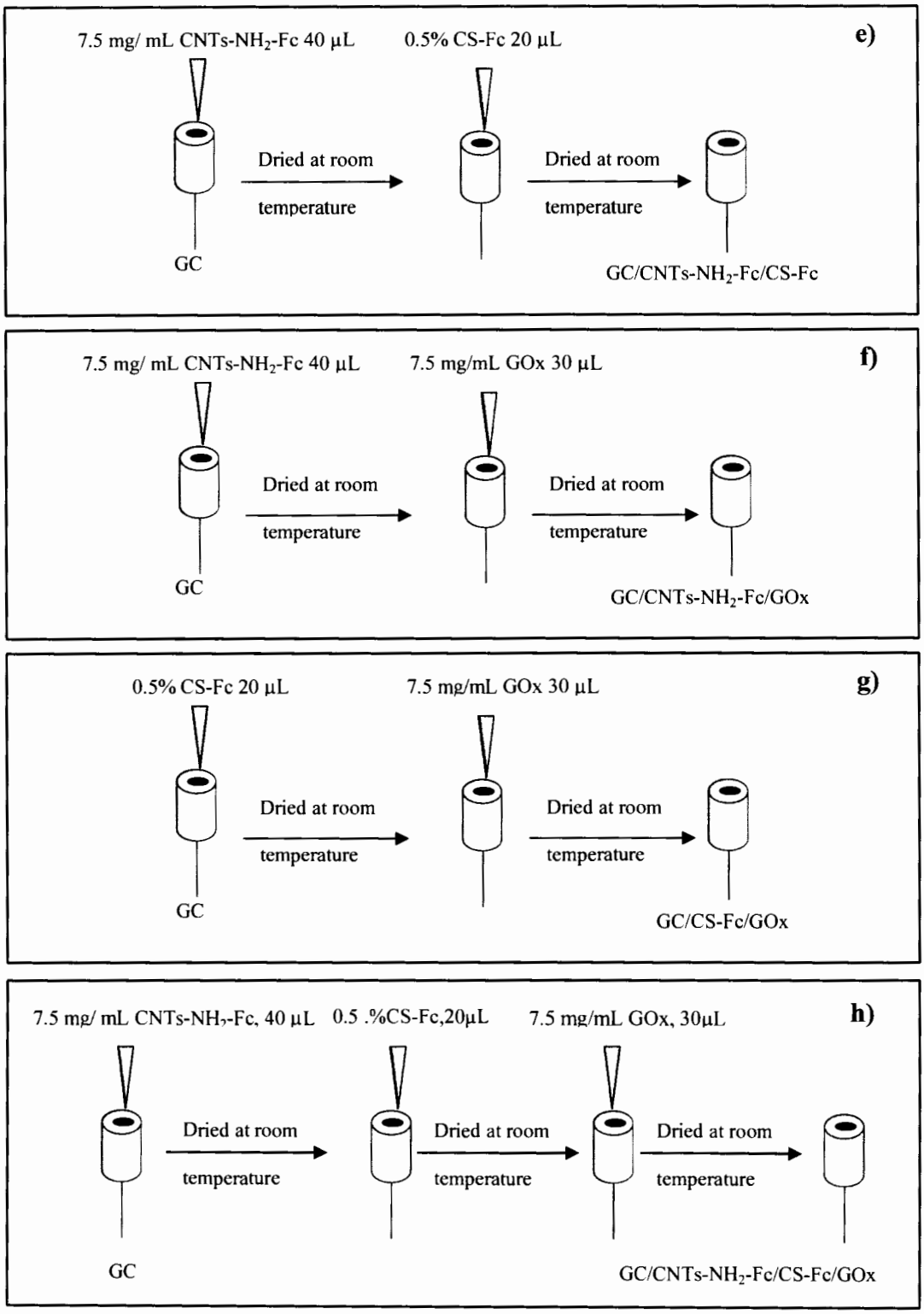


Figure 3.2 Preparation procedure of a) GC/CNTs-NH_2-Fc, b) GC/CS, c) GC/CS-Fc, d) GC/GOx, e) GC/CNTs-NH_2-Fc/CS-Fc, f) GC/CNTs-NH_2-Fc/GOx, g) GC/CS-Fc/GOx and h) GC/CNTs-NH_2-Fc/CS-Fc/GOx electrode (Continued).

3.3.3 Measurement procedures

3.3.3.1 Cyclic volatmmetry and amperometry

An eDAQ potentiostat (model EA161) equipped with e-Corder (model 210) with three electrodes system was used for all cyclic voltammetric studies. Electrochemical oxidation of glucose was studied the developed biosensor using cyclic voltammetry (CV). The modified electrode was used as a working electrode, Ag/AgCl as a reference electrode and Pt wire as a counter electrode. 0.1 M phosphate buffer pH 7.0 was used as a supporting electrolyte. Figure 3.3 shows the setup of the system used for all electrochemical studies.

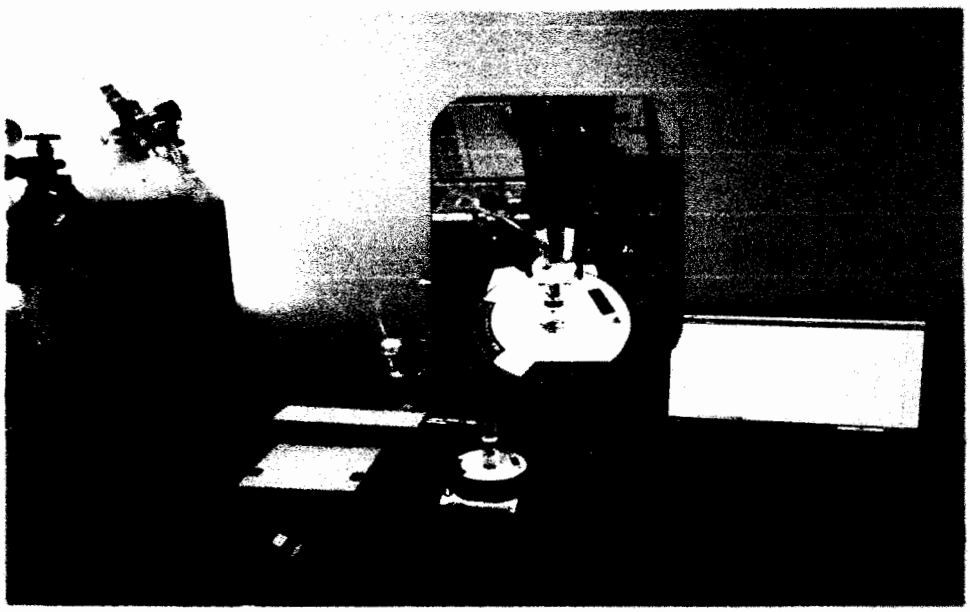


Figure 3.3 Photograph of the setup for cyclic volatammetric measurements using an eDAQ potentiostat with a 25 mL voltammetric cell.

3.3.3.2 Amperometric detection in flow injection system

The setup of flow injection analysis system (FIA) used for amperometric studies is shown in Figure 3.4. The FIA system, used for amperometric measurements, was comprised of a pump (model LC-10AD), an injection valve equipped with a 20 μL injection loop, an eDAQ potentiostat (model EA161), e-Corder (model 210) and a flow cell (CHI130) with three electrodes system. The modified electrode was used as a working electrode, Ag/AgCl as a reference electrode and a stainless steel tube as a counter electrode. 0.1 M phosphate buffer pH 7 was used as a carrier solution.

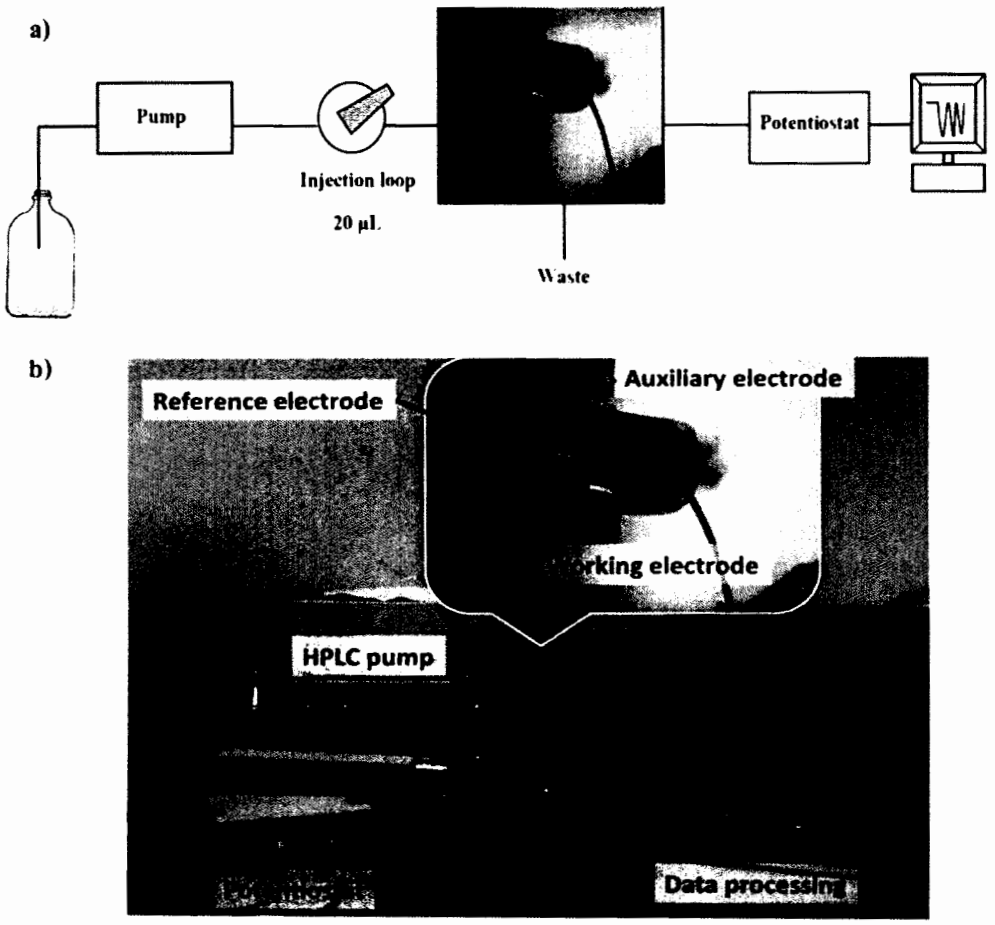


Figure 3.4 a) Manifold of FIA system, b) photograph of the setup for amperometric detection in the FIA system using thin layer flow cell coupled with the modified glassy carbon electrode as a working electrode.

3.3.4 Procedure

3.3.4.1 Electrochemical detection of glucose on biosensor

1) Cyclic voltammetric study of glucose oxidase

The unique electrochemical behavior of glucose oxidase was studied at different modified electrode materials. Cyclic voltammograms of a) bare GC and modified electrodes with b) GC/CNTs-NH_2-Fc, c) GC/CS, d) GC/CS-Fc, e) GC/GOx, f) GC/CNTs-NH_2-Fc/CS-Fc, g) GC/CNTs-NH_2-Fc/GOx, h) GC/CS-Fc/GOx and i) GC/CNTs-NH_2-Fc/CS-Fc/GOx in the absence and presence of glucose were measured using cyclic voltammetry. Electrode codes for bare and modified glassy carbon electrodes are shown in Table 3.4. Results are discussed in Section 4.1.1.1.

Table 3.4 Electrodes codes for bare and modified glassy carbon electrodes.

Electrode codes	Electrode
a	GC
b	GC/CNTs-NH_2-Fc
c	GC/CS
d	GC/CS-Fc
e	GC/GOx
f	GC/CNTs-NH_2-Fc/CS-Fc
g	GC/CNTs-NH_2-Fc/GOx
h	GC/CS-Fc/GOx
i	GC/CNTs-NH_2-Fc/CS-Fc/GOx

3.3.4.2 Studies parameters that affect the sensitivity of the glucose biosensor

1) Effect of CNTs-NH_2-Fc loading on the glucose response obtained from GC/CNT- NH_2-Fc/CS-Fc/GOx electrode.

Optimization of the CNTs-NH_2-Fc modified amount was investigated using cyclic voltammetry to find the most sensitive condition for glucose detection. At all investigated CNTs-NH_2-Fc amount at 0.0, 2.0, 5.0, 7.5, and 10 mg/mL in 0.1 M phosphate buffer solutions was used for a modified electrode of GC/CNT- NH_2-Fc/CS-Fc/GOx.

Results of the effect of CNTs-NH_2-Fc amount in the immobilized solution on the oxidation current were illustrated in Section 4.1.2.1.

2) Effect of CS-Fc loading (GC/CNTs-NH_2-Fc/CS-Fc/GOx)

The effect of CS–Fc concentration in a modified electrode was studied at the concentrations varied at 0.0, 0.1, 0.2, 0.5 and 1.0%. Results are discussed in Section 4.1.2.2.

3) Effect of glucose oxidase loading

The influence of enzyme glucose oxidase loading on the glucose oxidation response was studied at the concentrations varied at 2.0, 5.0, 7.5, and 10 mg/mL. Results are presented in Section 4.1.2.3.

4) Effect of buffer pH (0.1 M phosphate buffer solution)

The effect of pH on the performance of the GC/CNTs- NH_2-Fc/CS-Fc/GOx biosensor were varied at pH 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0 using 0.1 M phosphate buffer solution as supporting electrolyte. Results are presented in Section 4.1.2.4.

5) Electrochemical behavior of the GC/CNTs-NH_2-Fc/CS-Fc/GOx electrode under optimum conditions

The electrochemical behavior of glucose oxidation was studied at the developed glucose biosensor (GC/CNTs- NH_2-Fc/CS-Fc/GOx) using cyclic voltammetry (CV). The modified electrode was used as a working electrode, Ag/AgCl as a reference electrode and Pt wire as a counter electrode. 0.1 M phosphate buffer pH 7.0 was used as a supporting electrolyte. Results are presented in Section 4.1.2.5.

6) Optimum potential for amperometric detection

Optimization of the potential for amperometric detection of glucose oxidation was investigated using chronoamperometry (Figure 3.3). The potential for amperometric detection of glucose signal was studied at 0.1, 0.2, 0.3, 0.4 and 0.5 V in 0.1 M phosphate buffer solutions containing 0.5 mM glucose. Results are presented in Section 4.1.2.6.

3.3.4.3 Analytical features

1) Linearity

Calibration standards of glucose were prepared by diluting the appropriate amount of glucose solution in 0.1 M phosphate buffer pH 7.0 to give working solutions in the range of 2-80 mM. 20 µL of each standard was injected into the FIA system using a flow rate of 1.0 mL/min with 0.1 M phosphate buffer pH 7.0 as a carrier solution. Amperometric responses were recorded using an applied potential of 0.3 V. Results are presented in Section 4.1.3.1.

2) Limit of detection

In this study, the limit of detection was calculated from current peak signals from glucose standard solution at a concentration of 10 mM (10 replicates). The signal value of 3 times the standard deviation from injection of 10 mM glucose was converted to a concentration to give the limit of detection. Results are presented in Section 4.1.3.2.

3) Interference study

In this study, the effect of interference ions, including compounds that are likely to exist in the sample of interest was investigated. The concentration of the foreign species that provide a signal change greater than 5% was considered as the tolerance limit. The interference used in this study was fructose, sucrose, sodium chloride, dopamine and ascorbic acid. Results are discussed in Section 4.1.3.3.

4) Stability of the developed biosensor

20 µL of 10 mM glucose standard solution was repeatedly injected into the FIA system to evaluate of the responses stability (n = 50). Results are discussed in Section 4.1.3.4.

3.3.4.4 Characterization of the composites by FI-IR spectroscopy

Infrared spectroscopy is an important technique to identify the functional groups in molecules. Composites of the study including CS, CS-Fc and Fc were examined using FT-IR. FT-IR spectra were recorded on KBr pellets technique (100 mg of dried KBr and 2 mg of composites) with the Perkin-Elmer Spectrum RX I. The wavenumber range of measurement was between 500 to 4,000 cm^{-1}. Results are discussed in Section 4.1.4.

3.4 PartII: Development of glucose biosensor based on GC/CNTs-NH_2-Fc-BSA-GOx

3.4.1 Chemical preparation

Preparation of CNTs-NH_2-Fc composites

Preparation of CNTs-NH_2-Fc composites according to the reported method of Qiu et al. with a little modification [3]. Briefly, CNTs-NH_2 (30 mg) was dispersed in 10 mL of deionized water with ultrasonicating for 30 min, and then FcCHO aqueous solution (20 mL, 7 mg/mL) was added into the solution and continuously stirring for 1 h at room temperature. After that, 20 mg of $NaCNBH_3$ was added in the mixture, and stirring for 24 h. Then the CNTs-NH_2-Fc composites were centrifuged, and washed with methanol and water to remove the dissociative FcCHO or any physically adsorbed FcCHO from the surface of CNTs-NH_2 and dried at room temperature.

1% bovine serum albumin solution (BSA)

1% BSA solution was prepared by diluting 100 µL of BSA (20 mg/ mL) in 10 mL deionized water.

2.5% glutaraldehyde solution

2.5% Glu solution was prepared by diluting 500 µL of Glu (50%) in 10 mL deionized water.

7.5 mg/mL of CNTs-NH_2 solution

7.5 mg/mL of CNTs-NH_2 solution was prepared by dispersing 7.5 mg of CNTs-NH_2 in 1 mL of 0.1 M phosphate buffer pH 7.0 with ultrasonicating for about 1 h.

7.5 mg/mL of CNTs-NH_2 –Fc solution

7.5 mg/mL of CNTs-NH_2-Fc solution was prepared by dispersing 7.5 mg of CNTs-NH_2-Fc in 1 mL of 0.1 M phosphate buffer pH 7.0 with ultrasonicating for about 1 h.

Mixture of bovine serum albumin and glucose oxidase solution (BSA-GOx)

Mixtureof bovine serum albumin and glucose oxidase solution (BSA-GOx) was prepared by dissolving 7.5 mg of GOx in 500 µL of BSA solution (1% v/v). Then the resulted solution was mixed with 500 µL of Glu solution (2.5% v/v) and stored at 4 °C for 2 h.

Mixture of CNTs-NH_2-Fc-BSA-GOx solution

Mixture of CNTs-NH_2-Fc-BSA-GOx solution was prepared by pipetting 1,000 µL of BSA-GOx mixed with 1,000 µL of 7.5 mg/mL CNTs-NH_2-Fc solution (1:1).

0.1 M disodium hydrogen phosphate (Na_2HPO_4) solution

14.196 g di-sodium hydrogen phosphate (Na_2HPO_4) was dissolved and diluted with deionized water to 1.00 L in a volumetric flask to have 0.1 M Na_2HPO_4 solution.

0.1 M Sodium phosphate monobasic (NaH_2PO_4 H_2O) solution

13.799 g sodium phosphate monobasic (NaH_2PO_4 H_2O) was dissolved and diluted with deionized water to 1.00 L in a volumetric flask to have 0.1 M NaH_2PO_4 solution.

0.1 M Phosphate buffer pH 7.0

0.1 M phosphate buffer pH 7.0 prepared by mixing 59 mL of 0.1 M Na_2PO_4 and 41 mL of 0.1 M NaH_2PO_4 then the mixture was adjusted to pH 7.0 by addition of a small amount of 1 M NaOH or 3 M HCl.

3.4.2 Electrode preparation

Glassy carbon (GC) electrodes (diameter of 3mm, from CH Instrument inc.) were polished using 1.0, 0.3 and 0.05 μm alumina slurry, sequentially. The electrodes were rinsed with distilled water and then sonicated in deionized water for 5 min to remove any residual abrasive particles.

GC/CNTs-NH_2 electrode

A GC/CNTs-NH_2 electrode was prepared by dropping 40 μL of 7.5 mg/mL CNTs-NH_2 solution on the surface of a well be polished glassy carbon (GC) electrode, and then dried at room temperature. A schematic diagram for preparation of the GC/CNTs-NH_2 electrode is shown in Figure 3.5 a.

GC/CNTs-NH_2-Fc electrode

A GC/CNTs-NH_2-Fc electrode was prepared by dropping 40 μL of 7.5 mg/mL CNTs-NH_2-Fc solution on the surface of a well be polished the glassy carbon (GC) electrode, and then dried at room temperature. A schematic diagram for preparation of the GC/CNTs-NH_2-Fc electrode is shown in Figure 3.5 b.

GC/BSA electrode

A GC/BSA electrode was prepared by dropping 40 μL of 1% BSA solution on a glassy carbon (GC) electrode. Then the electrode was dried at room temperature. A schematic diagram for preparation of the GC/BSA electrode is shown in Figure 3.5 c.

GC/GOx electrode

A GC/GOx electrode was prepared by dropping 40 μL of 7.5 mg/mL GOx solution on a glassy carbon (GC) electrode. Then the electrode was dried at room temperature. A schematic diagram for preparation of the GC/CS electrode is shown in Figure 3.5 d.

GC/ BSA-GOx electrode

40 μL of the BSA-GOx solution was spread evenly onto a well-polished glassy carbon electrode (GC) surface and allowed to dry at 4 °C for overnight to form a BSA-GOx composites film. Figure 3.5 e shows a diagram for preparation of the GC/BSA-GOx biosensor.

GC/CNTs-NH_2-Fc-BSA-GOx electrode

40 μL of the CNTs-NH_2-Fc-BSA-GOx solution was spread evenly onto a well-polished glassy carbon electrode (GC) surface and allowed to dry at 4 °C for overnight to form a CNTs-NH_2-Fc-BSA-GOx composites film. Figure 3.5 f shows a diagram for preparation of the GC/CNTs-NH_2-Fc-BSA-GOx biosensor.

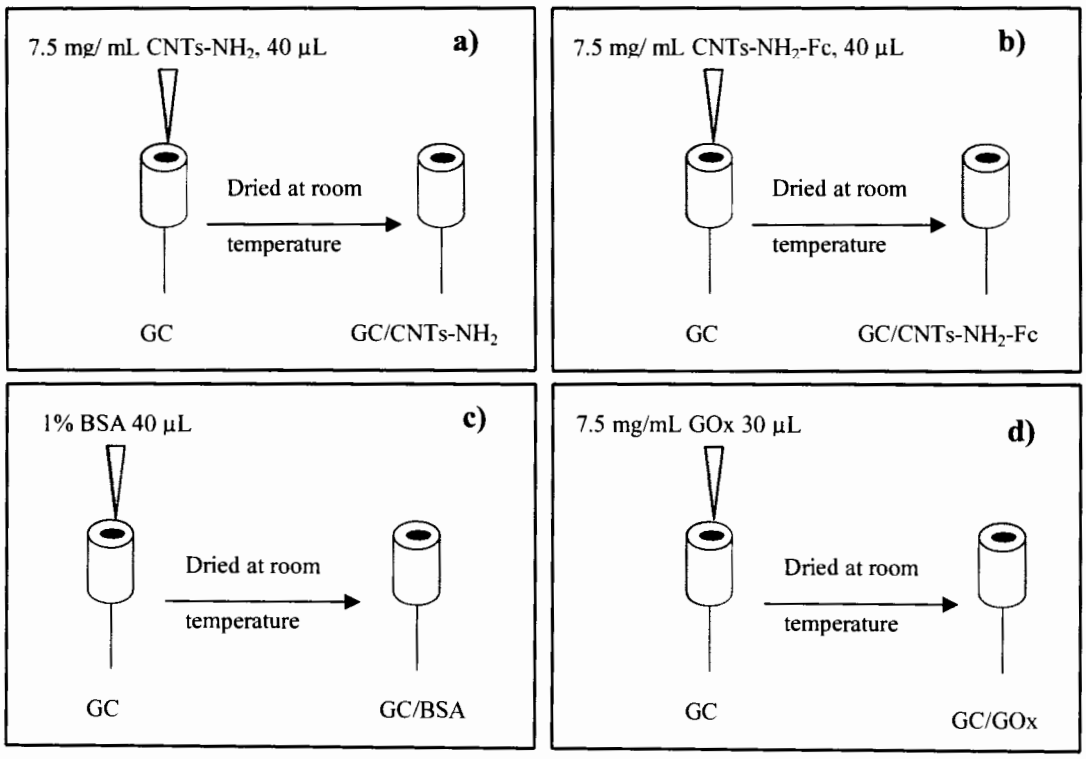


Figure 3.5 Preparation procedure of a) GC/CNTs-NH_2 b) GC/CNTs-NH_2-Fc, c) GC/BSA, d) GC/GOx, e) GC/BSA-GOx and f) GC/CNTs-NH_2-Fc-BSA-GOx electrodes.

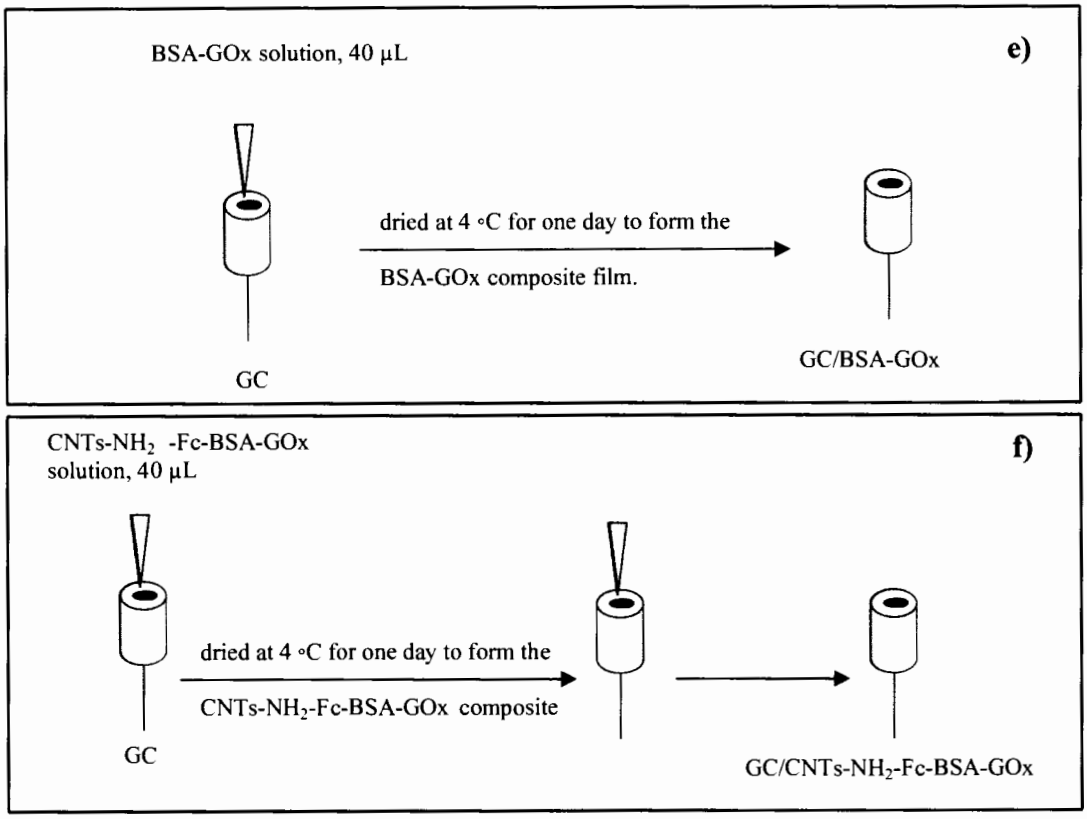


Figure 3.5 Preparation procedure of a) GC/CNTs-NH_2 b) GC/CNTs-NH_2-Fc, c) GC/BSA, d) GC/GOx, e) GC/BSA-GOx and f) GC/CNTs-NH_2-Fc-BSA-GOx electrodes (Continued).

3.4.3 Measurement procedures

Cyclic volatmmetry and amperometry for these studies are described in Section 3.3.3.1 (page 40).

Amperometric detection in flow injection system set up of the system was shown in Section 3.3.3.2 (page 41).

3.4.4 Procedure

3.4.4.1 Electrochemical detection of glucose on biosensor

1) Cyclic voltammetric study of glucose oxidase

The unique electrochemical behavior of glucose oxidase was studied using different modified electrode materials. Cyclic voltammograms of a) bare GC and modified electrodes of b) GC/CNTs-NH_2, c) GC/CNTs-NH_2-Fc, d) GC/BSA, e) GC/GOx, f) GC/BSA-GOx

and g) GC/CNTs-NH_2-Fc-BSA in the absence and presence of glucose were measured using cyclic voltammetry. Electrode codes for bare and modified glassy carbon electrodes are shown in Table 3.5. Results discussed in Section 4.2.1.1.

Table 3.5 Electrode codes for bare and modified glassy carbon electrode

Electrode codes	Electrode
a	GC
b	GC/CNTs-NH_2
c	GC/CNTs-NH_2-Fc
d	GC/BSA
e	GC/GOx
f	GC/BSA-GOx
g	GC/CNTs-NH_2-Fc-BSA-GOx

3.4.4.2 Studies of parameters that affect the sensitivity of the glucose biosensor

1) Effect of CNTs-NH_2-Fc amount on the glucose response

Optimization of the CNTs-NH_2-Fc modified amount was investigated using cyclic voltammetry to find the most sensitive condition for glucose detection. The amount of CNTs-NH_2-Fc at 0.0, 2.5, 5.0, 7.5 and 10.0 mg/mL was prepared by dispersing 0.0, 2.5, 5.0, 7.5 and 10 mg of CNTs-NH_2-Fc in 1 mL of 0.1 M phosphate buffer pH 7. Then the resulted solution was mixed with BSA-GOx solution (1:1) used to modify electrode of GC/CNTs-NH_2-Fc-BSA-GOx. Results of the amount of CNTs-NH_2-Fc amount in the immobilized solution were illustrated in Section 4.2.2.1.

2) Effect of bovine serum albumin concentration

The effect of bovine serum albumin used for the electrode modification was investigated at 0, 0.25, 0.5, 1, 1.5 and 2% using cyclic voltammetry to find the most sensitive condition for glucose detection. Results are presented in Section 4.2.2.2.

3) Effect of enzyme glucose oxidese modified on GC/CNTs-NH_2-Fc-BSA-GOx

The influence of enzyme glucose oxidase loading was studied at the concentration of 0, 2.5, 5, 7.5 and 10 mg/mL. Results are presented in Section 4.2.2.3.

4) Effect of buffer pH (0.1 M phosphate buffer solution)

The effect of buffer pH on oxidation peak current for glucose at the GC/CNTs- NH_2- Fc /CS-Fc/GOx electrode was investigated over the pHs of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by using 0.1 M phosphate buffer solution as supporting electrolyte. Cyclic voltammograms of glucose were recorded using electrolyte solutions of varied pH. Results are presented in Section 4.2.2.4.

5) Electrochemical behavior of the GC/CNTs-NH_2-Fc-BSA-GOx electrode under optimum conditions

The electrochemical behavior of glucose oxidation was studied at the developed glucose biosensor (GC/CNTs-NH_2-Fc-BSA-GOx) using cyclic voltammetry (CV). The modified electrode was used as a working electrode, Ag/AgCl as a reference electrode and Pt wire as a counter electrode. 0.1 M phosphate buffer pH 7.0 was used as a supporting electrolyte. Results are presented in Section 4.2.2.5.

3.4.4.3 Amperometric detection of glucose in the developed FIA

1) Optimum potential for amperometric detection

Optimization of the potential for amperometric detection of glucose was studies at 0.3, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7 and 0.8 V. Results are presented in Section 4.2.3.1.

2) Optimum flow rate for glucose detection

The influence of flow rate for detection of glucose signal was studied at 0.3, 0.5, 0.8, 1.0 and 1.5 mL/min. Results are presented in Section 4.2.3.2.

3.4.4.4 Analytical features

1) Linear concentration range

A calibration curve of glucose standards was investigated by diluting the appropriate amount of in 0.1 M phosphate buffer pH 7.0 to give working solutions in the range of 2 - 16 mM. 20 µL of each standard was injected into the FIA system using a flow rate

of 1.0 mL/min and 0.1 M phosphate buffer pH 7.0 as a carrier solution. Amperometric responses were recorded using an applied potential of 0.6 V. Results are presented in Section 4.2.4.1.

2) Limit of detection

In this study, the limit of detection was investigated by injecting standard solution at a low concentration for 7 times. The injection of 3 times the standard deviation from 2 mM glucose signals was converted to a concentration to give the limit of detection. Results are presented in Section 4.2.4.2.

3) Interference study

In this study, the effect of interference, including compounds that are likely to exist in the sample of interest was investigated. The concentration of the interference that provides a signal change greater than 5% was considered as the tolerance limit. The interference used in this study was sodium chloride, fructose, sucrose, dopamine and ascorbic acid. Results are discussed in Section 4.2.4.3.

3.4.4.5 Application of glucose biosensor in real samples

1) Samples preparation

Glucose samples of intravenous injection were obtained from A.N.B Laboratories co., LTD. A picture of samples used in this work is shown in Figure 3.6. This type of samples contains glucose. The samples were diluted (A) 150, (B) 200, (C) 300, (D) 500, and (E) 600 times with 0.1 M phosphate buffer pH 7.0 before measurement using the developed method. Results are presented in Section 4.2.4.4.

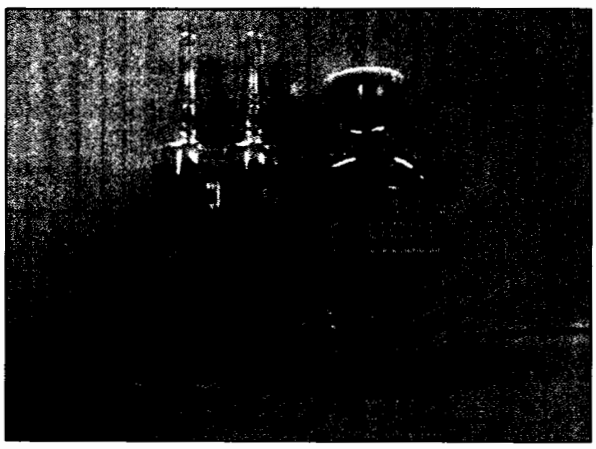


Figure 3.6 Samples for glucose determination in application of real samples.

3.4.4.6 Stability of the developed biosensor

20 µL of 10 mM glucose standard solution was repeatedly injected in to the FIA system to evaluation of the signal stability (n = 120). Results are discussed in Section 4.2.4.5.

3.4.4.7 Characterization of the composites by FI-IR spectroscopy

In this study nanocomposites including CNT-NH_2, CNT-NH_2-Fc and Fc were investigated. Perkin-Elmer Spectrum RX I was used to recorded FT-IR spectra by the KBr pellets technique. The wavenumber range of measurement was between 500 to 4,000 cm^{-1}. Results are discussed in Section 4.2.5.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Part I: Development of glucose biosensor based on GC/CNTs-NH_2-Fc/CS-Fc/GOx

4.1.1 Electrochemical detection of glucose oxidation on biosensor

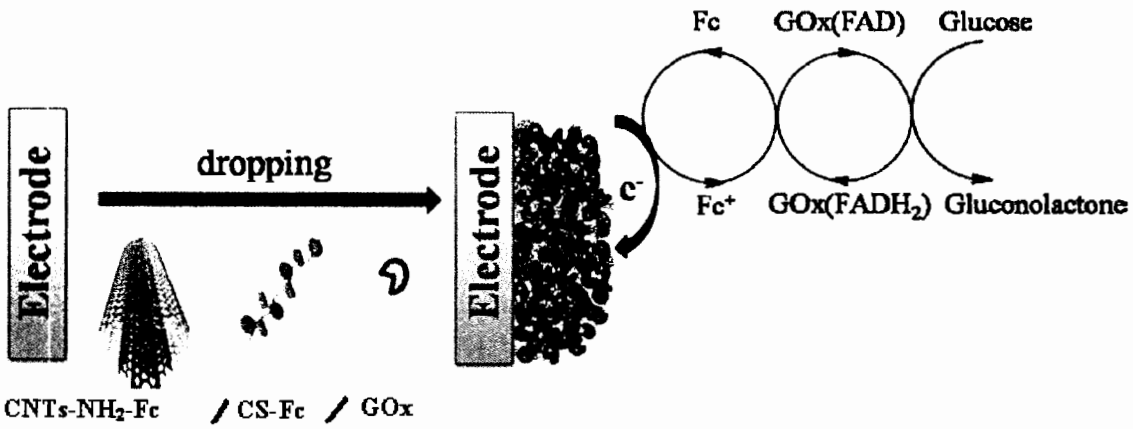
4.1.1.1 Cyclic voltammetric study of glucose oxidation

The unique electrochemical behavior of glucose oxidation was studied at different modified electrode materials using cyclic voltammetry (CV). The bare GC or GC modified electrode was used as a working electrode, Ag/AgCl and Pt wire as a reference and counter electrode, respectively. Cyclic voltammograms of glucose were measured in a 0.1 M phosphate buffer pH 7.0. Results are shown in Figure 4.1. The glucose oxidation waves obtained from GC, GC/CS, GC/GOx and GC CNTs- NH_2-Fc/GOx were not well defined peaks at a potential window of 0.0 to 1.0 V. The GC/CNTs-NH_2-Fc and GC/CNTs-NH_2-Fc/CS-Fc electrodes were observed with the low current signal of glucose oxidation at potentials of 0.35 and 0.30 V, respectively. High current signals from glucose oxidation were obtained from GC/CS-Fc, GC/CS-Fc/GOx and GC/CNTs-NH_2-Fc/CS-Fc/GOx. Moreover, these three modified electrodes enhanced the oxidation of glucose at lower potentials than 0.30 V. Results of anodic potentials and currents of 10 mM glucose obtained from the bare and modified electrodes are summarized in Table 4.1. The GC/CNTs-NH_2-Fc/CS-Fc/GOx electrode provides the highest sensitivity for glucose oxidase (Figure 4.1 i). This result indicated that this nanocomposie CNTs-NH_2-Fc/CS-Fc/GOx was the most effectively catalytic in glucose oxidation. This result showed that chemically bonded of ferrocene (Fc) to CNTs-NH_2 and CS could inhibit the leakage of Fc from electrode surface. CS-Fc could also improve the catalytic property of the composites film and it can be used as an electron transfer mediator to shuttle electrons between analytes and the electrode. In addition, CS-Fc was an effective supporting material for immobilization of the enzyme because of an excellent membrane-forming ability, high permeability toward water, good adhesion, non-toxicity, high mechanical strength and biocompatibility. This biosensor (GC/CNTs-NH_2-Fc/CS-Fc/GOx) exhibited good analytical performance towards the glucose

detection. The mechanism of glucose oxidation by the biosensor is illustrated in scheme 4.1. In this study, the GC/CNTs-NH_2-Fc/CS-Fc/GOx which provided the highest glucose oxidation current and lower potential was selected as the optimum modified electrode.

Table 4.1 Anodic potential ($E_{p,a}$) and current ($i_{p,a}$) of 10 mM glucose measured using bare and modified GC electrodes.

Electrode code	Electrode	$E_{p,a}$, (V)	$i_{p,a}$, (μA)
a	GC	> 1.0	-
b	GC/CNTs-NH_2-Fc	0.35	0.29
c	GC/CS	>1.0	-
d	GC/CS-Fc	0.3	0.79
e	GC/GOx	>1.0	-
f	GC/CNTs-NH_2-Fc/CS-Fc	0.3	0.32
g	GC/CNTs-NH_2-Fc/GOx	>1.0	-
h	GC/CS-Fc/GOx	0.27	1.40
i	GC/CNTs-NH_2-Fc/CS-Fc/GOx	0.3	8.90



Scheme 4.1 Schematic representation of the CNTs-NH_2-Fc/CS-Fc/GOx modified electrode and the mechanism of the oxidation of glucose catalyzed by GOx and mediated by Fc.

Where Fc and Fc^{+} represent reduced and oxidized forms of the ferrocene, GOx (FAD) and GOx ($FADH_2$) are the oxidized and reduced forms of glucose oxidase. In this process, initiation the GOx (FAD) catalytic oxidized glucose to gluconolactone and GOx(FAD) is converted to GOx ($FADH_2$). The GOx ($FADH_2$) is reoxidized to the GOx (FAD by the mediator, Fc^{+}. Then, the Fc is reoxidized to Fc^{+} directly at an electrode, giving a current signal (proportional to the glucose concentration). The current flowing through the electrode is an amperometric measurement for the glucose concentration.

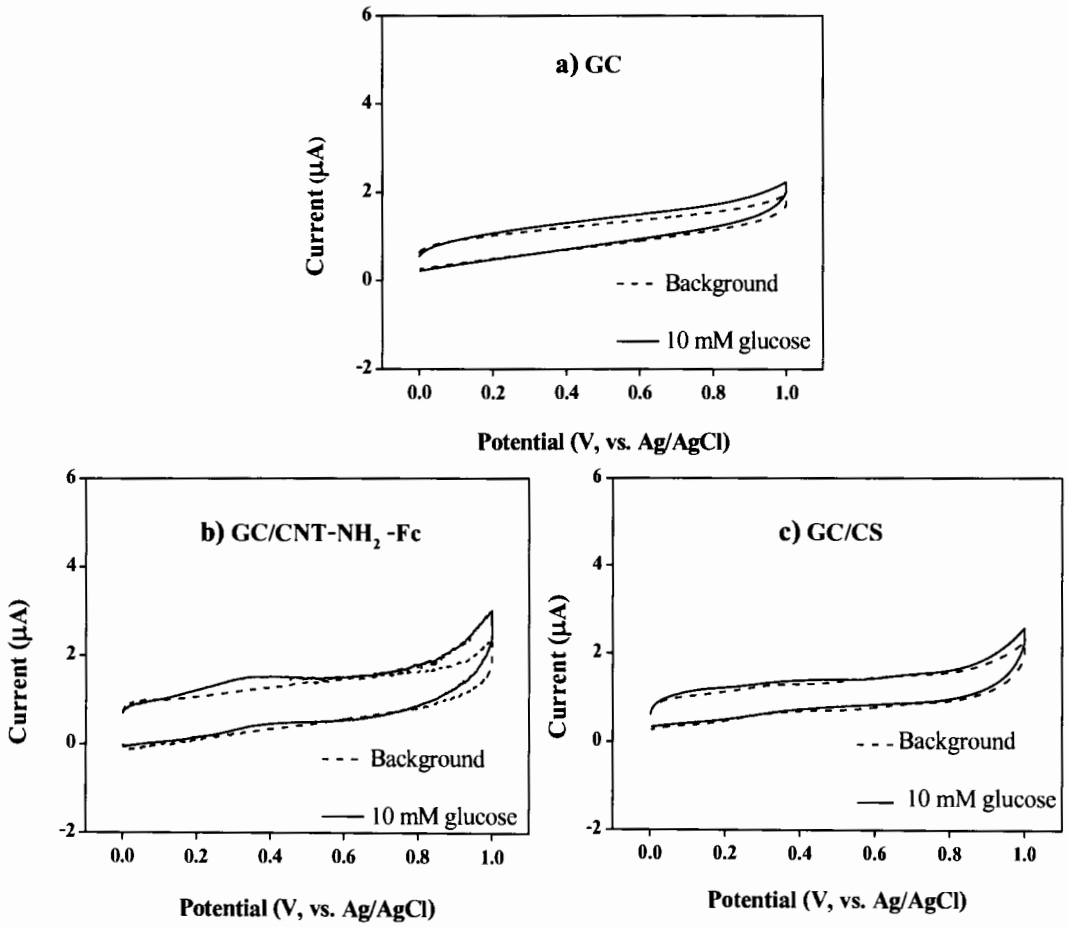


Figure 4.1 Cyclic voltammograms of a) bare glassy carbon electrode (GC) in the absent (dotted line) and present of 10 mM glucose (solid line), b) GC/CNTs-NH_2-Fc, c) GC/CS, d) GC/CS-Fc, e) GC/GOx, f) GC/CNTs-NH_2-Fc/ CS-Fc, g) GC/CNTs-NH_2-Fc/GOx, h) GC/CS-Fc/GOx, and i) GC/CNTs-NH_2-Fc/CS-Fc/GOx in 0.1 M phosphate buffer pH 7.0.

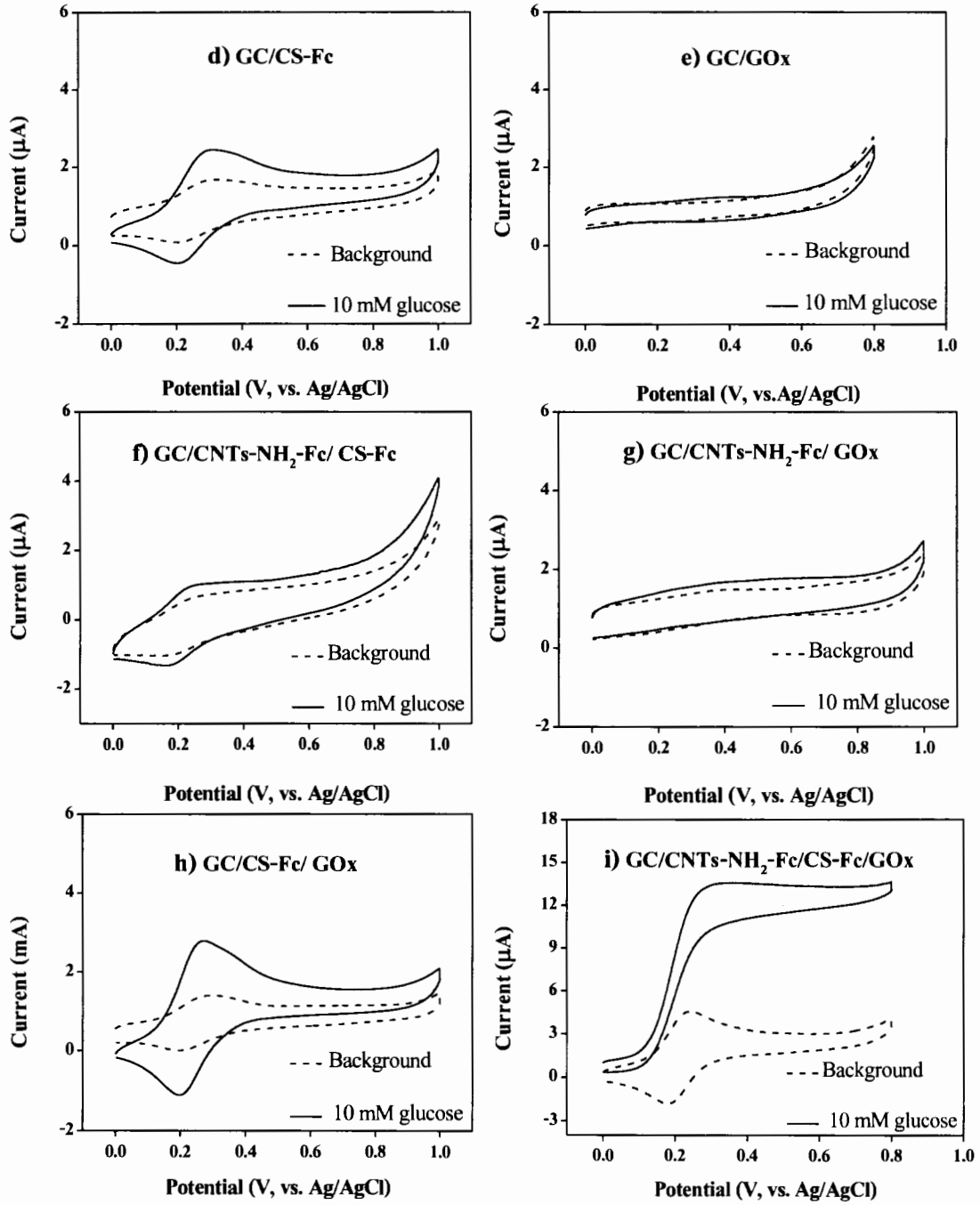


Figure 4.1 Cyclic voltammogramsof a) bare glassy carbon electrode (GC) in the absent (dotted line) and present of 10 mM glucose (solid line), b) GC/CNTs-NH_2-Fc, c) GC/CS, d) GC/CS-Fc, (e) GC/GOx, f) GC/CNTs-NH_2-Fc/CS-Fc, g) GC/CNTs-NH_2-Fc/GOx, h) GC/CS-Fc/GOx, and i) GC/CNTs-NH_2-Fc/CS-Fc/GOx in 0.1 M phosphate buffer pH 7.0 (Continued).

4.1.2 Studies of parameters that effect the sensitivity of biosensor

4.1.2.1 Effect of CNTs-NH_2-Fc loading on the glucose response obtained from GC/CNTs-NH_2-Fc/CS-Fc/GOx electrode

The effect of CNTs-NH_2-Fc loading from 0 to 10 mg/mL on the biosensor was investigated. As shown in Figure. 4.2, increasing of CNTs-NH_2-Fc amount from 0 -7.5 mg/mL resulted in response increasing. This result assumed that increasing of CNTs-NH_2-Fc amount can improve the conductive property of the composites film. The response current seems to decreasing at the concentrations above 7.5 mg/mL. Thus the CNTs-NH_2-Fc of 7.5 mg/mL was selected as the optimum concentration.

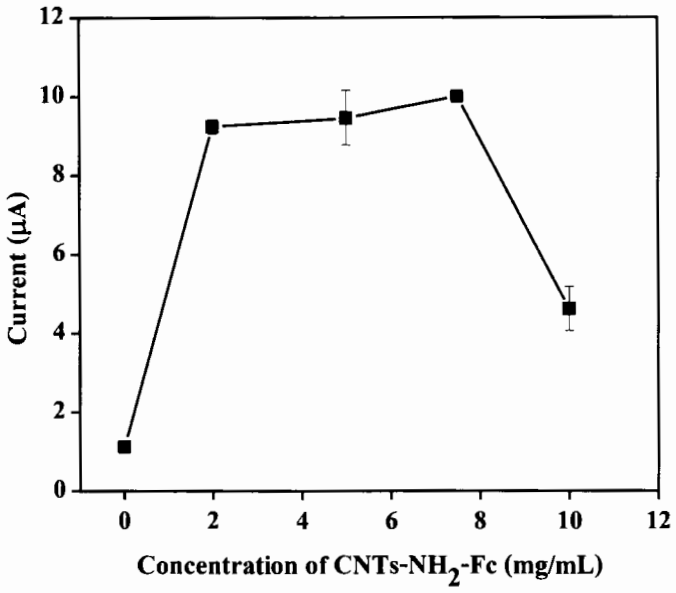


Figure 4.2 The effect of the concentration of CNTs-NH_2-Fc (mg/ mL) on oxidation current of 10 mM glucose solution in 0.1 M phosphate buffer solution pH 7.0.

4.1.2.2 Effect of CS-Fc loading

The effect of CS-Fc loading from 0 to 1% on the biosensor (GC/CNTs-NH_2-Fc/CS-Fc/GOx) was investigated. As shown in Figure. 4.3, the current response increased with increasing the amount of CS-Fc immobilized on the electrode and reached maximum at 0.5%. These results were assumed to be due to the chitosan was biocompatibility and contributed to the enzyme activity. Chitosan (CS) is convenient polymeric scaffold for enzyme immobilization and has been used previously for the immobilization of the other dehydrogenases

in an enzymatic reaction [60]. The result also demonstrated the benefit of using CS to enhance performance. Slightly decrease of the oxidation current when the concentration of CS-Fc was greater than 0.5% also observed. This could be ascribed to viscosity of CS if CS-Fc concentration was greater than 0.5%, the viscosity increased and the resistance to the electron transfer was also increased. In this study, the CS-Fc concentration of 0.5% which provided the maximum response current was selected as the optimum concentration.

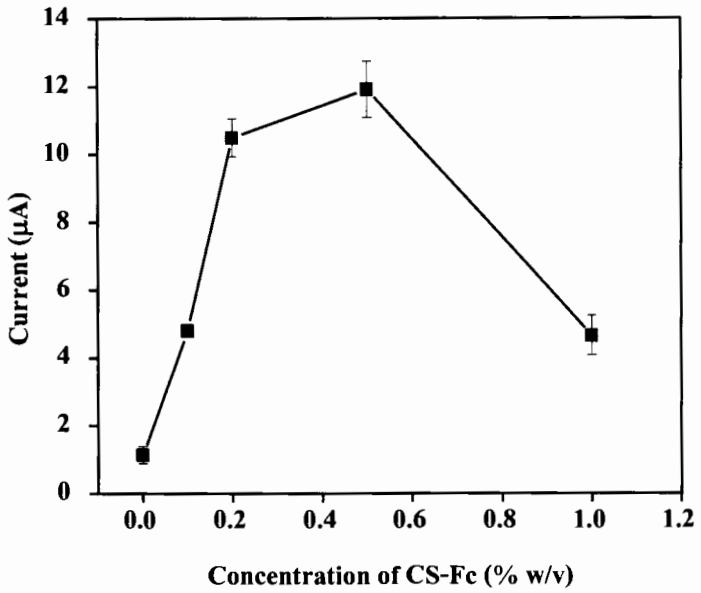


Figure 4.3 The effect of concentration of CS-Fc (% w/v) on the oxidation current of 10 mM glucose solution in phosphate buffer solution pH 7.0.

4.1.2.3 Effect of enzyme glucose oxidase loading

The redox-active material, Fc, enhances electron transfer efficiency between the redox center of GOx and the surface of the working electrode. The enzymatic process occurs according to the following reaction [61]:

$$\text{Glucose} + \text{GOx (FAD)} \longrightarrow \text{Gluconolactone} + \text{GOx (FADH}_2\text{)} \quad (3.1)$$

$$\text{GOx (FADH}_2\text{)} + 2\text{Fc}^{+} \longrightarrow \text{GOx (FAD)} + 2\text{Fc} + 2\text{H}^{+} \quad (3.2)$$

$$\text{Fc} \rightleftharpoons \text{Fc}^{+} + \text{e}^{-} \quad (3.3)$$

Where Fc and Fc^- represent reduced and oxidized forms of the ferrocene, GOx (FAD) and GOx ($FADH_2$) are the oxidized and reduced forms of glucose oxidase. In this process, the GOx (FAD) catalytic oxidized glucose to gluconolactone and GOx (FAD) is converted to GOx ($FADH_2$) (Eq (3.1)); electrons are transferred from the GOx ($FADH_2$) to the Fc sites (Eq (3.2)), and the electrons are then transferred through the Fc^+ / Fc sites to the electrode surface (Eq (3.3)). These electrons can then be transferred from GOx ($FADH_2$) to the mediator, which is then oxidized at the electrode surface producing a current that is directly proportional to the concentration of glucose in solution.

According to the catalytic reaction above, the biosensor performance is related to the enzyme loading amount. Hence, the effect of enzyme that immobilized on the biosensor is very essential to the biosensors sensitivity. The effect of GOx loading on the current response shows in Figure 4.4. The current response gradually increased with increasing the amount of enzyme loading from 2 to 7.5 mg/mL. These results were assumed that due to the GOx is a catalyses of the oxidation of glucose. With increasing amount of enzyme loading increases the rate of glucose oxidation as a result, the current response gradually increased. The current response decreased significantly when the amount of GOx was increased further than 7.5 mg/mL, which could be attributed to an increase of film thickness, leading to an increase of interface electron transfer resistance. The increasing of film thickness may cause the electron transfer more difficulty. Therefore the concentration of 7.5 mg/mL of GOx was selected as the optimum for subsequent experiments.

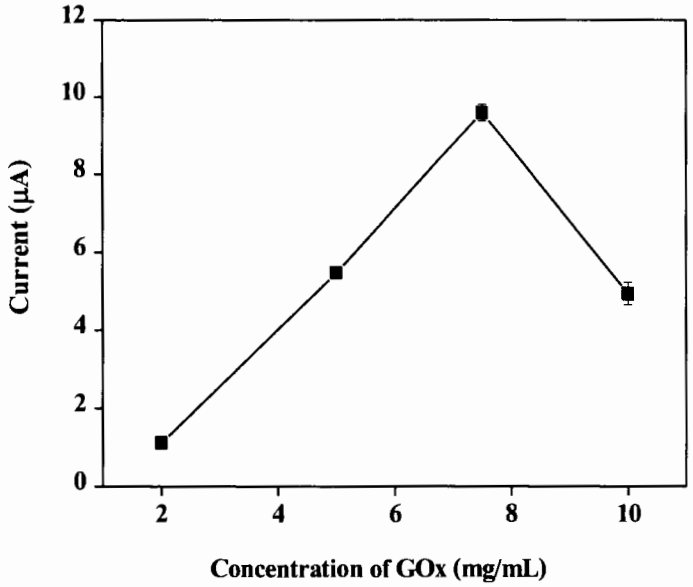


Figure 4.4 The effect of GOx concentration (mg/ mL) on the current response obtained from 10 mM glucose in phosphate buffer solution (0.1 M, pH 7.0).

4.1.2.4 Effect of buffer pH (0.1 M phosphate buffer solution)

The pH of supporting electrolyte can affect the performance of the biosensor. It is generally known that the bioactivity of GOx and the stability of CS are pH dependent. Alkaline solution will decrease the enzyme activity. On the contrast, strong acidic medium will decrease both of CS stability and GOx bioactivity leading to the decreasing of biosensor performance [12]. Thus, the effect of pH was examined in the range of pH 5.0 to 8.0 in 0.1 M phosphate buffer solution using cyclic voltammetry. Figure 4.5 a shows the signal of 10 mM glucose at various pH. The relationship between the oxidation peak potential and pH was shown in Figure 4.5 b. It can be seen that the oxidation peak potential decreased with an increase of pH value. The developed biosensor showed a maximum response current at pH 7.0 (Figure 4.5 c), which is in good agreement with the previous studies [12, 61-63].

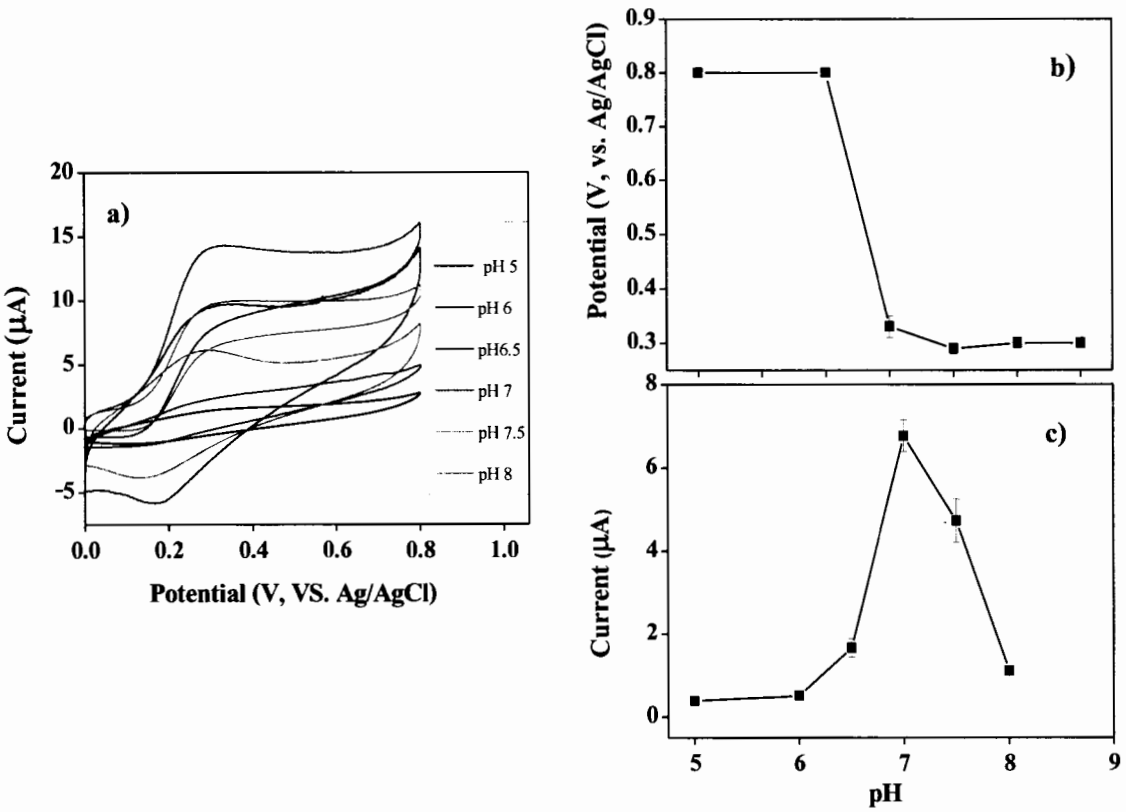


Figure 4.5 a) Cyclic voltammetric responses of 10 mM glucose at various buffer pH and the dependence of buffer pH on b) peak potential, c) peak current obtained from the biosensor (GC/CNTs-NH_2-Fc/CS-Fc/GOx).

4.1.2.5 Electrochemical behavior of the GC/CNTs-NH_2-Fc/CS-Fc/GOx electrode under optimum conditions

The electrochemical behavior of glucose oxidation was studied at the developed glucose biosensor (GC/CNTs-NH_2-Fc/CS-Fc/GOx) using cyclic voltammetry (CV). Experiments were performed in 0.1 M phosphate buffer under the optimum conditions. The modified electrode exhibits high sensitivity and stability. The matrix of CNTs-NH_2-Fc/CS-Fc can be used as mediator for electron transfer between analytes and the electrode and also as biocompatibility environment for GOx immobilization. Figure 4.6 shows the CVs of the developed biosensor in the absence and presence of glucose in 0.1 M PBS. In the absence of glucose, the reversible electrochemical behavior of Fc was observed on the GC/CNTs-NH_2-Fc/CS-Fc/GOx modified electrode (Figure. 4.6 a). After addition of 10 and 20 mM glucose, the anodic peak current increased with concentration increased (Figure 4.6 band c, respectively), which

clearly showed the catalytic properties of modified electrode to glucose. The catalytic effect of CS-Fc via glucose oxidation was similar to GC/CS-Fc/GOx developed by Yang et al. [42]. Yang et al. reported GOx immobilized in CS-Fc to prevent GOx leakage from an electrode.

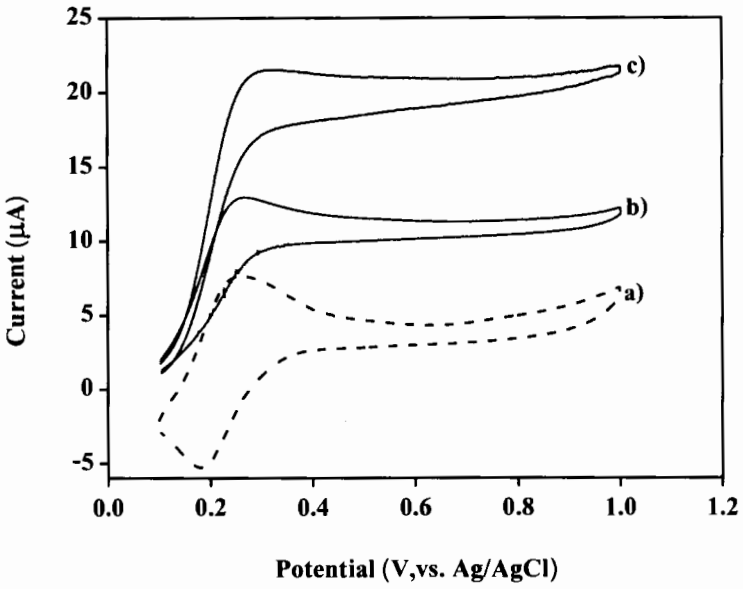


Figure 4.6 Cyclic voltammograms obtained from the glucose biosensor (GC/CNTs-NH_2-Fc/CS-Fc/GOx) in a) 0.1 M phosphate buffer b) 10 and c) 20 mM glucose.

4.1.2.6 Optimum potential for amperometric detection

The detection potential affects the sensitivity of current signal obtained from analytes. The optimal potential was studied in the applied potential from 0.1 to 0.5 V by using chronoamperometry. The effect of applied potential on the glucose response of the biosensor was shown in Figure 4.7. The electrode response to glucose increases with the change of applied potential from 0.1 to 0.3 V. The significant increase of oxidation current upon changing the potential in the above range is attributed to the increased driving force for the oxidation of ferrocene to ferriciniumium [63]. The applied potential is higher than 0.3 V, the current response decrease rapidly, presumably due to the rate-limiting process of the enzyme kinetics and substrate diffusion. The potential at 0.3 V provided the maximum response and therefore selected as the optimum potential. This represents a significant improvement with respect to other biosensors for glucose determination using ferrocene as mediator reported in the

literature: The optimal potential at 0.3 V reported by Qiu et al. [61], their developed amperometric biosensor for glucose based on chitosan-ferrocene/graphene oxide/glucose oxidase (CS-Fc/GO/GOx) nanocomposites film was cast on the surface of GCE. 0.35 V reported by Qiu et al. [3], their developed amperometric biosensor for glucose was developed by entrapping glucose oxidase (GOx) in a new composites doped with CNTs-Fc and chitosan (CS) modified GCE, Yilma et al. [49], their developed biosensor for detecting glucose based on immobilization of GOx onto Chitosan-ferrocene (CS-Fc) hybrid synthesized through covalent modification then coated on GCE, Yang et al. [63], their developed biosensor for detecting glucose based on covalent immobilization of an enzyme GOx onto a carbon sol–gel silicate composites surface as a biosensing platform and Kase et al. [64], their developed amperometric biosensor for glucose based on plasma-polymerized thin film of dimethylaminomethylferrocene (DMAMF) on a sputtered gold electrode. 0.4 V reported by Qiu et al. [12], their fabricate glucose biosensor based on a homogeneous chitosan-ferrocene/Au nanoparticles/glucose oxidase (CS-Fc/AuNPs/GOx) and Yang et al. [42], their fabricate glucose biosensor by using CS-Fc/GOx films coated on GCE.

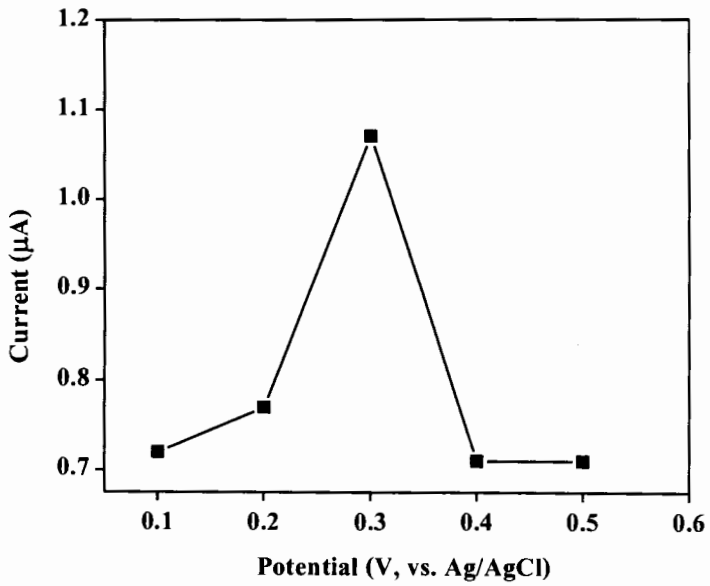


Figure 4.7 Effect of the applied potential on glucose detection using the GC/CNTs-NH_2-Fc/CS-Fc/GOx electrode. The current obtained from 0.5 mM glucose in phosphate buffer (0.1 M, pH 7.0).

4.1.3 Analytical feature

4.1.3.1 Linear concentration range

In order to produce a linear concentration range of the analytical system, it was necessary to inject several different concentrations of standard glucose into the stream. The analytical performance of FIA method with amperometric detection using the developed glucose biosensor was examined. As shown in Figure 4.8, an anamperometric detector yielded a fast response to glucose and the response current returned to the baseline within 138 s. A calibration curve of glucose shows a linear range of 2 - 80 mM and the regression equation is $y = 0.005x - 0.067$ ($r^2 = 0.997$). This linear range is wider than the ranges previous reported by Yilmaz et al. [49], their studied the glucose biosensor based on covalent immobilization of ferrocene on a chitosan modified GCE, which provided the linear range of 2-16 mM and Gomathi et al. [8], their fabricationthe glucose sensor based on immobilization of glucose oxidase in multiwalled carbon nanotubes grafted chitosan nanowire, repored the linear range of 1.0 - 10.0 mM.

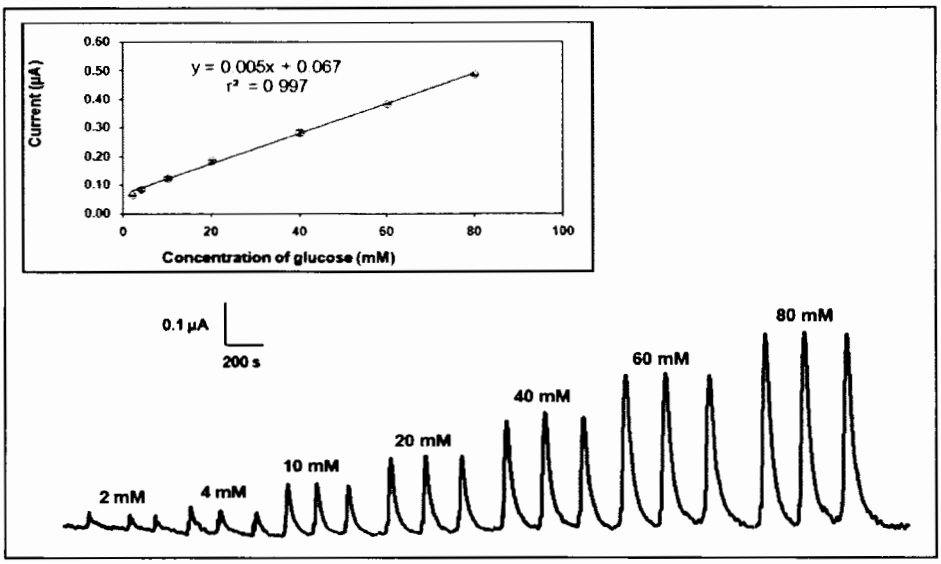


Figure 4.8 An FIA diagram of injected glucose concentration within 2 - 80 mM into phosphate buffer solution pH 7.0, operating potential 0.3 V vs Ag/AgCl, flow rate 1 mL/min, injection volume of 20 μL. The inset shows the linear relationship between the signal of glucose and the concentration.

4.1.3.2 Limit of detection

The limit of detection was injected by 10 mM glucose with ten replicate injections into a carrier solution of 0.1 M phosphate buffer pH 7.0. Results are shown in Figure 4.9. The limit of detection (S/N = 3) is 1.86 mM. The system provides an impressively good precision (% RSD = 3.0). The developed system demonstrates good stability of the immobilized enzyme in spite of the hydrodynamic condition. Sample throughput of the developed method is 42 samples/h.

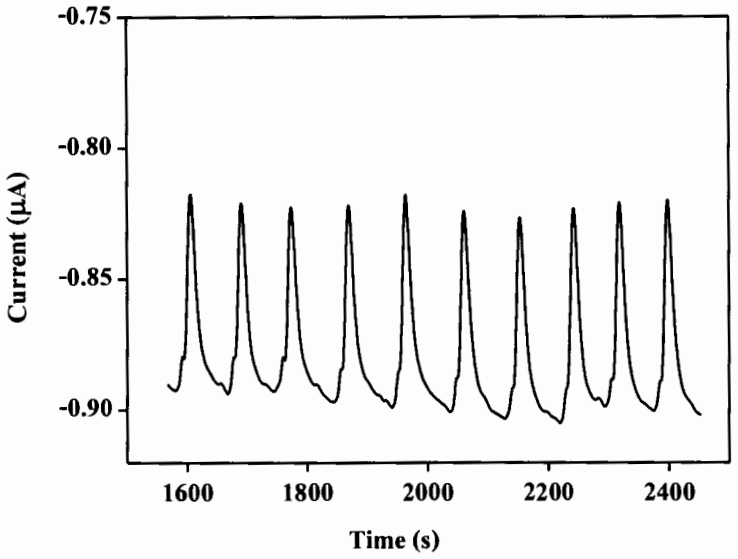


Figure 4.9 An FI amperometric response obtained from the developed glucose biosensor for 10 repetitive injection of 10 mM glucose. Carrier solution; 0.1 M phosphate buffer pH 7.0, applied potential; 0.3 V, flow rate; 1.0 mL/min.

4.1.3.3 Interference study

In this study, the effect of interference, including compounds that are likely to exist in the sample of interest was investigated. The concentration of the interference that provides signal change greater than 5% was considered as the tolerance limit. The interference used in this study was sodium chloride (NaCl), fructose, sucrose, and ascorbic acid (AA). The results are summarized in Table 4.2.

Table 4.2 Effect of interferences on amperometric signals obtained from replicate injections (n=3) of 10 mM standard glucose.

Interferences	**Investigated concentration (mM)**	**Tolerance limit[a](mM)**
ascorbic acid(AA)	2-4	2
fructose	2-20	2
sucrose	2-80	4
sodium chloride	2-200	4

[a] greater than ± 5% signal alteration is classified as an interfering condition.

The results from Table 4.2 show the tolerance limit; of NaCl, fructose, sucrose, and AA were 4, 2, 4 and 2, respectively. Ascorbic acid strongly interferes glucose analysis. This is because AA is an organic compound that easily to be oxidized and the oxidation potentials is close to glucose. Fructose and sucrose also strongly interfere glucose analysis. This result was assumed that fructose and glucose have the same molecular formula, $C_6H_{12}O_6$, sucrose is a disaccharide chemically composed of equal parts glucose and fructose. Therefore, the oxidation potentials are close to glucose. NaCl is an electrolyte substance that provides positive and negative charges when dissolved. In this case, the produced charged were interferes increase conductivity and the current signal of glucose.

4.1.3.4 Stability of the developed biosensor

In this study operational and stability of the developed electrode was investigated. The stability was carried out using the amperometric measurement in flow injection analysis (Figure 3.4). The operational stability of the modified electrode was investigated by uninterruptedly measuring the glucose response from repeated injections of 10 mM glucose. Relationship between (%) relative responses and number of injection (times) was shown in Figure 4.10. The responses showed good stability after 40 injections. The current response remained at 90% of its initial response. The excellent analytical performance and long-term stability of the developed biosensor can be attributed to three aspects: Firstly, biopolymer of chitosan provides a biocompatible microenvironment around the enzyme molecules to stabilize its biological activity to a large extent. Secondly, the formation of the CNTs-NH_2-Fc and CS-Fc

conjugate prevents the Fc leakage out of the film. Lastly, CNTs-NH_2 provided a conduction pathway to accelerate electron transfer due to their excellent conductivity.

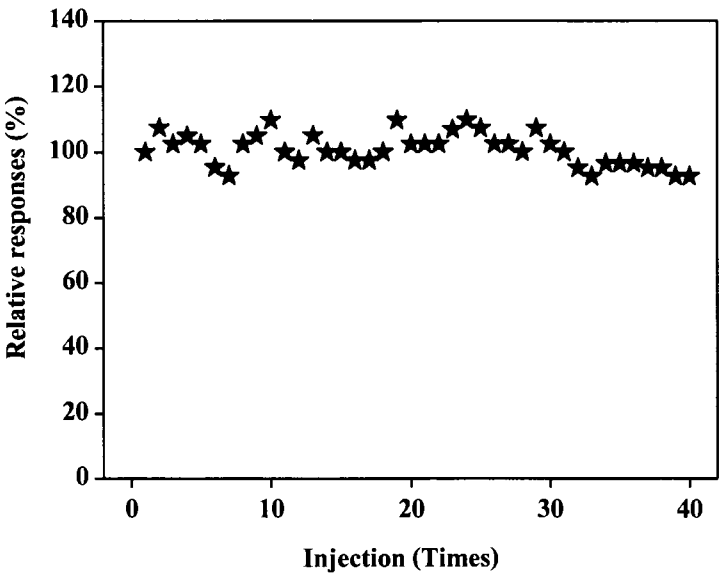


Figure 4.10 Operational stability of the CNTs-NH_2-Fc/CS-Fc/GOx glucose biosensor to repeatedly injections of 10 mM glucose.

4.1.4 Characterization of CS-Fc by FI-IR spectroscopy

CS-Fc biopolymer or supporting material used in this work was synthesized according to the procedure proposed by Yilmaz *et al.* [49]. In the first step of preparation a Schiff base was formed by using the reaction between aldehyde group of FcCHO and the amino group of CS. In the second step, the Schiff base was reduced by $NaCNBH_3$. The formation of CS-Fc was confirmed by FT-IR spectra (Figure 4.11). CS shows characteristic peaks at 1,655 and 1,596 cm^{-1}, which are assigned to the amide I and II bands of CS (Figure 4.11 a), respectively. It is should be note that the intensity of the peak at 1,596 cm^{-1} for primary amine N-H bending decreases in the spectrum of CS-Fc (Figure 4.11 b), as a result of N-alkylation at the glucosamine unit. On the other hand, the characteristic peak of FcCHO at 1,680 cm^{-1} (ν, C-O, Figure 4.11c) disappears in the CS-Fc spectrum, because this synthetic process consumes the aldehyde groups of FcCHO. Simultaneously, the CS-Fc spectrum displays a new absorption band at about 486 cm^{-1}, attributed to M-ring stretch and ring tilt of ferrocenyls [42]. In addition, another new absorption

band at 819 cm^{-1}, for C-H, in the spectra also indicates that the ferrocenyls exist in the CS-Fc biopolymer.

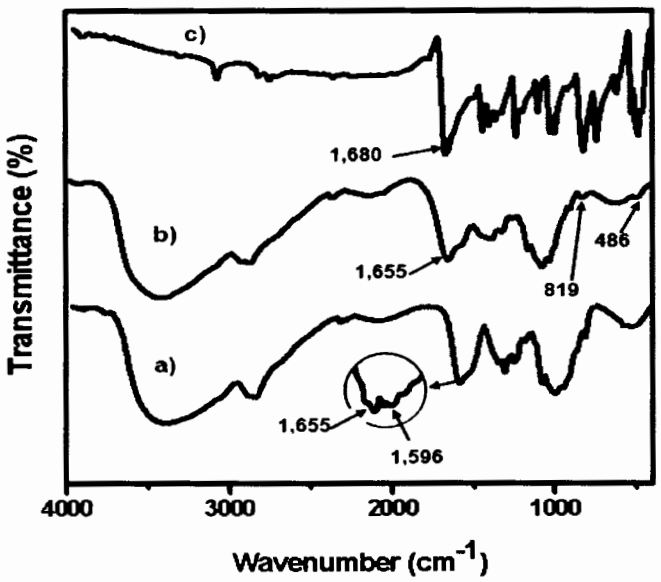


Figure 4.11 FT-IR spectra of a) CS, b) CS-Fc, and c) FcCHO.

4.2 Part II: Development of glucose biosensor based on GC/CNTs-NH_2-Fc-BSA-GOx electroed

4.2.1 Electrochemical detection of glucose oxidation on the developed biosensor

4.2.1.1 Cyclic voltammetric study of glucose oxidation

The electrochemical behavior of glucose oxidation was studied at different modified electrode materials using cyclic voltammetry (CV). The GC or GC modified electrode was used as a working electrode, Ag/AgCl as reference electrode and Pt wire as a counter electrode. Cyclic voltammograms of glucose were measured in a 0.1 M phosphate buffer pH 7.0 and the results show in Figure 4.12. Glucose oxidation waves obtained from GC, GC/CNTs-NH_2, GC/BSA, GC/GOx and GC/BSA-GOx electrodes were not well defined at the potential window from 0.0 to 1.0 V. As show in Figure 4.12 (c, g) the oxidation of glucose from GC/CNTs-NH_2-Fc and GC/CNTs-NH_2-Fc-BSA-GOx were observed at potential of 0.35 and 0.6 V, respectively. Results of anodic potential and current of 10 mM glucose at these electrodes are summarized in Table 4.3 The GC/CNTs-NH_2-Fc-BSA-GOx electrode provides the highest glucose oxidation current (Figure 4.12 g). CNTs-NH_2-Fc could improve the conductive property of the composites film and it can be used as electron transfer mediator to shuttle electrons between analytes and the electrode. The results indicated that electrode modified with the conjugation of mediator Fc to CNTs- NH_2 structure could inhibit Fc leakage from electrode surface. In addition, BSA plays an important role in enhancing the activity of the immobilized enzyme. This biosensor exhibited good analytical performance towards the quantification of glucose oxidation.

Table 4.3 Anodic potential ($E_{p,a}$) and current ($i_{p,a}$) of 10 mM glucose measured using bare and modified GC electrodes.

Electrode code	Electrode	($E_{p,a}$), V	($i_{p,a}$), μA
a	GC	> 1.0	-
b	GC/CNTs-NH_2	> 1.0	-
c	GC/CNTs-NH_2-Fc	0.35	0.32
d	GC/BSA	> 1.0	-
e	GC/GOx	>1.0	-
f	GC/BSA-GOx	> 1.0	-
g	GC/CNTs-NH_2-Fc-BSA-GOx	0.6	7.03

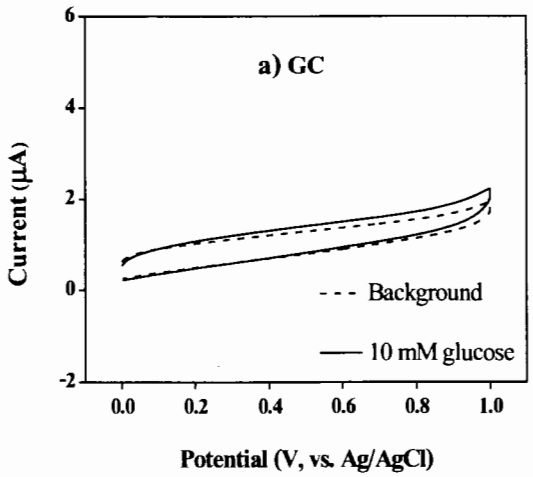


Figure 4.12 Cyclic voltammograms for a) bare glassy carbon electrode (GC) in the absent (dotted line) and present of 10 mM glucose (solid line), b) GC/CNTs-NH_2, c) GC/CNTs-NH_2-Fc, d) BSA, e) GC/GOx, f) GC/BSA-GOx and g) GC/CNTs-NH_2-Fc-BSA-GOx in 0.1 M phosphate buffer pH 7.0.

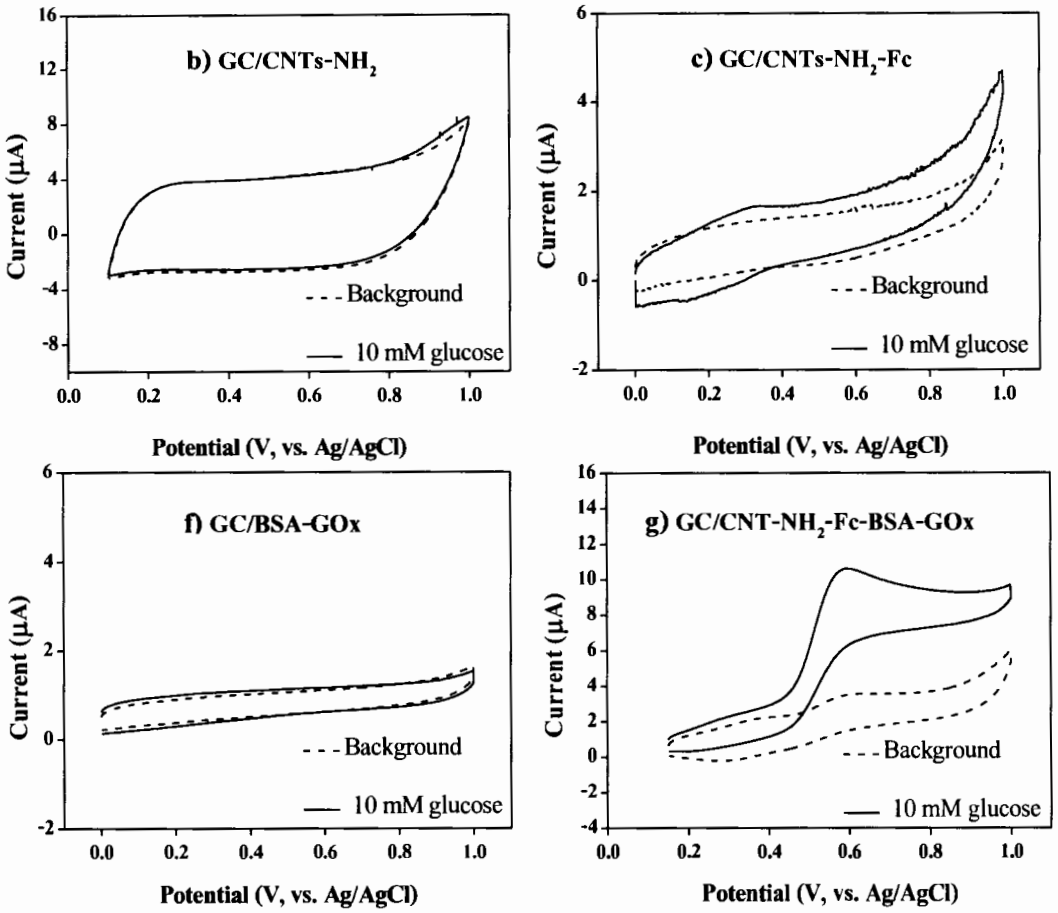


Figure 4.12 Cyclic voltammograms for a) bare glassy carbon electrode (GC) in the absent (dotted line) and present of 10 mM glucose (solid line), b) GC/CNTs-NH_2, c) GC/CNTs-NH_2-Fc, d) BSA, e) GC/GOx, f) GC/BSA-GOx and g) GC/CNTs-NH_2-Fc-BSA-GOx in 0.1 M phosphate buffer pH 7.0 (Continued).

4.2.2 Studies of parameters that effect the sensitivity of biosensor

4.2.2.1 Effect of CNTs-NH_2-Fc amount on the glucose response

The effect of CNTs-NH_2-Fc loading on the biosensor from 0 to 10 mg/mL was investigated. As shown in Figure 4.13, increasing of CNTs-NH_2-Fc amount could improve the conductive property of the composites film, which increased response of the resulting biosensor from 0.0 -5.0 mg/mL and seems to saturate at the concentration above 7.5 mg/mL. Thus the CNTs-NH_2-Fc of 7.5 mg/mL was selected as the optimum concentration.

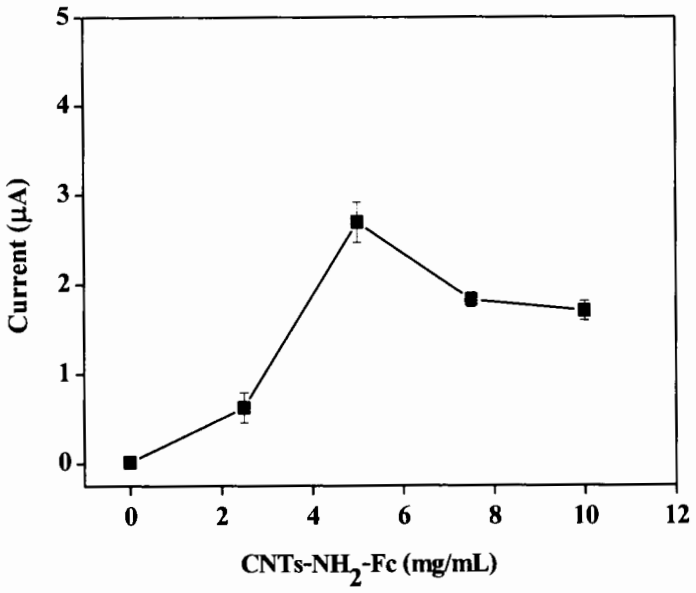


Figure 4.13 The effect of the concentration of CNTs-NH_2-Fc (mg/ mL) on the cyclic voltametry response of oxidation current to 10 mM glucose solution in phosphate buffer solution pH 7.0 .

4.2.2.2 Effect of BSA loading(GC/CNTs-NH_2-Fc-BSA-GOx)

The effect of BSA loading on the biosensor from 0 to 2% was investigated. As shown in Figure 4.14, the current response increased with increasing amount of BSA and reached maximum at about 1%. BSA contributes current response increased, it can be seen that the current response obtained from 0% BSA only about 1.5 μA while 1% BSA provide current response about 3.5 μA which for 2 times higher than that obtained from without BSA. These results assume that due to BSA was biocompatibility. It is concluded thus that BSA plays an important role in enhancing the activity of the immobilized enzyme. The slightly decrease of reduction current when the concentration of BSA greater than 1% was also observed. This assumed that because the resistance was increased and therefore the electron transfer was blocked. BSA contributes current response increased, it can be seen that the current response obtained from 0% BSA only about 1.5 μA while 1% BSA provide current response about 3.5 μA which 2 times higher than that obtained from without BSA.

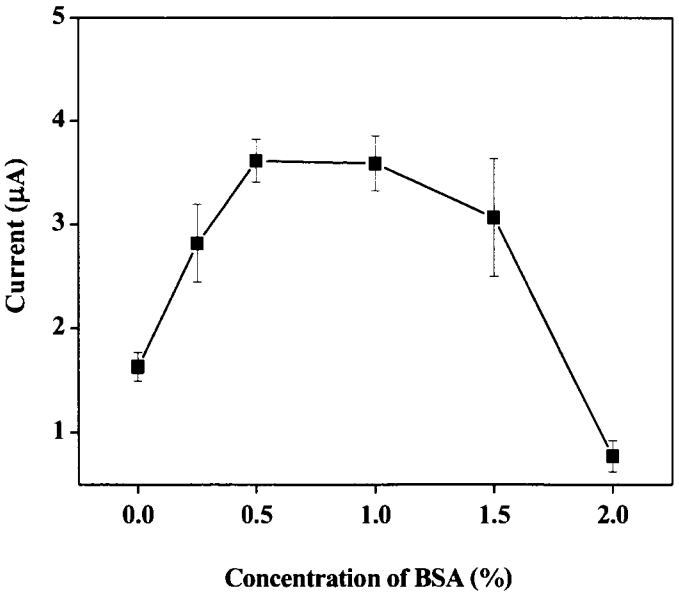


Figure 4.14 The effect of the concentration of BSA (%) on the cyclic voltametry response of oxidation current to 10 mM glucose solution in phosphate buffer solution pH 7.0

4.2.2.3 Effect of enzyme glucose oxidase loading

The concentration of enzyme in composites was also an important factor affecting the current response of the biosensor. The effect of enzyme loading on the biosensor from 0.0 to 10.0 mg/mL was investigated. As show in Figure 4.15, the current response increased with increasing amount of glucose oxidase immobilized on the electrode and reached the maximum at 7.5 mg/mL. These results were assumed that due to the GOx is a catalyzed the oxidation of glucose with increasing amount of enzyme loading increases the rate of glucose oxidation as a result, the current response gradually increased. The current response decreased significantly when the amount of GOx increased further than 7.5 mg/mL. The response then reduced slowly due to the increased protein concentration in composites, which restricted the approach of the substrate to the immobilized enzyme and attributed to the increase of film thickness, leading to an increase of interface electron transfer resistance, making the electron transfer more difficult, which was consistent with other previous studies [16, 65]. Therefore, 7.5 mg/mL glucose oxidase immobilized on the electrode was chosen for all subsequent experiments.

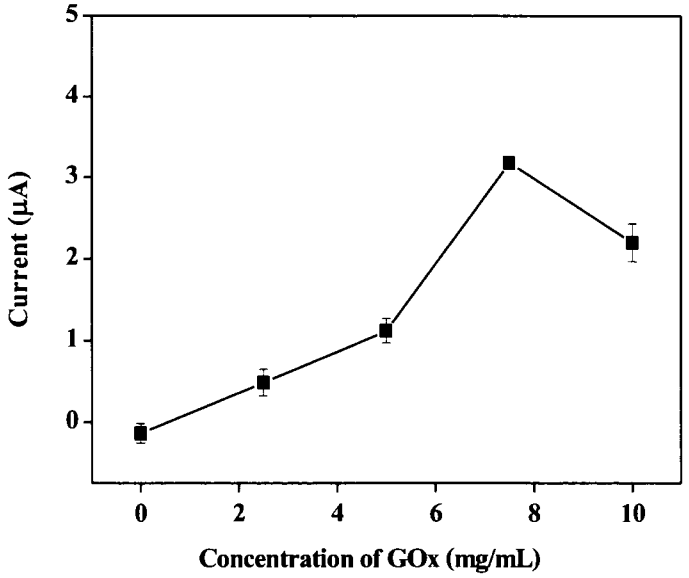


Figure 4.15 The effect of the concentration of GOx (mg/ mL) on the cyclic voltametry response of oxidation current to 10 mM glucose solution in phosphate buffer solution pH 7.0.

4.2.2.4 Effect of buffer pH (0.1 M phosphate buffer)

The influence of the buffer pH is very essential to the sensitivity of the biosensors, because the pH affects not only the electrochemical behavior of Fc but also the bioactivity of GOx. The optimal pH reported for GOx is usually in the range of 6.5 - 7.5 [3, 65-66], which varies with immobilization method and micro-environment around the enzyme. Thus the effect of pH was examined in the range of pH 4.0 to 9.0. Figure 4.16 a shows the cyclic voltammogram of 10 mM glucose at various pH. The relationship between the oxidation peak potential and pH was shown in Figure 4.16 b. With the pH increased from 4.0 - 8.0, the peak potential values remain constant. The further increase of buffer pH led to decrease of the potential values. Figure 4.16 c shows the relationship between the current response of the biosensor and pH. According to Figure 4.16 c, oxidation peak current increased with increasing pH from 4.0 to 7.0. The further increase of buffer pH led to decrease of the response. Decrease in the response at high pH was possibly due to the decrease of enzymatic activity. This biosensor showed a maximum response at pH 7.0, which was consistent with other previous studies [12, 16, 61-62]. As a consequence, the performance of the glucose biosensor was evaluated at pH 7.0 for the further work.

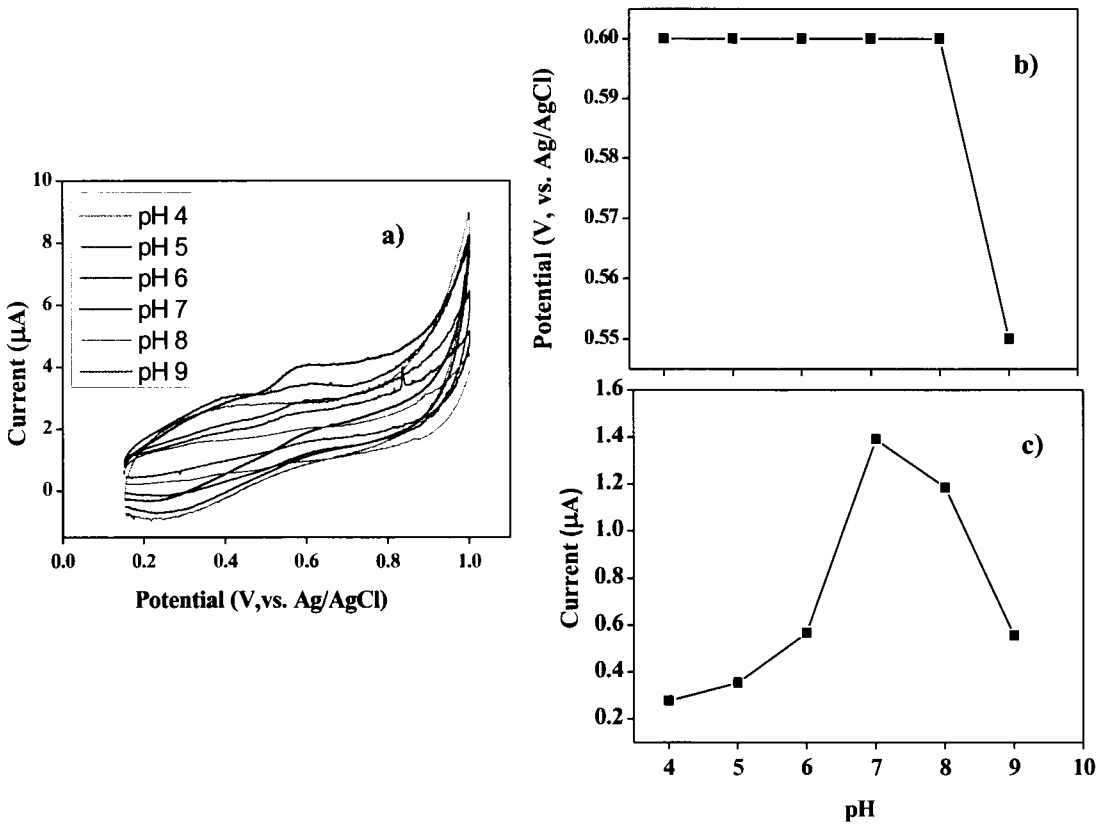


Figure 4.16 a) Cyclic voltammetric responses of 10 mM glucose at various buffer pH and the dependence of buffer pH on the b) peak potential, c) peak current obtained from the biosensor.

4.2.2.5 Electrochemical behavior of the GC/CNTs-NH_2-Fc-BSA-GOx electrode under optimum conditions

The electrochemical behavior of glucose oxidation was studied at the developed glucose biosensor (GC/CNTs-NH_2-Fc-BSA-GOx) using cyclic voltammetric experiments were performed in 0.1 M phosphate buffer. Figure 4.17shows the electrocatalytic response of the CNTs-NH_2-Fc-BSA-GOx modified electrodes to glucose. In the absence of glucose, the reversible electrochemical behavior of Fc was observed on the electrode (Figure 4.17 a)). Figure 4.17 b, c and d displayed the cyclic voltammogram of 5, 10, 15 mM glucose which anodic peak current increased corresponding to the increasing concentration. The results clearly showed the catalytic properties of modified electrode to glucose. This reaction could be described by the well known following mechanism in [67].

$$\text{Glucose} + GOx_{(ox)} \longrightarrow \text{Gluconolactone} + GOx_{(red)} \quad (4.1)$$

$$GOx_{(red)} + 2Fc^{+} \longrightarrow GOx_{(ox)} + 2Fc \quad (4.2)$$

$$2Fc + 2e^{-} \longrightarrow 2Fc^{+} \text{ (on electrode)} \quad (4.3)$$

In the presence of glucose and enzyme GOx, glucose is oxidized to gluconolactone and the coenzyme $GOx_{(ox)}$ is converted to $GOx_{(red)}$. The resulting reduced form of the enzyme $GOx_{(red)}$ is then reoxidized by the ferrocene ion, yielding ferrocene, which in turn is reoxidized at the electrode.

These facts suggested that the BSA and ferrocene bound CNT-NH_2 could facilitate electron communication between the active site of the immobilized enzyme and the electrode surface. Therefore, sensitive and stable biosensors can be fabricated using these nanocomposites.

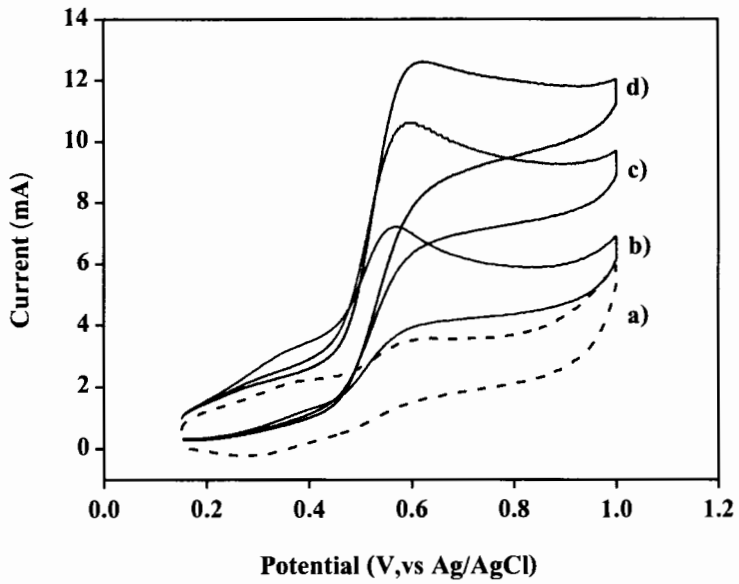


Figure 4.17 Cyclic voltammograms of the glucose biosensor (GC/CNTs-NH_2-Fc-BSA-GOx) a) 0.1 M phosphate buffer, b) 5, c) 10 and d) 15 mM of glucose.

4.2.3 Amperometric detection of glucose in the developed FIA

4.2.3.1 Optimum potential for amperometric detection

The detection potential affects the sensitivity of current signal of an analyte. In order to obtain the optimal potential for amperometric detection in FIA, hydrodynamic voltammetric behavior of glucose was investigated at various potential from 0.3 to 0.8 V. As shown in Figure 4.18, the response current increased with the increase of applied potential until the potential reached 0.6 V, and then decreased when the of applied potential was above. Therefore, a potential of 0.6 V was chosen for further studies. The results indicated that electrocatalytic activity of enzyme GOx incorporated with the matrix CNTs- NH_2-Fc-BSA toward the oxidation of glucose enables the biosensor can be effectively detect at potential 0.6 V. This potential was similar to previous work reported by Koide et al. [68], their developed glucose biosensor based on glucose oxidase (GOx) immobilized to ferrocene-modified polyallylamine hydrochloride and bovine serum albumin by using glutaraldehyde (Glu) before coated on a glassy carbon (GC) electrode.

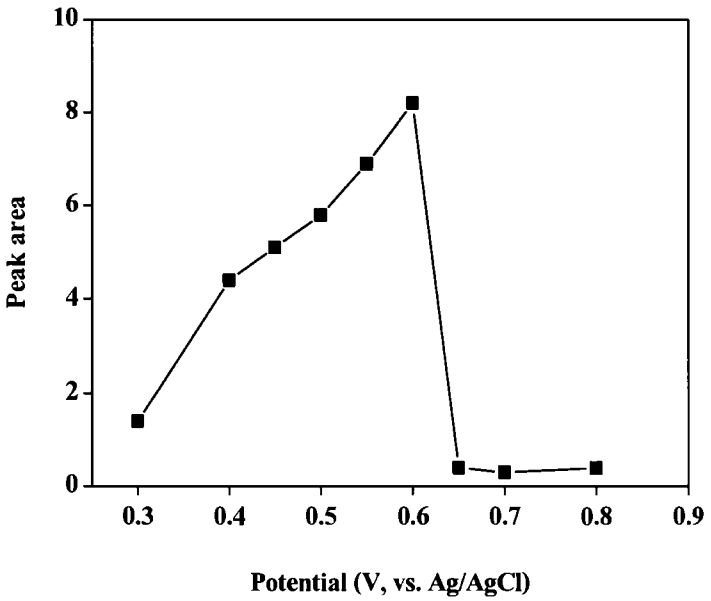


Figure 4.18 Effect of operating potential on the biosensor response studied by amperometric measurement of 10 mM glucose in 0.1M phosphate buffer (pH 7.0).

4.2.3.2 Optimum flow rate for glucose measurements using FIA system.

The flow rate used for glucose measurements was an important parameter since the process involved the enzymatic reaction kinetics and the sensor response time toward oxidation of glucose. The effect of flow rate on the biosensor response was optimized by injection of 10 mM of glucose into the carrier stream to optimize sensitivity and sample throughput. The flow rate was studied between 0.3 to 1.5 mL/min. Figure 4.19 shows that the response decreases with the increasing of flow rate. If the higher flow rate was applied, it is likely that carrier stream containing samples passed by the detector has not adequate time to react at the surface of electrode which correspond to the previous report [59]. However, increasing flow rate increase sample throughput. To balance between response and sample throughput, the flow rate of 1.0 mL/min was selected as the future experimental.

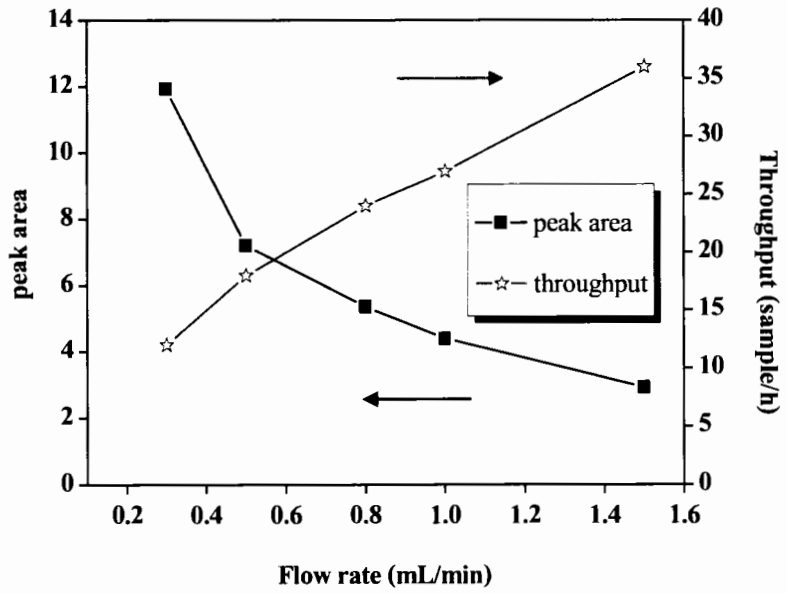


Figure 4.19 Effect of the flow rate on glucose biosensor response and sample throughput.

4.2.4 Analytical feature

4.2.4.1 Linear concentration range

The current response of the GC/CNTs-NH_2-Fc-BSA-GOx biosensor was investigated after injection glucose standard solution into the carrier solution under optimized condition. As shown in Figure 4.20. The calibration curve of the biosensor shows a linear range of 2 -16 mM for glucose with the regression equation of y = 0.3235 x - 0.6655 and a correlation coefficient of 0.9996. The sensitivity of the biosensor obtained from this method is approximately 323 nA/mM. This linear response range is wider than the ranges for the glucose biosensor based on immobilizing glucose oxidase (GOx) on poly(methy l methacrylate)–bovine serum albumin (PMMA-BSA) core–shell nanoparticles modified GCE (0.2-9.1 mM) [13] and the glucose sensor prepared using ferrocene modified polysiloxane/chitosan composites (1.0-6.0 mM) [10].

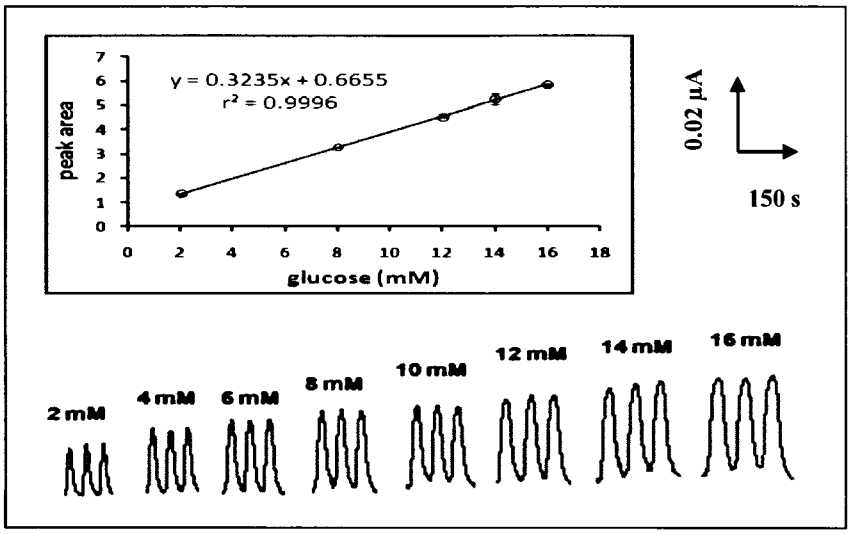


Figure4.20 FIA diagram of injected glucose concentration within 2 - 16 mM in to phosphate buffer solution pH 7.0, operating potential 0.6 V vs Ag/AgCl, flow rate 1 mL/min, injection volume of 20 µL. The inset shows the linear relationship between the signal of glucose and the concentration.

4.2.4.2 Limit of detection

The limit of detection was investigated by injection by 2 mM glucose with the seven replicates into a carrier solution of 0.1 M phosphate buffer pH 7.0. The results show in Figure 4.21. The detection limit was calculated to 0.65 mM that was obtained from the signal-to-noise characteristics of these data (S/N = 3). A relative standard deviation (% RSD) of 1.9 for the measurement was obtained. These results indicate that the measurement has high precision for the biosensor. Sample throughput is 72 samples /h.

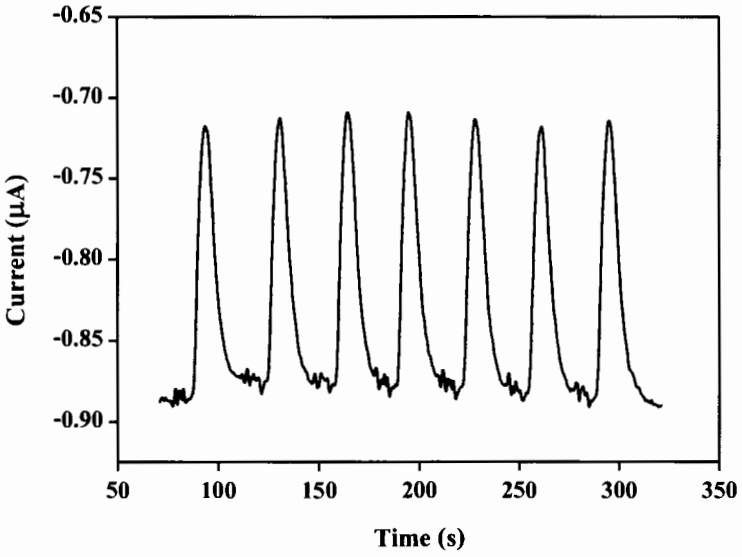


Figure 4.21 FI amperometric response obtained from the developed sulfite biosensor for 7 repetitive injection of 2 mM glucose. Carrier solution; 0.1 M phosphate buffer pH 7.0, applied potential 0.6 V, flow rate; 1.0 mL/min.

4.2.4.3 Interference study

In this study, the effect of interference, including compounds that are likely to exist in the sample of interest was investigated. The concentration of the interference that provides signal change greater than 5 % was considered as the tolerance limit. The interference used in this study was sodium chloride (NaCl) fructose, sucrose, dopamine, and ascorbic acid (AA). The results are summarized in Table 4.4. The results from this study could be roughly divided in to two groups.

Group I: very low interfering potentials. In this group, tolerance limit were in between 4 to 10 mM these foreign were including NaCl, fructose and sucrose.

Group II: low interfering potentials. In this group, tolerance limit was less than 2 mM which were dopamine and AA.

Table 4.4 Effect of interference on amperometric signal obtained from replicate injections (n=3) of standard glucose 10 mM.

Interference	Investigated concentration (mM)	Tolerance limit[a] (mM)
sodium chloride (NaCl)	2-100	10
fructose	2-200	4
sucrose	2-200	10
dopamine	2	2
ascorbic acid(AA)	2-4	2

[a] greater than ± 5% signal alteration is classified as interfering condition.

4.2.4.4 Performance on real sample applications

In an attempt to explore the CNTs-NH_2-Fc-BSA-GOx composites electrode for practical application, the biosensor was applied to determine glucose in sample of glucose for diabetes. Five samples including: A (diluted sample 150 times), B (diluted sample 200 times), C (diluted sample 300 times), D (diluted sample 500 times) and E (diluted sample 600 times) were diluted with 0.1 M phosphate buffer pH7.0 then investigated using our developed method. The concentration of glucose in samples determined from calibration of the FIA system (Figure 4.22). As show in Table 4.5, the concentration of glucose in samples determined by the developed glucose biosensor provide the accurate values with the relative error (calculated from the labeled concentration) was less than 1.4%, which indicates that the CNTs-NH_2-Fc-BSA-GOx composites electrode is promising for practical application in determination of glucose.

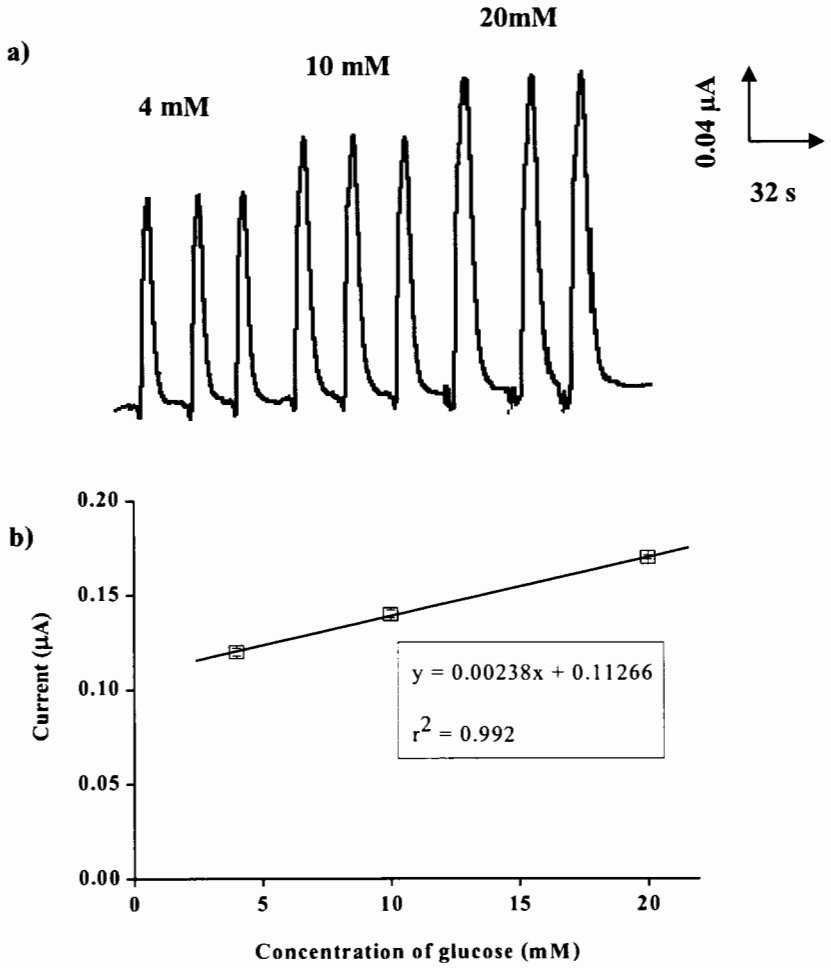


Figure 4.22 Response to standard glucose a) and the corresponding standard glucose calibration curve b). Flow rate 1.0 mL/min, applied potential 0.6 V.

Table 4.5 Determination of glucose in real sample with the biosensor.

Sample code	Signal (current, µA)	Determined by biosensor (mM)	[a]Labeled content (mM)	Relative error (%)
A	0.157 ± 0.002	2,808.50± 0.002	2,770.00	1.39
B	0.146 ± 0.002	2,767.36± 0.002		0.10
C	0.135 ± 0.001	2,803.36±0.001		1.20
D	0.126 ± 0.001	2,773.66±0.001		0.13
E	0.124 ± 0.002	2,808.40±0.002		1.39

[a]Glucose for intravenous injection in diabetes (volume 50 mL, glucose 5 g/10 mL).

4.2.4.5 Stability of the developed biosensor

The modified electrodes were investigated for their operational stability. The operational stability of the modified electrode was investigated by uninterruptedly measuring the glucose response from the repeated injections of 10 mM glucose. Relationship between of (%) relative responses and injection (times) was shown in Figure 4.23. The responses showed good stability, at 50 injections the current response still remained at 90 % of its initial response. The excellent analytical performance and response stability of the fabricated biosensor can be attributed to three aspects: First, BSA provides a biocompatible microenvironment around the enzyme molecules to stabilize its biological activity. Second, the formation of the CNTs -NH_2-Fc conjugant prevents the Fc from leakage out of the film. Lastly, CNTs-NH_2 provided a conduction pathway to accelerate electron transfer due to their excellent conductivity.

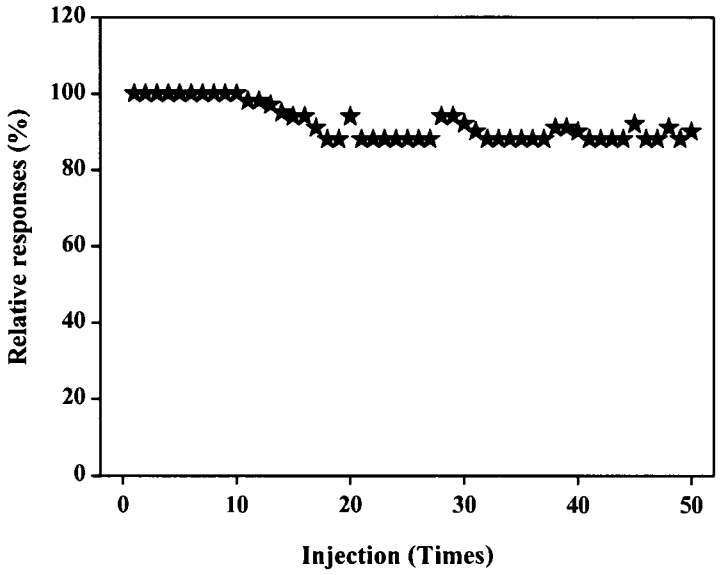


Figure 4.23 Operational stability of the CNTs-NH_2-Fc-BSA-GOx glucose biosensor to repeated injections of 10 mM glucose.

4.2.5 Characterization of CNTs-$NH_2$by FI-IR spectroscopy

Figure 4.24 shows the FT-IR spectra of CNTs-NH_2 (curve a), CNTs-Fc (curve b), and pure FcCHO (curve c). Results from Figure 4.24 displays the characteristic peaks of N-H stretching vibration at 3,417 cm^{-1} and in-plane scissoring vibration at 1,647 cm^{-1} from primary amine, C–H stretching vibration at 2,868, 2,935 cm^{-1}. The FTIR spectrum of FcCHO (curve c) shows the peak at 1,680 cm^{-1} which is ascribed to the stretching vibration of carbonyl from the aldehyde group. The aldehyde group can easily react with the -NH_2 group on CNTs-NH_2 through the Schiff-based reaction and subsequently forms the CNTs-Fc by the reduction of $NaCNBH_3$. In the spectrum of the CNTs-NH_2-Fc (curve b), displays the evident of FcCHO completely reacted with the amino groups of CNTs- NH_2. This evident was confirmed by the disappeared of all peaks at 1,647 cm^{-1} and 1,680 cm^{-1}. IR-spactrum obtained from this work corresponded to the CNTs-NH_2 spectrum reported by Qiu et al. and Liu et al. [3, 62].

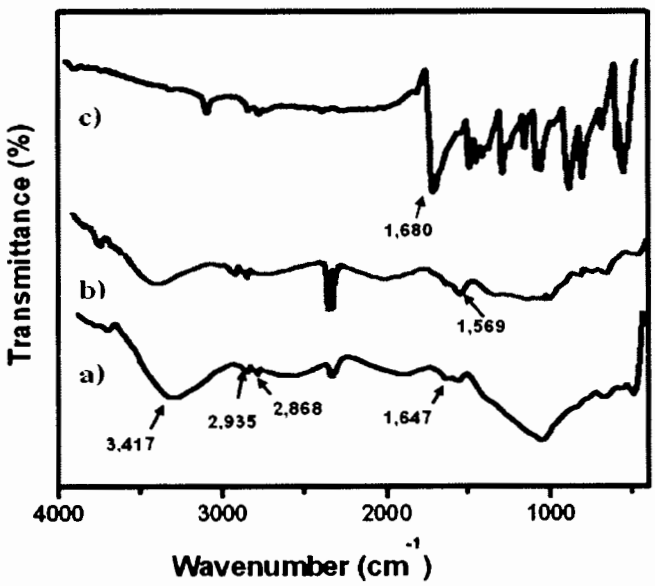


Figure 4.24 FT-IR spectra of a) CNTs-NH_2, b) CNTs-NH_2-Fcand c) FcCHO.

4.2.6 Comparison the performance characteristics of biosensor GC/CNTs-NH_2-Fc/CS-Fc/GOx with GC/CNTs-NH_2-Fc-BSA-GOx electrode

In this work consists of two methods for the construction of novel amperometric glucose biosensors. The first method is based on glucose oxidase (GOx) entrapped on composites of ferrocene covalently bound onto the NH_2-functionalized multiwall carbon nanotubes (CNTs-NH_2-Fc) and ferrocene bound chitosan (CS-Fc) coated on GC electrode (GC/CNTs-NH_2-Fc/CS-Fc/GOx). The second method is based on glucose oxidase (GOx) immobilized to ferrocene-modified NH_2-functionalized multiwalled carbon nanotubes and bovine serum albumin (CNTs-NH_2-Fc -BSA) composites film by using glutaraldehyde (Glu) before coated on a glassy carbon (GC) electrode (GC/CNTs-NH_2-Fc-BSA-GOx). The performance characteristics of the amperometric response assessed in terms of linear concentration range, sensitivity and limit of detection from both methods are summarized in Table 4.6. A wider linear range was obtained in the GC/CNTs-NH_2-Fc/CS-Fc/GOx biosensor, when compared to the GC/CNTs-NH_2-Fc-BSA-GOx biosensor. The limit of detection was obtained in the GC/CNTs-NH_2-Fc-BSA-GOx biosensor lower that of the GC/CNTs-NH_2-Fc/CS-Fc/GOx about 3 times. The GC/CNTs-NH_2-Fc-BSA-GOx biosensor showed a satisfactory sensitivity, which was revealed by the value of 323 nA/mM when compared the GC/CNTs-NH_2-Fc/CS-Fc/GOx biosensor was sensitivity about 5.0 nA/mM. The GC/CNTs-NH_2-Fc-BSA-GOx biosensor showed good stability at 50 injections the current response still remained at 90 % of its initial response with the GC/CNTs-NH_2-Fc/CS-Fc/GOx biosensor showed stability, at 40 injections.

These all results were assumed that due to immobilization of an enzyme results in a considerable change in the microenvironment of the enzyme and may affect the properties of the enzyme, as well as changes in the physical and kinetic properties. These changes may affect their usefulness in biochemical analysis. The first method is based on physical entrapment of GOx molecules in the composites. There is no modification of the biological element so that the activity of the enzyme is preserved during the immobilization process. Biosensors based on physically entrapped enzymes are often characterized by increased operational. However, limitations such as leaching of biocomponent and possible diffusion barriers can restrict the performances of the systems. The second method is based on GOx molecules cross-linked by glutaraldehyde on the BSA. This method is attractive due to its simplicity and the strong chemical

binding achieved between biomolecules. The main drawback is the possibility of activity losses due to the distortion of the active enzyme conformation and the chemical alterations of the active site during cross-linking.

Table 4.6 Comparison of the present GC/CNTs-NH_2-Fc/CS-Fc/GOx with GC/CNTs-NH_2-Fc-BSA-GOx electrode.

Electrode	Detection potential	Sensitivity (nA/mM)	Linear range (mM)	Limit of detection (mM)	Sample throughput (samples/h)	[a]Stability (injection)
GC/CNTs-NH_2-Fc/CS-Fc/GOx	0.3 V	5.0	2-80	1.86	42	40
GC/CNTs-NH_2-Fc-BSA-GOx	0.6 V	323	2-16	0.65	72	50

[a] The current response remained at 90% of its initial response.

CHAPTER 5

CONCLUSIONS

This work presented the development of glucose biosensor for amperometric determination of glucose using a simple flow injection system. This work consists of two main parts.

5.1 Part I: Development of glucose biosensor based on GC/CNTs- NH_2-Fc/CS-Fc/GOx

In this work biosensor were developed based on glucose oxidase (GOx) entrapped on composites of i) ferrocene covalently bound onto the NH_2-functionalized multiwall carbon nanotubes (CNTs-NH_2-Fc) and ii) ferrocene bound chitosan (CS-Fc) to prevent ferrocene leakage from the matrix and retain its activity as an electron mediator before coated on a glassy carbon (GC) electrode. The electrocatalytic activity of CNTs-NH_2-Fc remarkably improves the electron relays for activation of oxidation of glucose and accelerate electrochemical reaction. The CS-Fc provides a favorable microenvironment to keep the bioactivity of GOx. The developed glucose biosensor (GC/CNTs-NH_2-Fc/CS-Fc/GOx) was applied in the flow injection analysis system for amperometric detection of glucose using solution of 0.1 M phosphate buffer (pH 7.0) as a carrier and applying a potential of 0.3 V. The proposed glucose biosensor exhibits linear calibration over the range of 2-80 mM for glucose response with the regression equation $y = 0.005 x - 0.067$ and a correlation coefficient of 0.997. The limit of detection, based on a signal-to-noise ratio (S/N = 3) of three, was 1.86 mM. The developed biosensor also provides good precision (% RSD = 3.0) for glucose signal (2 mM, n = 10) with rapid sample throughput (42 samples/h).

5.2 Part II: Development of glucose biosensor base on GC/CNTs-NH_2-Fc-BSA-GOx

In this part, a high sensitive and rapid measurement of glucose biosensor was developed for amperometric detection in flow injection system. The biosensor was developed based on ferrocene (Fc) covalently bound onto the NH_2-functionalized multiwall carbon nanotubes (CNTs-NH_2) to prevent ferrocene leakage from the matrix and retain its activity as an electron mediator. Glucose oxidase (GOx) was immobilized to ferrocene-modified NH_2-functionalized multiwalled carbon nanotubes and bovine serum albumin (CNTs-NH_2-Fc-BSA) composites film by using glutaraldehyde (Glu) before coated on a glassy carbon (GC) electrode. In CNTs-NH_2-Fc -BSA) composites film, BSA provided a biocompatible microenvironment for the GOx to retain its bioactivity and CNTs-NH_2-Fc possessed excellent inherent conductivity to enhance the electron transfer rate. The developed glucose biosensor (GC/CNTs-NH_2-Fc-BSA-GOx) was applied in the flow injection analysis system for amperometric detection of glucose using solution of 0.1 M phosphate buffer (pH 7.0) as a carrier and applying a potential of 0.6 V. The proposed glucose biosensor exhibits linear calibration over the range of 2-16 mM for glucose response with the regression equation y = 0.3235x-0.6655 and a correlation coefficient of 0.9996. The sensitivity of the biosensor obtained from the slop of this linear part of the calibration is approximately 323 nA /mM. The limit of detection, based on a signal-to-noise ratio (S/N = 3) of three, was 0.65 mM. The developed biosensor also provides good precision (% RSD = 1.9) for glucose signal (2 mM, n = 7) with rapid sample throughput (72 samples/h). The fabrication of amperometric glucose biosensors both methods exhibits good reproducibility, sensitive, selectivity, stability, fast response time and ease of preparation and low cost.

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APPENDICES

APPENDIX A

Part I: Electrochemical detection of glucose on glucose biosensor

(GC/CNTs-NH_2-Fc/CS-Fc/GOx)

Effect of CNTs-NH_2-Fc loading on the glucose response obtained from GC/CNTs-NH_2-Fc/CS Fc/GOx electrode (raw data for Figure 4.2)

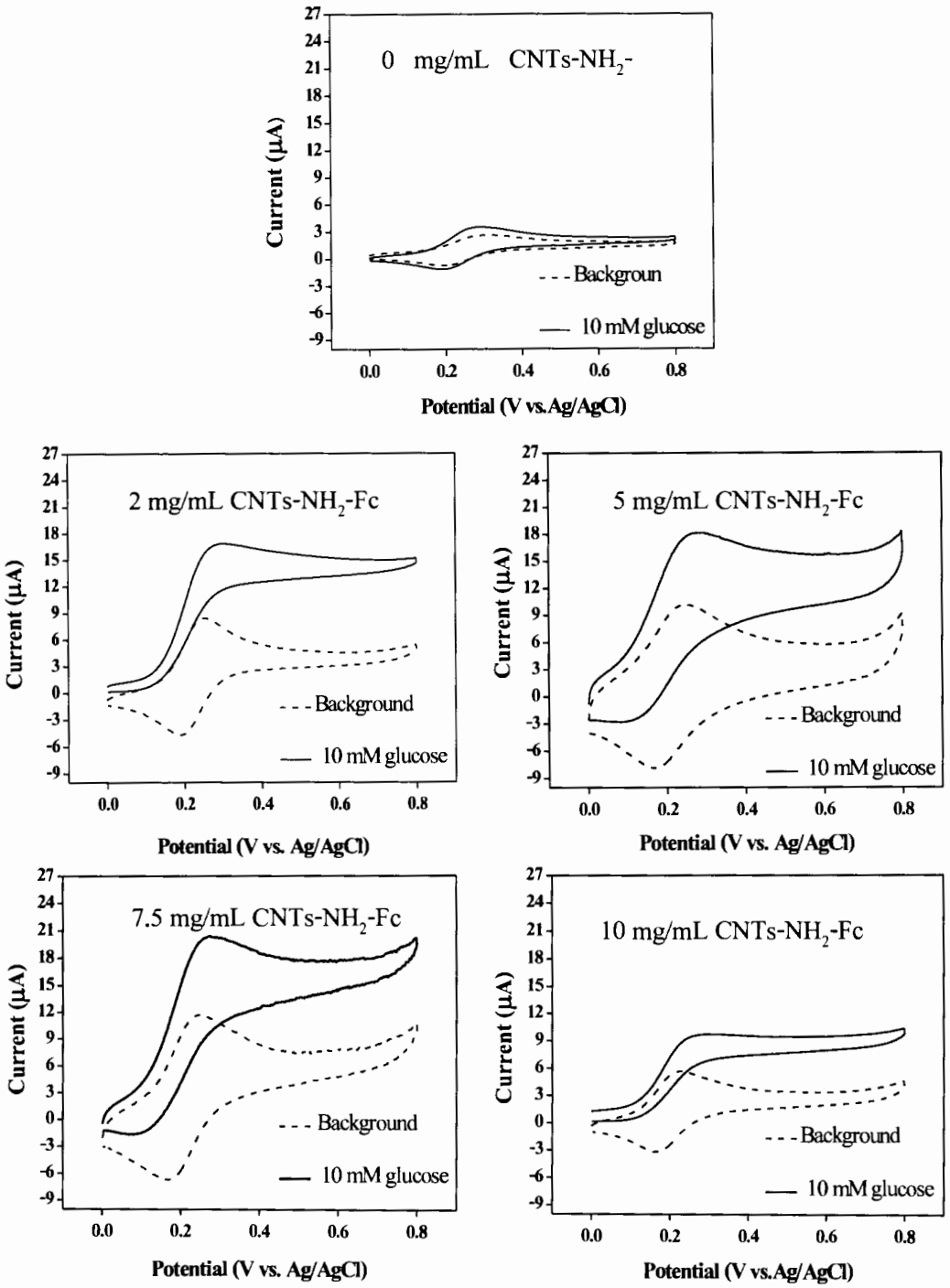


Figure A.1 Cyclicvoltammetric responses of 10 mM glucose at various concentration of CNTs-NH_2-Fc (mg/mL).

Effect of CS-Fc loading (raw data for Figure 4.3)

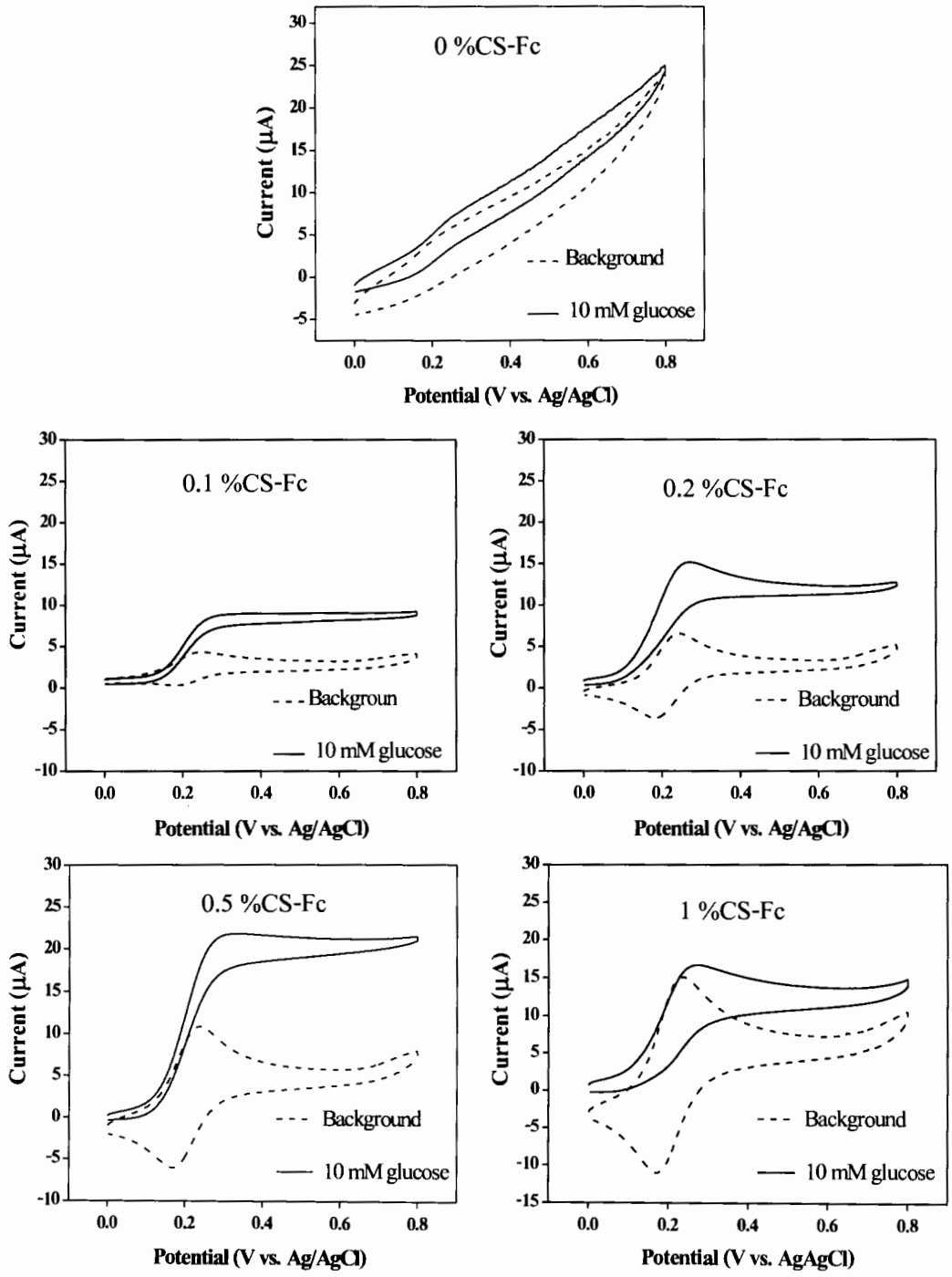


Figure A.2 Cyclic voltammetric responses of 10 mM glucose CS–Fc concentration in modified in electrode.

Effect of enzyme glucose oxidase loading(raw data for Figure 4.4)

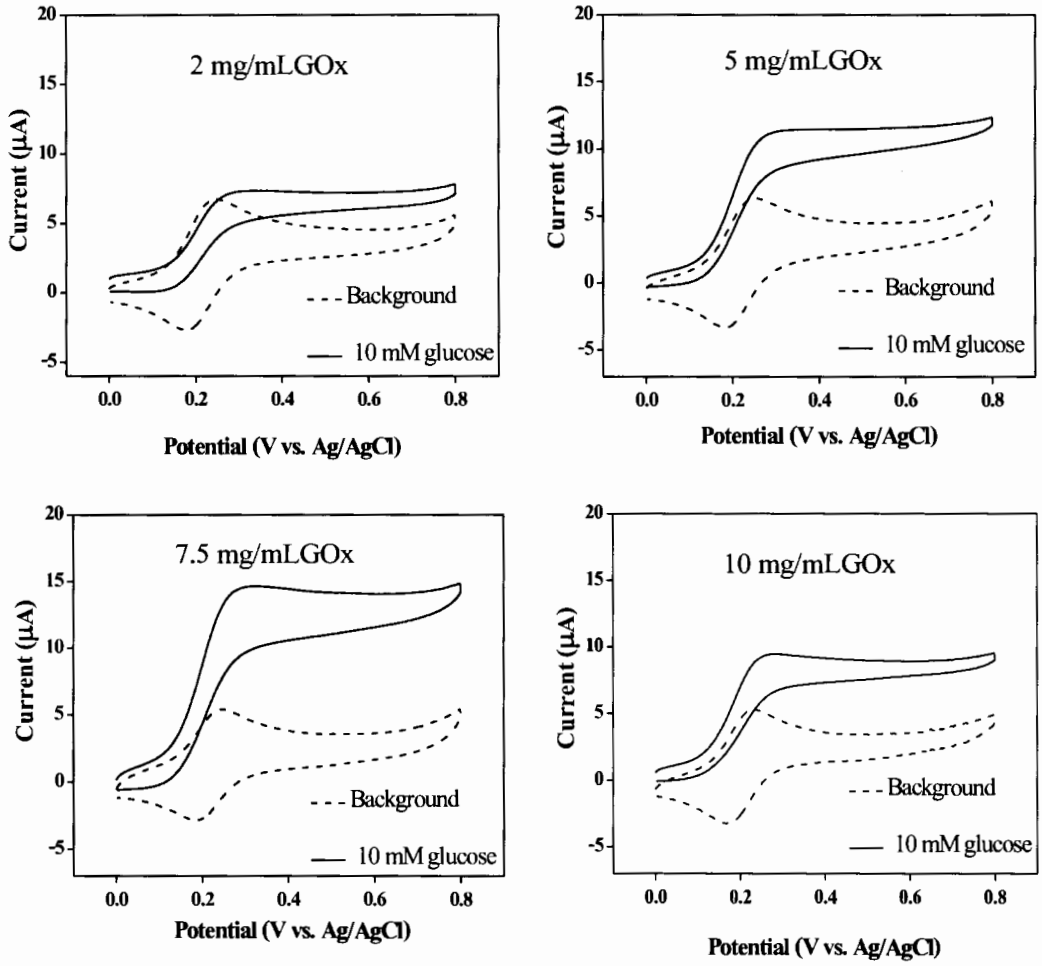


Figure A.3 Cyclic voltammetric responses of 10 mM glucose on GOx concentration in modified in electrode.

Optimum potential for amperometric detection (raw data for Figure 4.7)

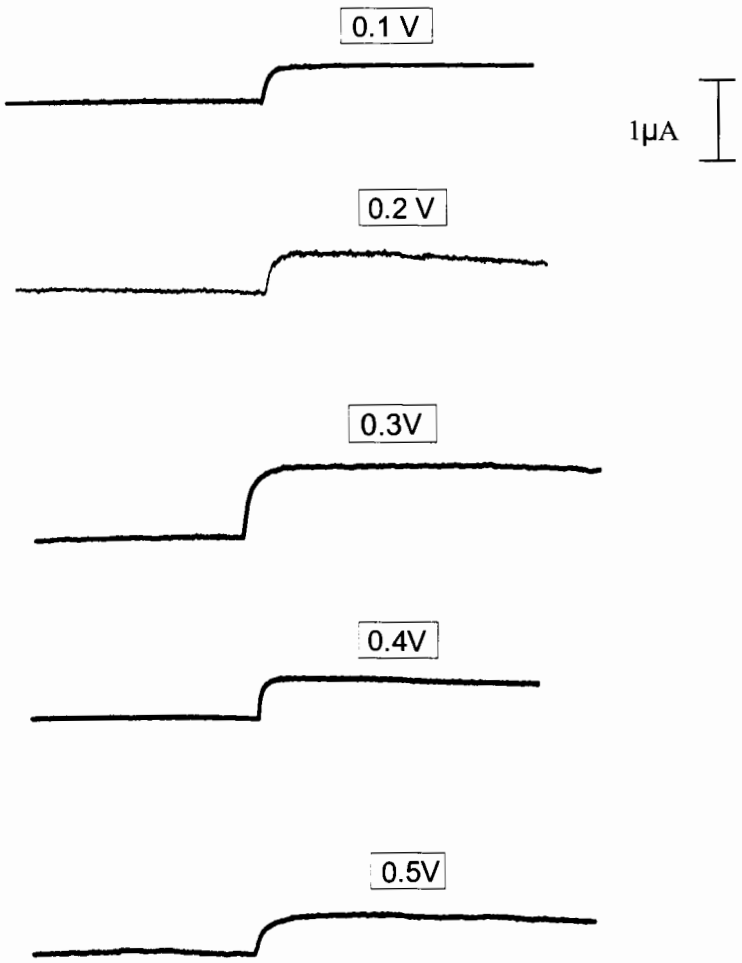
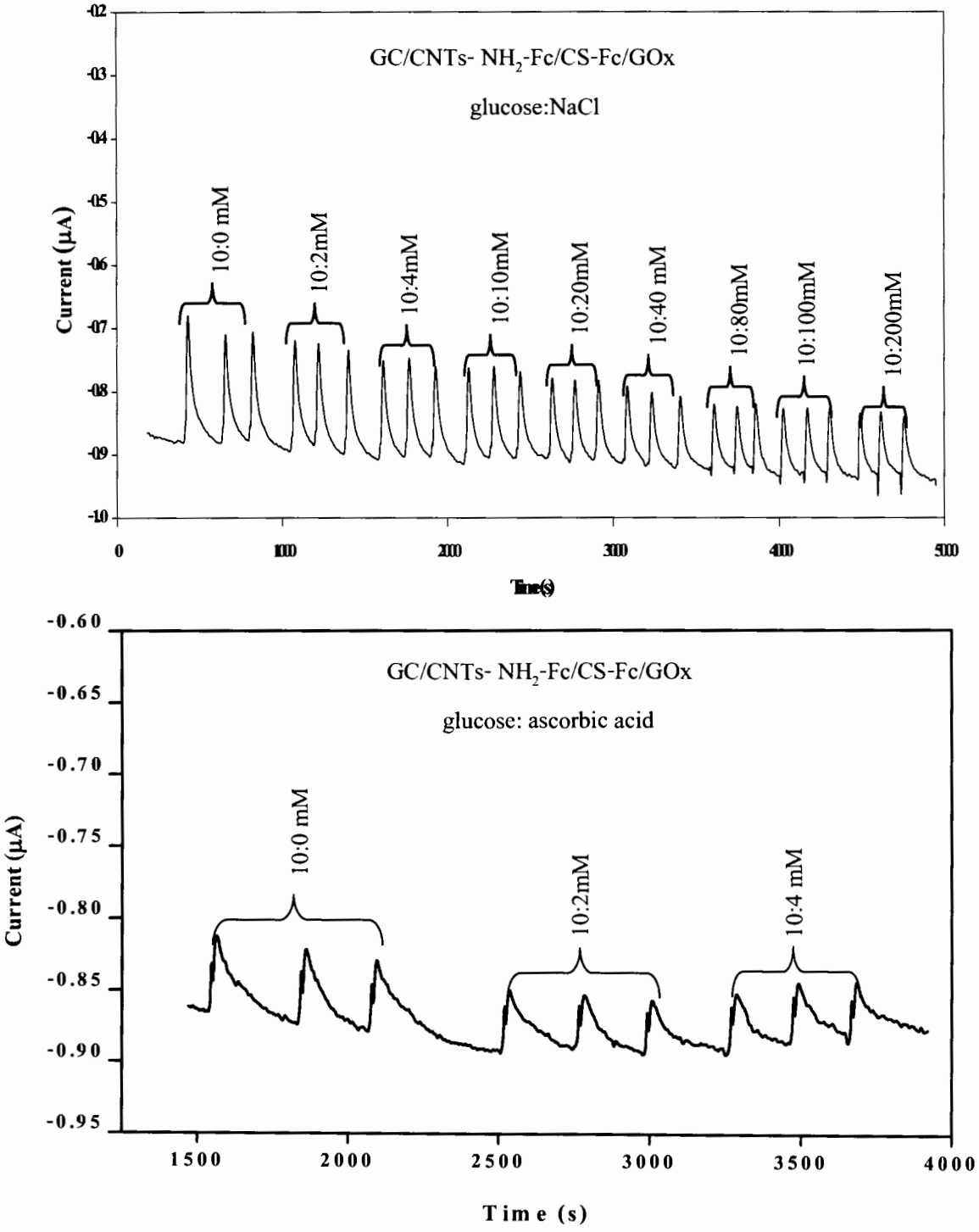
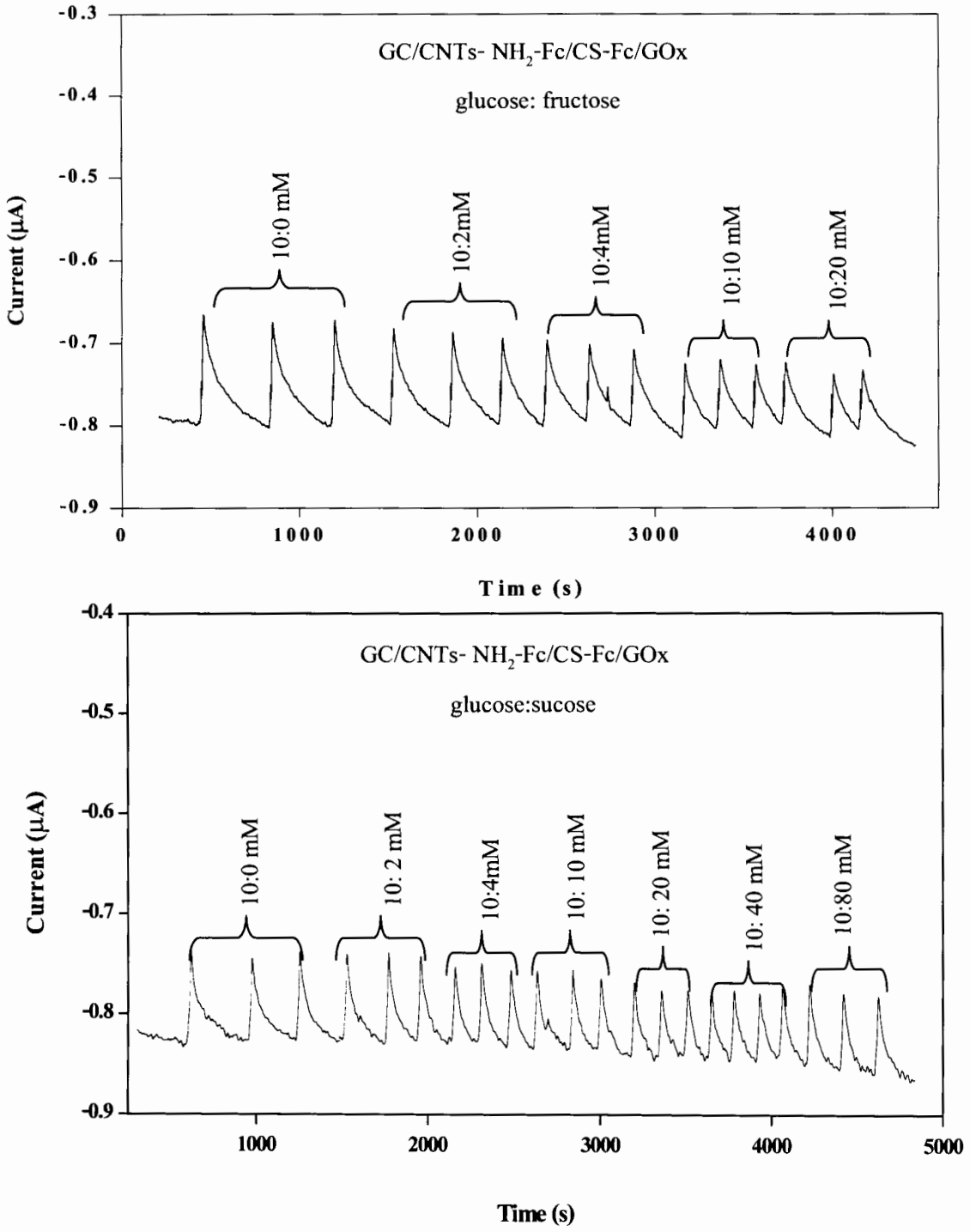


Figure A.4 Amperogram of the applied potential on the GC/CNTs-NH_2-Fc/CS-Fc/GOx biosensor response 0.5 mM glucose.

Interference study (raw data for Table 4.2)

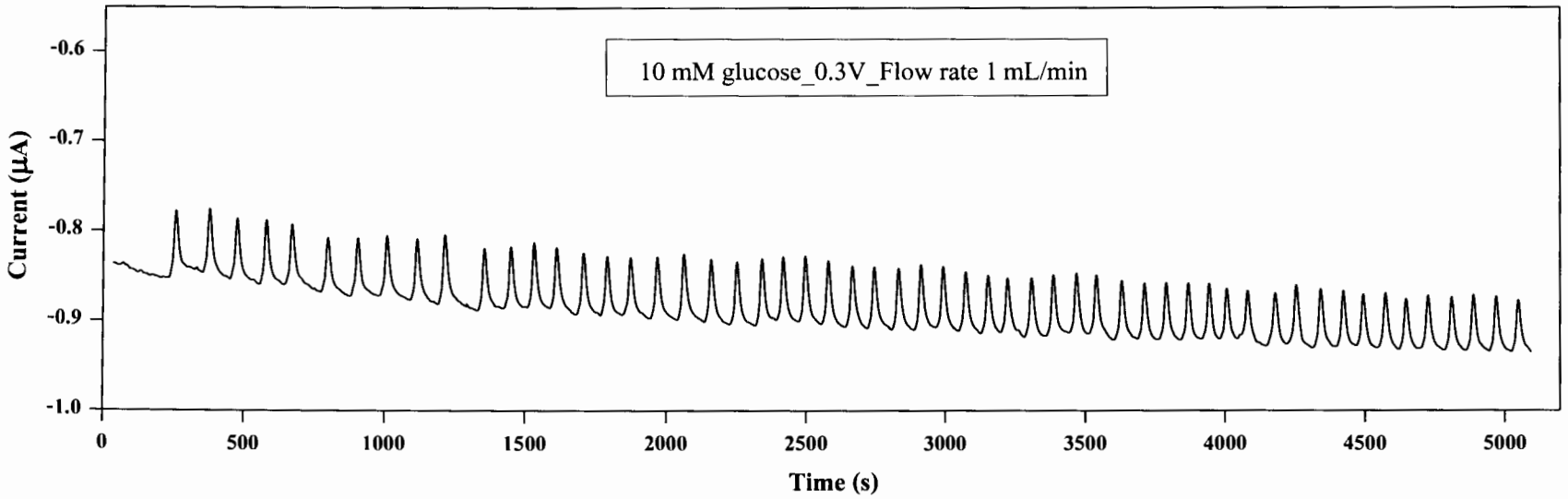


Figrure A.5 FIA gram of the interference on the GC/CNTs-NH_2-Fc/CS-Fc/GOx biosensor.



Figrure A.5 FIA gram of the interference on the GC/CNTs-NH_2-Fc/CS-Fc/GOx biosensor (Continued).

Stability of the develop biosensor (raw data for Figure 4.10)



Figrure A.6 FIA gram of stability on the GC/CNTs-NH_2-Fc/CS-Fc/GOx biosensor.

APPENDIX B

Part II: Electrochemical detection of glucose on glucose biosensor

(GC/CNTs-NH_2-Fc-BSA-GOx)

Effect of CNTs-NH_2-Fc amount on the glucose response(raw data for Figure 4.13)

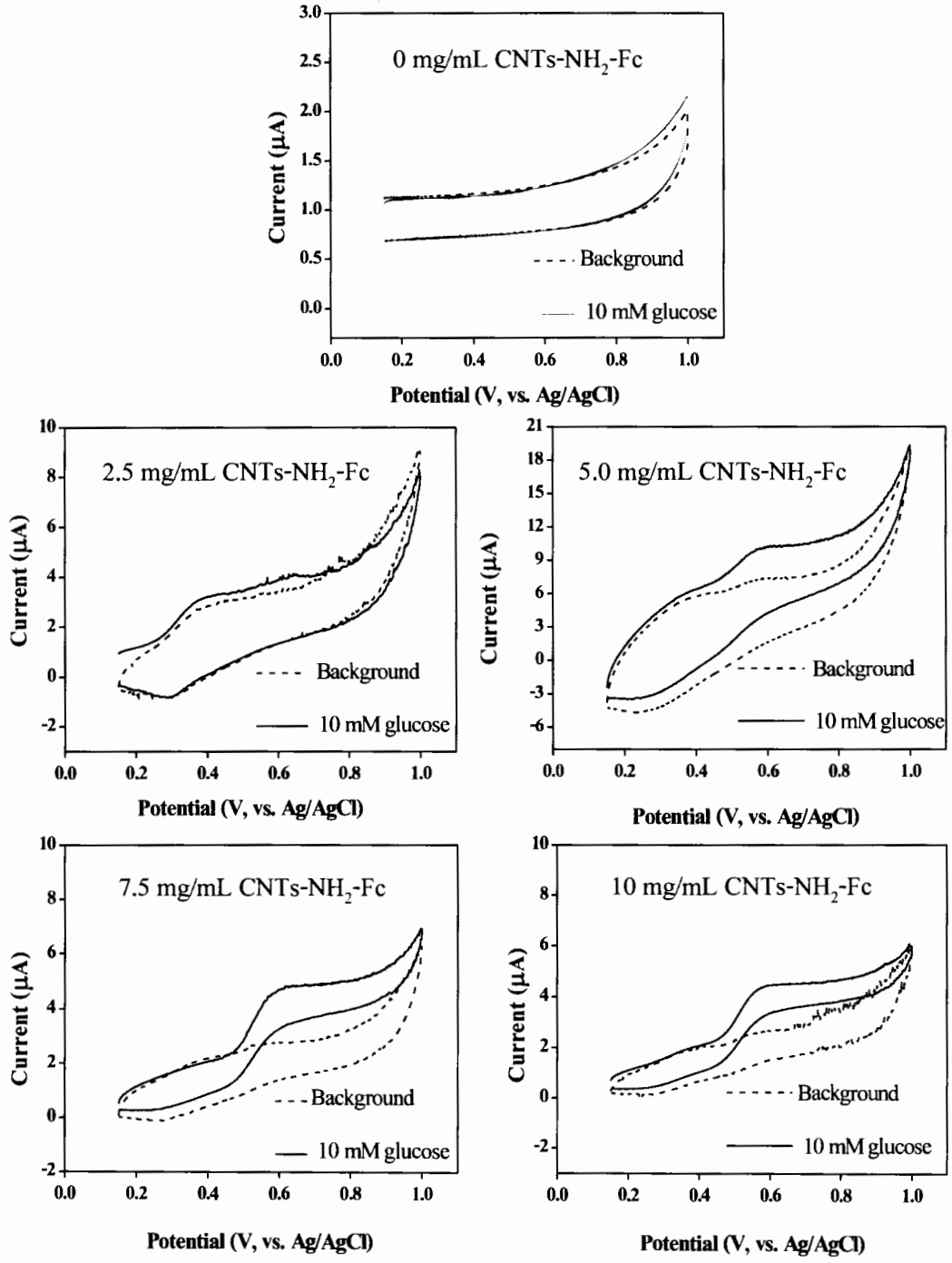


Figure B.1 Cyclicvoltammetric responses of 10 mM glucose on CNTs-NH_2-Fc concentration in modified in electrode.

Effect of BSA loading (GC/CNTs-NH_2-Fc-BSA-GOx) (raw data for Figure 4.14)

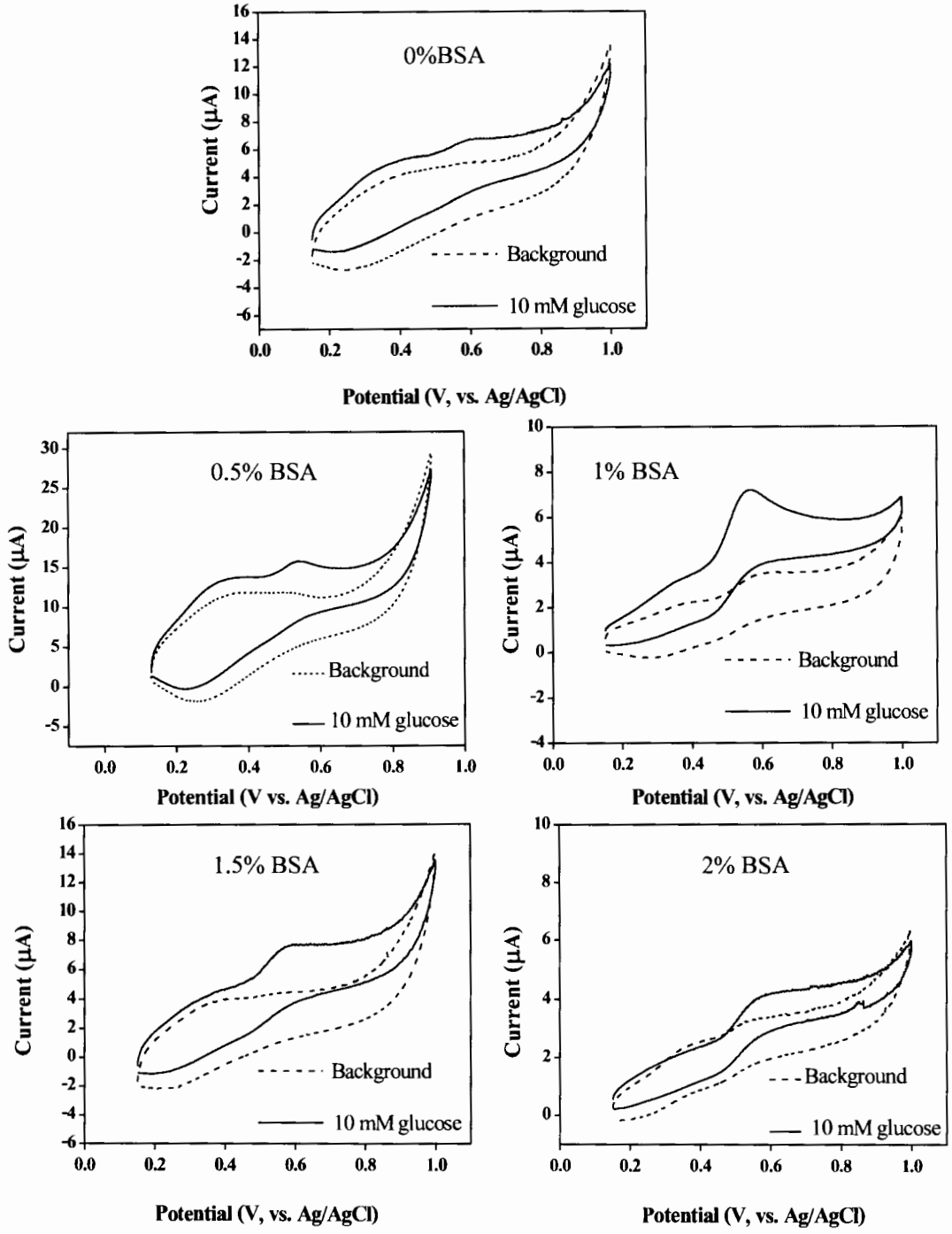


Figure B.2 Cyclic voltammetric responses of 10 mM glucose on BSA concentration in modified in electrode.

Effect of enzyme glucose oxidase loading (raw data for Figure 4.15)

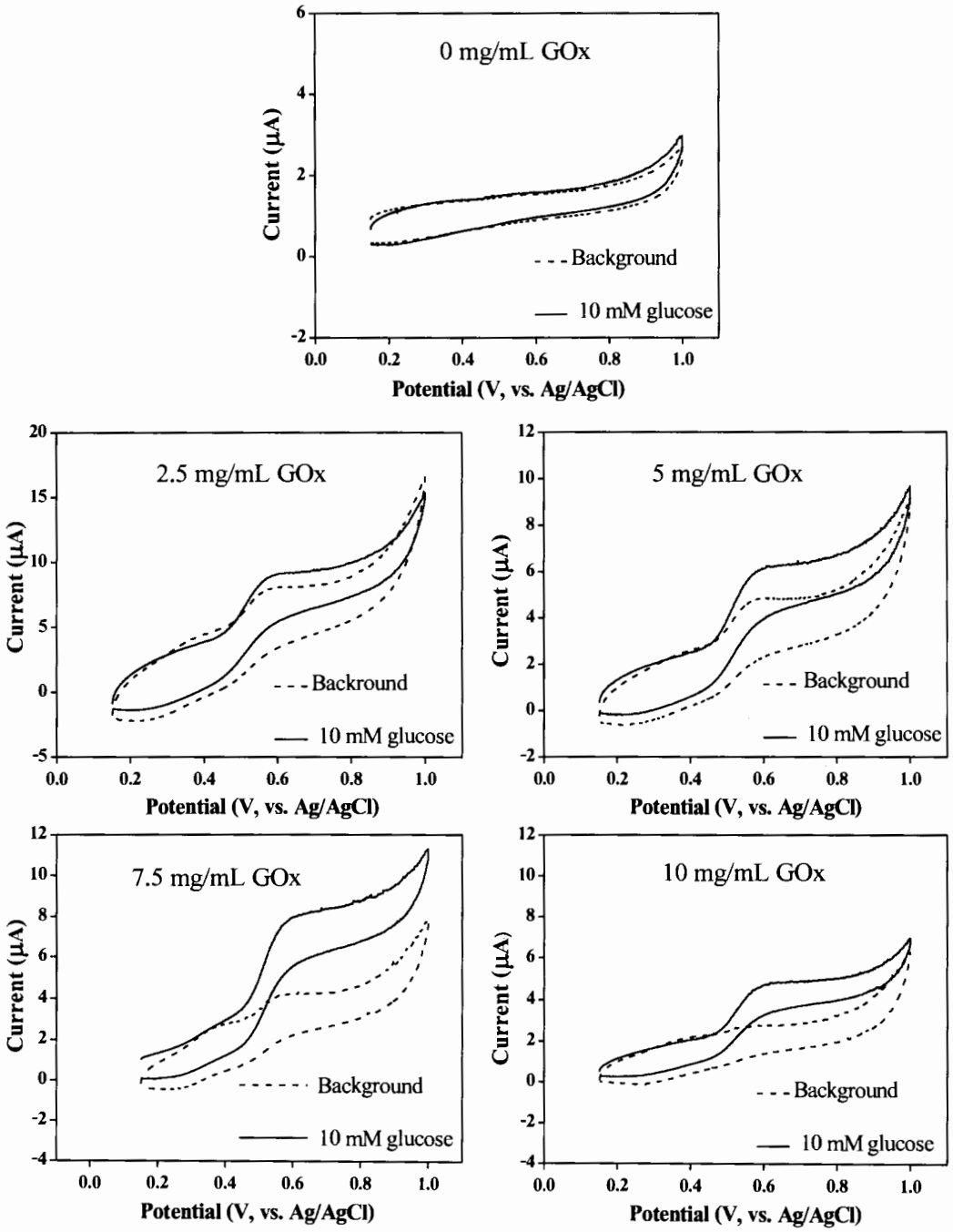
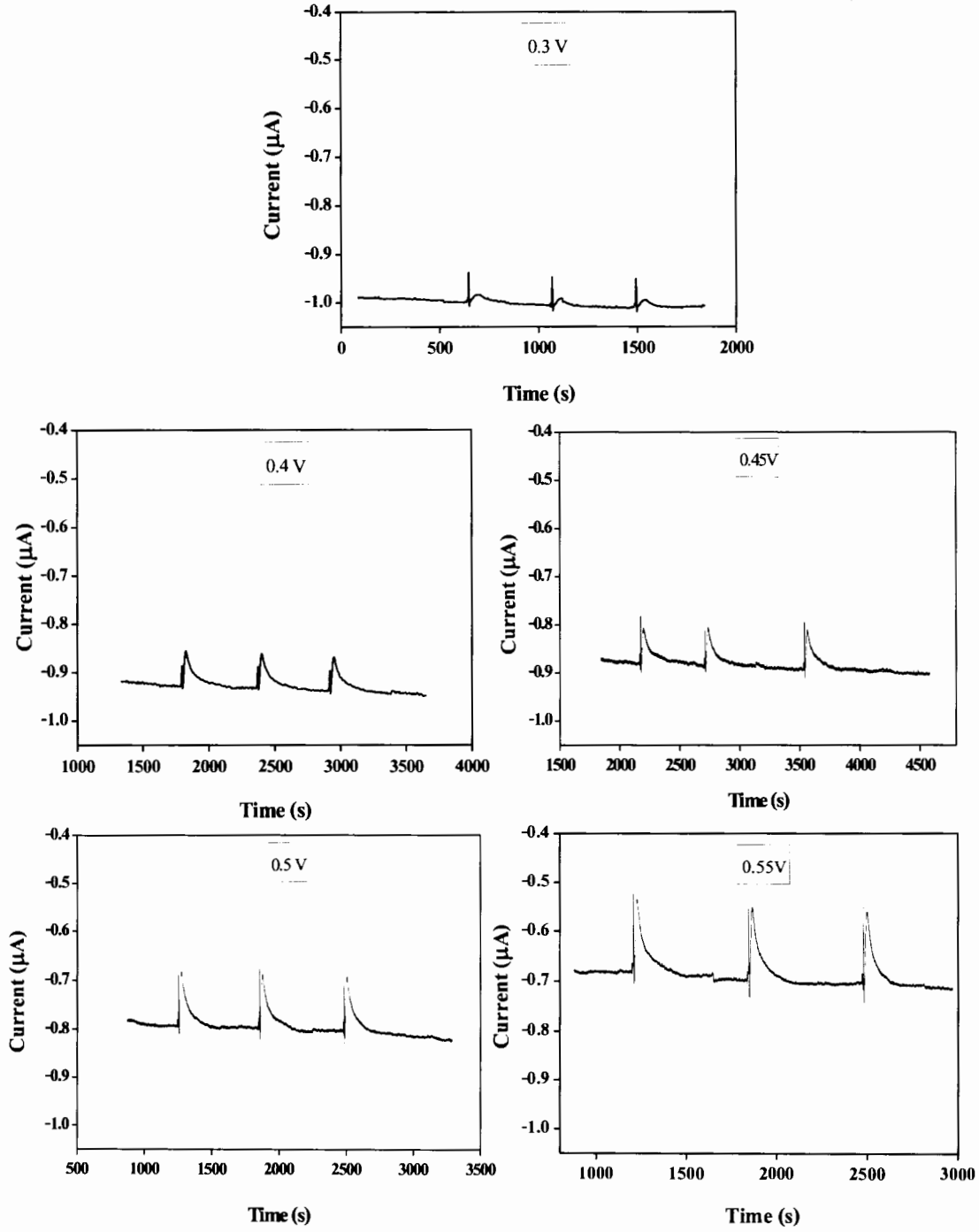
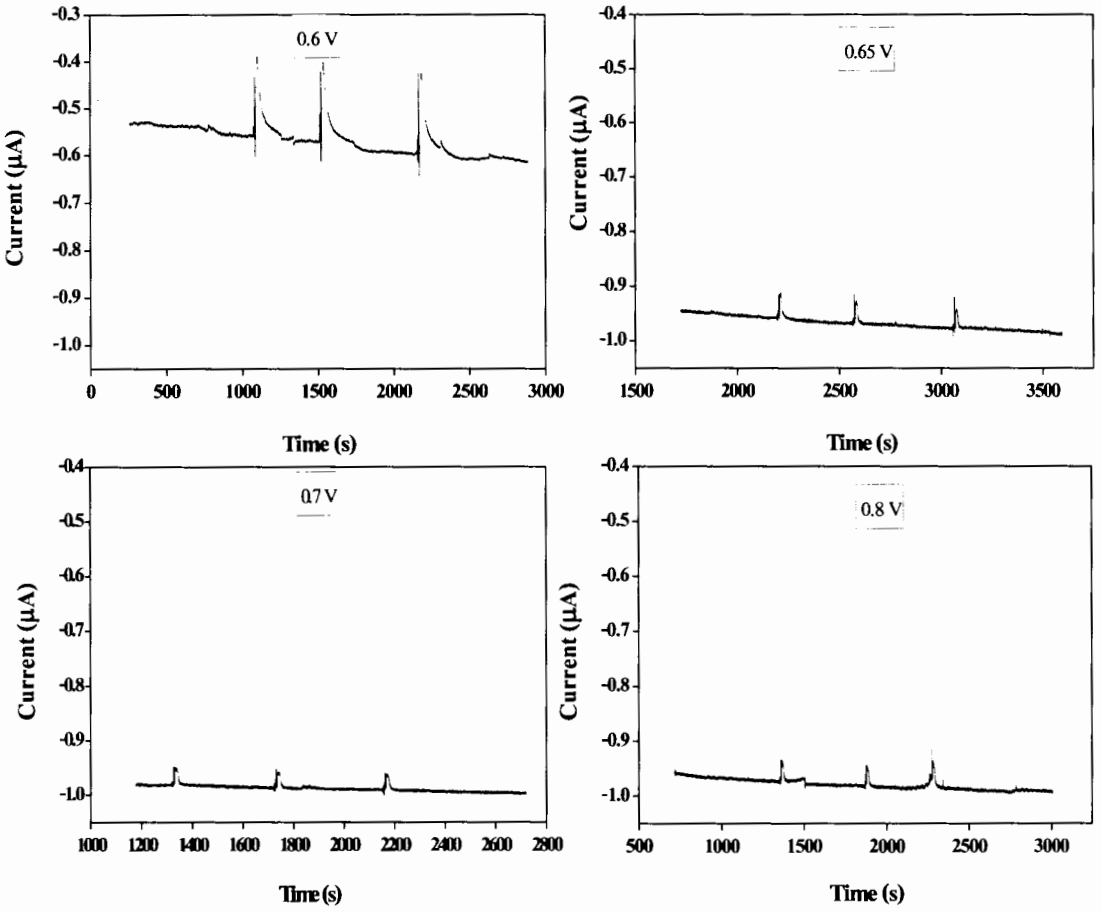


Figure B.3 Cyclic voltammetric responses of 10 mM glucose on glucose oxidase (GOx) concentration in modified in electrode.

Optimum potential for amperometric detection (raw data for Table 4.18)

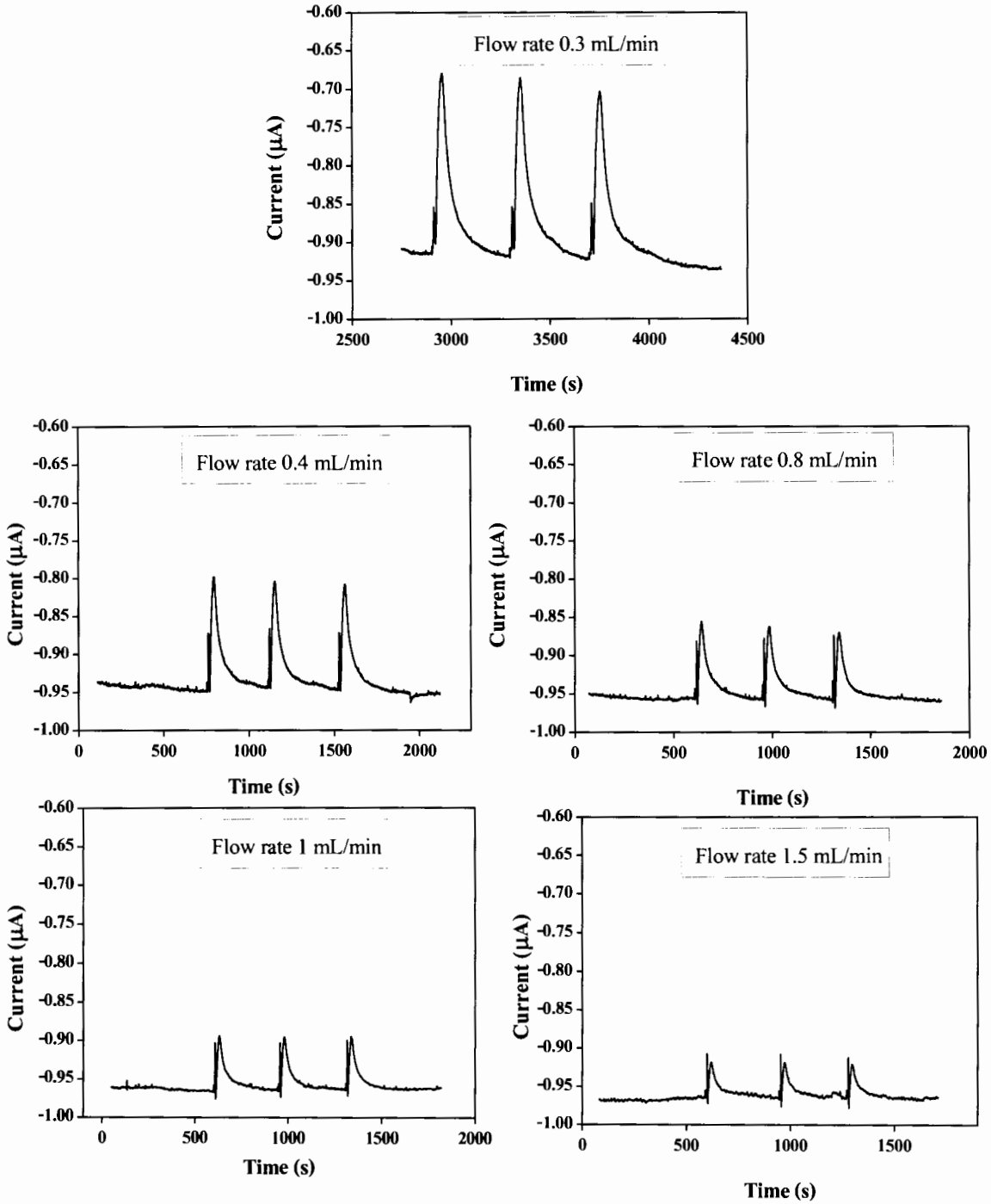


Figrure B.4 FIA gram of the applied potential on the GC/CNTs-NH_2-Fc-BSA-GOx biosensor response 10 mM glucose, flow rate 1 mL/min.



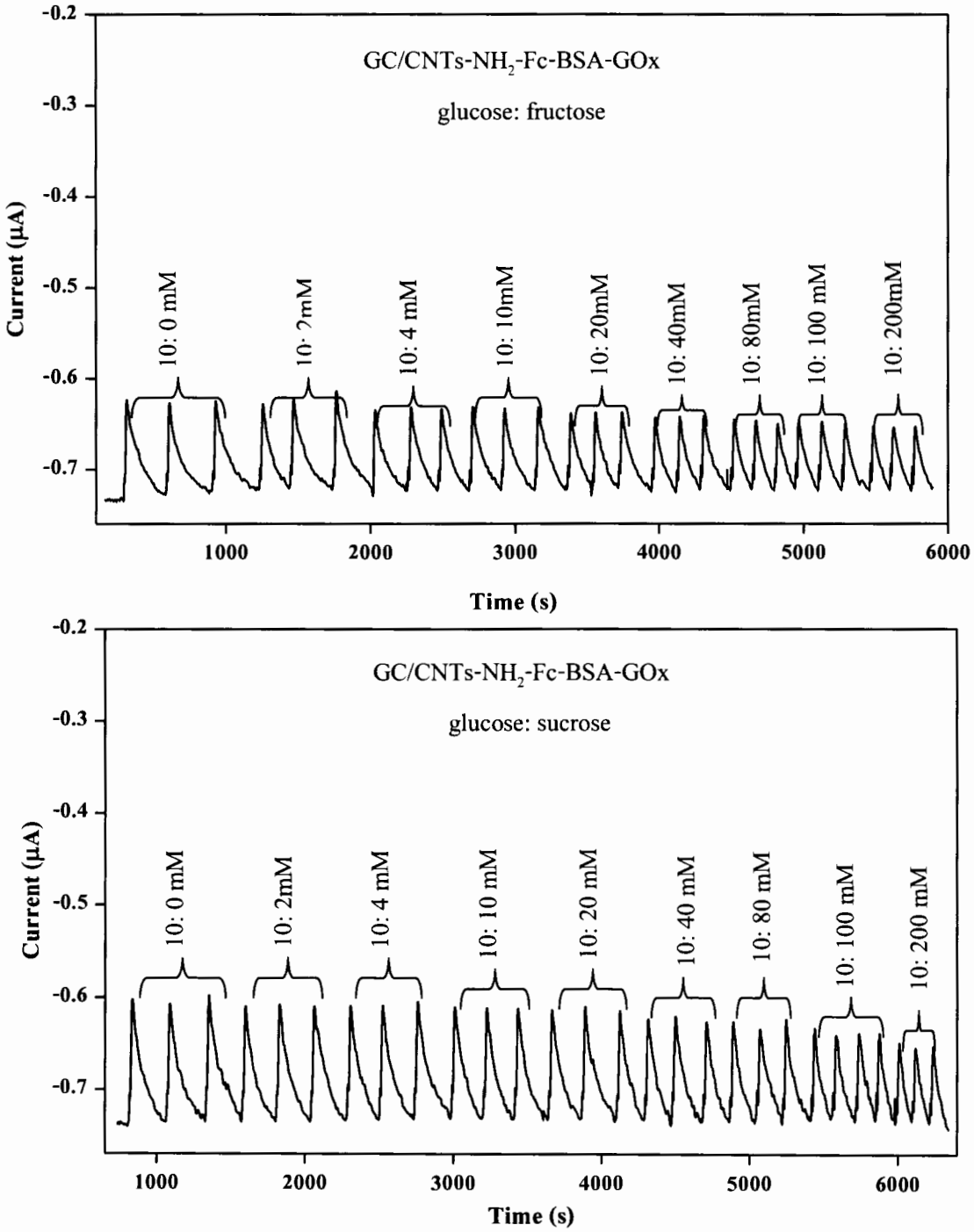
Figrure B.4 FIA gram of the applied potential on the GC/CNTs-NH_2-Fc-BSA-GOx biosensor response 10 mM glucose, flow rate 1 mL/min (Continued).

Optimum flow rate for glucose measurements using FIA system (raw data for Table 4.19)

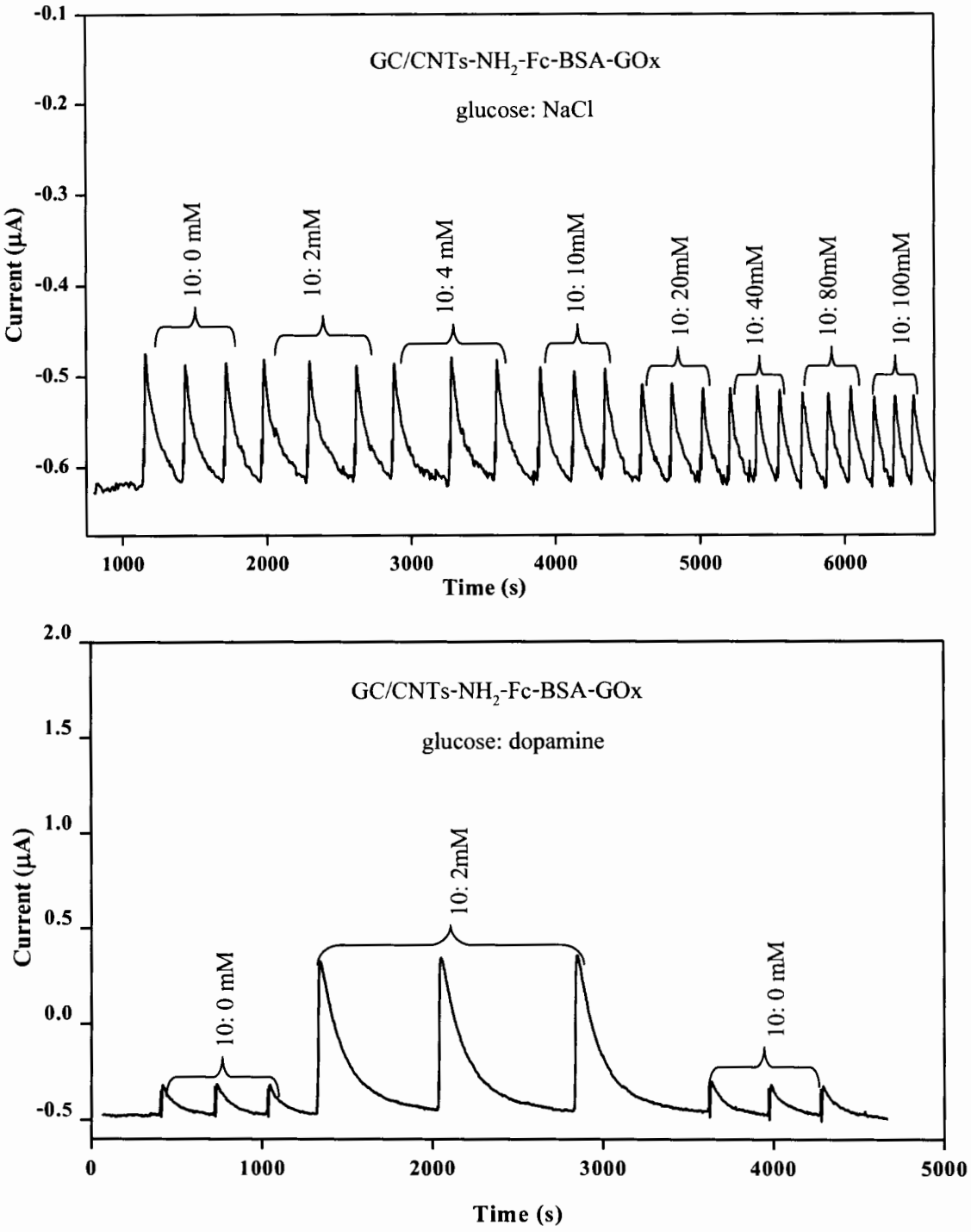


Figrure B.5 FIA gram of flow rate on the GC/CNTs-NH_2-Fc-BSA-GOx biosensor response 10 mM glucose, applied potential at 0.6 V.

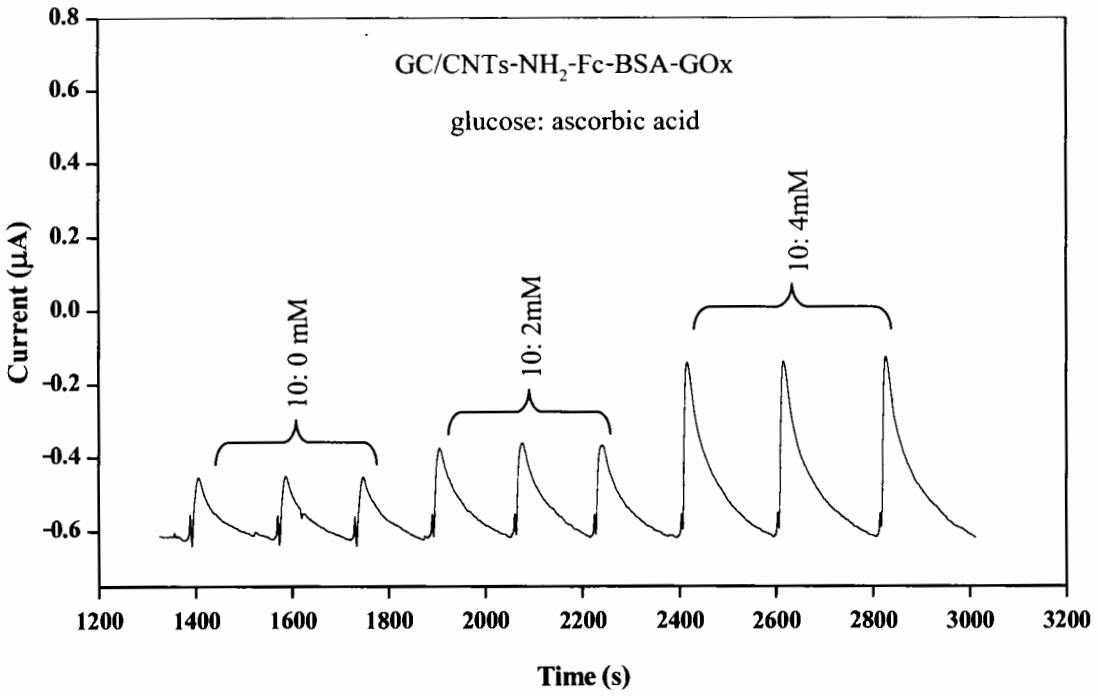
Interference study (raw data for Table 4.4)



Figrure B 6 FIA gram of the interference on the GC/CNTs-NH_2-Fc-BSA-GOx biosensor at 0.6 V, flow rate 1 mL/min.

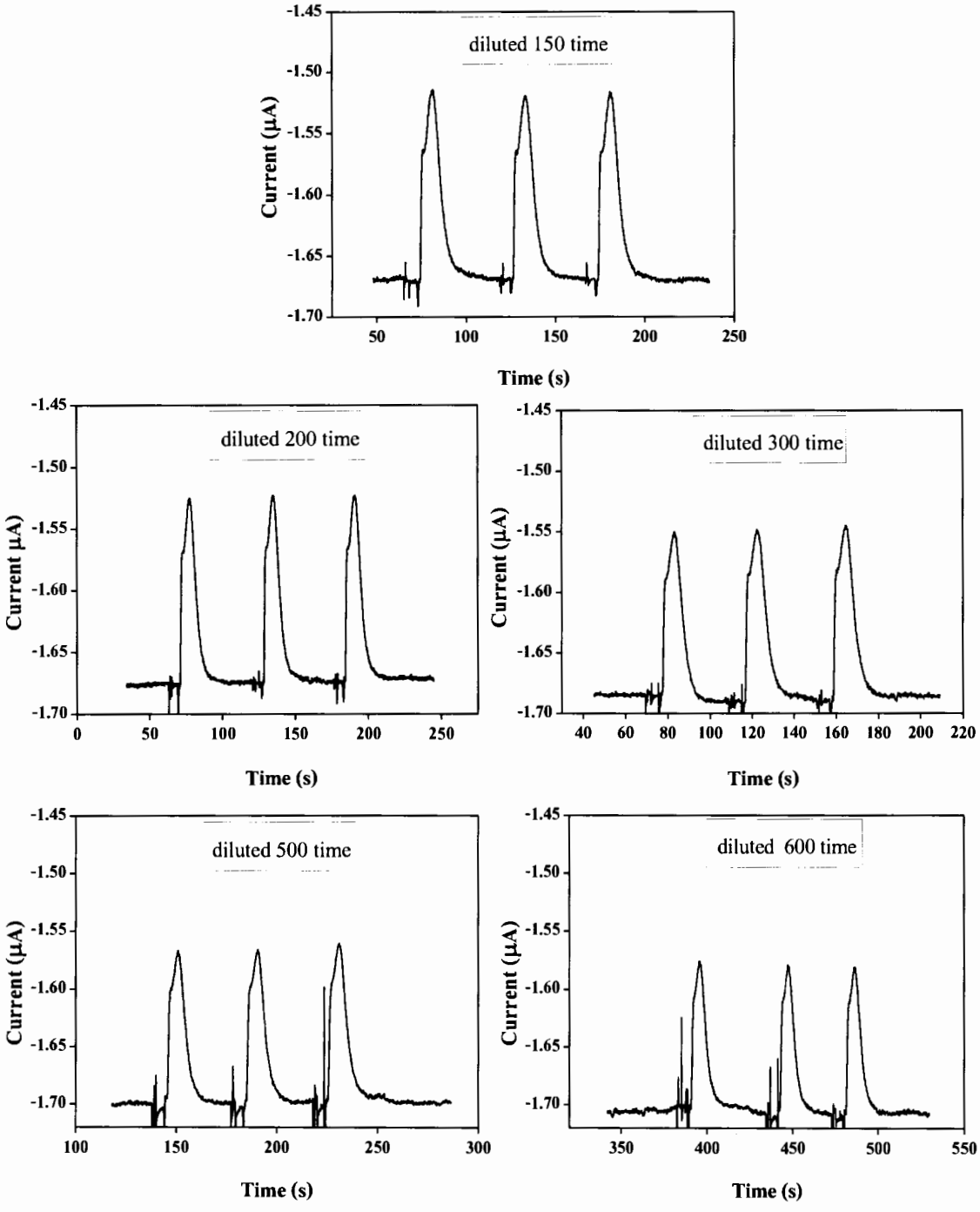


Figrure B 6 FIA gram of the interference on the GC/CNTs-NH_2-Fc-BSA-GOx biosensor at 0.6 V, flow rate 1 mL/min (Continued).



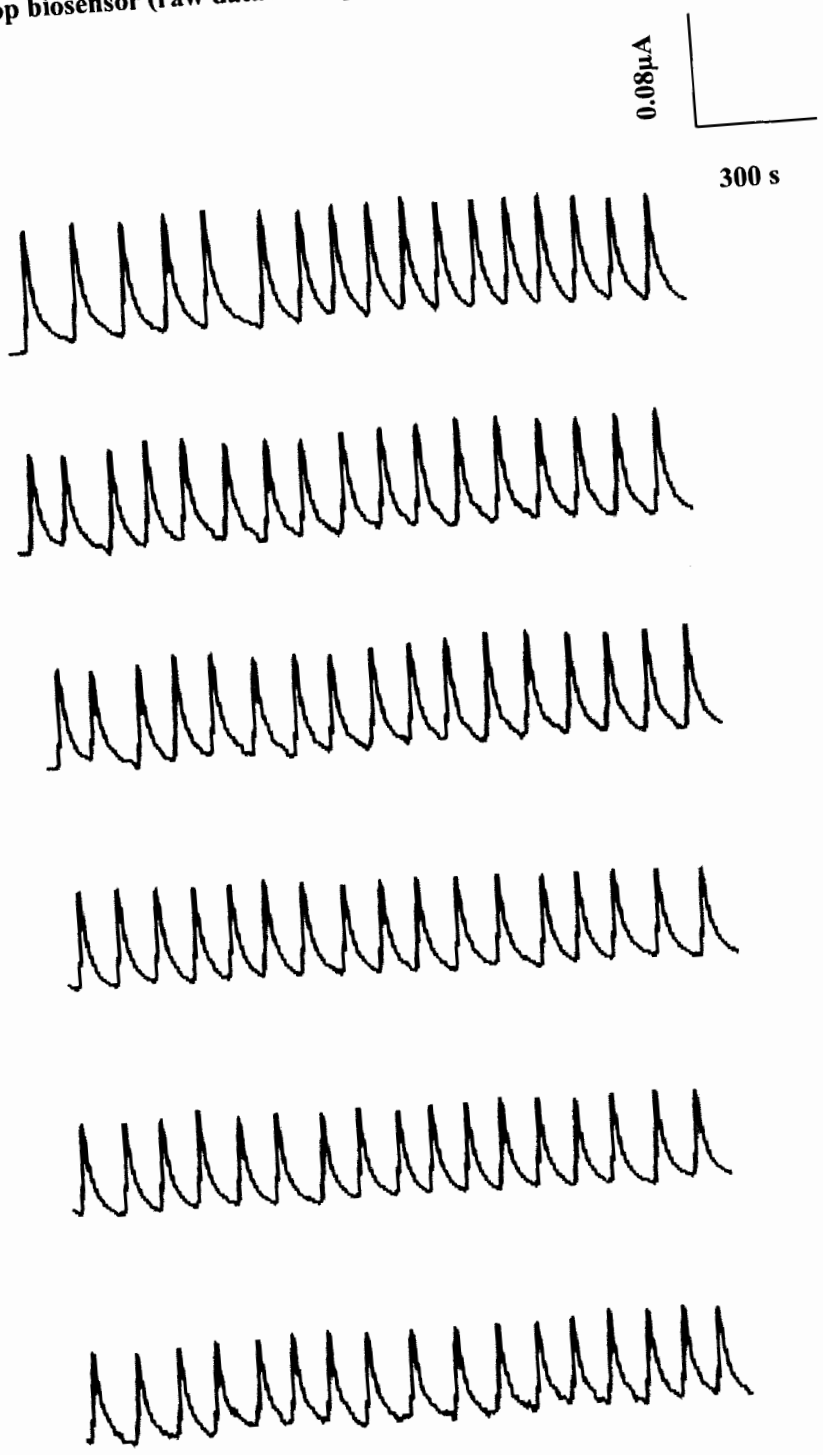
Figrure B 6 FIA gram of the interference on the GC/CNTs-NH_2-Fc-BSA-GOx biosensor at 0.6 V, flow rate 1 mL/min (Continued).

Sample determination (raw data for Table 4.5)



Figrure B.7 FIA gram of the sample on the GC/CNTs-NH_2-Fc-BSA-GOx biosensor at 0.6 V, flow rate 1 mL/min.

Stability of the develop biosensor (raw data for Figure 4.23)



Figrure B.8 FIA gram of stability on the GC/CNTs-NH_2-Fc-BSA-GOx biosensor.

APPENDIX C

CONFERENCES

CONFERENCES

Poster presentation

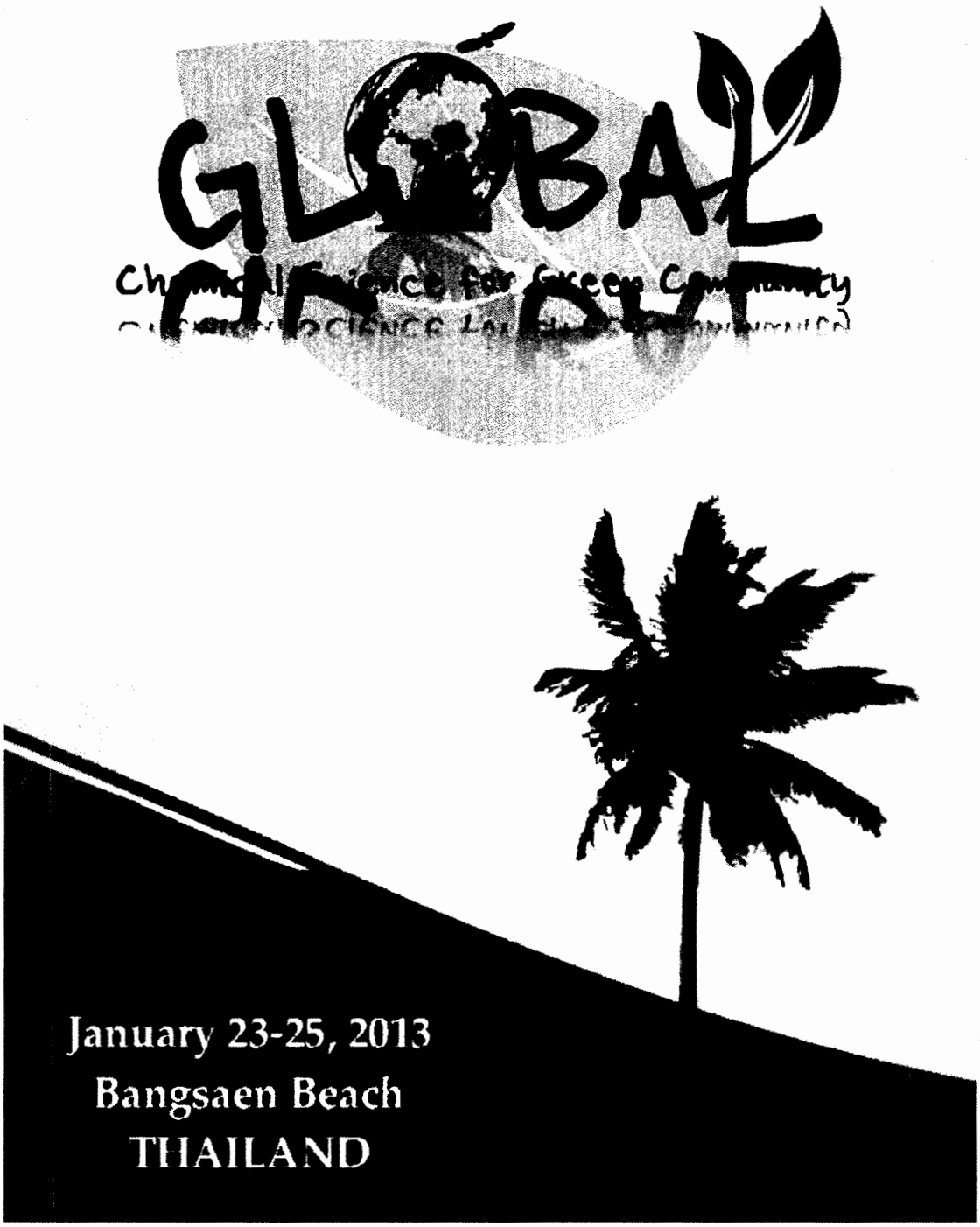
1. Uangporn Sompong, Wongduan Sroysee, Maliwan Amatatongchai. Development of flow injection system with amperometric detection on the biosensor modified with composite films of Fc-NH_2-MWCNTs-BSA-GOx for glucose determination. PURE AND APPLIED CHEMISTRY INTERNATIONAL CONFERENCE, January 23-25, 2013, Bangsaen Beach, Chonburi, Thailand.

2. Uangporn Sompong, Wongduan roysee, Maliwan Amatatongchai. Development of flow injection system for glucose detection by amperometric detection on Fc-NH_2-MWCNTs-BSA-GOx modified-glassy carbon electrode. PERCH-CIC CONGRESS VIII, May, 5-8, 2013, Pattaya, Chonburi, Thailand.

Publication

1. Uangporn Sompong, Wongduan Sroysee, Maliwan Amatatongchai. Flow injection system for amperometricdetection of glucose using MWCNTs-NH_2-FcCHO-BSA-GOx nanocomposite modified glassy carbon electrode. Journal of Science& Technology, UbonRatchathani University, Volume: 15, Issue: 3, Pages: 53-61, September- December, 2013.

PURE AND APPLIED CHEMISTRY INTERNATIONAL CONFERENCE 2013 (PACCON2013)



ANC P 047 DISCRIMINAION OF THE GEOGRAPHICAL ORIGIN OF THAI CHILI PEPPERS (*capsicum annuum L.*) USING CHROMATOGRAPHIC AND SPECTROSCOPIC PROFILES COMBINATION WITH CHEMOMETRIC TECHNIQUES

Worraluck Meemak, Kanet Wongravee, Soparat Yudthavorasit, Natchanun Leepipatpiboon

Faculty of Science, Chulalongkorn University,Thailand

ANC

Keywords Chili peppers; Chemometric techniques; Discrimination; Geographical origin

ANC P 048 DEVELOPMENT OF FLOW INJECTION SYSTEM WITH AMPEROMETRIC DETECTION ON THE BIOSENSOR MODIFIED WITH COMPOSITE FILMS OF FC-NH_2-MWCNTS-BSA-GOX FOR GLUCOSE DETERMINATION

Uangporn Sompong, Wongduan Sroysee, Maliwan Amatatongchai

Faculty of Science, Ubon Ratchathani University, Thailand

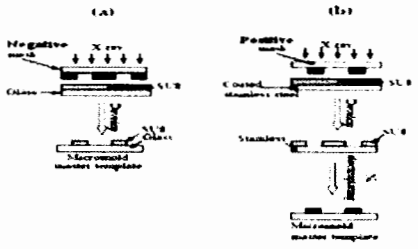
A flow injection system with amperometric detection on glucose biosensor was developed for sensitive and rapid measurement of glucose. The biosensor was developed based on cross-linking of glucose oxidase (GOx) by glutaraldehyde with ferrocene-modified NH_2-functionalized multiwalled carbon nanotubes (Fc- NH_2-MWCNTs) and bovine serum albumin (BSA) composite film coated on a glassy carbon (GC) electrode. The covalently bonded of redox mediator ferrocene group (Fc) with NH_2-MWCNTs could prevent the leakage of Fc from the matrix and retain its electrochemical acitivity. The developed biosensor (Fc-NH_2-MWCNTs-BSA-GOx/GC) was used in the amperometric... read more in Abstract book.

Keywords Glucose; Biosensor; NH2-MWCNT; Flow injection

ANC P 049 DEVELOPMENT OF MICROMOLD MASTER TEMPLATE FOR PDMS MICROFLUIDIC DEVICE FABRICATION BY DEEP X-RAY LITHOGRAPHY

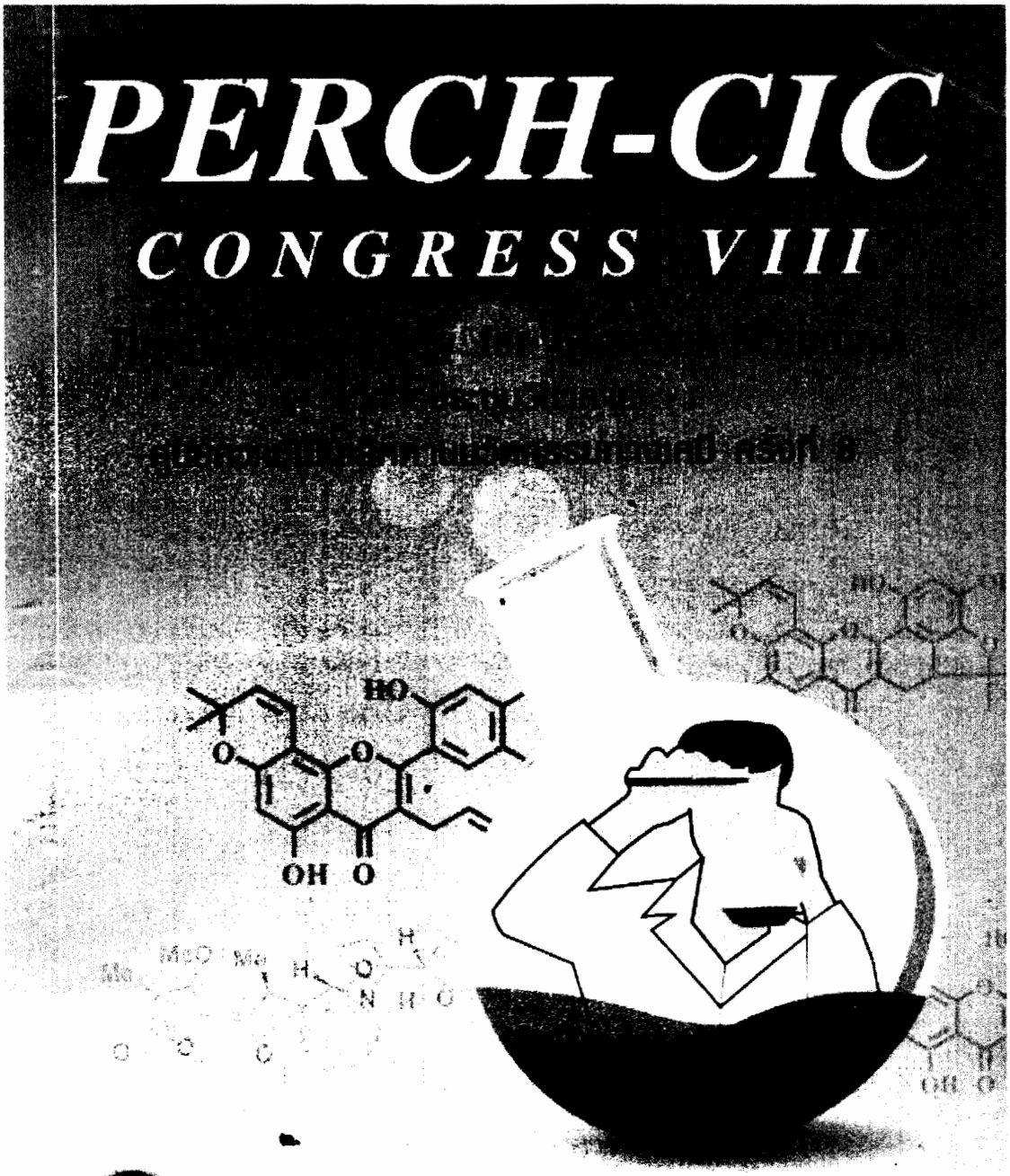
U. Petprapai, J. Rattana, M. Tongtem, R. Phatthanakun, W. Threeprom

Mahidol University, Kanchanaburi Campus, Thailand



Deep X-ray lithography schematics: (a) SU-8 Glass mold and (b) Ni-Stainless steel mold.

Keywords Microfluidic device; Lithography; X-ray synchrotron



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S1-P15

Development of Flow Injection System for Glucose Detection by Amperometric Detection on Fc-NH_2-MWCNTs-BSA-GOx Modified-Glassy Carbon Electrode

Uangporn Sompong, Wongduan Sroyseea and Maliwan Amatatongchai

Department of Chemistry and center of Excellent for Innovation in Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani , 34190, Thailand.

Introduction and Objective

In this research, development of a flow injection system with amperometric detection on Fc-NH_2-MWCNTs-BSA-GOx modified-glassy carbon electrode was proposed for sensitive and selective measurement of glucose.

Methods

The glucose sensor was developed based on cross-linking of glucose oxidase (GOx) by glutaraldehyde with ferrocene-modified NH_2-functionalized multiwalled carbon nanotubes (Fc- NH_2-MWCNTs) and bovine serum albumin (BSA) composite film coated on a glassy carbon (GC) electrode. The covalently bonded of redox mediator ferrocene group (Fc) with NH_2-MWCNTs could prevent the leakage of Fc from the matrix and retain its electrochemical acitivity. Optimization of the analytical conditions were studied using cyclic voltammetry (CV).

The FIA with amperometric detection can be easily constructed using a HPLC system. The system consisted of a pump, an injection port equipped with a 20 µLsample loop, and the electrochemical detector.

Results

In order to improve sensitivity for glucose detection, GOx, Fc- NH_2-MWCNTs and BSA composite film was coated onto the glassy carbon working electrode of the HPLC electrochemical detector. When the developed glucose sensor was employed for amperometric detection (at +0.55 vs Ag/AgCl), highly reproducible responses was observed (RSD = 2.5 %). The linear working range was 2-16 mM, with the detection limit of 0.36 mM (3σ of blank).

Conclusion

The developed biosensor (Fc-NH_2-MWCNTs-BSA-GOx/GC) was used in the amperometric detection of glucose in a solution of 0.1 M phosphate buffer, pH 7.0, by applying a potential of +0.55 V at the working electrode. The proposed Fc-NH_2-MWCNTs-BSA-GOx/GC electrode performed good electrocatalytic oxidation for glucose. A linear calibration graph is obtained over range 2-16 mM of glucose with slope of 300 nA/mM and correlation coefficient of 0.9996. The detection limit, based on a signal-to-noise ratio (S/N) of three, was 0.36 mM.

Keywords: glucose, biosensor, NH_2-MWCNT, flow injection

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ระบบโฟลอินเจคชันสำหรับตรวจวัดกลูโคสแบบแอมเพอร์โรเมทรีที่ขั้วไฟฟ้ากลาสซีคาร์บอนที่ดัดแปรด้วยวัสดุเชิงประกอบของท่อนาโนคาร์บอนแบบผนังหลายชั้นที่มีหมู่อะมิโน-เฟอร์โรซีน-โบวีนซีรัมอัลบูมิน-กลูโคสออกซิเดส

Flow Injection System for Amperometric Detection of Glucose using MWCNTs-NH_2-FcCHO-BSA-GOx Nano-composite Modified Glassy Carbon Electrode

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บทคัดย่อ

งานวิจัยนี้ได้พัฒนากลูโคสไบโอเซนเซอร์ที่มีสภาพไวและสามารถตรวจวัดได้อย่างรวดเร็วมาใช้เป็นตัวตรวจวัดกลูโคสด้วยเทคนิควิเคราะห์แอมเพอร์โรเมทรีในระบบโฟลอินเจคชัน การเตรียมกลูโคสไบโอเซนเซอร์อาศัยการสร้างพันธะโคเวเลนต์ของเฟอร์โรซีนคาร์บอกซัลดีไฮด์กับท่อนาโนคาร์บอนแบบผนังหลายชั้นที่มีหมู่อะมิโนเพื่อช่วยป้องกันการหลุดออกของเฟอร์โรซีนออกจากเมทริกซ์และรักษาสมบัติของสารนำส่งอิเล็กตรอน จากนั้นเอนไซม์กลูโคสออกซิเดสจะถูกตรึงโดยอาศัยการเชื่อมไขว้ด้วยกลูตารัลดีไฮด์ในวัสดุเชิงประกอบท่อนาโนคาร์บอนแบบผนังหลายชั้นที่มีหมู่อะมิโน-เฟอร์โรซีน-โบวีนซีรัมอัลบูมิน แล้วนำไปเคลือบบนขั้วไฟฟ้ากลาสซีคาร์บอน กลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้น (GC/MWCNTs-NH_2-FcCHO-BSA-GOx) นำมาประยุกต์ใช้เป็นตัวตรวจวัดกลูโคสแบบแอมเพอร์เมทรีในระบบโฟลอินเจคชันที่ใช้สารละลายฟอสเฟตบัฟเฟอร์พีเอช 7 เป็นสารละลายตัวพา และให้ศักย์ไฟฟ้า +0.6 โวลต์ ผลการศึกษาพบว่าขั้วกลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้นให้กราฟมาตรฐานที่มีการตอบสนองแบบเส้นตรงอยู่ในช่วง 2-16 มิลลิโมลาร์ ค่าความชันเท่ากับ 323 นาโนแอมแปร์ต่อมิลลิโมลาร์ ค่าสัมประสิทธิ์สหสัมพันธ์ (r^2) เท่ากับ 0.9996 ขีดจำกัดต่ำสุดในการตรวจวัดเท่ากับ 0.36 มิลลิโมลาร์ (S/N=3) ค่ากระแสที่ได้จากการวัดมีความเที่ยงของการวิเคราะห์กลูโคสเข้มข้น 0.6 มิลลิโมลาร์ซ้ำ 7 ครั้ง (%RSD) เท่ากับ 2.5% และสามารถวิเคราะห์ได้รวดเร็วถึง 72 ตัวอย่างต่อชั่วโมง

คำสำคัญ: กลูโคสไบโอเซนเซอร์ มัลติวอลล์คาร์บอนนาโนทิวบ์ที่มีหมู่อะมิโน เฟอร์โรซีน แอมเพอร์โรเมทรี โฟลอินเจคชันอะนาลิซิส

Abstract

In this work, a highly sensitive and rapid measurement of a glucose biosensor was developed for amperometric detection in a flow injection system. The biosensor was developed based on ferrocene carboxaldehyde (FcCHO) co-valently bound onto the NH_2-functionalized multi-walled carbon nano-tubes (MWCNTs-NH_2) to prevent ferrocene leakage from the matrix and retention of its activity as an electron mediator. Glucose oxidase (GOx) was immobilized to ferrocene-modified NH_2-functionalized multi-walled carbon nano-tubes and bovine serum albumin (MWCNTs-NH_2-FcCHO -BSA) composite film by using glutaraldehyde (Glu) before being coated on a glassy carbon (GC) electrode. The developed glucose biosensor (GC/MWCNTs-NH_2-FcCHO-BSA-GOx) was applied in the flow injection analysis system for amperometric detection of glucose using a solution of 0.1 M phosphate buffer (pH 7.0) as a carrier and applying a potential of +0.6 V. The proposed glucose biosensor exhibited linear calibration over the range of 2-16 mM of glucose with a slope of 323 nA/mM and a correlation coefficient of 0.9996. The detection limit, based on a signal-to-noise ratio (S/N) of three, was 0.36 mM. The developed biosensor also provided good precision (%RSD=2.5) for a glucose signal (0.6 mM, n=7) with a rapid sample throughout (72 samples/h).

Keywords: Glucose biosensor, MWCNTs-NH_2, Ferrocene, Amperometry: Flow injection analysis

1. บทนำ

การวิจัยเพื่อพัฒนากลูโคสไบโอเซนเซอร์ให้มีความถูกต้อง แม่นยำและมีสภาพไวสูงได้รับความสนใจเป็นอย่างมาก เนื่องจากปริมาณกลูโคสในปัสสาวะและเลือดสามารถบ่งชี้ถึงสภาวะของโรคเบาหวาน (diabetes) [1], [4] โดยปกติร่างกายของมนุษย์จะควบคุมระดับกลูโคสในเลือดอย่างเข้มงวดซึ่งเป็นส่วนหนึ่งของการรักษาสมดุลของร่างกาย ให้มีค่าอยู่ที่ประมาณ 3.6-5.8 มิลลิโมลาร์ หรือ 64.8-104.4 มิลลิกรัมต่อเดซิลิตร [5] สมาคมโรคเบาหวานแห่งสหรัฐอเมริกาแนะนำว่าระดับน้ำตาลในเลือดหลังรับประทานอาหารควรจะอยู่ที่ระดับน้อยกว่า 10 มิลลิโมลาร์ (180 มิลลิกรัมต่อเดซิลิตร) และระดับกลูโคสในพลาสมาก่อนรับประทานอาหารควรอยู่ที่ระดับ 5.0-7.2 มิลลิโมลาร์ (90-130 มิลลิกรัมต่อเดซิลิตร) [5] การวินิจฉัยว่าเป็นโรคเบาหวานจะใช้เมื่อระดับน้ำตาลในเลือดหลังการอดอาหารอย่างน้อย 8 ชั่วโมง สูงกว่า 7.0 มิลลิโมลาร์ (126 มิลลิกรัมต่อเดซิลิตร) การตรวจวัดปริมาณกลูโคสนอกจากจะใช้ในการตรวจและวินิจฉัยทางการแพทย์แล้ว ในขบวนการหมักและผลิตภัณฑ์อาหารปริมาณกลูโคสก็มีความสำคัญ เนื่องจากสามารถใช้เป็นดัชนีในการชี้บอกคุณภาพของอาหารและผลิตภัณฑ์ [4]

กลูโคสออกซิเดส (glucose oxidase; GOx) เป็นเอนไซม์ที่ใช้เป็นตัวเร่งปฏิกิริยาออกซิเดชันของกลูโคสที่ได้รับความสนใจและนิยมนำมาใช้สร้างกลูโคสไบโอเซนเซอร์เนื่องจากมีความจำเพาะเจาะจงต่อกลูโคสสูง [6] การปรับปรุงประสิทธิภาพของไบโอเซนเซอร์ที่อาศัยการตรึง GOx ในสภาพแวดล้อมที่เข้ากันได้ดีทางชีวภาพและองค์ประกอบอื่นที่ช่วยส่งเสริมการถ่ายโอนอิเล็กตรอนระหว่างผิวหน้าขั้วไฟฟ้ากับ GOx เป็นสิ่งจำเป็นอย่างยิ่ง [4], [7] วัสดุที่นิยมนำมาใช้เป็นเมท-ริกซ์ (matrix) ในการปรับปรุงประสิทธิภาพของกลูโคสไบโอเซนเซอร์ ได้แก่ วัสดุที่มีความสามารถส่งเสริมการถ่ายโอนอิเล็กตรอน เช่น คาร์บอนนาโนทิวบ์ (carbon nanotube; CNTs) และเฟอร์โรซีน (ferrocene; Fc) และวัสดุที่มีคุณสมบัติทางชีวภาพที่เข้ากันได้กับเอนไซม์ เช่น โบวีนซีรัมอัลบูมิน (bovine serum albumin; BSA) และไคโตซาน (chitosan; CS) [7], [12]

CNTs ได้รับความสนใจเป็นอย่างมากในการนำมาดัดแปรขั้วไฟฟ้า เนื่องจากมีพื้นที่ผิวมาก นำไฟฟ้าได้ดี และโดยเฉพาะอย่างยิ่งสามารถเข้ากันได้ดีกับเอนไซม์ CNTs จึงกลายเป็นวัสดุที่เหมาะสมสำหรับช่วยสนับสนุนการถ่ายโอนอิเล็กตรอนระหว่าง GOx กับผิวหน้าของขั้วไฟฟ้า [3],[4], [8], [10] สำหรับการประยุกต์ใช้งาน CNTs สามารถนำมาดัดแปรให้มีคุณสมบัติที่ต้องการโดยการสร้างหมู่ฟังก์ชัน -OH, -COOH หรือ -NH_2 เพื่อปรับปรุงความสามารถในการตรึงสารทางชีวภาพ ได้แก่ โปรตีน เอนไซม์ หรือ ดีเอนเอ BSA เป็นโปรตีนที่มีมากในพลาสมา เป็นสารชีวภาพที่ไม่มีพิษ สามารถเข้ากันได้ดีกับเอนไซม์และช่วยส่งเสริมการทำงานของเอนไซม์ จึงนิยมนำมาใช้สร้างไบโอเซนเซอร์อย่างกว้างขวาง [13],[17]

Fc และอนุพันธ์เป็นอีกวัสดุหนึ่งที่เป็นที่รู้จักกันดีและถูกนำมาใช้เป็นสารนำส่งอิเล็กตรอนในการพัฒนาไบโอเซนเซอร์ เนื่องจากสามารถเกิดปฏิกิริยาเคมีแบบผันกลับได้ และช่วยให้ปฏิกิริยาออกซิเดชันเกิดที่ศักย์ไฟฟ้าต่ำๆ ได้ [18],[19] ในการนำ Fc และอนุพันธ์มาดัดแปรขั้วไฟฟ้าโดยกระบวนการดูดซับจะทำให้ขั้วไบโอเซนเซอร์ที่ได้มีความเสถียรค่อนข้างต่ำ เนื่องจากแรงยึดเหนี่ยวบนผิวขั้วไฟฟ้าค่อนข้างอ่อน ทำให้ Fc หลุดออกมาในสารละลายเมื่อใช้ไบโอเซนเซอร์อย่างต่อเนื่อง ส่งผลกระทบต่อประสิทธิภาพการทำงานของไบโอเซนเซอร์ทำให้ได้สัญญาณค่ากระแสต่ำลงงานวิจัยนี้ได้เสนอวิธีวิเคราะห์กลูโคสด้วยกลูโคสไบโอเซนเซอร์ชนิดใหม่ที่สะดวก รวดเร็วและมีประสิทธิภาพ โดยการนำเทคนิคโฟลอินเจคชันมาใช้ร่วมกับการตรวจวัดแบบแอมเพอร์โรเมทรีที่ขั้วกลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้น ไบโอเซนเซอร์จะตรวจวัดกลูโคสโดยอาศัยการนำเฟอร์โรซีนคาร์บอกซัลดีไฮด์ หรือ FcCHO มาสร้างพันธะโคเวเลนต์กับ MWCNTs-NH_2 เพื่อป้องกัน Fc หลุดออกจากเมทริกซ์และรักษาแอคทิวิตีทางเคมีไฟฟ้า และใช้ BSA เพื่อช่วยส่งเสริมการทำงานของเอนไซม์ GOx โดยอาศัยการเชื่อมไขว้ (cross link) ของ GOx ด้วยกลูตารัลดีไฮด์เพื่อให้เอนไซม์ถูกตรึงไว้ในวัสดุเชิงประกอบ MWCNTs-NH_2-FcCHO-BSA-GOx ก่อนนำไปเคลือบบนขั้วไฟฟ้าชนิดกลาสซีคาร์บอน เพื่อใช้เป็นตัวตรวจวัดกลูโคสในระบบโฟลอินเจคชัน ทำให้ได้เทคนิควิเคราะห์กลูโคสที่มีข้อดีคือใช้สารปริมาณน้อยช่วยลดค่าใช้จ่ายและสามารถตรวจวัดได้อย่างต่อเนื่อง รวดเร็วและมีความเที่ยงสูง

2. วัสดุอุปกรณ์และวิธีการวิจัย

2.1 สารเคมี

โบวีนซีรัมอัลบูมิน (BSA, non acetylated, 20 mg/mL ในน้ำ) จาก Sigma-Aldrich (USA), ท่อนาโนคาร์บอนแบบผนังหลายชั้นที่มีหมู่ฟังก์ชันอะมิโน (MWCNTs-NH_2, บริสุทธิ์ > 95%, เส้นผ่านศูนย์กลาง 15 + 5 นาโนเมตร, ยาว 1-5 ไมครอน) จาก NanoLab inc. (USA), เอนไซม์

กลูโคสออกซิเดส (GOx type X-S จาก Aspergillus niger), เฟอร์โรซีนคาร์บอกซัลดีไฮด์(Ferrocene-carboxaldehyde; FcCHO, บริสุทธิ์ 98%) กลูตารัลดีไฮด์ (Glu, บริสุทธิ์ 50%) และ กลูโคส (β-D(+)-glucose) จาก Sigma-Aldrich, โซเดียมไซยาโนบอโรไฮด์ไดร์ ($NaCNBH_3$ บริสุทธิ์ 95%) จาก Acros organics

2.2 อุปกรณ์

เครื่องมือและอุปกรณ์ที่นำมาใช้ในการตรวจวัดทางเคมีไฟฟ้าประกอบด้วย เครื่องโพเทนชิโอสแตท รุ่น EA 161, e-corder 210 (บริษัท eDAQ, Australia) ระบบ 3 ขั้วไฟฟ้า คือขั้วไฟฟ้าใช้งาน (working electrode) ชนิดกลาสซีคาร์บอน (ขนาดเส้นผ่าศูนย์กลาง 3 มิลลิเมตร), ขั้วไฟฟ้าอ้างอิงซิลเวอร์-ซิลเวอร์คลอไรด์ (Ag/AgCl) และขั้วไฟฟ้าช่วย (counter electrode) ชนิดลวดแพลทินัม (Pt) (บริษัท CH Instruments, USA.)

ระบบโฟลอินเจคชันที่พัฒนาขึ้นเพื่อใช้ในการตรวจวัดปริมาณกลูโคสด้วยเทคนิคแอมเพอร์โรเมทรีที่ขั้วกลูโคสไบโอเซนเซอร์ประกอบด้วย ปั๊มรุ่น LC-10A D (บริษัท Shimadzu, Japan) สารละลายตัวพา (ฟอสเฟตบัฟเฟอร์ พีเอช 7.0 ความเข้มข้น 0.1 โมลาร์) injection valve ที่มี sample loop ขนาด 20 ไมโครลิตร (บริษัท Rheodyne, USA) thin layer flow cell ระบบ 3 ขั้วไฟฟ้า คือขั้วไฟฟ้าใช้งานกลูโคสไบโอเซนเซอร์ ขั้วไฟฟ้าอ้างอิงซิลเวอร์-ซิลเวอร์คลอไรด์ (Ag/AgCl) และขั้วไฟฟ้าช่วยชนิด stainless steel tube (บริษัท CH Instruments, USA.) และระบบตรวจวัดทางเคมีไฟฟ้า

2.3 การสังเคราะห์วัสดุเชิงประกอบของคาร์บอนนาโนทิวบ์ที่มีหมู่อะมิโน-เฟอร์โรซีน (MWCNTs-NH_2-FcCHO)

MWCNTs-NH_2-FcCHO เตรียมโดยพัฒนามาจากวิธีของ Qiu และคณะ [3] โดยนำ MWCNTs-NH_2 30 มิลลิกรัมมา ทำให้กระจายตัว ในน้ำกลั่นปริมาตร 10 มิลลิลิตร ด้วยเครื่องอัลตราโซนิก (รุ่น CT360D, บริษัท Scientific promotion) เป็นเวลา 30 นาที นำมาเติมสารละลาย FcCHO ความเข้มข้น 7 มิลลิกรัมต่อมิลลิลิตร ปริมาตร 20 มิลลิลิตร แล้วนำไป กวนที่อุณหภูมิห้องเป็นเวลา 1 ชั่วโมงจากนั้นเติม $NaCNBH_3$ 20 มิลลิกรัมแล้วนำไปกวนต่อที่อุณหภูมิห้องเป็นเวลา 24 ชั่วโมง ทำเซนตริฟิวจ์และล้างตะกอนด้วยเมทานอล 10 มิลลิลิตร ตามด้วยน้ำปราศจากไอออน 10 มิลลิลิตร ทิ้งให้แห้งที่อุณหภูมิห้อง จะได้วัสดุเชิงประกอบ MWCNTs-NH_2-FcCHO

2.4 การเตรียมกลูโคสไบโอเซนเซอร์ GC/MWCNTs-NH_2-FcCHO-BSA-GOx

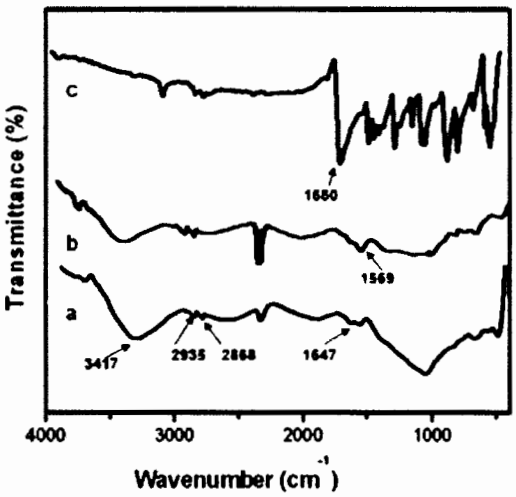
นำขั้วไฟฟ้ากลาสซีคาร์บอนมาทำความสะอาดโดยขัดผิวหน้าของขั้วไฟฟ้ากับผ้าสักหลาดโดยใช้ผงอะลูมินาขนาด 1.0, 0.3, และ 0.05 ไมครอนตามลำดับ จากนั้นล้างให้สะอาดด้วยน้ำกลั่น และล้างด้วยน้ำปราศจากไอออนโดยใช้เครื่องอัลตราโซนิกเป็นเวลา 5 นาที แล้วเป่าให้แห้ง การเตรียมสารละลายตัดแปรขั้วไฟฟ้าเริ่มจากนำ GOx 7.5 มิลลิกรัม เติมลงในสารละลาย 1% BSA ปริมาตร 500 ไมโครลิตร จากนั้นเติม Glu ความเข้มข้น 2.5% ปริมาตร 500 ไมโครลิตร นำสารละลายที่ได้ไปเก็บที่อุณหภูมิ 4 องศาเซลเซียส เป็นเวลา 2 ชั่วโมง หลังจากนั้นเติมสารละลาย MWCNTs-NH_2-FcCHO เข้มข้น 7.5 มิลลิกรัมต่อมิลลิลิตร ปริมาตร 1,000 ไมโครลิตร แล้วนำไปสั่นผสมด้วยเครื่องอัลตราโซนิก เป็นเวลา 5 นาที จะได้สารละลายสำหรับดัดแปรขั้วไฟฟ้า (MWCNTs-NH_2-FcCHO-BSA-GOx) กลูโคสไบโอเซนเซอร์เตรียมได้โดยปิเปตสารละลายสำหรับดัดแปรขั้วไฟฟ้า ปริมาตร 40 ไมโครลิตร หยดลงบนผิวหน้าขั้วไฟฟ้าที่ทำความสะอาดแล้วทิ้งให้แห้งที่อุณหภูมิ 4 องศาเซลเซียส เป็นเวลา 1 คืน จะได้กลูโคสไบโอเซนเซอร์ (GC/MWCNTs-NH_2-FcCHO-BSA-GOx)

3 ผลการวิจัยและวิจารณ์ผล

3.1 คุณลักษณะของ MWCNTs-NH_2-FcCHO

รูปที่ 1 แสดง FTIR สเปคตรัม ของ MWCNTs-NH_2 (กราฟ a), MWCNTs-NH_2-FcCHO (กราฟ b) และ FcCHO (กราฟ c) จาก กราฟ a ในรูปที่1 พีคที่ 3,417 cm^{-1} เป็นลักษณะการสั่นแบบยืด-หด (stretching) ของหมู่ N-H และการสั่นแบบกรรไกรในระนาบ (In-plane scissoring vibration) ที่ 1,647 cm^{-1} จากหมู่เอมีนปฐมภูมิ (primary amine) ส่วนพีคที่ 2,868, 2,935 cm^{-1} เกิดจากการสั่นแบบยืด-หด ของ C-H ใน FTIR สเปคตรัมของ FcCHO (กราฟ c) พีคที่ 1,680 cm^{-1} เกิดจากการสั่นของ C=O จากหมู่อัลดีไฮด์ FTIR สเปคตรัม (กราฟ b) ของสารประกอบ MWCNTs-NH_2-FcCHO เกิดจาก C=O จากหมู่อัลดีไฮด์ เข้าทำปฏิกิริยากับ $-NH_2$ บน MWCNTs-NH_2 ที่ผ่านปฏิกิริยา Schiff-base และตามด้วยปฏิกิริยารีดักชันโดยใช้ $NaCNBH_3$ จะได้สารประกอบในรูป MWCNTs-NH_2-FcCHO ซึ่งยืนยันได้ด้วยผลจากสเปคตรัม (กราฟ b) จะไม่ปรากฏพีคที่ 1,647 cm^{-1} ของ MWCNTs-NH_2 (เปรียบเทียบกับกราฟ a) และ พีคที่ 1,680 cm^{-1} ของ FcCHO (เปรียบเทียบกราฟ c) เนื่องจากเกิดปฏิกิริยา Schiff-base ระหว่าง -NH_2 และหมู่อัลดีไฮด์ สอดคล้องกับผลการทดลองของ Qiu และคณะ

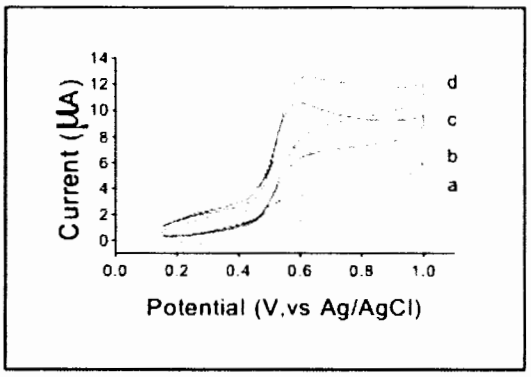
[3] แสดงให้เห็นว่า FcCHO ถูกตรึงลงบนวัสดุเชิงประกอบ MWCNTs-NH_2-FcCHO ได้เป็นผลสำเร็จ



รูปที่ 1 แสดง FTIR spectra ของ MWCNTs-NH_2 (กราฟ a), MWCNTs-NH2-FcCHO (กราฟ b) และ FcCHO (กราฟ c)

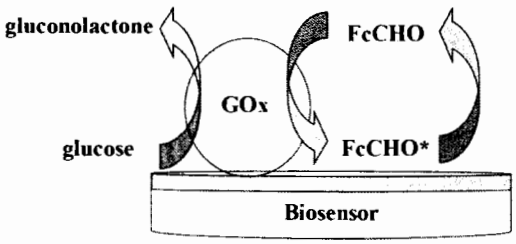
3.2 การตอบสนองทางเคมีไฟฟ้าของขั้วกลูโคสไบโอเซนเซอร์ (GC/MWCNTs-NH_2-FcCHO-BSA-GOx) ต่อกลูโคส

ผลการทดลองจากเทคนิคไซคลิกโวลแทมเมทรี (cyclic voltammetry) ในการศึกษาการตอบสนองของขั้วกลูโคสไบโอเซนเซอร์ GC/MWCNTs-NH_2-FcCHO-BSA-GOx แสดงดังรูปที่ 2



รูปที่ 2 แสดงไซคลิกโวลแทมโมแกรมของกลูโคส ความเข้มข้น (a) 0, (b) 5, (c) 10 และ (d) 15 มิลลิโมลาร์ ที่ขั้วกลูโคสไบโอเซนเซอร์ (GC/MWCNTs-NH_2-FcCHO-BSA-GOx) ในสารละลายฟอสเฟตบัฟเฟอร์ เข้มข้น 0.1 โมลาร์ พีเอช 7.0 (ภายใต้บรรยากาศก๊าซอาร์กอน)

จากรูปไซคลิกโวลแทมโมแกรม (CV) ที่ขั้วกลูโคสไบโอเซนเซอร์ (รูปที่ 2) พบว่า CV ของไบโอเซนเซอร์ที่มีการเติมกลูโคส 5 มิลลิโมลาร์ ลงในสารละลาย (กราฟ b) ทำให้กระแสอาโนดิกเพิ่มขึ้นอย่างเห็นได้ชัดเมื่อเทียบกับสารละลายที่ไม่มีกลูโคส (กราฟ a) และค่ากระแสจะเพิ่มขึ้นตามความเข้มข้นของกลูโคสที่เพิ่มขึ้น (กราฟ c-d) กลูโคสสามารถเกิดออกซิเดชันได้โดยมีค่าศักย์ที่ยอดพีคอาโนดิก ($E_{p,a}$) ประมาณ 0.6 โวลต์ แสดงว่าวัสดุเชิงประกอบ (MWCNTs-NH_2-FcCHO-BSA) ทำให้ GOx สามารถเร่งปฏิกิริยาออกซิเดชันของกลูโคสได้อย่างมีประสิทธิภาพ และสามารถตรวจวัดปริมาณกลูโคสได้ที่ศักย์ต่ำๆ โดยมี Fc-CHO ที่สร้างพันธะกับ MWCNTs เป็นเมดิเอเตอร์ช่วยนำส่งอิเล็กตรอนระหว่าง active site ของเอนไซม์กับผิวหน้าขั้วไฟฟ้า ซึ่งสอดคล้องกับงานวิจัยของ Cass และคณะ [20] และ Liu และคณะ [11]



รูปที่ 3 กลไกปฏิกิริยาของไบโอเซนเซอร์ในการตรวจวัดกลูโคส

สมการที่เกี่ยวข้อง แสดงได้ดังนี้

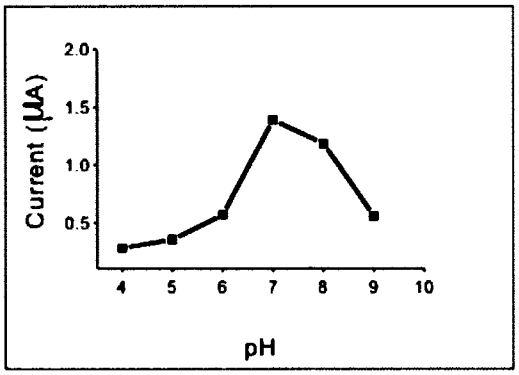
$$glucose + GOx_{(ox)} \rightarrow gluconolactone + GOx_{(red)} \quad (1)$$

$$GOx_{(red)} + 2FcCHO^{+} \rightarrow GOx_{(ox)} + 2FcCHO + 2H^{+} \quad (2)$$

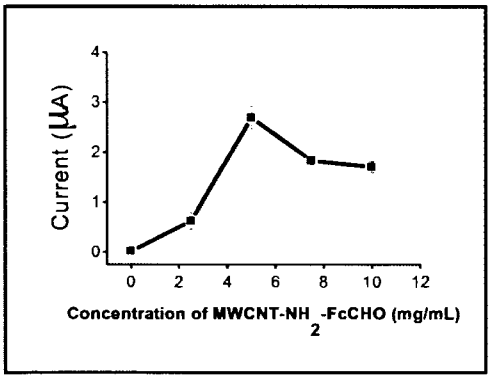
$$2FcCHO \rightleftarrows 2FcCHO^{+} + 2e^{-} \quad (3)$$

3.3 การศึกษาหาพีเอชของสารละลายอิเล็กโทรไลต์ที่เหมาะสม

ค่าพีเอชของสารละลายอิเล็กโทรไลต์มีผลอย่างมากต่อสภาพไวของไบโอเซนเซอร์ เนื่องจากค่าพีเอชมีผลต่อพฤติกรรมทางเคมีไฟฟ้าของ Fc และไบโอแอคทิวิตี (bio-activity) ของ GOx จากรายงานวิจัยที่ผ่านมาค่าพีเอชที่เหมาะสมต่อการทำงานของ GOx มักอยู่ที่ช่วงพีเอช 6.5-7.5 [21-22] ทั้งนี้ขึ้นอยู่กับวิธีและวัสดุที่ใช้ในการตรึงเอนไซม์ การทดลองนี้จึงทำการศึกษาผลของพีเอชในช่วง 4.0-9.0 ผลการทดลองแสดงดังรูปที่ 4



รูปที่ 4 ความสัมพันธ์ระหว่างค่ากระแสจากปฏิกิริยาออกซิเดชันของกลูโคส กับค่าพีเอชของสารละลายอิเล็กโทรไลต์ ฟอสเฟตบัฟเฟอร์ เข้มข้น 0.1 โมลาร์



รูปที่ 5 ความสัมพันธ์ระหว่างค่ากระแสจากปฏิกิริยาออกซิเดชันของกลูโคสกับความเข้มข้นของวัสดุเชิงประกอบMWCNTs-NH_2-FcCHO ที่ใช้ในการดัดแปรกลูโคสไบโอเซนเซอร์

ผลการทดลองจากรูปที่ 4 พบว่าเมื่อพีเอชของสารละลายสูงขึ้น ค่ากระแสจะเพิ่มสูงขึ้นจนถึงพีเอช 7.0 และลดลงเมื่อพีเอชของสารละลายมากขึ้น จากการทดลองแสดงให้เห็นอย่างชัดเจนว่าพีเอชของสารละลายมีผลต่อการทำงานของเอนไซม์ GOx กลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้นนี้ให้ค่ากระแสสูงสุดที่พีเอช 7.0 สอดคล้องกับงานวิจัยก่อนหน้านี้ [12], [17], [23],[24] ดังนั้นงานวิจัยนี้จึงเลือกค่าพีเอช 7.0 เป็นพีเอชของสารละลายอิเล็กโทรไลต์ที่เหมาะสม

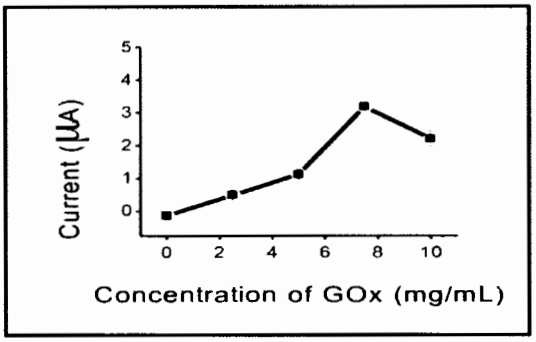
3.4 การศึกษาหาความเข้มข้นของวัสดุเชิงประกอบ MWCNTs-NH_2-FcCHO ที่เหมาะสม

ในการศึกษาหาปริมาณวัสดุเชิงประกอบที่เหมาะสมในการเตรียมกลูโคสไบโอเซนเซอร์ ผลของปริมาณวัสดุเชิงประกอบ MWCNTs-NH_2-FcCHO ต่อการตรวจวัดปฏิกิริยาออกซิเดชันของกลูโคสแสดงดังรูปที่ 5 ค่ากระแสออกซิเดชันในรูป เป็นค่าเฉลี่ยจากขั้วไฟฟ้าที่เตรียมขึ้น 3 ขั้วไฟฟ้า ส่วนแถบความคลาดเคลื่อน (error bar) แสดงค่าเบี่ยงเบนมาตรฐาน (S.D.) จากการวัด

จากผลการทดลองในรูปที่ 5 พบว่าเมื่อปริมาณวัสดุเชิงประกอบ MWCNTs-NH_2-FcCHO เพิ่มขึ้นจาก 0-5 มิลลิกรัมต่อมิลลิลิตรค่ากระแสที่ได้เพิ่มขึ้น ซึ่งเป็นผลมาจากวัสดุเชิงประกอบ MWCNTs-NH_2-FcCHO ช่วยปรับปรุงคุณสมบัติการนำไฟฟ้าของฟิล์มคอมโพสิทให้ดีขึ้น ค่ากระแสที่ได้จึงมีค่าสูงขึ้น และให้ค่ากระแสสูงสุดที่ความเข้มข้น 5 มิลลิกรัมต่อมิลลิลิตร จากนั้นค่าลดลงและเริ่มคงที่ที่ความเข้มข้น 7.5 มิลลิกรัมต่อมิลลิลิตร ดังนั้นในงานวิจัยนี้จึงเลือกวัสดุเชิงประกอบ MWCNTs-NH_2-FcCHO ความเข้มข้น 7.5 มิลลิกรัมต่อมิลลิลิตร ที่ค่ากระแสเริ่มคงที่เป็นสภาวะที่เหมาะสมในการทดลองต่อไป

3.5 การศึกษาผลของปริมาณกลูโคสออกซิเดส

ความเข้มข้นของเอนไซม์ที่ใช้สร้างไบโอเซนเซอร์เป็นอีกปัจจัยหนึ่งที่มีความสำคัญต่อผลการดอบสนองของไบโอเซนเซอร์ ผลการศึกษาความเข้มข้นของ GOx ที่ใช้สร้างกลูโคสไบโอเซนเซอร์ต่อสภาพไวของไบโอเซนเซอร์แสดงดังรูปที่ 6

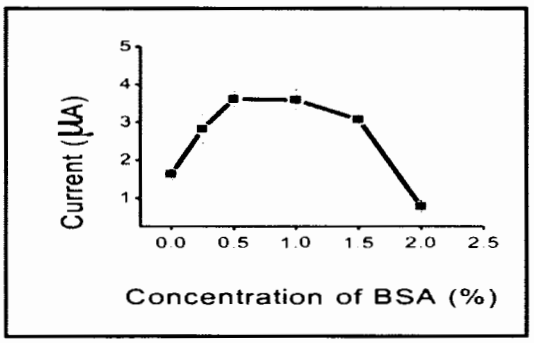


รูปที่ 6 ความสัมพันธ์ระหว่างค่ากระแสจากปฏิกิริยาออกซิเดชันของกลูโคส กับความเข้มข้นของเอนไซม์กลูโคสออกซิเดส (GOx)

จากรูปที่ 6 เมื่อเพิ่มความเข้มข้นของเอนไซม์ GOx จาก 0-7.5 มิลลิกรัมต่อมิลลิลิตร จะให้ค่ากระแสออกซิเดชันของกลูโคสที่สูงขึ้นจนถึงที่ความเข้มข้น 7.5 มิลลิกรัมต่อมิลลิลิตร แต่เมื่อเพิ่มความเข้มข้นของ GOx ให้สูงขึ้นอีกค่ากระแสกลับลดลง ซึ่งอาจเกิดจากเมทริกซ์ที่ใช้ตรึงเอนไซม์มีพื้นที่จำกัดจึงสามารถตรึงเอนไซม์ได้จำนวนหนึ่งเท่านั้น เมื่อเติมเอนไซม์ที่มากเกินไปส่งผลให้ความสามารถในการนำไฟฟ้าของวัสดุเชิงประกอบลดลง ซึ่งสอดคล้องกับผลการศึกษาของ Tripathi และคณะ [17], [21] ดังนั้นในงานวิจัยนี้จึงเลือกความเข้มข้นของเอนไซม์กลูโคสออกซิเดสที่ 7.5 มิลลิกรัมต่อมิลลิลิตร ใช้ในการทดลองต่อไป

3.6 การศึกษาผลของปริมาณสารละลายโบวีนซีรัมอัลบูมิน

ผลของปริมาณสารละลาย BSA ที่ใช้ในการตัดแปรกลูโคสไบโอเซนเซอร์ต่อค่ากระแสออกซิเดชันของกลูโคส แสดงดังรูปที่ 7



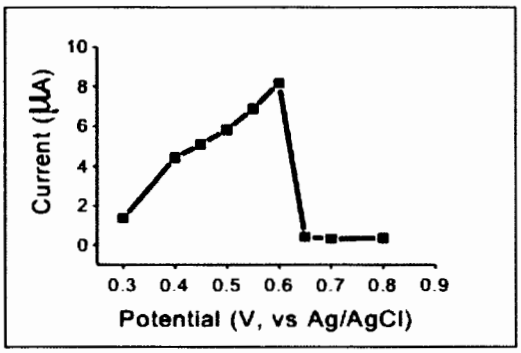
รูปที่ 7 ความสัมพันธ์ระหว่างค่ากระแสจากปฏิกิริยาออกซิเดชันของกลูโคส กับความเข้มข้นของสารละลายโบวีนซีรัมอัลบูมิน (BSA) ที่ใช้ในการเตรียมกลูโคสไบโอเซนเซอร์

เมื่อเพิ่มความเข้มข้นของสารละลาย BSA จาก 0 จนถึง 1% ค่ากระแสที่ได้จะเพิ่มขึ้นและให้ค่าสูงสุดที่ความเข้มข้น 0.5% แสดงว่า BSA สามารถช่วยส่งเสริมการทำงานของเอนไซม์ GOx ได้ สอดคล้องกับผลการทดลองของ Kulys และคณะ [15] ซึ่งพบว่าอัลบูมินสามารถช่วยส่งเสริมการทำงานของเอนไซม์แลคเคสในการเร่งปฏิริยาการเกิดออกซิเดชันของสารประกอบฟีโนลิคได้ จากนั้นค่ากระแสจะเริ่มคงที่ที่ความเข้มข้น 1% และค่อยลดลงเมื่อความเข้มข้นเพิ่มขึ้น ทั้งนี้อาจเป็นผลเนื่องมาจากการเพิ่มปริมาณของ BSA ซึ่งเป็นโปรตีนโมเลกุลใหญ่ที่มากเกินไปส่งผลให้ค่าการนำไฟฟ้าลดลง ค่ากระแสที่ได้จึงลดลง ดังนั้นจึงเลือกใช้ 1% BSA ซึ่งเป็นสภาวะที่ค่ากระแสคงที่ได้เริ่มคงที่ในการดัดแปรขั้วไฟฟ้า

3.7 ศึกษาการตรวจวัดปริมาณกลูโคสแบบเอมเพอร์โรเมทรีในระบบโฟลอินเจคชัน

3.7.1 ศึกษาหาศักย์ไฟฟ้าที่เหมาะสมในการตรวจวัดกลูโคส

การนำเทคนิคโฟลอินเจคชันมาใช้ร่วมกับการตรวจวัดแบบแอมเพอร์โรเมทรีที่ขั้วกลูโคสไบโอเซนเซอร์ที่พัฒนาขึ้นจะทำให้ได้เทคนิควิเคราะห์ที่สะดวก รวดเร็วและมีประสิทธิภาพในการตรวจวัดกลูโคส ในการศึกษาหาศักย์ไฟฟ้าที่เหมาะสมในการตรวจวัดกลูโคสที่ขั้วกลูโคสไบโอเซนเซอร์ผลการทดลองดังรูปที่ 8



รูปที่ 8 ความสัมพันธ์ระหว่างค่ากระแสจากปฏิกิริยาออกซิเดชันของกลูโคสกับศักย์ไฟฟ้าที่ให้แก่ขั้วกลูโคสไบโอเซนเซอร์ในระบบโฟลอินเจคชัน

จากผลการทดลองในรูปที่ 8 พบว่าเมื่อเพิ่มศักย์ไฟฟ้าตั้งแต่ +0.3 ถึง +0.6 โวลต์ ค่ากระแสที่ได้จะเพิ่มขึ้น แสดงให้เห็นถึงการตอบสนองที่ดีขึ้นของขั้วกลูโคสไบโอเซนเซอร์ที่ถูกควบคุมโดยปฏิกิริยาออกซิเดชันของกลูโคส

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