University of Alberta

Human Trials of an Implantable Glucose Sensor

by

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A thesis submitted to the Faculty of Graduate Studies and Research
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Human Trials of an Implantable Glucose Sensor

submitted by **Chunman Yu** in partial fulfillment of the requirements for the degree of **Master of Science.**

Dr. D. Jed Harrison
(supervisor)

Dr. James A. Plambeck

Dr. Ray V. Rajotte

Date: May 28/96

ABSTRACT

The research undertaken during the course of this program was toward the improvement of an implantable glucose sensor and its human trials.

An implantable glucose sensor was made with high sensitivity and selectivity to glucose, good biocompatibility and biostability. The sensor was coated with a inner polymer, 1,3-diaminobenzene with a new coating method, in which the 1,3-DAB was coated last in the coating procedure.

The sensor with high sensitivity and selectivity was subsequently evaluated *in vivo* in human . A total of 16 sensors was tested on 15 human volunteers. The majority of the sensors responded consistently with blood plasma glucose values acquired during the same period of oral glucose tolerance test (OGTT). The mean *in vivo* sensitivity of the set of sensors was 5.84±3.16 nA/mM (n=11).

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TABLE OF CONTENTS

CHAPTER **PAGE**

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

A sensor can be broadly defined as an input transducer that converts physical or chemical information into an electrical signal [1]. Some familiar examples are accelerometers used to trigger airbags in cars, glass electrode which measures pH, etc. A biosensor, according to some authors, is a self-contained analytical device that responds selectively and reversibly to the concentration or activity of chemical species in biological samples [2]. Any sensor physically or chemically operated in biological samples can be considered a biosensor. The modern concept of a biosensor owes much to the ideas presented by Leland C. Clark Jr. and his co-workers [3] in 1962. They proposed that enzymes could be immobilized at electrochemical detectors to form 'enzyme electrodes' which could expand the analyte range of the base sensor, which in their case was an oxygen sensor. A diagram of a biosensor is shown in Figure 1.1.

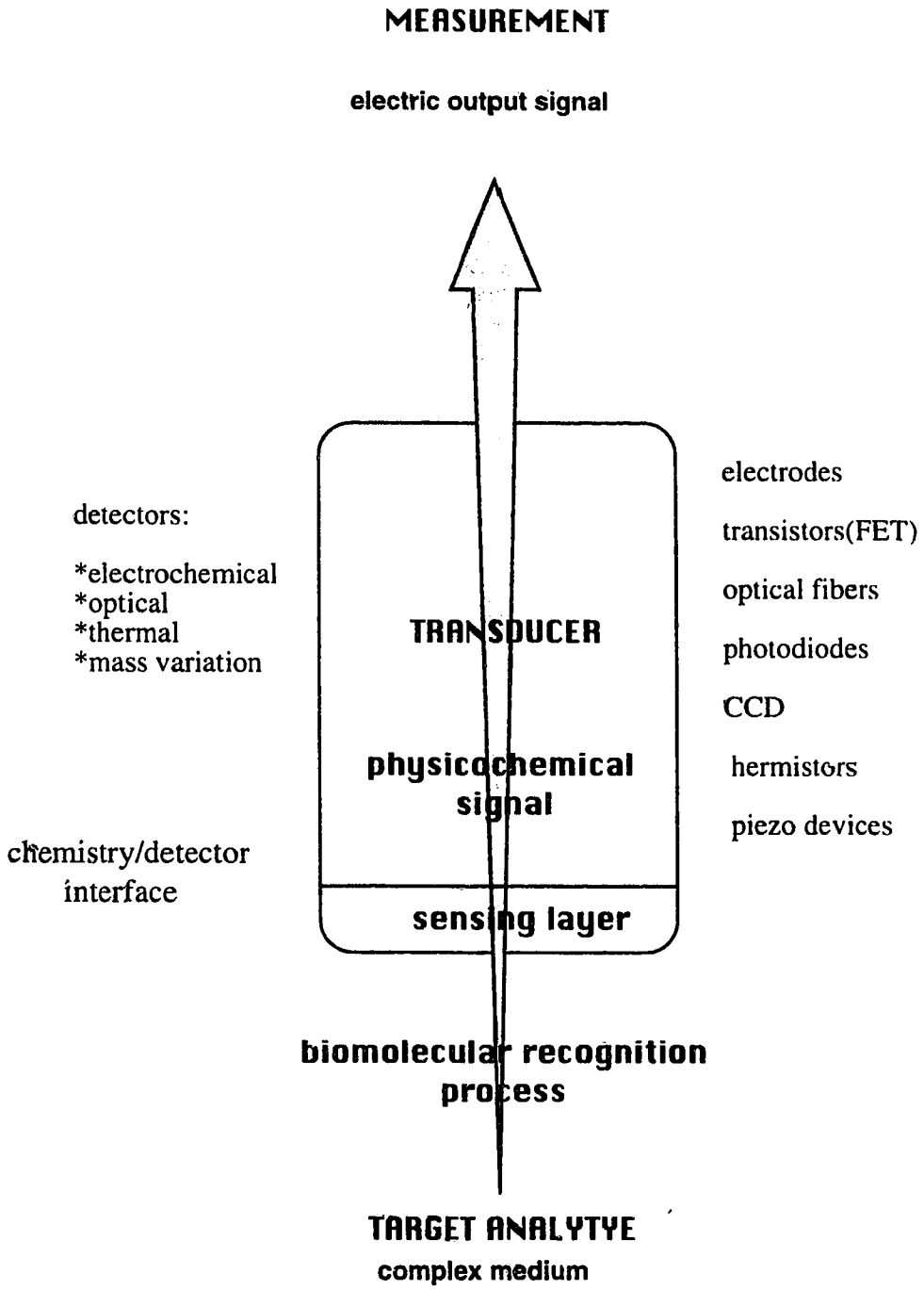

Fig.1.1 Conceptual Configuration of a biosensor [2]

1.1 Diabetes and Current Treatment

Diabetes is the third leading cause of death in Canada. The most severe form of the disease is called Idiopathic Diabetes Mellitus----Type I, or Insulin-Dependent Diabetes Mellitus (IDDM), which is observed in three to seven individuals per 1000 [4]. All therapeutic measures are aimed at limiting the disease and relieving the patient's symptoms [5]. Conventional treatment of Type I Diabetes usually involves a strict regimen of dietary management and daily prescribed doses of exogenous insulin. Insulin is the most powerful and reliable antidiabetic agent known. Current insulin therapies, however, are ineffective in maintaining normal blood glucose levels because of the lack of continuous feedback for endogenous insulin levels. Insulin delivery can be via discrete injections [6,7] or by continuous pumping at a given flow rate. Because glucose levels are affected by external factors such as meals and stress, patients must test their blood sugar (glucose) at least four times a day [8]. Without a continuous means of sensing fluctuations in glucose levels, it is not possible to maintain a physiologically optimum concentration of insulin in bodily tissues. This need has spurred development of the glucose biosensor.

Continuous glucose sensing would be particularly important in the detection and management of hypoglycemia. Such sensing would also allow for early detection of hyperglycemia and provide a basis for insulin administration at more appropriate dosages. Further developments could include an artificial pancreas, which would include not only a glucose sensor but also an insulin delivery system that automatically administers

the amount of insulin based on the feedback signal from the sensor [1,9-13]. The flow rate of the pump would be controlled by the continuous estimation of blood glucose concentration by the sensor.

1.2 Glucose Sensor

A glucose sensor is, of course, a sensor that can selectively respond to glucose. As early as 1962, Clark and Lyons [3] described the determination of glucose by oxidizing it using a glucose oxidase enzyme, which is now the basis of virtually all glucose sensors and analyzers. Glucose is catalytically oxidized to gluconic acid by an oxidant in the presence of the enzyme. The oxidant is usually dissolved oxygen.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{GOx} \text{Gluconic acid} + H_2O_2 \quad \text{.....[1.1]}$$

This is a two substrate reaction [14]which can be rewritten as:

$$E_{ox} + G \xrightarrow{k1} E_{red}P_1 \xrightarrow{k2} E_{red} + P_1 \quad \text{.....[1.2]}$$

$$E_{red} + O_2 \xrightarrow{k3} E_{ox}P_2 \xrightarrow{k4} E_{ox} + P_2 \quad \text{.....[1.3]}$$

where G stands for glucose, P_1 for gluconic acid, and P_2 for H_2O_2. Therefore, the entire reaction rate can be described as:

$$\frac{1}{V} = \frac{k_2 + k_4}{k_2 k_4} + \frac{1}{k_1[G]} + \frac{1}{k_3[O_2]} \quad \text{.....[1.4]}$$

where $\frac{k_2 + k_4}{k_2 k_4} = \frac{1}{V_{max}}$ and V_{max} is the rate with infinite concentrations of glucose and oxygen, $\frac{V_{max}}{k_1} = K_m$ for glucose and K_m is the Michaelis constant, a characteristic of a given enzyme/substrate system obeying Michaelis-Menton kinetics [15]; similarly, K_m for oxygen is defined as $K_m = \frac{V_{max}}{k_3}$. For Gox, reported values of these constants at 27°C are V_{max} = 1150 s-1 M, K_m for glucose is 0.11 M, and K_m for oxygen is 0.48 mM [16].

Since $K_m (O_2)$ is about three orders less than K_m (Glucose), the last term of equation 1.4 can be omitted and the rate can be rewritten as:

$$V = \frac{V_{max}[G]}{K_m + [G]} \quad \text{.....[1.5]}$$

where V is the rate of formation of the product in equation 1.1; K_m is the Michaelis constant for glucose.

The least complicated and most commonly employed detection method for miniaturized glucose sensors is to determine the rate of hydrogen peroxide produced in reaction 1.1 [1,17-20]. Hydrogen peroxide can be electrochemically measured at a potential of about +0.7 V versus a saturated calomel electrode (SCE), as shown in equation 1.6 below.

$$H_2O_2 \xrightarrow{+0.7V} O_2 + 2H^+ + 2e^- \quad \text{..... [1.6]}$$

If the electrochemical oxidation of hydrogen peroxide described by reaction 1.6 is assumed to proceed at a much faster rate than V, the

dependence of the electrode current on glucose concentration can be approximated by:

$$I \propto \frac{V}{V_{max}} \propto \frac{[G]}{Km + [G]} \quad \text{.....[1.7]}$$

which is hyperbolic in the concentration of glucose [G]. Because the value of K_m represents the glucose concentration at which the electrode current is one half of the maximum current, for concentrations small compared to K_m, the current will be approximately linear in glucose concentration. Consequently, the linear dynamic range of the sensor depends on the value of K_m.

Another possible detection method is to determine the consumption of oxygen. This mechanism is employed by an oxygen-based enzyme electrode sensor [21,22], which has a membrane containing immobilized glucose oxidase coupled to a gas permeable membrane-covered electrochemical oxygen sensor. Glucose and oxygen diffuse into the enzyme layer and react, resulting in a reduction in the amount of oxygen that would otherwise be detected by the oxygen sensor. The signal current is subtracted from that of a similar reference oxygen sensor without the enzyme, and a glucose-dependent difference in the current results.

In addition to the above two types of glucose sensor, other designs include mediator-based enzyme electrode sensors [23-25], microdialysis sampling systems [26,27], membrane-covered catalytic electrode sensors [28,29], non-invasive optical glucose sensors [30] and others [31].

Presently, detecting H_2O_2 is the least complicated and most commonly employed detection mechanism for miniaturized glucose sensors [1]. Sensors of this type have a much broader dynamic range than oxygen uptake sensors. Oxygen uptake sensors rely on differential measurements where both the signal and background level can change significantly, whereas hydrogen peroxide sensors measure a signal relative to a fairly constant background level. As a consequence, it is somewhat less complicated to reduce the sensitivity of a hydrogen peroxide electrode to dissolved oxygen.

Based on the above discussion, detecting H_2O_2 was chosen as the method of detection utilized throughout this research.

1.3 Implantable Glucose Sensors

The goal of developing an implantable glucose sensor for marketing has not yet been achieved. Such a product, however, would provide continuous sensing in addition to convenience for a diabetic patient. It would eliminate the need for patients to prick their fingers several times a day to obtain blood samples.

Implantable sensors require an appropriate choice of site for the implant. Possible sites include intravascular, subcutaneous and within the peritoneal cavity. Because of potential hazards with intravascular glucose sensing [32], for example, the possibility of clot formation or vascular wall damage, most studies have focused on the development of glucose sensors for subcutaneous glucose monitoring [33-36]. The glucose concentration in subcutaneous tissue has been shown to closely follow

plasma glucose concentrations under stationary conditions [37,38]. Changes in blood glucose concentration are transmitted to the subcutaneous tissue within 5 min [4]. Examples of studies evaluating subcutaneously implanted sensors exist in the literature [38,39].

A clinically useful implantable glucose sensor requires two major developments: miniaturization and improved lifespan [13]. A small size would make the sensor easily implantable, while a long lifespan would eliminate the need for frequent replacement. The size is dependent on the sensor configuration and design, while the lifespan is dependent on the sensor components (*i.e.* enzyme lifetime, the sensor's biocompatibility and the sensor's resistance to fouling).

A common design for an implantable glucose sensor is the needle type design. Needle-type sensors are usually micro-electrodes having a platinum core (anode) isolated from an external silver/silver chloride cathode reference and counter-electrode. The Pt electrode may be coated with an inner membrane, an enzyme layer and an outer membrane [4]. Both the inner and outer membranes should be selective, *i.e.*, permeable to the chemical of interest, while eliminating transport of interfering compounds and thus preventing fouling. In addition the outer membrane should be biocompatible. When such a sensor is implanted, glucose and oxygen in the body first diffuse through the outer membrane and are catalyzed by the enzyme into hydrogen peroxide, which then permeates through the inner membrane and is detected at the Pt electrode. The inner layer needs to be selective to hydrogen peroxide, while the outer layer

must be selective to glucose and oxygen. These coatings are discussed in more detail below.

Alternative designs for implantable glucose sensors are the planar-geometry type and the vessel-shaped type. Plane geometry sensors consist of a plane surface support containing the metal working electrode and the reference and counter electrodes coated by various combinations of enzymatic and non-enzymatic, hydrophilic and hydrophobic membranes. The membranes provide a support for the enzyme, an environment for the chemical reaction, and a diffusion barrier assuring the optimal concentrations of glucose and oxygen in this environment. A vessel-shaped device through which the blood can flow was originally described by Kondo *et al.* [39]. Oxygen electrodes coupled with glucose sensitive membranes are disposed around the wall of the 'vessel'.

Several groups have developed miniature glucose sensors, which have been evaluated in rats, dogs, and humans [4,37]. Recently, we are doing research on improving the sensor selectivity and sensitivity. We also evaluate our sensors *in vivo* in humans.

1.4 Polymer Coatings

A concern of paramount importance to analytical chemistry is selectivity. Incorporation of membranes into the sensor design is necessary to screen out interference in samples with a complex matrix, and to give appropriate sensitivity to the analyte (glucose). The membrane used for the outer layer of an implantable glucose sensor is especially important because it must prevent degradation of the enzyme and electrode in the

biological environment, be permeable to glucose and oxygen and be biocompatible as well. Biocompatibility has been defined as the ability to perform with an appropriate host response in a specific application [4]. In other words, after implantation, a biostable environment should be rapidly formed that will be compatible with the sensor's function. For example, a common reaction of the body to a foreign object such as a sensor is encapsulation of the sensor by tissue. Such a process inhibits or destroys sensor performance.

Several materials, such as polyurethane [11,13,33,34,39,41], Teflon [41], cellulose and cellulose acetate [19,42,43], and the perfluorinated ionomer Nafion [1,37,44-46] have been selected as the outer membrane for implantable glucose sensors. Nafion-encapsulated glucose sensors were pioneered by Harrison *et al.* and have demonstrated superior performance both *in vitro* and *in vivo* to other sensors in terms of response time, reproducibility and bio-compatibility. The Nafion polymer has since been used by several other investigators as a membrane/ encapsulation material for biosensor applications [47-50].

The molecular structure of the Nafion monomer is shown in Fig. 1.2 below.

This Nafion's structure is quite similar to that of Teflon, except for the sulfonate groups. These sulfonate groups play an important role in the selectivity of the Nafion membrane, conferring a negative charge to the membrane which attracts cations and expels anions. Many interfering compounds in blood, such as ascorbic acid, are anions at physiological pH, and so are rejected by Nafion. The charged sites also provide a pathway

for water, ions and other solutes through the membrane, allowing it to transport charge and glucose.

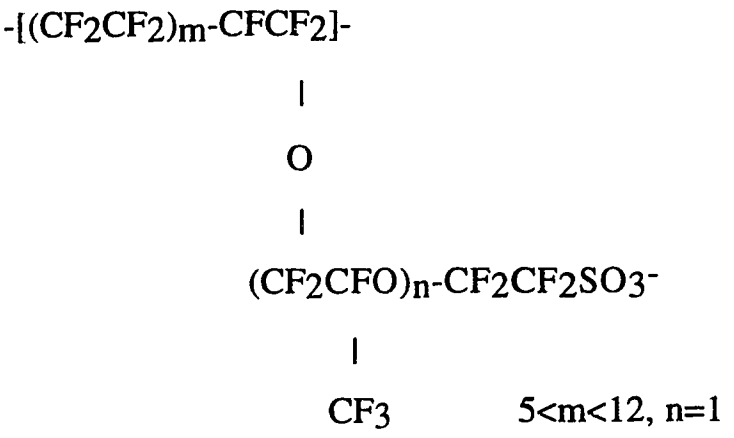

Figure 1.2 The molecular structure of Nafion monomer

Nafion films are commonly deposited from a dissolved form for ease of film preparation. However, Nafion prepared in this manner has substantially different characteristics than the solid form supplied by the manufacturer [51-54]. It has been determined that the diffusion coefficients in the recast film are greater than in the solid form received from DuPont [55], and the recast film is slightly water soluble unless treated.

Nafion has been used as a protective or selective coating material for a number of other applications. For example, a carbon electrode was coated with Nafion for neurotransmitter determination in cerebral fluids [56] and for metal ions in whole blood [57]. As well, an enzyme electrode for blood glucose testing [45] was coated with Nafion to get better protection to the body fluids.

An inner membrane layer is usually needed to coat the working electrode of a glucose sensor in order to screen out small, electroactive ions and neutral compounds. Some polymers used as inner coatings for sensors are poly(ether sulfone)(PES), polyphenolic film [58] formed by electropolymerization of phenol and phenyl/allylphenol mixtures, poly(1,2-diaminobenzene) [37,59,60] formed by electropolymerization, and polymeric mercaptosilane [61] from (3-mercaptopropyl) tri-methoxysilane. We and others have recently employed electrodepositon of 1,3-diaminobenzene (1,3-DAB). This polymer has successfully screened out acetaminophen, which is a major interferent [38,62].

1.5 Summary

The research undertaken during the course of this program was directed toward improving the selectivity and sensitivity of an implantable glucose sensor. After successful *in vitro* studies, the sensor was evaluated *in vivo* in dogs and humans.

Chapter 2 discusses results from *in vitro* studies on the improvement of performance of an implantable glucose sensor. For selectivity, prevention of fouling, biocompatibility, and optional reaction conditions, the glucose sensor is coated with multiple polymers. Specifically, studies on inner coating polymers of 1,2- and 1,3-diaminobenzene (DAB) and their coating order are presented. In addition the effect of the thickness of the outer Nafion coating was studied. These studies resulted in a sensor with improved sensitivity and selectivity.

After optimizing the sensor performance *in vitro*, we evaluated our glucose sensor *in vivo* by inserting it subcutaneously in the abdominal region of humans. Chapter 3 presents the evaluation of the sensor in humans for short-term (hours and days) experiments. After implantation and upon obtaining a steady state response from the sensor, a standard oral glucose tolerance test (OGTT) was performed. Sensor response was monitored for 2 hours after the OGTT. During this same period blood was sampled for analysis in the biochemistry laboratories at the University of Alberta Hospitals. A comparison was made between the sensor output and the measured glycemia. In addition to excellent agreement between the sensor response and the glycemia, it was determined that the sensor worked well, with ideal sensitivity and reasonable stability in short-term experiments and exhibited good biocompatibility over this period.

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CHAPTER 2

IMPROVED SENSITIVITY AND SELECTIVITY OF IMPLANTABLE GLUCOSE SENSORS

2.1 Introduction

In principle, many biological compounds can be monitored in situ with implantable sensors, yet glucose biosensors have been investigated the most intensively [1,2]. This research is a direct result of the potential improvements in care that could be given to diabetics as a result of a successful method for continuous glucose monitoring.

A working glucose sensor must selectively respond to glucose, have a linear response for easy calibration and have a high sensitivity to glucose in the low mM range. Previous research in this laboratory resulted in the development of a needle-type sensor that utilized a trilayer membrane system [3]. A schematic diagram and an electron micrograph of this sensor are shown in Figures 2.1a and 2.1b. This sensor consisted of a Pt wire which was first coated with an inner layer of electropolymerized 1,2-diaminobenzene (1,2-DAB). Next the enzyme, glucose oxidase, was added in an albumin/glutaraldehyde matrix to immobilize the enzyme. Finally, the outer biocompatible Nafion layer was applied. The sensor also had a built in Ag/AgCl reference electrode, which was a silver wire coiled on the supporting copper wire, and then anodized at 0.4 V in 0.1 M

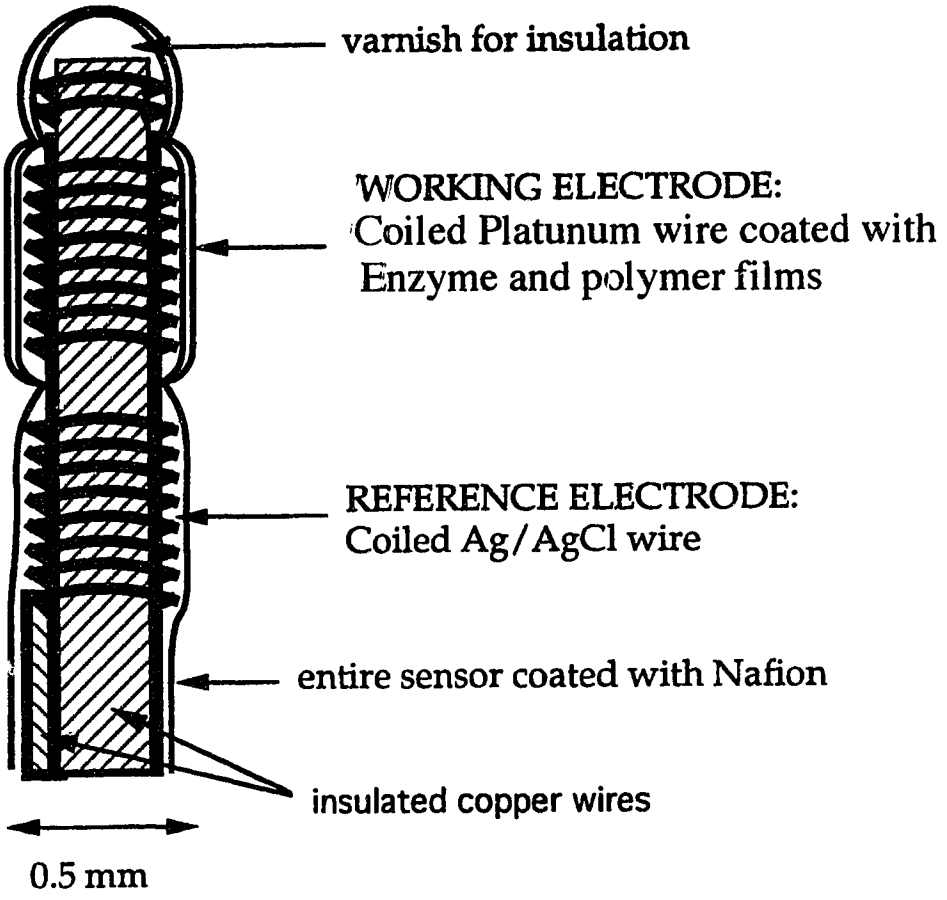

Figure 2.1a A schematic graph of a glucose sensor

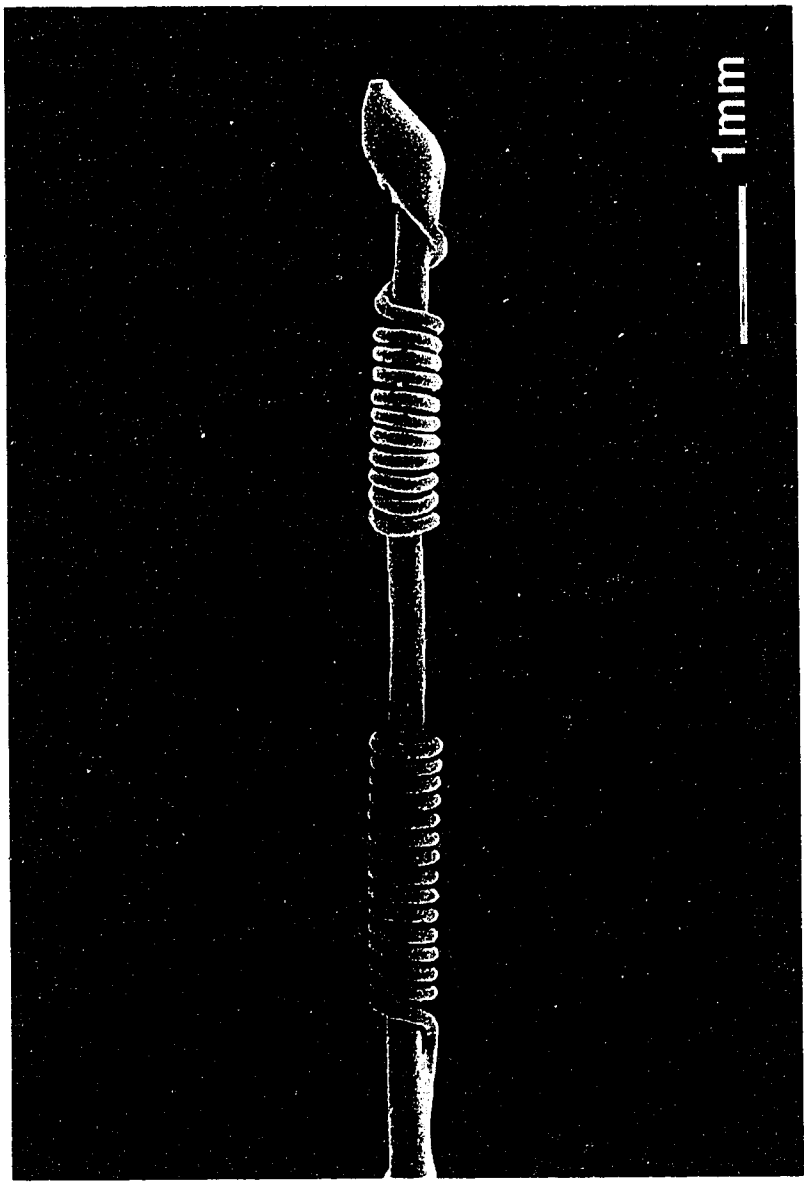

Figure 2.1b SEM micrograph of the sensor

HCl for half an hour. Once the coating process was finished the entire sensor was heat cured at 120°C.

This sensor had demonstrated good sensitivity to glucose in both *in vitro* and *in vivo* studies [4], however was also susceptible to interferents such as acetaminophen and uric acid.

The goal of the research discussed within this chapter was to improve upon the previous sensor design. Specifically, this work was aimed toward eliminating the sensor's response to interferents and improving upon the sensitivity to glucose.

In this Chapter we report two new developments. First, we have eliminated the interference of acetaminophen and uric acid by using an inner polymer formed by electropolymerizing 1,3-diaminobenzene (1,3-DAB) instead of 1,2-DAB. Second, by changing the order of coating deposition we have obtained better sensitivity and selectivity to glucose. Not only do these modifications result in the elimination of interferents, but the sensitivity is improved.

2.2 Experimental Section

2.2.1 Equipment

Cyclic voltammetry and amperometry were performed using a modified Pine RDE-4 (Pine Instrument Company, Grove City, PA) or a BAS CV-27 potentiostat (Bioanalytical Systems Inc., West Lafayette, IN) or a LC-3D Petit Ampere(Bioanalytical Systems, Inc., West Lafayette, IN). A combined potentiostat and telemetry system made by the

Electronics shop in the Department of Chemistry at University of Alberta was also used. The data was recorded using either an x-y-t-BD 91 recorder (Kipp & Zonen, Delft, Holland) or a strip chart recorder (Johns Scientific Inc., Toronto). For the *in vitro* experiments, a three neck round bottom flask was used as the electrochemical cell. Stirring was provided by an air-driven magnetic stirrer. The reference electrode was a saturated calomel electrode (SCE) while the counter electrode was a Pt mesh.

2.2.2 Material

High-purity glucose-oxidase (GOx) (Aspergillus Niger) was obtained from both Calbiochem (La Jolla, CA) and Sigma (St. Louis, MO) (type x-s). Bovine serum albumin (Fraction V, 98-99% albumin), and glutaraldehyde (25% aqueous solution) were obtained from Sigma and used as received. All other chemicals used were of reagent grade quality. Solutions were prepared from double distilled, deionized water. A stock, pH 7.4 buffer solution was prepared from 56.2 mM di-sodium hydrogen orthophosphate (Na_2HPO_4), 5.61 mM sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot H_2O$), 0.1 M sodium chloride with 5 mM sodium benzoate and 1 mM EDTA as preservatives.

2.2.3 Procedures

2.2.3.1 Electrode Preparation

Most of these studies utilized coiled Pt wire electrodes such as in the sensor design, although some measurements were made with a planar

Pt electrode (A=0.2733 cm^2) which was prepared by polishing with 0.3 and 0.05 mm Al_2O_3 (Buehler, Lake Bluff, IL) and then washing with doubly distilled water. The coiled Pt electrodes were made from 0.1 mm thick platinum wire (Alfa Puratronic, Johnson Mattey, MA), which was coiled about 10 turns around a 0.25 mm varnished copper wire, as shown in the sensor design in figure 2.1a. The crimp connection at the tip was sealed with insulating varnish (GC electronics, Rockford, IL). After being dried overnight, the coiled Pt electrodes were washed in an ultrasonic bath with doubly distilled water. Both types of electrodes were then anodized at +1.9 V *vs.* SCE and then cycled between -0.26 and +1.1 V *vs.* SCE in 0.5 M H_2SO_4. Next, the electrodes were connected together in parallel in a three-neck flask using a three electrode system for deposition of DAB. Both the 1,2- and 1,3-DAB were in a solution of pH 5.5 acetate buffer solution at a concentration of 5 mM. Before electropolymerization, the solution was flushed with nitrogen for 5 minutes and was kept under a nitrogen environment for the remainder of the experiment. The DAB was then coated onto the electrode surface through electropolymerization at +0.65 V *vs.* SCE for 5 minutes. The current was monitored using a strip chart recorder. Typical current responses are shown in Figure 2.2.

2.2.3.2 Comparison of 1,2-DAB and 1,3-DAB

1,2- and 1,3-DAB were deposited under the conditions given above. The effectiveness of the coating was tested by measuring the response of the sensor to the following chemicals: acetaminophen (0.17 mM), uric acid (0.48 mM) and hydrogen peroxide (0.5 mM). All the *in*

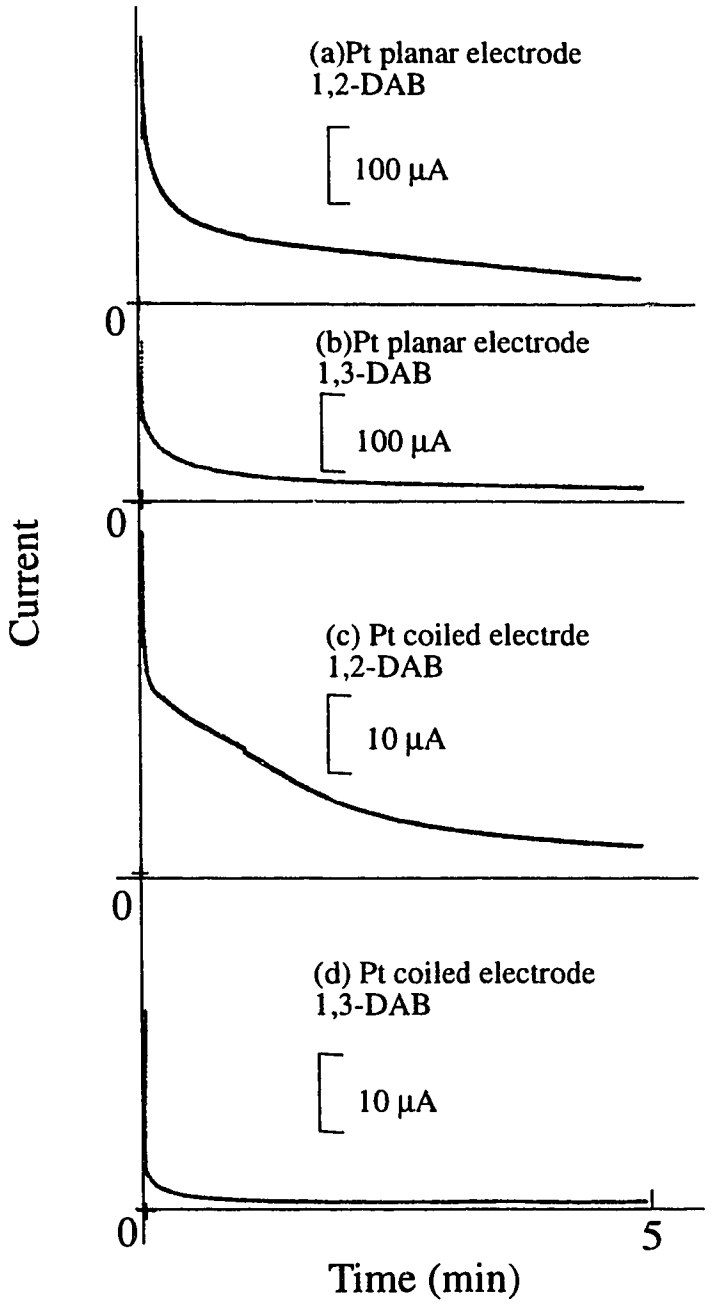

Figure 2.2 Current/time curves for polymerization of 1,2- and 1,3-DAB on Pt planar and coiled electrodes. 1,2- and 1,3 DAB were electrodeposited on the electrodes at an applied potential of 0.65 V vs. SCE. The concentration of both DAB was 5 mM in pH 5.5 acetate buffer solution. The solution was purged by nitrogen then kept under a nitrogen environment.

vitro measurements were carried out in pH 7.4 phosphate buffer saline (PBS) and stirred with an air driven magnetic stir. An external SCE was used instead of the coiled Ag/AgCl reference electrode. A potential of 0.70 V was applied. A steady-state current was obtained before any addition of interferents.

The electrodes were modified and tested throughout the fabrication process. Different steps in this process will be referred to as stage 1, 2, 3, etc.; these are defined below. After stage 1, in which the electrodes were coated with DAB and tested with acetaminophen and uric acid then dried in air, the Pt coiled electrodes were aged three days in air (stage 2) and tested again with acetaminophen and H_2O_2. They were then dried in air, coated with a layer of glucose oxidase which was mixed with glutaraldehyde and bovine serum albumin (BSA), and dried again in air (stage 3). The electrodes were then tested with acetaminophen and glucose in order to determine any interference from acetaminophen on the glucose response. The electrodes were then dried in air and coated with Nafion films (1 layer 0.5 wt%, 1 layer 3 wt% and 3 layers 5 wt%). After drying in air (stage 4), the electrodes were tested again with acetaminophen and glucose. Finally, the electrodes were heat cured in an oven at 120°C for 60 min (stage 5), then tested again with acetaminophen and glucose.

To obtain some information on the structures of poly-1,2- and 1,3-DAB, IR spectra were performed at Ames Laboratory/Iowa State University in the laboratory of Prof. Marc Porter. 1,2- or 1,3-DAB was electrodeposited on a 1.5 cm x 2.0 cm Pt substrate. Pt substrates were prepared by evaporation of 2 nm of Ti followed by 200 nm of Pt on clean

glass microscope slides. This procedure was performed at Alberta Microelectronic Center (AMC). The polymerization process was performed in a pH 5.5 acetate buffer solution containing 5 mM DAB by cycling between 0 and +0.85 V *vs* Ag/AgCl reference electrode for 10 hours.

2.2.3.3 Effects of the Order of Deposition of the layers

Two methods of electrode coating were used. One group of coiled wire electrodes were coated with 1,3-DAB, then enzyme and finally Nafion as described in the previous section. Another group of coiled wire electrodes were made by first depositing the enzyme layer directly onto the bare Pt electrode, then coating with Nafion, then electropolymerizing the 1,3-DAB through these coatings onto the Pt surface. These different electrodes were characterized *in vitro* in PBS using an SCE reference electrode.

To evaluate sensitivity, the steady-state current at an applied potential of +0.7 V *vs* SCE was measured as a function of added glucose concentration. For selectivity studies, an acetaminophen solution in pH 7.4 PBS containing 5.66 mM glucose was added to a 5.66 mM glucose solution after a steady-state current in the glucose solution was first obtained.

2.2.3.4 Most effective Nafion Thickness

Several groups of sensors were made using both DAB coating orders as described in Section 2.2.3.3, but the number of 5 wt% Nafion

layers was varied. The sensitivity to glucose and selectivity to acetaminophen were measured in the same way as above.

2.2.3.5 Acid Treatment Experiment

Treatment of the poly (1,3-DAB) film with methanesulfonic acid (MSA) was performed in order to alter the film's permeability. MSA solutions were 0.045 M and 0.136 M MSA in 1:1 (v:v) isopropanol:water

Coiled Pt electrodes was first coated by electrodeposition of 1,3-DAB at 0.65 V for 5 min, then dried in air. Three groups of electrodes were made. Two groups were dipped into MSA solutions twice with an interval of 30 min in air. The other group was dipped into a solution with only isopropanol:water. The electrodes were tested in PBS solution with additions of H_2O_2 and then an acetaminophen solution containing the same concentration of H_2O_2 to avoid peroxide dilution.

2.2.3.6 Blood and Dog Tests

For blood and dog tests, the sensor used all coatings, as described below. The Pt wire was coiled and cleaned as in Section 2.2.3.1. A silver wire (0.1 mm diameter) was coiled as shown in Figure 2.1. The silver wire was then anodized in a 0.5 M HCl solution at 0.4 V *vs* SCE for 30 min. The bare Pt wire was then coated with glucose oxidase and then the whole sensor (both Pt and Ag/AgCl) was coated with one layer each of 0.5 wt%, 3 wt% and then 4 layers of 5 wt% Nafion. Finally 1,3-DAB was electropolymerized onto the Pt electrode. The sensors were then heat cured at 120°C for 120 min. Another group of sensors which were also

used in the dog tests were made by first coating 1,2-DAB on the coiled wire Pt electrode then applying glucose oxidase and finally the Nafion with one layer each of 0.5 wt%, 3 wt%, then 4 layers of 5 wt%. The whole sensor was heat cured at 120°C for only 60 min.

The sensors were tested *in vitro* in blood. Blood samples were taken from a non-diabetic dog or diabetic person and then mixed with heparin. Two milliliters of blood were put in a 5 ml beaker, after which the sensor was inserted into the blood, making sure both electrodes of the sensor were immersed in the solution. A small stirring bar was put into the beaker, which was stirred by an air driven magnetic stir. A potential of 0.7 V was applied between the working electrode and Ag/AgCl reference electrode incorporated on the needle-type sensor. After a steady state current was obtained, several standard additions of glucose were made to test the sensitivity. A long-term study was done by leaving the sensor in a blood solution with 5.66 mM glucose for 24 to 48 hours. The current was monitored continuously.

The sensor was also tested *in vivo* in dogs by implanting subcutaneously in a halothane-anesthetized healthy dog through an incision on the neck. A silastic catheter was also inserted in a jugular vein to allow collection of blood samples. Immediately after the surgery, a 0.7 V potential was applied between the working electrode (Pt with coatings) and the reference electrode (Ag/AgCl incorporated on needle-type sensor). After a steady state current was obtained (about 40 min after the potential was applied), a bolus of glucose (0.5 g/kg body weight) was injected through the venous catheter, and blood was sampled at 0, 1, 5, 10, 15, 30,

60 and 90 min after glucose administration. The glucose concentration in the blood samples was determined using a Beckman glucose analyzer at the Biological laboratory of the Surgical Medical Research Institute at University of Alberta. The blood glucose was compared with the sensor's output current.

2.3 Results and Discussion

2.3.1 Comparison of 1,2-DAB with 1,3-DAB

Figure 2.3 shows a typical i/t curve obtained from the interference measurements. Tables 2.1-2.3 give a comparison between the performance of 1,2- and 1,3-DAB in terms of acetaminophen and uric acid interferences relative to 0.5 mM H_2O_2 or 5.66 mM glucose. Table 2.1 shows the behavior initially after film formation (stage 1). The much higher percent errors for both uric acid and acetaminophen for electrodes coated with 1,2-DAB indicate that this film is more permeable to these interferents. Thus coating with 1,3-DAB gives better screening against both acetaminophen and uric acid.

The electrodes were tested again after 3 days of storage in air (stage 2). The data listed in Table 2.2 show that the 1,3-DAB coating continued to give approximately 3 times better selectivity.

After being coated with glucose oxidase, the electrodes were tested for interference by acetaminophen relative to the basal glucose level of 5.66 mM (stage 3). Table 2.3 shows the results. Again, coating with 1,3-

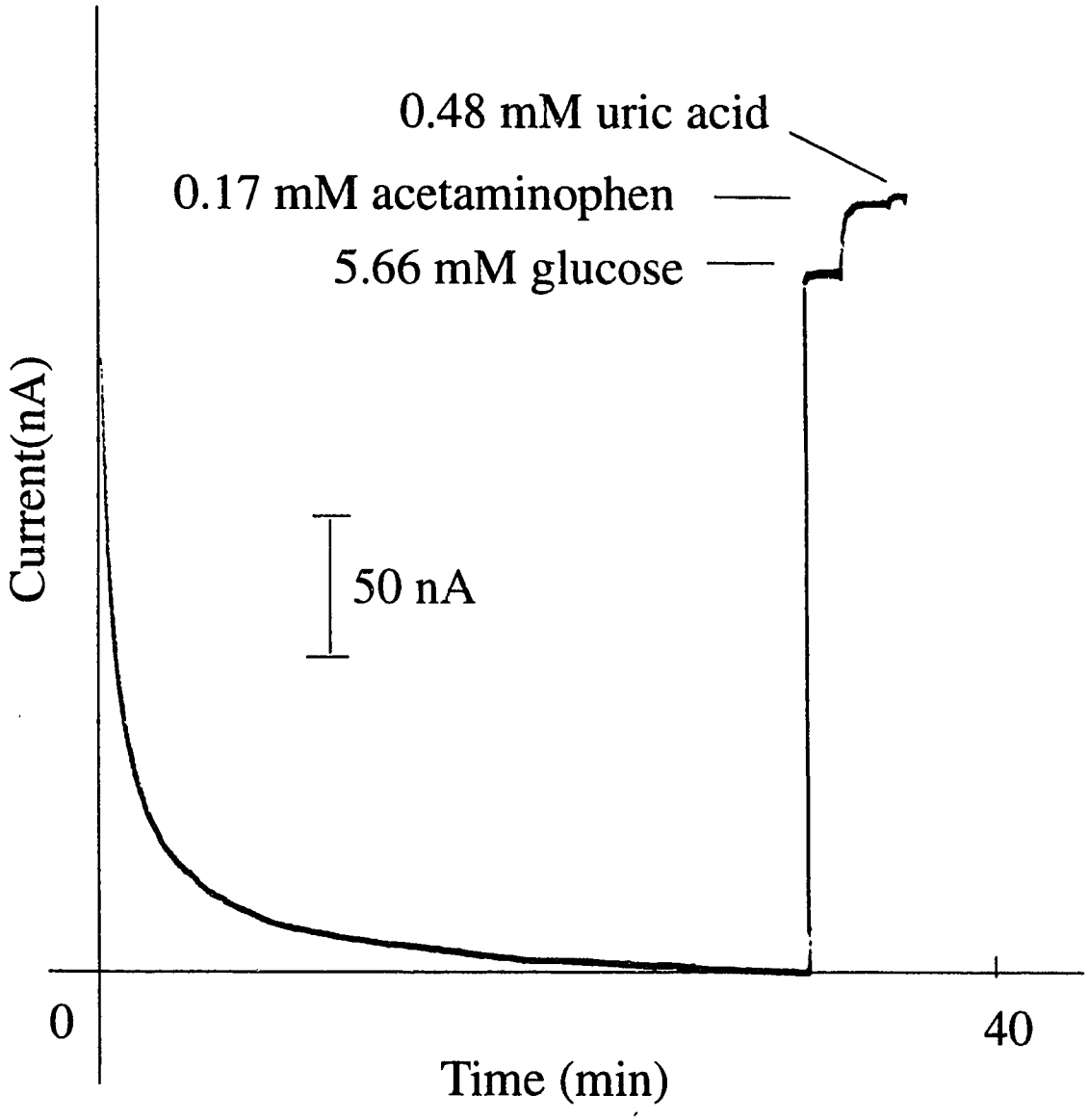

Figure 2.3 Current/time curve of acetaminophen and uric acid vs glucose.

Sensor #	Coating method	Response (H_2O_2) nA/mM	Response (aceto) nA/mM	Response (UA) nA/mM	% error (aceto)	% error (UA)
1	1,2-DAB planar Pt	45.5	44.1		33	
2	"	44.0	14.7		11.4	
3	"	52.0	29.4		19.2	
4	"	41.0	51.2	8.96	42.4	21
5	"	53.0	67.5	20.83	43.3	37.4
6	"	27.6	16.8	1.77	20.6	6.2
average	"	43.85	37.28	10.52	28.32	21.4
7	1,2-DAB coiled Pt	4.8	4.71		33	
8	"	2.0	1.47		25	
9	"	5.8	2.06		12.1	
10	"	2.88	0.559	0.271	6.6	9.0
11	"	5.92	1.44	1.42	8.3	23.0
12	"	1.80	0.882	0.583	16.7	31.1
average	"	3.87	1.86	0.76	16.95	21.03

Table 2.1a Comparison of 1,2- and 1,3-DAB in terms of the interference of acetaminophen and uric acid. The electrodes are Pt planar and Pt coiled electrodes. 1,2- or 1,3-DAB is electropolymerized onto the electrode by applying a potential of 0.65 V *vs* SCE in a pH 5.5 acetate buffer solution with 0.5 mM DAB.

Sensor #	Coating method	Response (H_2O_2) nA/mM	Response (aceto) nA/mM	Response (UA) nA/mM	% error (aceto)	% error (UA)
1	1,3-DAB planar Pt	15.0	2.82		6.4	
2	"	21.4	3.94		6.3	
3	"	17.6	2.35		4.5	
4	"	31.0	0.588	0.312	0.6	1.0
5	"	25.6	0.059	0.021	0.0	0.8
6	"	41.3	0.765	0.250	0.6	0.6
average	"	25.32	10.52	0.194	3.07	0.8
7	1,3-DAB coiled Pt	2.96	0.412		4.7	
8	"	5.50	1.294		8.0	
9	"	6.20	1.765		9.7	
10	"	3.14	0.235	0.083	2.5	2.5
11	"	2.48	1.0	0.177	13.7	6.8
12	"	4.50	0.312	0.071	2.4	1.5
average	"	4.13	0.84	0.11	6.83	3.6

Table 2.1b Comparison of 1,2- and 1,3-DAB in terms of the interference of acetaminophen and uric acid. The electrodes are Pt planar and Pt coiled electrodes. 1,2- or 1,3-DAB is electropolymerized onto the electrode by applying a potential of 0.65 V vs SCE in a pH 5.5 acetate buffer solution with 0.5 mM DAB.

Sensor #	Coating method	Response (H2O2) nA/mM	Response (aceto) nA/mM	% error (aceto)
1	1,2-DAB coiled Pt	7.2	14.06	66.4
2	"	4.2	5.294	42.8
3	"	7.2	8.235	47.0
4	"	5.32	4.353	27.8
5	"	7.26	6.824	31.9
6	"	4.04	3.247	22.8
average	"	6.00	7.00	39.78
7	1,3-DAB coiled Pt	7.0	2.206	10.7
8	"	7.3	5.000	23.3
9	"	8.08	2.059	8.7
10	"	4.34	1.294	10.1
11	"	4.96	1.765	12.1
12	"	7.02	1.971	9.5
average	"	6.45	2.38	12.4

Table 2.2 Comparison of 1,2- and 1,3-DAB in terms of aging the interference of acetaminophen over H_2O_2. The electrodes are Pt coiled electrodes which were tested previously with acetaminophen and uric acid giving the data listed in table 2.1. 1,2- or 1,3-DAB is electropolymerized onto the electrode by applying a potential of 0.65 V vs SCE in a pH 5.5 acetate buffer solution with 0.5 mM DAB.

Sensor #	Coating method	Response (Glucose) nA/mM	Response (aceto) nA/mM	% error (aceto)
1	1,2-DAB coiled Pt	8.569	4.412	15.5
2	"	5.035	2.529	15.1
3	"	9.011	4.118	13.7
4	"	6.007	4.706	23.5
5	"	7.067	3.824	16.2
6	"	5.477	2.647	14.5
7	"	5.654	2.941	15.6
8	"	6.890	2.647	11.5
average	"	6.71	3.48	15.7
9	1,3-DAB coiled Pt	9.717	5.882	18.2
10	"	8.481	2.353	8.3
11	"	8.657	2.059	7.1
12	"	6.184	1.176	5.7
13	"	4.417	1.176	8.0
14	"	6.625	1.471	6.7
15	"	5.565	0.882	4.8
16	"	4.329	0.882	6.1
average	"	9.00	1.98	8.11

Table 2.3 Comparison of 1,2- and 1,3-DAB in terms of the interference of acetaminophen over glucose on Pt coiled electrtodes. The electrodes had been tested previously, giving the data listed in Tables 2.1 and 2.2 and then were coated with glucose oxidase without an outer layer of Nafion.

DAB gives electrodes with much better selectivity than coating electrodes with 1,2-DAB.

Figure 2.4 summarizes the above data with the addition of an evaluation of electrode performance after Nafion coating (stage 4) and heat curing (stage 5). Initially, 1,3-DAB was more selective. Both 1,2- and 1,3-DAB declined in selectivity after being stored in air for 3 days, with 1,2-DAB exhibiting a much larger decline in selectivity. When tested with glucose after being coated with glucose oxidase, 1,3-DAB continued to give better selectivity. Coating with Nafion decreased selectivity of both 1,2- and 1,3-DAB. While the 1,3-DAB retained better selectivity, the difference in selectivity relative to 1,2-DAB was not as great as before coating with Nafion. Heat curing further decreased selectivity of both 1,2- and 1,3-DAB, however, 1,3-DAB remained more selective.

In summary, the 1,3-DAB coating consistently gives a higher selectivity over the 1,2-DAB coating at every stage of the electrode fabrication process.

Based on these data, it is reasonable to propose that 1,3-DAB forms a more dense and uniform film than 1,2-DAB. Both monomers polymerize to form insulating films. Thus, during the polymerization process, the current due to polymer formation drops off because of the formation of an insulating layer at the electrode surface. The current/time curves in Figure 2.2 for the polymerization of 1,2- and 1,3-DAB show that the current drops much faster for 1,3-DAB electropolymerization. These data indicate that the polymer from 1,3-DAB is less permeable.

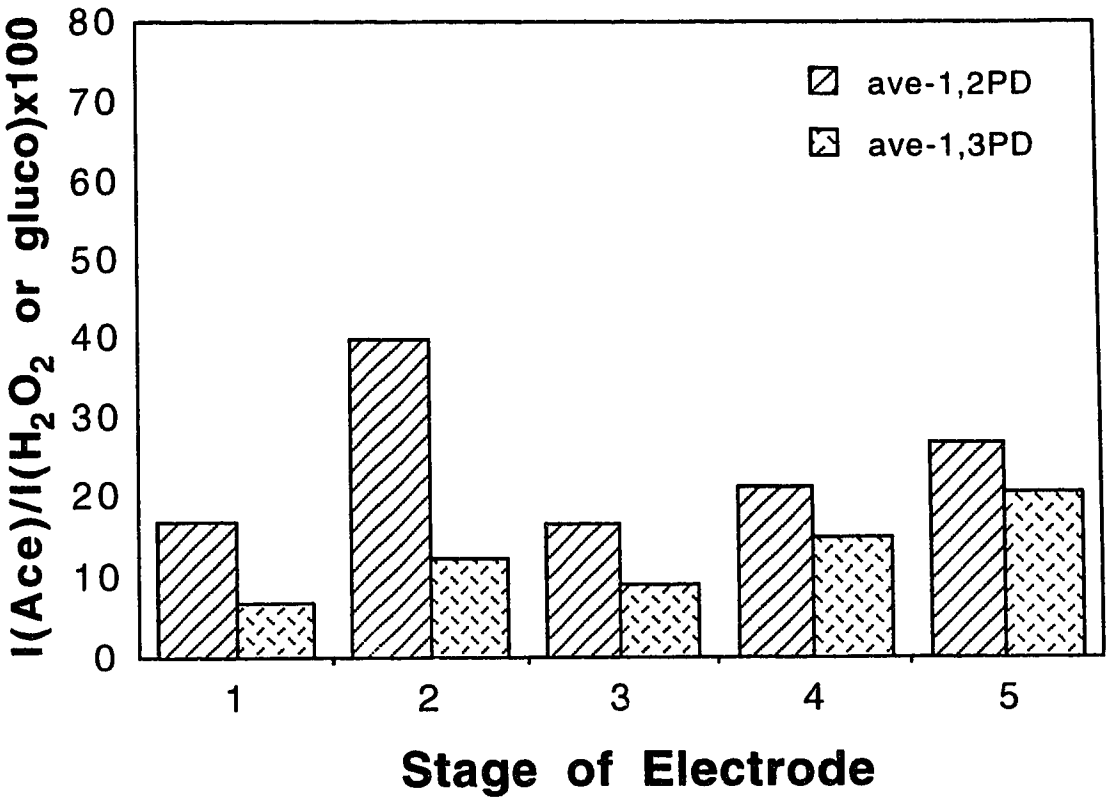

Figure 2.4 Comparison of 1,2- and 1,3-DAB on different electrode stages in term of interference of acetaminophen. All values were averaged from 6 or 8 coiled Pt electrodes. Tests were performed at room temperature.

Electrode stages:

1. With only DAB coating and testing with acetaminophen vs H_2O_2;
2. Same test 3 days later while the electrodes were kept in air;
3 Coating with glucose oxidase and testing with acetaminophen vs glucose;
4. Coating with Nafion and testing with acetaminophen vs glucose;
5. Heat curing at 120°C for 60 min and testing with acetaminophen vs glucose.

A proposed mechanism for polymerization of 1,3-DAB at pH 5,5 is shown in Figure 2.5. The process of the polymerization involves an electrophilic reaction, which is favored to occur at an ortho- or a para-position of an activating NH_2 group on an aromatic ring. For 1,3-DAB, there are 3 positions which are activated by both NH_2 groups. However, for 1,2-DAB, there are four positions which are activated by only one NH_2 group.

In this mechanism, 1,3-DAB forms a more stable conjugated, resonance-stabilizing polymer in its doped form. For 1,2-DAB, a similar pathway can be proposed [5], however the end product is a less stable conjugated polymer chain. Based on this mechanism, one would expect a better-formed film for 1,3-DAB *vs* 1,2-DAB.

IR spectra (see Figures 2.6a and 2.6b) confirm that there are NH_2 groups on the polymer chain from both 1,2- and 1,3-DAB. Peaks around 3300 cm^{-1} are very characteristic of a free primary amino group [6]. In addition, IR spectra obtained on polymers formed from 1,2- and 1,3-DAB are quite different. However, comparison of the polymer spectra with reference spectra [7] of the two monomer precursors indicates that these differences arise from the polymers' similarities to their respective monomers. These preliminary IR spectra thus indicate that the two polymer films, although different from each other, retain structural similarities to their respective monomers.

2.3.2 Effects of the Order of Deposition of the Layers and Nafion Thickness

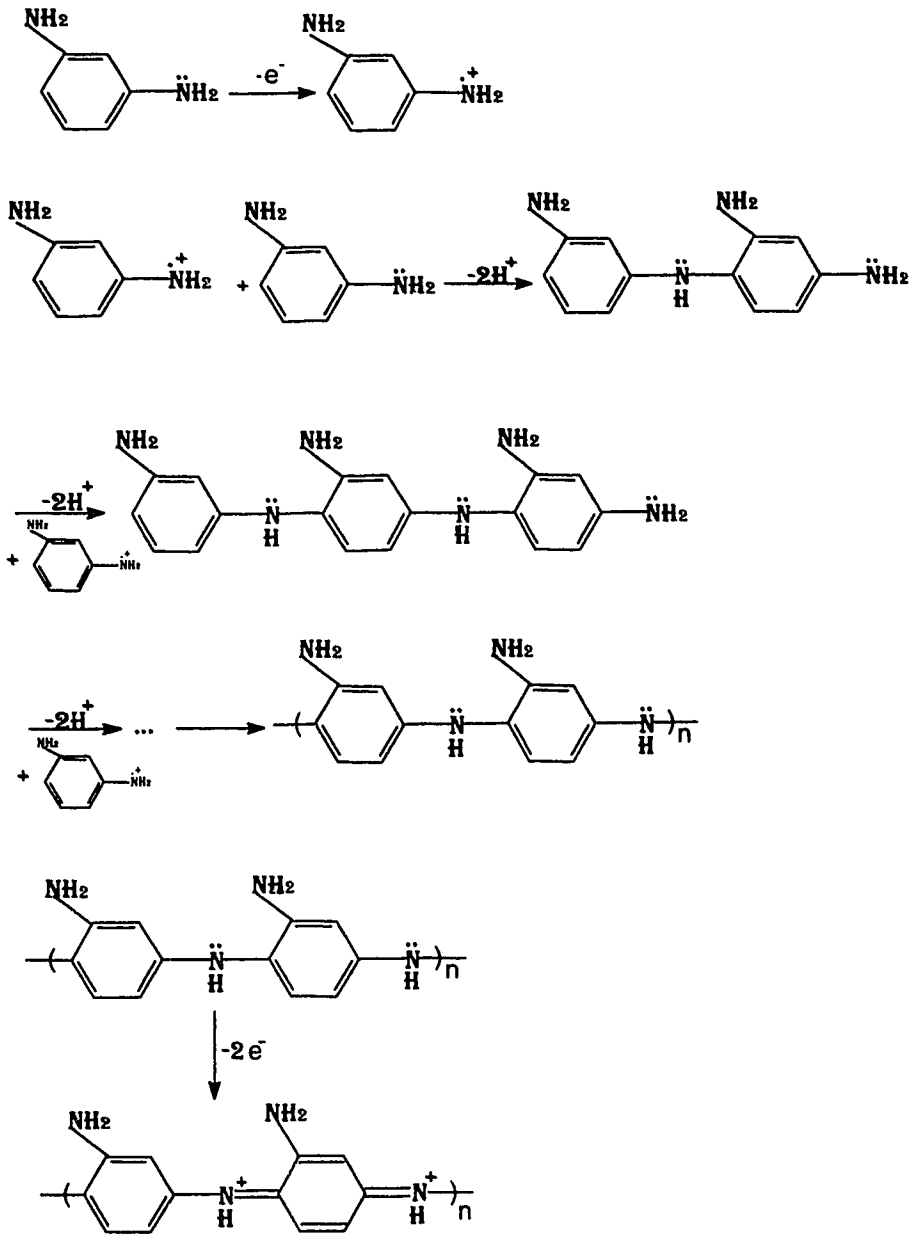

Figure 2.5 A proposed mechanism for electropolymerization of 1,3-DAB in pH 5.5 acetate buffer solution

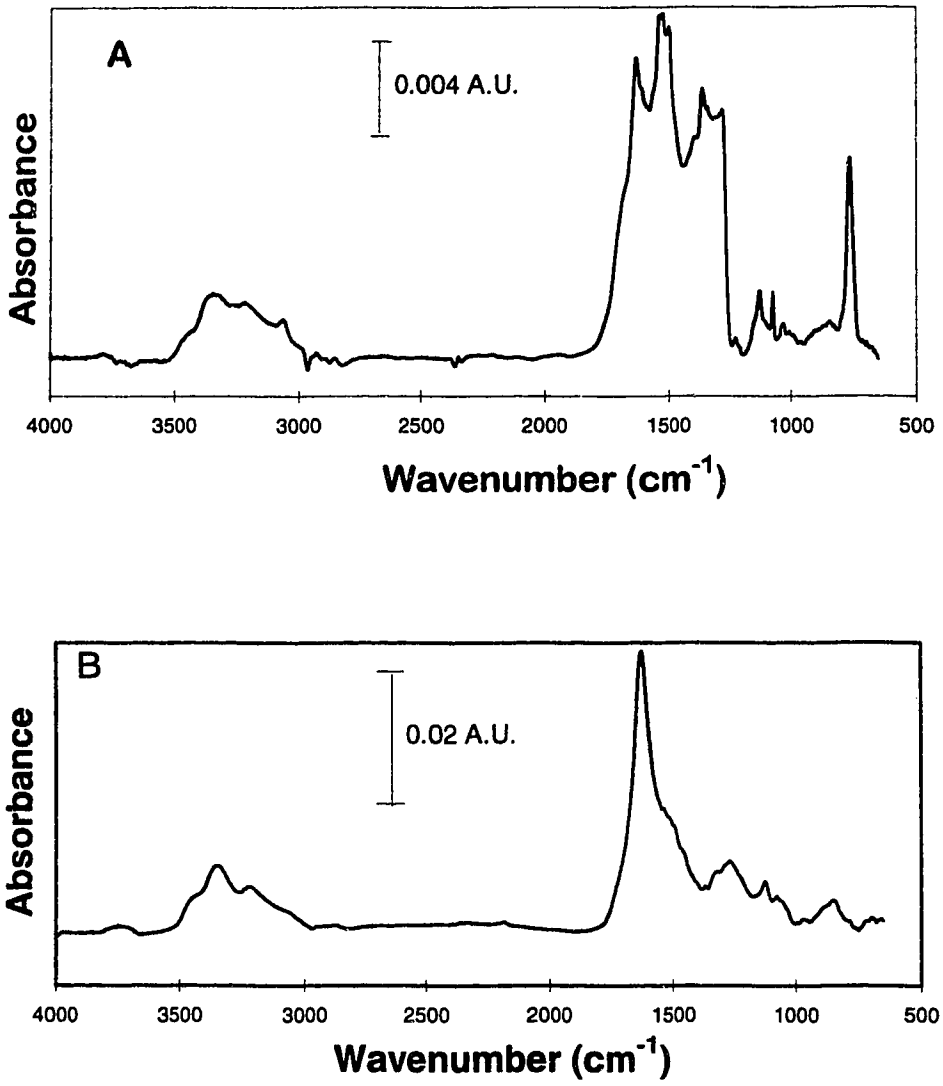

Figure 2.6 IR spectra of poly-1,2-DAB and poly-1,3-DAB. 1,2- or 1,3-DAB was electrodeposited on a 1.5 cm x 2.0 cm Pt substrate. Pt substrates were prepared by evaporation of 2 nm of Ti followed by 200 nm of Pt on clean glass microscope slides. The polymerization process was performed in a pH 5.5 acetate buffer solution containing 5 mM DAB by cycling between 0 and 0.85 V *vs* Ag/AgCl reference electrode for 10 hours. (A)1,2-DAB (B)1,3-DAB

During the course of evaluating the 2 different polymers at various stages of electrode fabrication, it became apparent that the thickness of the Nafion coating affected the sensitivity to glucose (Figure 2.7). Although this figure shows that the sensitivity does not change significantly with 1,2- *vs* 1,3-DAB, the thickness of the Nafion layer has a very strong influence on sensitivity. As might be expected, the sensitivity decreases with increasing thickness of the Nafion coating. However, the selectivity had also been shown to decrease with the addition of Nafion layers (stage 5). These results led us to evaluate how modifications in the order of the film coating procedure affected the sensor performance.

For all of the data presented above, the DAB was deposited before the GOx and Nafion layers as described in Section 2.2.1. Since DAB can permeate both of the outer layers (GOx and Nafion), it is also possible to deposit it after the Nafion layer is coated. Because our earlier studies showed that 1,3-DAB did not give the same selectivity against acetaminophen or the same sensitivity to glucose after depositing Nafion as it did before the Nafion coating, we examined the effect of depositing the DAB after the Nafion. Figure 2.8 shows the comparison of the sensitivity and selectivity produced by the two different coating orders. The sensor with the later 1,3-DAB electrodeposition gives both higher sensitivity and better selectivity. Even after heat curing for 1h at 120°C with a 4-layer Nafion coating, necessary for good sensor durability, the sensor shows good sensitivity and selectivity.

Coating the DAB after the Nafion gave electrodes for which the sensitivity did not change very much with increasing Nafion thickness, as

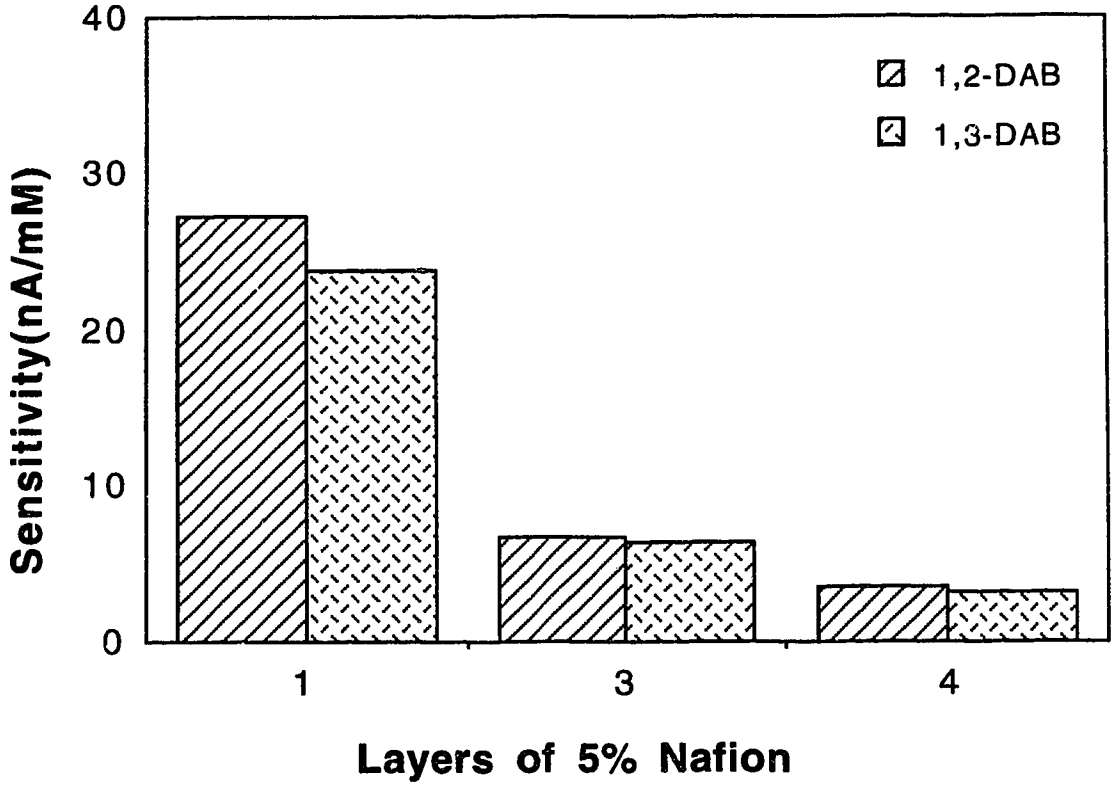

Figure 2.7 Comparison of 1,2- and 1,3-DAB with various Nafion coatings in term of sensitivity to glucose. Coiled Pt electrodes were coated with DAB/GOx/Nafion and heat cured at 120°C for 60 min. Tests were performed at room temperature in PBS by various glucose additions after a steady-state current was obtained. Potential applied was 0.7 V *vs* SCE. Average from 6 electrodes

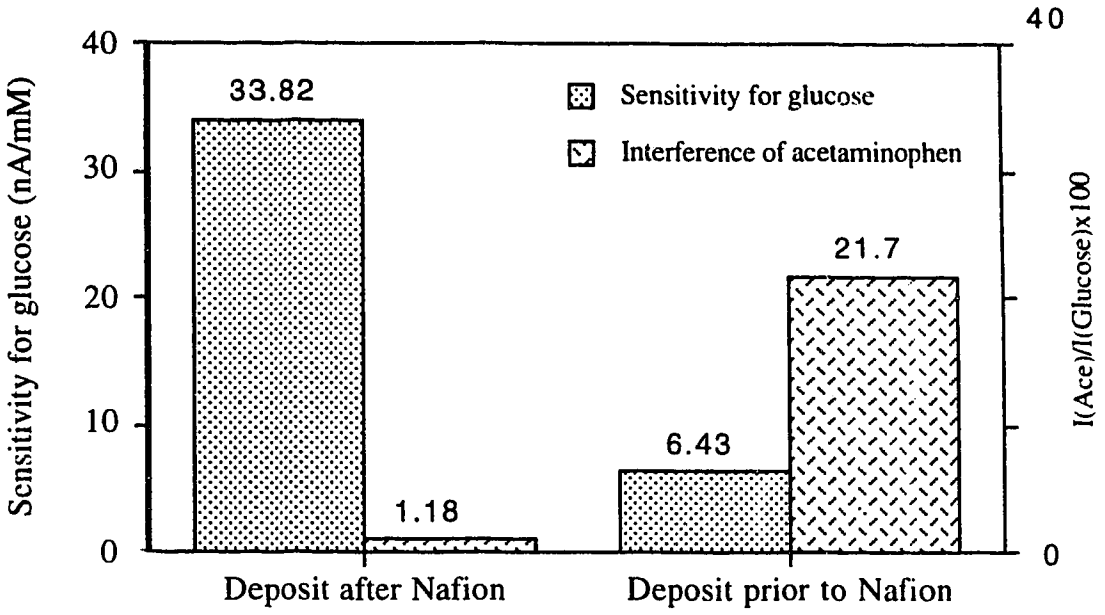

Figure 2.8 Effect of coating orders of 1,3-DAB on sensitivity and selectivity on Pt coiled electrode. The electrodes were heat cured at 120°C for 60 min.

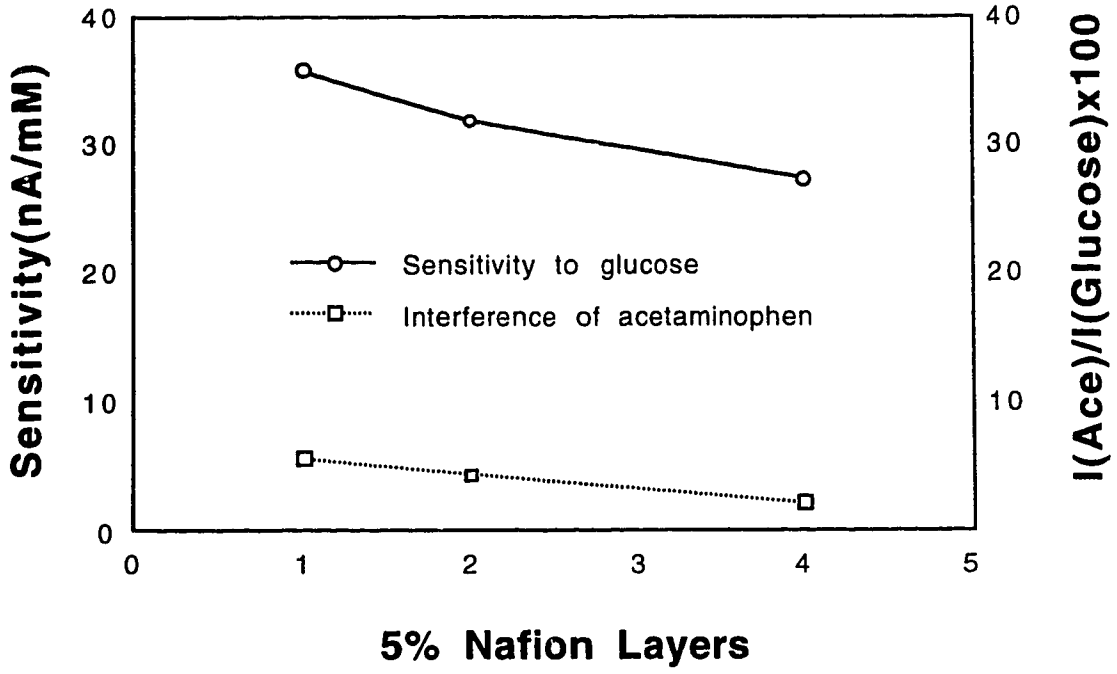

Figure 2.9a Effect of the thickness of Nafion layers on sensitivity and selectivity on Pt coiled electrodes. The electrodes were coated with GOx/Nafion/1,3-DAB and then heat cured at 120°C for 60 min.

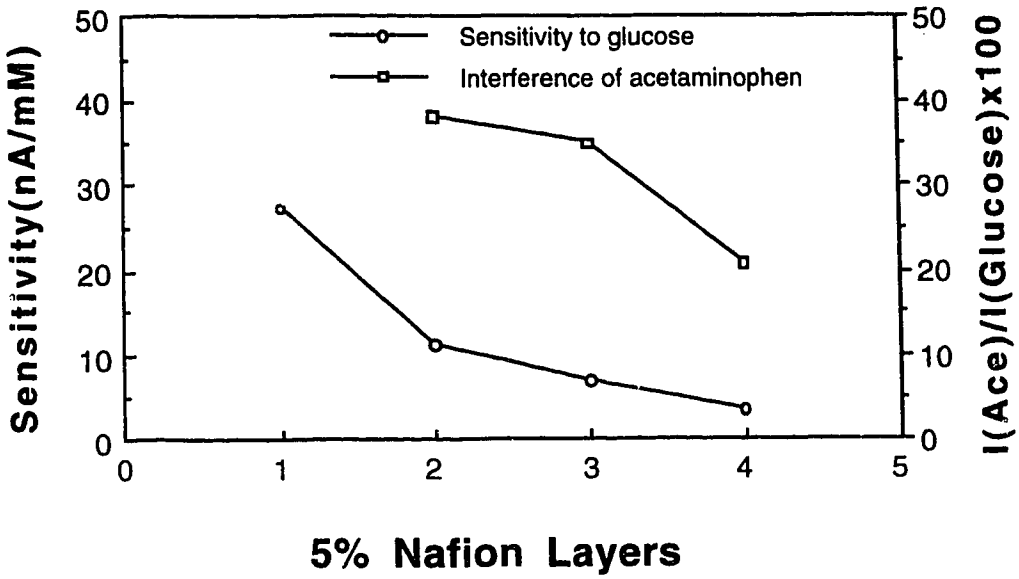

Figure 2.9b Effect of the thickness of Nafion layers on sensitivity and selectivity on Pt coiled electrodes. The electrodes were coated with 1,2-DAB/GOx/Nafion and then heat cured at 120°C for 60 min.

seen in Figure 2.9a. These results contrast sharply with the data shown in Figures 2.7 an 2.9b, in which the DAB was deposited first and showed a substantial decrease in sensitivity with increasing Nafion thickness.

Nafion Permeability

Comparison of Figures 2.7 and 2.9 shows that the permeability of the Nafion top layer is much less relevant when 1,3-DAB is electroplated onto the Pt as the last step. These results indicate that the 1,3-DAB forms a less selective film when Nafion is deposited second, indicating that the deposition of Nafion somehow changes the 1,3-DAB permeability. Coating with Nafion introduces two features which may affect the DAB film, the first is the use of an organic solvent, the second is the acidity of the Nafion solution, which increases as it dries.

To understand the degradation of DAB by Nafion deposition we treated a 1,3-DAB film with isopropanol:H_2O and found no change in the permeability to H_2O_2 and acetominophen. When we treated the film with methanesulphonic acid, however, there was a significant increase in permeability, as illustrated in Table 2.4. Chemically treating the films with acid results in an increased permeability to both H_2O_2 and acetaminophen, depending on the concentration of acid. Using the ratios of i_{H2O2}/i_{aceto} as an indicator of selectivity, it can be seen that the film becomes less selective with increasing permeability.

These above results indicate that the film should also show an increased sensitivity with increasing permeability. This, however, contrasts with the results obtained in the previous section when coating the

DAB film last, since the sensors show a sensitivity increase as selectivity increases. Resolution of this issue probably requires detailed knowledge of the electrochemically-formed film structure. For instance, the increased selectivity may be a result of the film excluding acetaminophen because of size or structure (aromaticity), while H_2O_2 is allowed to pass through.

Table 2.4 Permeability of treated 1,3-DAB films.

Film of 1,3-DAB	I(H2O2)(mA)	I(Aceto)(mA)	I(H2O2)/I(Aceto)
Untreated	2.06	0.222	9.28(n=6)
Acid treated(0.045 M)	3.08	0.516	5.97(n-6)
Acid treated(0.136 M)	3.20	1.04	3.08(n=9)

2.3.4 Blood and Dog Tests

Based on the above results, the sensors for *in vivo* studies were fabricated with 1,3-DAB coated during the last step. The sensors were found to work well in dog blood and human blood, giving good linear responses to standard additions of glucose. Figures 2.10 and 2.11 show traces of current output of the sensor and its calibration curve in dog blood and human blood, respectively. The sensors also gave stable responses after a 2-hour period in dog blood and human blood. The signal changed after this period because the concentration of glucose in the blood began to change. Figures 2.12 and 2.13 show the trace of the sensor output current for 24 hours in dog blood and human blood, respectively.

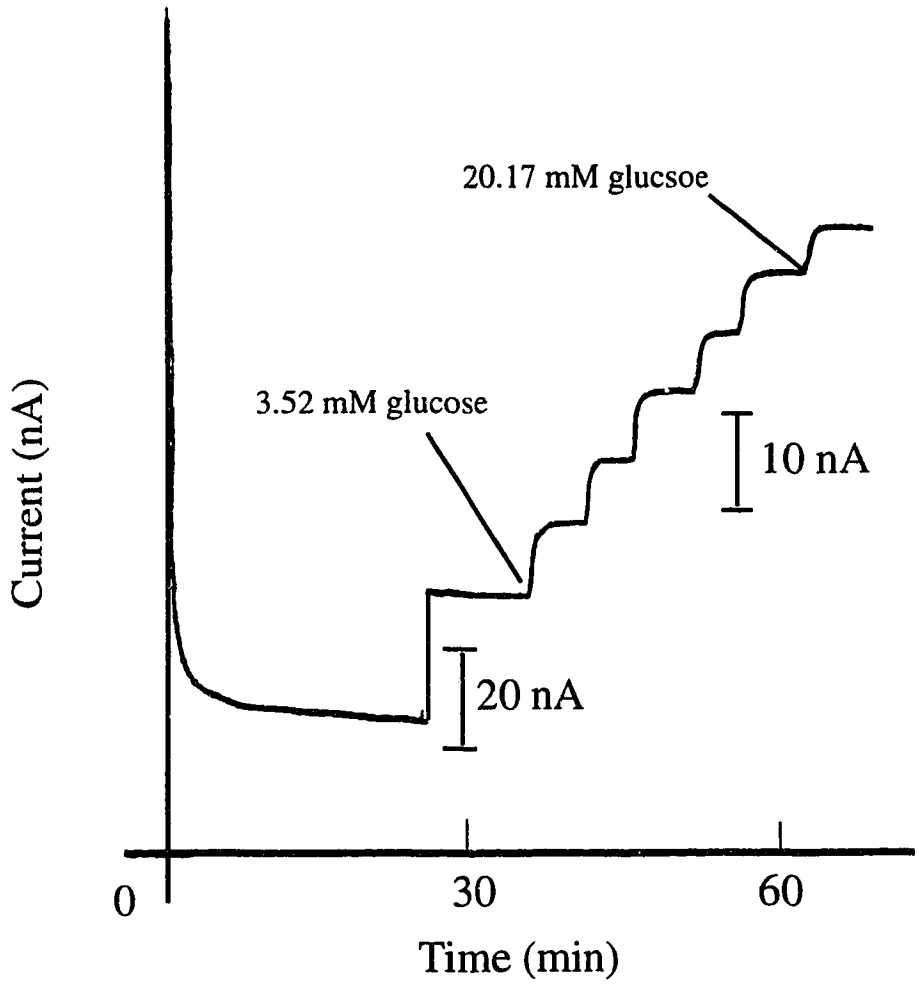

Figure 2.10a Time record of sensor output current response to changes in glucose concentration in dog blood.

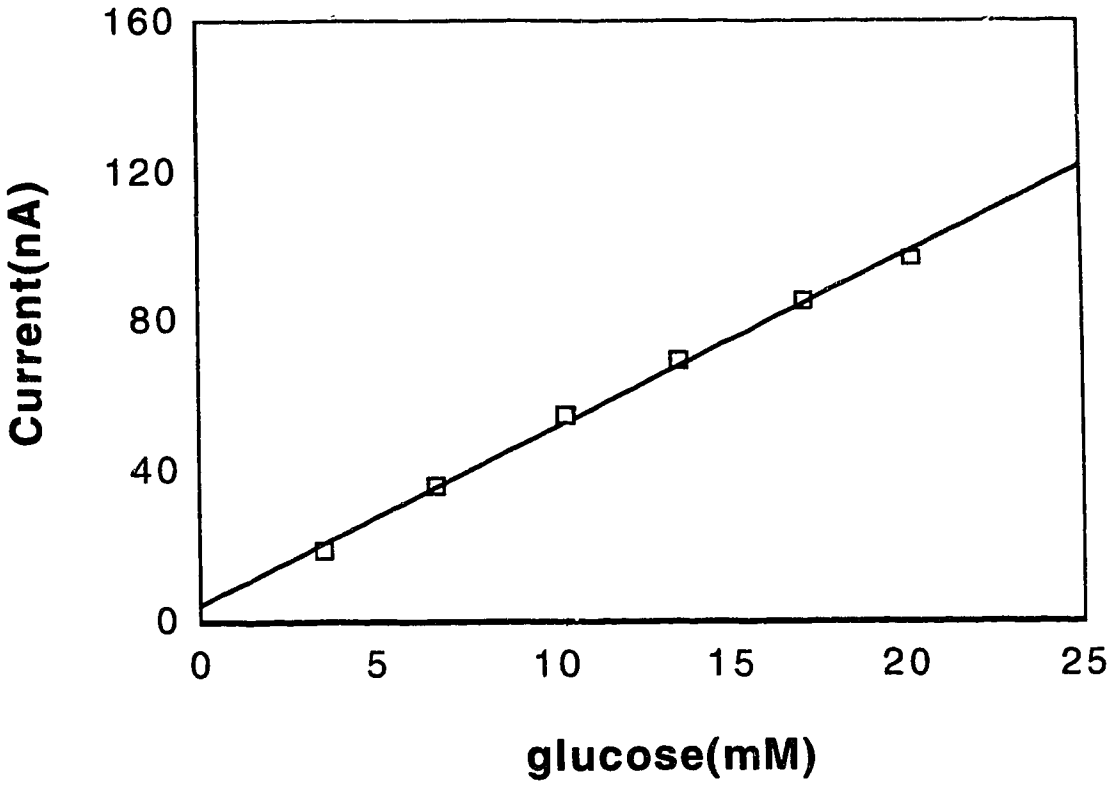

Figure 2.10b Calibration curve in dog blood of the sensor shown in Figure 2.10a.

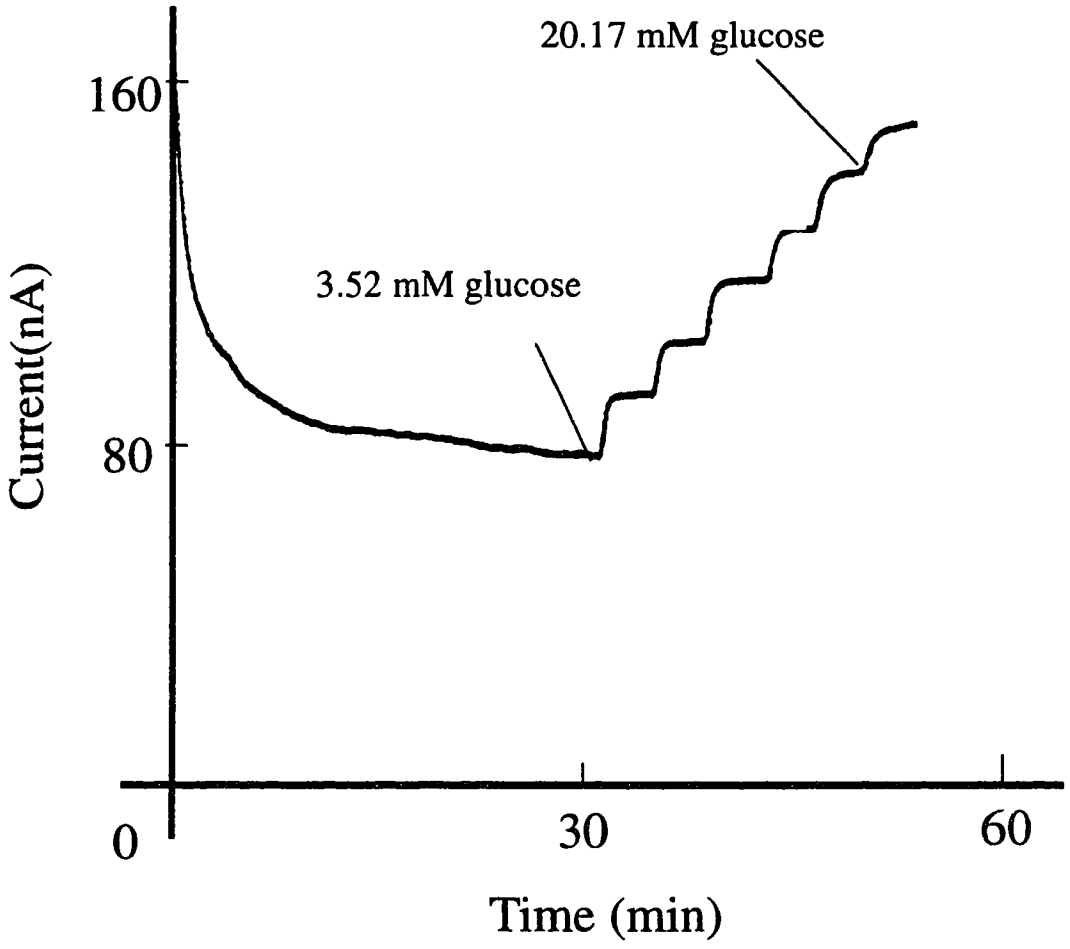

Figure 2.11a Time record of sensor output current response to changes in glucose concentration in human blood.

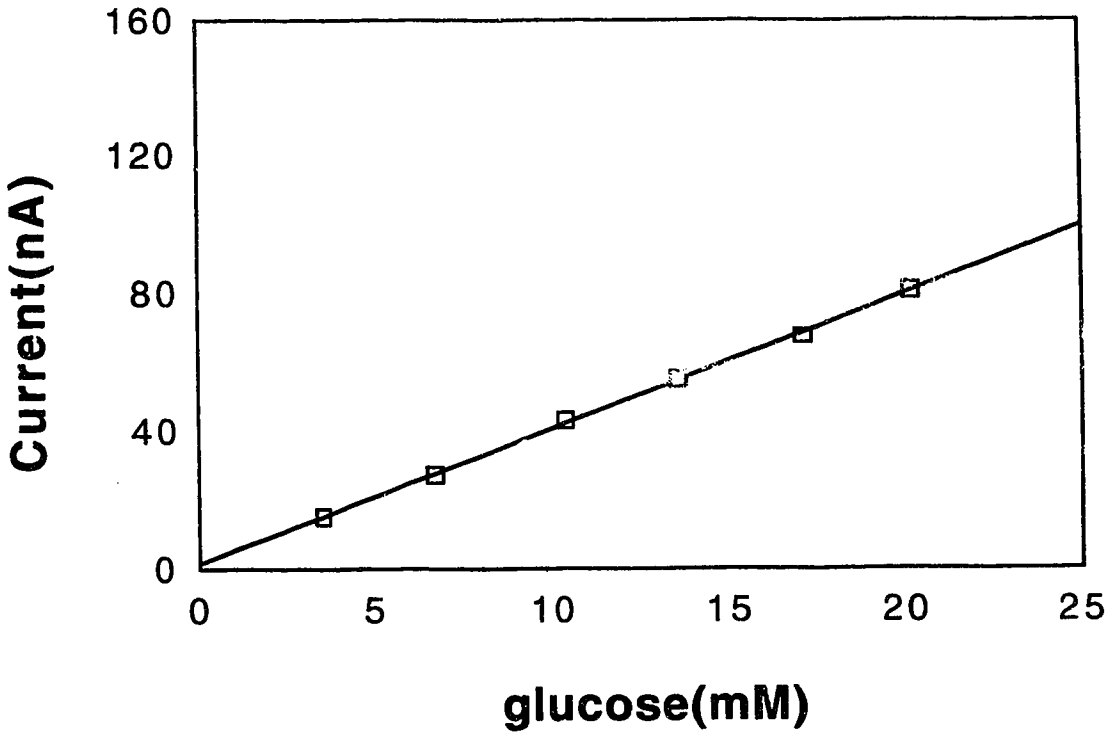

Figure 2.11b Calibration curve in human blood of the sensor shown in Figure 2.11a.

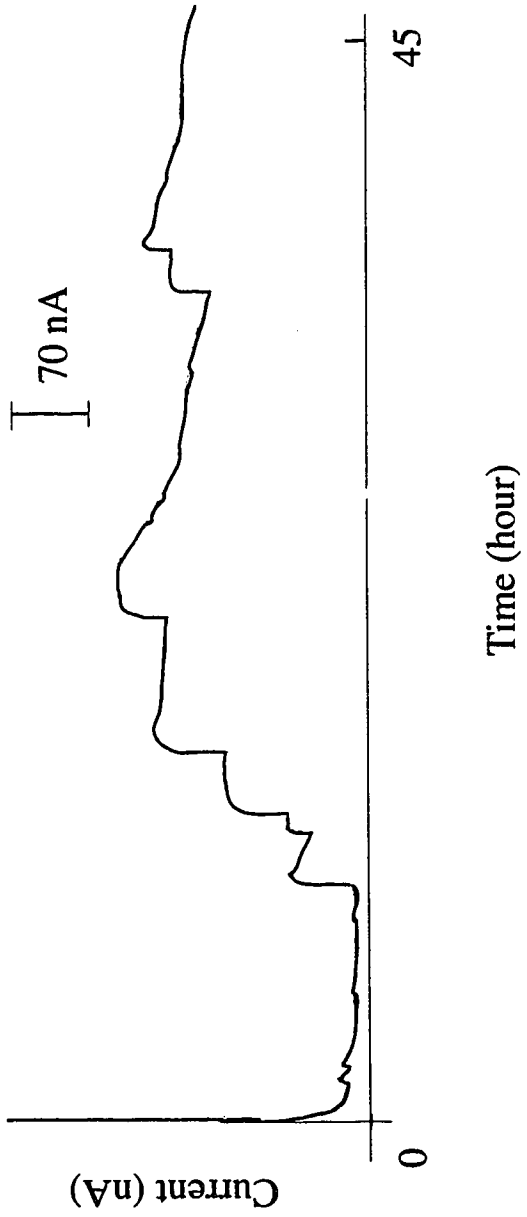

Figure 2.12 Time record of sensor output in dog blood versus additions of glucose. Test was performed at room temperature with the solution being stirred.

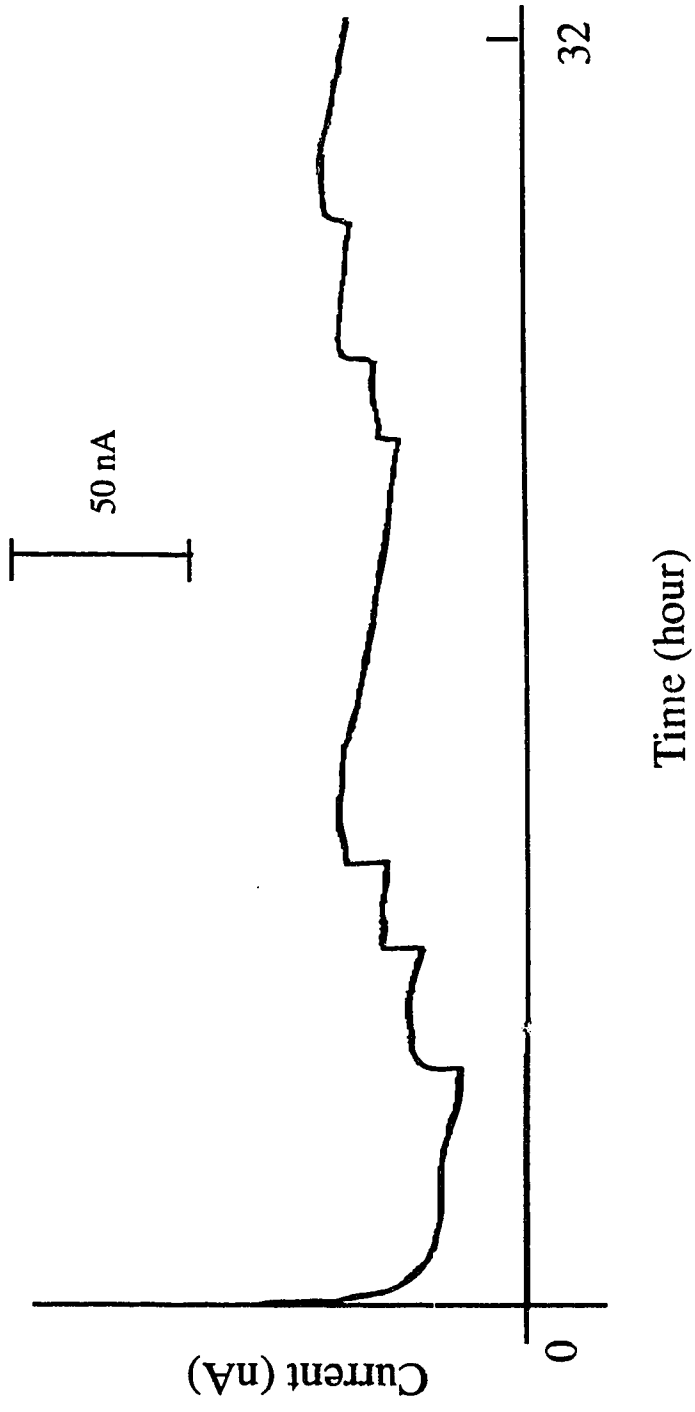

Figure 2.13 Time record of sensor output in human blood versus additions of glucose. Test was performed at room temperature with the solution being stirred.

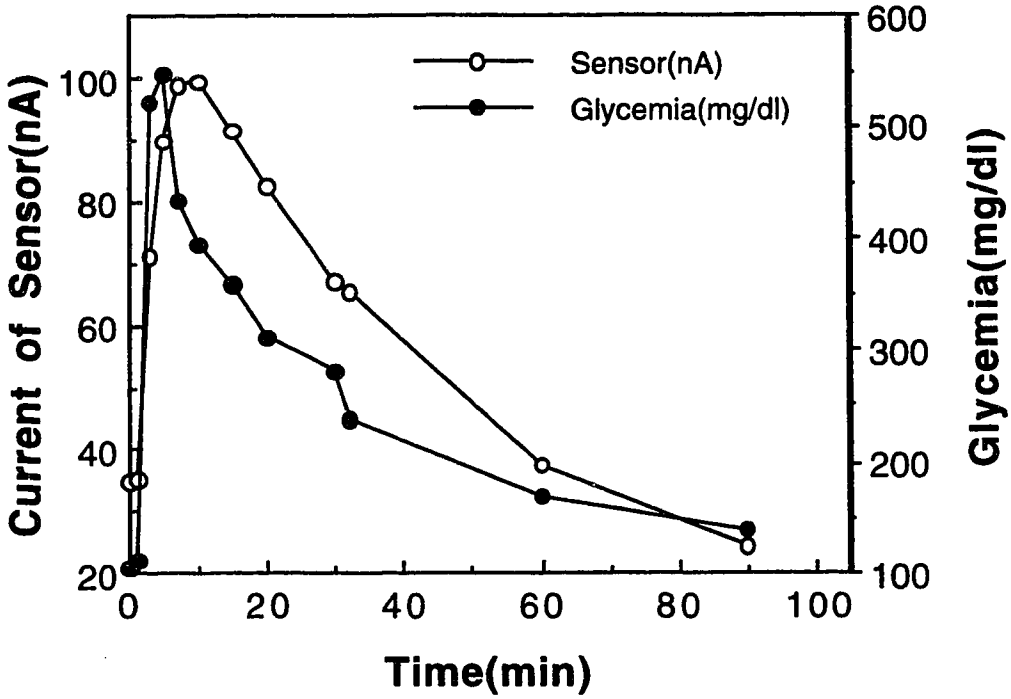

Figure 2.14 Chronic *in vivo* test in a dog. Sensor output vs glycemia in an active, non-diabetic dog after a rapid glucose injection once a steady-state current was obtained with a 0.7 V potential applied. Sensor was coated with GOx/Nafion/1,3-DAB and heat cured at 120°C for 120 min.

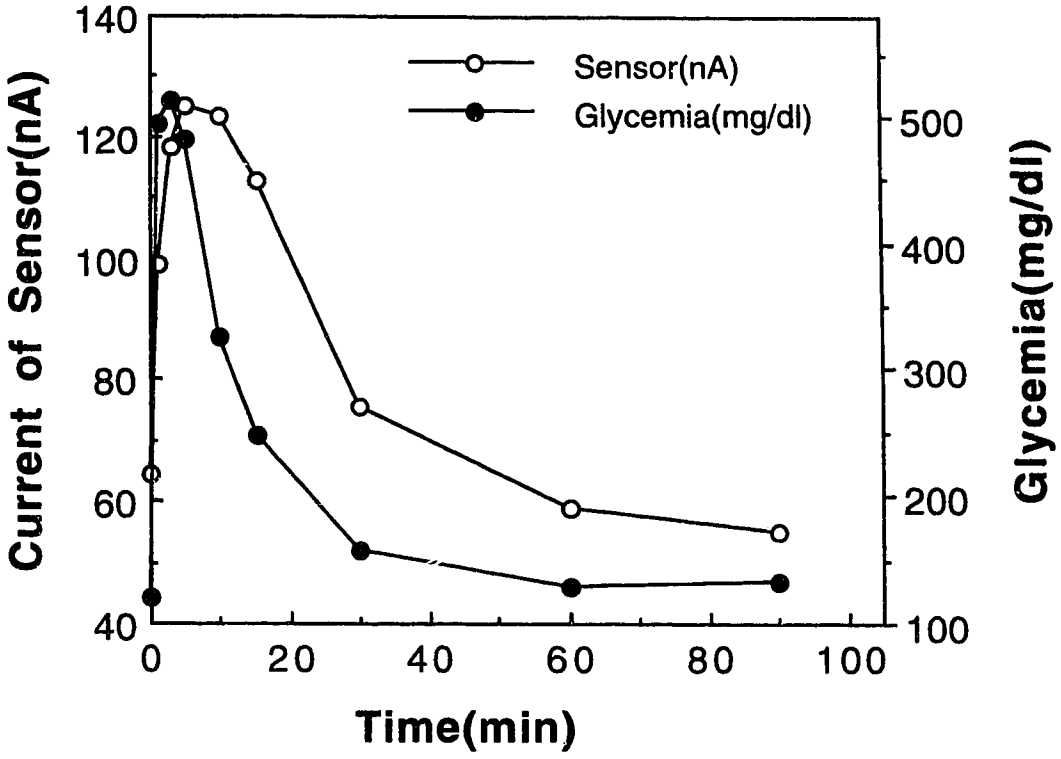

Figure 2.15 Chronic *in vivo* test in a dog. Sensor output vs glycemia in an active, non-diabetic dog after a rapid glucose injection once a steady-state current was obtained with a 0.7 V potential applied. Sensor was coated with 1,2-DAB/GOx/Nafion, then heat cured at 120°C for 60 min.

When the sensor was implanted in a dog, about 40 to 60 min was required for the current to stabilize (defined as less than 0.5% change per minute) after a 0.7 V potential was applied. After stabilization, a bolus intravenous injection of glucose was made and sensor output was monitored. Blood was sampled from an in-dwelling catheter to determine venous glucose levels. Figures 2.14 and 2.15 show the typical sensor response to the injection of a glucose bolus with the accompanying glycemia measurement. The sensor signal matched the glycemia of the dog very well. The 5-10 min delay between the maximum in blood glucose and the sensor signal corresponds to the known lag time between blood and subcutaneous glucose levels [8].

2.4 Conclusions

Sensitivity and selectivity are important characteristics of a biosensor. By electrodepositing a more highly selective polymer film (1,3 DAB), and using the proper coating order, we obtain a highly sensitive and selective sensor compared to the use of 1,2-DAB. The sensor worked and was stable in blood and dogs. Further examination of the sensor must be performed in human studies.

2.5 References

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CHAPTER 3

HUMAN TRIALS OF AN IMPLANTABLE GLUCOSE SENSOR

3.1 Introduction

Some investigators are confident that intravenous glucose sensors are already a reality [1], although their effectiveness for short term monitoring of glucose remains to be confirmed through widescale application. Some obstacles encountered in the application of an intravenous glucose sensor include the uneasiness doctors may feel with a new intravenous device and the potential risk of infection. Because of these issues, the development of a reliable subcutaneous sensor, both for short- and long-term monitoring of glucose in diabetic persons is being investigated.

The needle-type subcutaneous glucose sensor based on a tri-membrane configuration employing Nafion, poly(1,3-Diamino-benzene) and immobilized glucose oxidase had been tested successfully *in vitro* and *in vivo* at subcutaneous sites in dogs prior to the modifications discussed in the previous chapter [2-3]. The sensor was proven to have very good selectivity and accuracy when it was tested on dogs during an intravenous glucose tolerant test (IVGTT) over the course of ten day implant studies. Short-term evaluation of the sensor in humans is the objective of the present study, to demonstrate that the results of the animal studies can be reproduced in humans and that the sensor performance is suitable for

short-term subcutaneous, and potentially long-term, monitoring of glucose.

3.2 Materials and Methods

3.2.1 Volunteers' Characteristics

A study was performed on ten Type-2 diabetic volunteers (six male and four female) who were not on insulin; and five non-diabetic (one male and four female) volunteers. One non-diabetic female volunteer participated twice. Patients' characteristics are shown in Table 3.2. The mean age of the 15 subjects was 44.47±8.36 (range 30-56 years, n=15), and the mean body mass index (BMI), which is defined as Body weight (kg) over the square of height (m), was 30.62±8.05 (range 22.1-48.0 kg/m^2, n=15).

HbA1c value, which is a good indicator of glycemia control [4], was checked for persons with diabetes or who exhibit a diabetic OGTT curve. The criteria for determing the glucose tolerance of the individual volunteers was the diagnostic standard for Diabetes Mellitus/Impaired Glucose Tolerance/Normal Glucose Tolerance issued by the National Diabetes Data Group (NDDG). These criteria are given in Table 3.1 and well used to assign the status indicated in Table 3.2.

The volunteers were given instructions to fast overnight, and avoid any kind of medication.

Table 3.1 Criteria for the diagnosis of diabetes mellitus, normal glucose tolerance and impaired glucose tolerance in nonpregnant adults[a].

	Venous Plasma Glucose mM (mg/dl)		
	Diagnosis of Diabetes Melitus	**Diagnosis of Normal Glucose Tolerance**	**Diagnosis of Impaired Glucose Tolerance**
Fasting	>7.8 (140)	<6.4 (115)	<7.8 (140)
	or		and
OGTT (½, 1, or 1½h)	and one other value >11.1 (200)	<11.1 (200)	>11.1 (200)
			and
OGTT (2h)	>11.1 (200)	<7.8 (140)	7.8-11.1 (140-199)
	or		
Random	'gross and unequivocal elevation' of glucose levels with classical symptoms of uncontrolled diabetes		

a: From the NDDG=National Diabetes Data Group (NDDG): "Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* **1979**, 28, 1039

Table 3.2.Volunteers' characteristics and OGTT results

							Glucose level (mM)											
#	sex	age	Weight kg	Height m	BMI kg/m^2	Status /HbA1c mmol/l	base-line	min 5	10	15	30	45	60	90	120	Pattern of OGTT	sensor's response	sensors' batch
1	F	51	94.0	1.72	31.7	D/0.101	12.1	13.2	14.4	14.8	17.1	n/a	19.6	21.8	22.0	D	nil	1
2	F	37	126.0	1.62	48.0	N	4.7	4.1	4.4	5.1	6.5	n/a	7.2	6.9	6.0	N	nil	1
3	F	41	58.2	1.52	25.1	N	4.1	4.5	5.0	5.9	8.6	n/a	12.5	13.0	8.9	IGT	nil	1
4	M	56	69.7	1.73	23.2	N/0.048	5.1	5.8	10.3	14.6	17.4	n/a	13.5	9.1	6.8	D**	√	2
5	F	41	88.5	1.69	30.9	D/0.059	6.1	6.2	n/a	6.4	9.6	12.4	13.6	15.0	15.8	D	√	2
6	F	45	59.3	1.61	22.8	N	4.3	4.5	n/a	4.9	6.2	6.8	10.5	8.8	7.4	N	√	2
7	M	33	90.4	1.83	26.9	N	4.6	4.8	n/a	6.0	7.7	7.8	7.2	7.5	7.1	N	√	3
8	M	36	134.0	1.69	46.9	D/0.099	11.1	10.4	n/a	11.5	14.0	17.4	17.5	21.8	15.3	D	√	3
9	M	54	61.1	1.66	22.1	D/0.083	9.6	9.5	n/a	10.5	12.8	15.6	16.8	19.3	hem	D	√	3
10	F	30	87.9	1.67	31.5	N	4.6	4.5	n/a	5.9	6.7	7.9	7.9	5.9	6.0	N	nil	4
11 *	F	30	87.9	1.67	31.5	N	4.9	4.5	n/a	6.4	9.5	hem	8.8	7.3	6.7	N	√	5
12	F	42	85.3	1.58	34.1	D/0.059	5.6	5.8	n/a	6.3	8.3	10.2	11.7	12.3	10.6	IGT	√	5
13	M	51	93.5	1.84	27.6	D/0.069	5.4	6.2	n/a	7.4	10.0	12.1	13.4	14.7	14.6	D	√	5
14	M	43	105.6	1.71	36.1	D/0.073	12.7	11.5	n/a	12.4	15.3	17.2	19.7	21.3	21.1	D	√	5
15	M	55	83.6	1.49	26.1	D/0.081	8.8	9.4	n/a	10.4	13.5	14.6	16.1	17.6	17.5	D	nil	5
16	F	52	62.0	1.58	24.8	D/0.084	10.3	10.6	n/a	14.3	18.5	21.8	22.8	24.4	20.6	D	√	5

D=Diabetic, N=Normal, IGT =Impaired Glucose Tolerance; hem=sample hemolyzed; * Same as #10; **Gastrectomy

3.2.2 Sensor Fabrication

A varnished copper wire with 0.2 mm diameter was used as the supporting element of the needle-type sensor (see figure). Electrical contact with a platinum wire (0.1 mm diameter) (Puratronic, Johnson Matthey) was made at one end by removal of 1 mm of varnish. The platinum wire was coiled 10 times around the insulated copper wire. One mm from the coiled platinum wire, a silver wire (0.1 mm diameter) (Puratronic, Johnson Matthey) was coiled 15 times and was connected to another varnished copper wire (0.15 mm diameter). The sensor was washed with 0.1 M HCl then D.D.water in an ultrasonic bath. The exposed copper wire connected with platinum was then insulated using first green then red GLPT insulating varnish (GC electronics, Rockford, Illinois). The exposed copper wire connected with silver then was insulated with Red GLPT insulating varnish only. The sensor was dried in air overnight. It was small enough to fit through a 20 gauge catheter.

The coiled platinum wire was anodized at +1.8 or +1.9 V *vs* SCE for 5 min to have a fresh surface and cycled between -0.26 and +1.1 V vs. SCE for 5 min in 0.5 M H_2SO_4. Silver chloride was then formed on the silver wire by anodizing galvanostatically at +0.4 V vs. SCE for 30 min in stirred 0.1 M HCl.

About 1 mL of acetate-buffered (pH 5.5) solution of glucose oxidase, bovine serum albumin (BSA) and glutaraldehyde was deposited onto the platinum electrode by passing the electrode part of the sensor through the drop formed in a V-shape silver wire previously dipped in the

enzyme solution. The enzyme solution was made by first making 1 mL glutaraldehyde acetate buffer solution (5 mg/mL glutaraldehyde), then dissolving 73.2 mg BSA into this 1 mL solution. Finally, 3.9 mg of glucose oxidase was added to 200 ml aliquots of the above solution.

The sensor was dried in air for 1h at room temperature. Then the entire sensor (Pt and Ag/AgCl) was dip coated with Nafion with one layer of each 0.5 wt% and 3 wt% and 4 layers of 5 wt%. Later, 1,3-DAB was electropolymerized onto the Pt electrode in an acetate buffered solution (pH 5.5) from 5 mM 1,3-DAB at +0.65 V SCE. The solution was purged and then blanketed with nitrogen. After being carefully rinsed and dried, the sensor was sterilized by being heated in an oven at 120°C for 120 min.

3.2.3 *In vitro* Evaluation

The sensor was evaluated *in vitro* by being placed in PBS solution, dog blood and human blood with an applied potential of 0.7 V between the working electrode (Pt) and the reference electrode (Ag/AgCl). After a steady state current was obtained, aliquots of glucose were added, and the current response with concentration of glucose was recorded. (see chapter 2)

3.2.4 Sensor Implantation

An 18-gauge, 2 inch long IV catheter (Baxter healthcare Corp., Deerfield, IL) was inserted subcutaneously in the lower lateral abdominal wall through the disinfected skin. No local anesthesia was used. The inner needle was then removed and the sterile glucose sensor was inserted

through the cannula. Light pressure was applied by the fingertips of the investigator on the skin over the top of the cannula in order to keep the sensor in place, and the cannula was then withdrawn.

No sensor was re-used in humans after it had been removed from the body [6].

The sensor was secured with tape and connected to a 0.7 V DC voltage source (BAS CV 27 or a LC-3D potentiostat, CSA approved), which was applied continuously. A strip chart recorder (Johns Scientific Inc., Toronto), which was connected to the potentiostat, was used to record the sensor's signal.

3.2.5 OGTT

As soon as the sensor recorded a constant baseline, an IV line was inserted into the cephalic vein on the arm of the overnight fasted human volunteer, and normal saline was continuously infused to keep the vein patent. Blood was withdrawn from the IV site at set times throughout the experiment for comparison of plasma glucose levels with sensor response. A sample to determine baseline venous whole blood glucose was obtained initially. An oral glucose tolerance test using 75 grams of glucose (Trutol 75g, Custom Laboratories, Inc., Baltimore, MA) was then performed. Blood samples were taken five, fifteen, thirty, forty-five, sixty, ninety and one-hundred-and-twenty minutes after the volunteer started drinking the glucose solution. The blood samples were sent to the hospital laboratory to determine glucose levels during the OGTT, using a Hitachi glucose

analyzer. The criteria of NDDG was used in order to describe a curve as diabetic, non-diabetic, or as impaired glucose tolerance (Table 3.1).

3.3 Results

Although statistical averages are typically used to determine how closely sensor responses correlate with blood glucose concentrations [6-10], because this type of sensor is being tested in humans for the first time, we preferred to present all the available data. This complete set of data will allow for interpretation of the signals of individual sensors.

3.3.1 OGTT

Of the ten diabetic volunteers, nine produced a diabetic OGTT curve and one had an impaired glucose tolerance (IGT) pattern. Of the five non-diabetic volunteers, one had an IGT pattern without any known risk factors for diabetes mellitus, and one had a diabetic-like curve. The latter person had undergone gastrectomy three years before the experiment. He had not been previously aware of any diabetic symptoms and his HbA1c value was normal.

3.3.2 Sensor Responses

A total of 16 sensors were tested on humans, 11 of the 16 sensors showed a response to the 75g Glucose OGTT. For the 5 sensors that failed to respond, the baseline current remained constant throughout the entire test period. The first three of these failed sensors did not respond in humans, but were found to work well when they were subsequently

tested in PBS solution, dog blood and human blood. The fourth failed sensor (utilized in the tenth trial on humans) was from a newly fabricated batch of sensors which was subsequently shown to have a low sensitivity to glucose *in vitro*. This low sensitivity was probably due to poorly coated with glucose oxidase, where the enzyme solution may have gelled prior to coating the sensor. The fifth failed sensor, in the fifteenth trial, was due to incorrect connection of the electrodes where the leads were connected backwards. Thus, while no obvious reason was found for failure of the first three sensors, each subsequent problem was readily explained.

Figures 3.1a-c show sensor responses and plasma glucose levels during the course of the OGTT in individual trials. To match the sensor output to the glycemia scale, the sensitivity of the sensor was calibrated by the blood glucose levels. We plotted the current from the sensor verses the glucose concentration in the blood and obtained a linear relationship (Table 3.3), in order to calibrate the *in vivo* sensor response.

It is apparent from the data that the majority of the sensors showed the expected response after an OGTT. In addition, these sensors gave glucose concentrations that closely matched the measured blood glucose levels, indicating that the sensors could be used successfully in short-term subcutaneous glucose monitoring.

After each test in humans, the sensor was tested again in PBS solution. All successful *in vivo* sensors worked well, with linear responses to additions of glucose up to 20 mM. Table 3.3 shows the summary of sensor responses. The statistics, R^2 values and the linearities of the in vitro calibration curves, are satisfactory. Note that the sensitivities *in vitro*

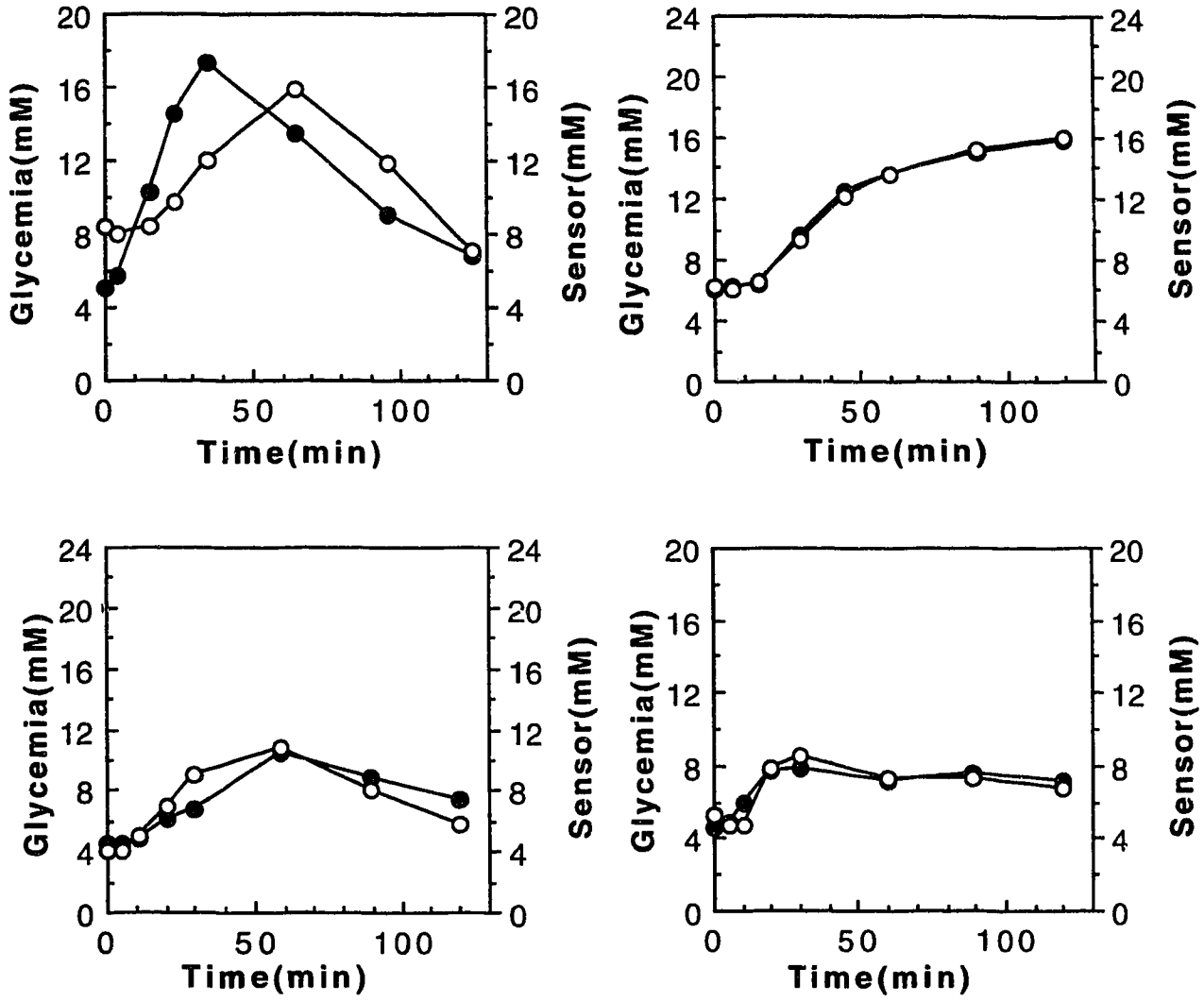
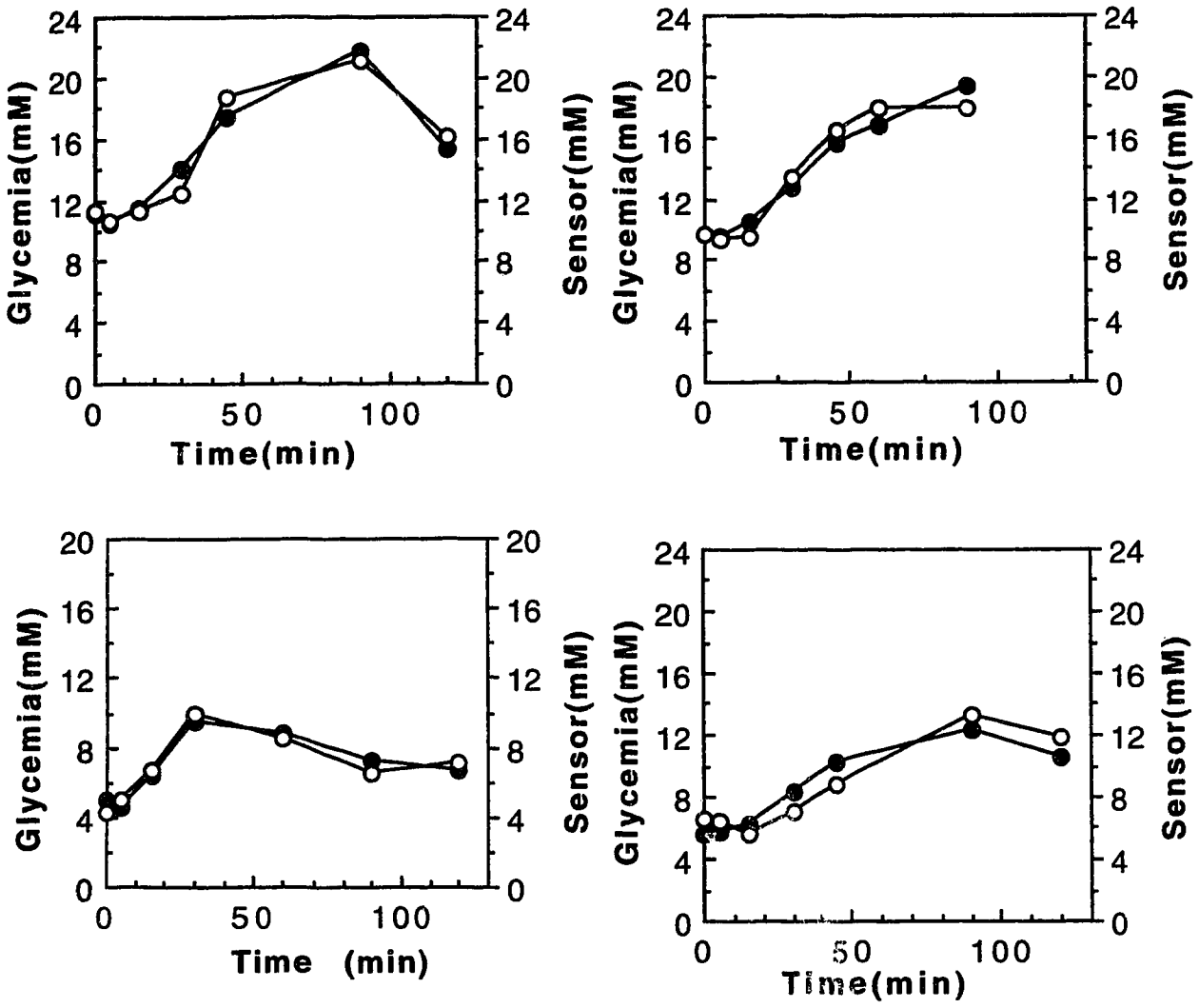
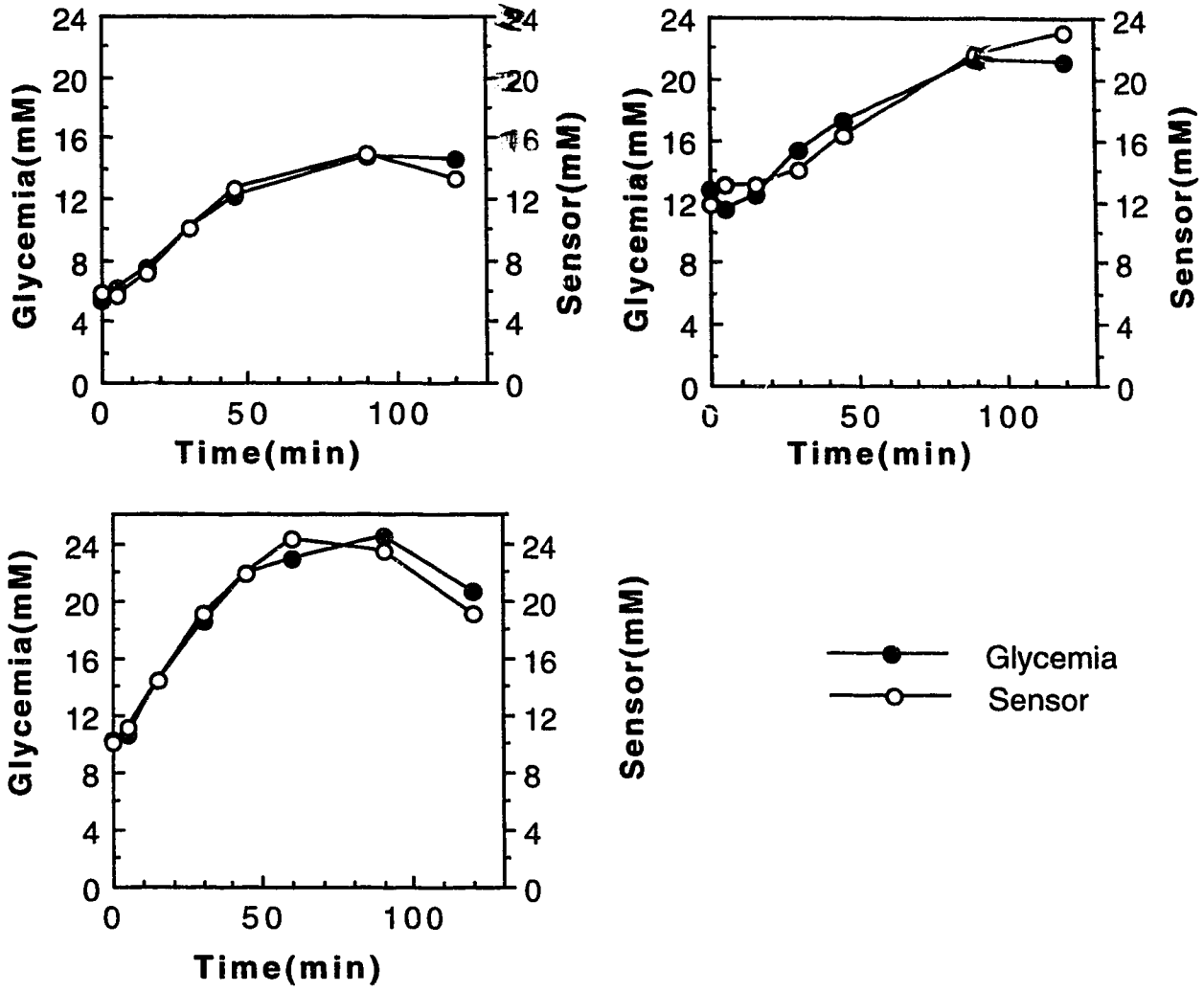


Figure 3.1a Variation in time of glycemia induced in humans by OGTT correlated with the changes in current for subcutaneously implanted glucose sensors (cured 2 hours at 120°C). The glucose solution was drunk at time zero. Volunteers were fasted overnight. Glycemia was measured in blood taken through an in-dwelling venous catheter. The numbers in the box identify the patient trials described in Table 3.2. The solid circles stand for the glucose levels from blood and the open ones for glucose levels measured by the sensor.



8	9
11	12

Figure 3.1b Variation in time of glycemia induced in humans by OGTT correlated with the changes in current for subcutaneously implanted glucose sensors (cured 2 hours at 120°C). The glucose solution was drunk at time zero. Volunteers were fasted overnight. Glycemia was measured in blood taken through an in-dwelling venous catheter. The numbers in the box identify the patient trials described in Table 3.2. The solid circles stand for the glucose levels from blood and the open ones for glucose levels measured by the sensor.



13	14
16	

Figure 3.1c Variation in time of glycemia induced in humans by OGTT correlated with the changes in current for subcutaneously implanted glucose sensors (cured 2 hours at 120°C). The glucose solution was drunk at time zero. Volunteers were fasted overnight. Glycemia was measured in blood taken through an in-dwelling venous catheter. The numbers in the box identify the patient trials described in Table 3.2. The solid circles stand for the glucose levels from blood and the open ones for glucose levels measured by the sensor.

Table 3.3. The sensor responses.

sensor		*in vivo*			*in vitro* (after *in vivo*)			Ratio (*in vivo*/ *in vitro*)	
#	series No.	sensitivity (nA/mM)	intercept (nA)	R^2	sensitivity(nA/mM)	intercept (nA)	R^2	sensitivity	intercept
4	508262	1.024	28.746	0.824	5.828	-2.989	0.996	5.693	-0.104
5	508263	3.162	21.640	0.997	7.655	-2.280	0.998	2.421	-0.105
6	508264	2.759	14.990	0.786	2.574	0.657	0.995	0.933	0.044
7	509201	6.506	46.405	0.818	14.416	1.673	1.000	2.216	0.036
8	509202	6.447	139.67	0.950	23.793	16.837	0.995	3.691	0.121
9	509204	10.559	66.953	0.945	15.468	-0.371	0.988	1.465	-0.006
11	510111	6.546	11.166	0.925	11.762	10.875	0.998	1.797	0.974
12	510112	4.102	46.404	0.860	8.993	4.179	0.999	2.192	0.090
13	510113	10.828	49.492	0.965	13.303	6.455	0.999	1.229	0.130
14	510114	8.224	9.857	0.908	10.004	2.282	1.000	1.216	0.231
16	510116	4.104	134.6	0.973	1.169	0.111	1.000	0.285	0.001

(after testing *in vivo* in humans) are almost always higher than those determined *in vivo*. The average ratio of $\frac{invivo}{invitro}$ sensitivities of 11 values is 2.10. These results indicate that the sensors have different sensitivities *in vivo* and *in vitro*. This is consistent with previous tests of these sensors in dogs and in blood samples, for which a reduction of sensitivity has been seen.

One of the requirements for an implantable glucose sensor is that the current must be a function of glucose concentration and behave in a predictable manner. Thus the sensor must be relatively insensitive to external factors such a movement. Two sets of raw data are given in Figures 3.2 and 3.3. One of the sensors gives a smooth response and the other gives a response that changes with body movement.

The concern that movement affects sensors has been mentioned in a recent review of the development of implantable glucose sensors [1]. Consider for example the current trace shown in Figure 3.3. The many "spikes" seen in the data are a result of patient movement. In many cases these correspond to temporary noise, and the signal returns to its basal level. However, in some cases, such as at the end of the trace in Figure 3.3, movement results in both spikes and a shift in the basal level. Clearly the latter effect is a much more significant problem. Movement may affect the sensor by either influencing the delivery of glucose to the sensor through the surrounding tissue, changing the sensors position in the subcutaneous tissue, so as to change the rate of glucose transport, or through random generation of noise. The data indicate that absolute

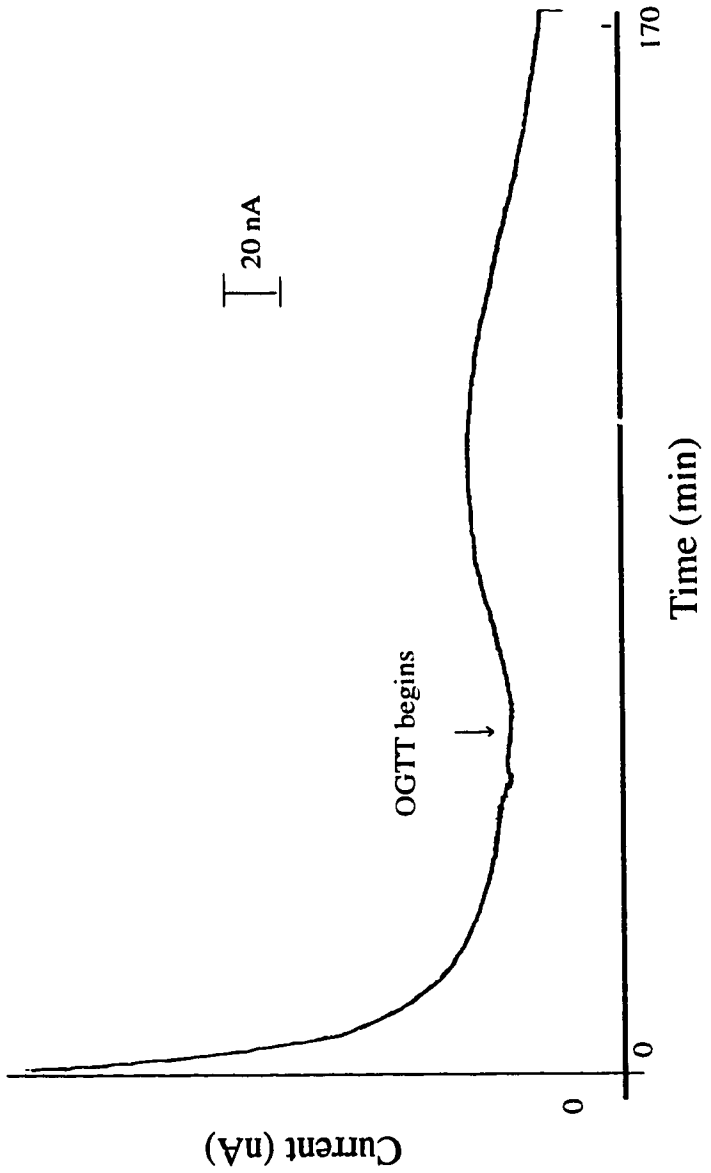


Figure 3.2 Time record of sensor output current response to changes in glucose level in subcutanous site of a non-diabetic human subject. A potential of 0.7 V was applied between the working and reference electrodes

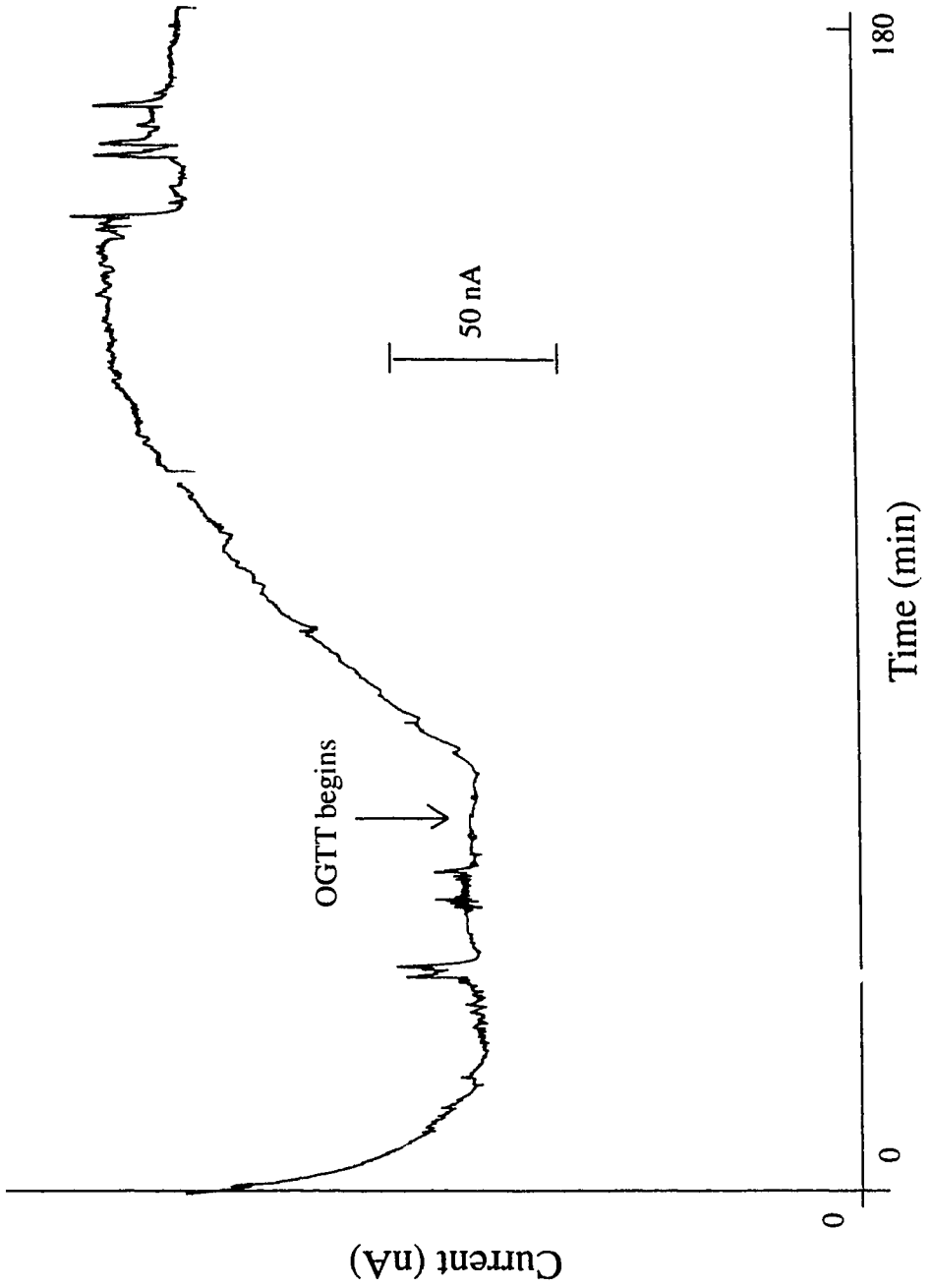


Figure 3.3 Time record of sensor output current response to changes in glucose level in subcutanous site of a diabetic hunman subject. A potential of 0.7 V was applied between the working and reference electrodes.

changes in glucose supply can result from movement. This is most likely caused by permanent shifts in the sensors position in the tissue.

Since these sensors are just freshly placed *in vivo* before testing, the surrounding tissue will not have begun to heal at all. It is possible that after a period of time recuperation at the incision sit would result in the sensor being more tightly fixed in place. Preliminary study of a sensor implanted in a diabetic person for 24 hours showed that sensitivity to motion is significantly decreased. However, the sensitivity to glucose was also decreased by about half. Further testing is required.

3.4 Clinical Accuracy Evaluation

A clinical accuracy evaluation has been done by applying the error grid analysis (EGA) developed by Clarke *et al.* [11]. In Figure 3.4, the x-axis is defined as the reference blood glucose and the y-axis is the value generated by the sensors. Zone A represents glucose values that deviate from the reference by no more than 20% or are in the hypoglycemic range (<70 mg/dl) when the reference is also <70 mg/dl. Values falling in this range would lead to a clinically correct treatment decision. Upper and lower Zone B represents values that deviate from the reference by >20% but would lead to benign or no treatment. Values in Zones A and B are clinically acceptable. Zones C, D and E are considered as clinically inaccurate zones. Values in Zones C, D and E are potentially dangerous and therefore are clinically significant errors [12].

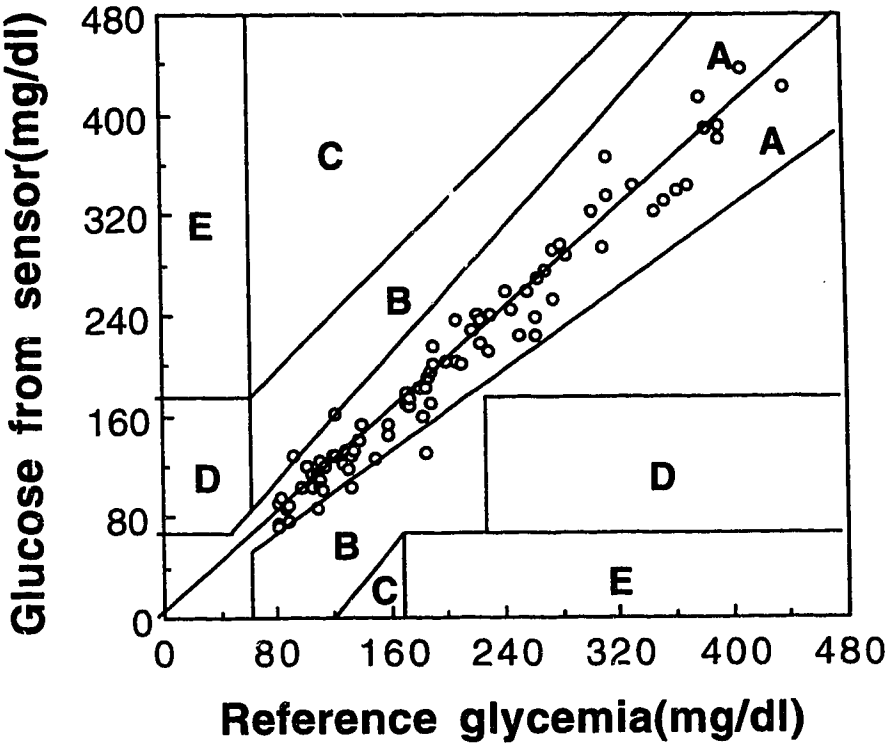


Figure 3.4 Error Grid Analysis of the sensor output. Data from eleven working sensors are included.

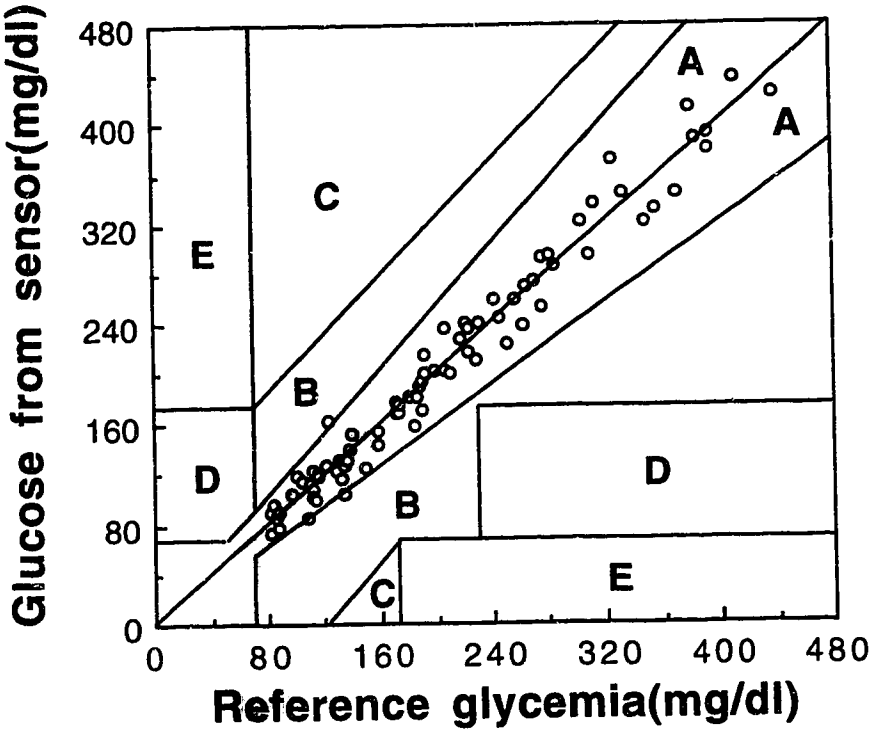
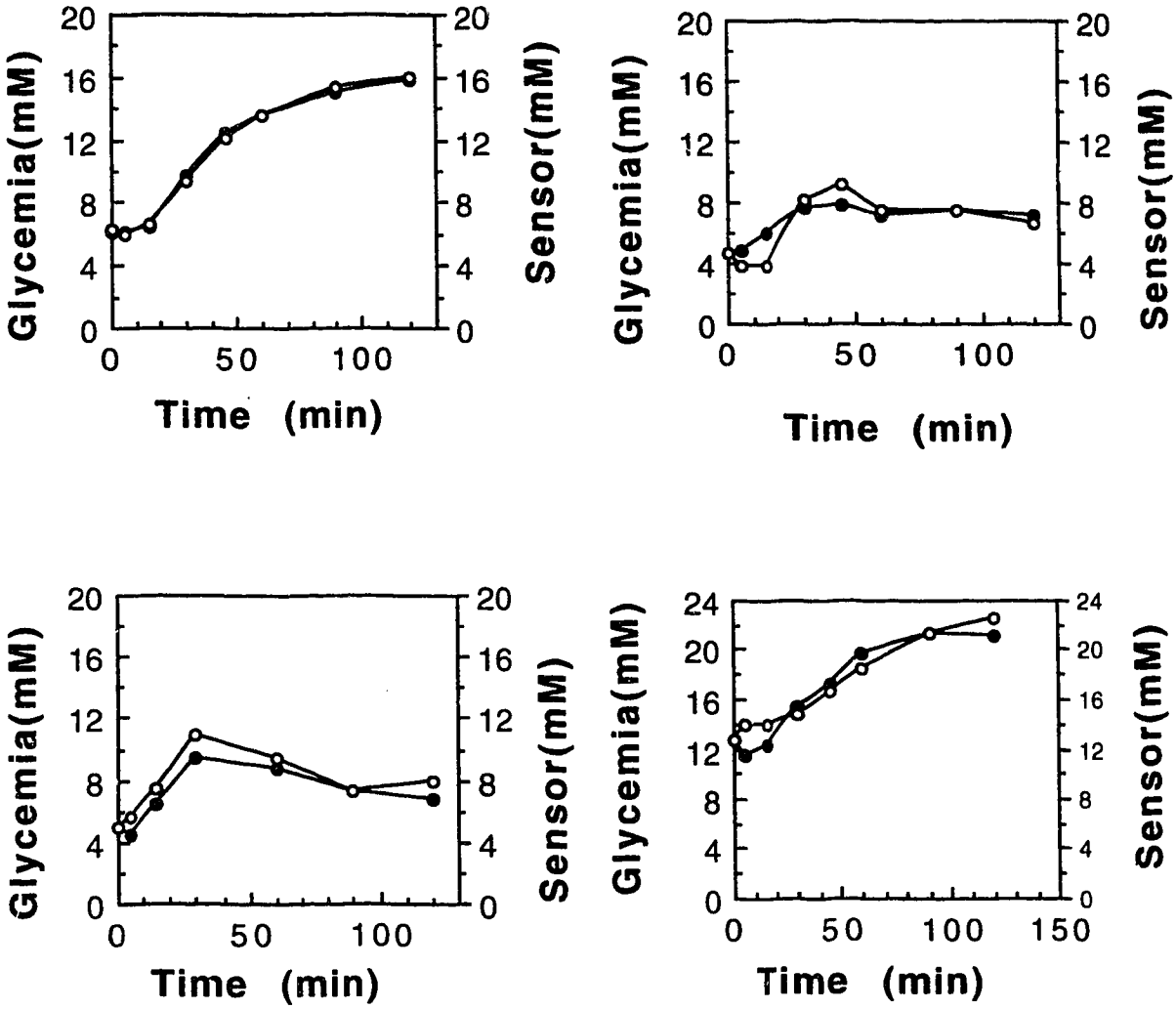


Figure 3.5 Error Grid Analysis of the sensor output. Data from ten working sensors are included. Sensor no.4, which has extra long time lag between the sensor output and the glycemia, is excluded.



5	7
11	14

Figure 3.6 Variation in time of glycemia induced in humans by OGTT correlated with the changes in current for subcutaneously implanted glucose sensors (cured 2 hours at 120°C). The glucose solution was drunk at time zero. Volunteers were fasted overnight. Glycemia was measured in blood taken through an in-dwelling venous catheter. The sensor output current was transformed from two point calibration method. The numbers in the box identify the patient trials described in Table 3.2. The solid circles stand for the glucose levels from blood and the open ones for glucose levels measured by the sensor.

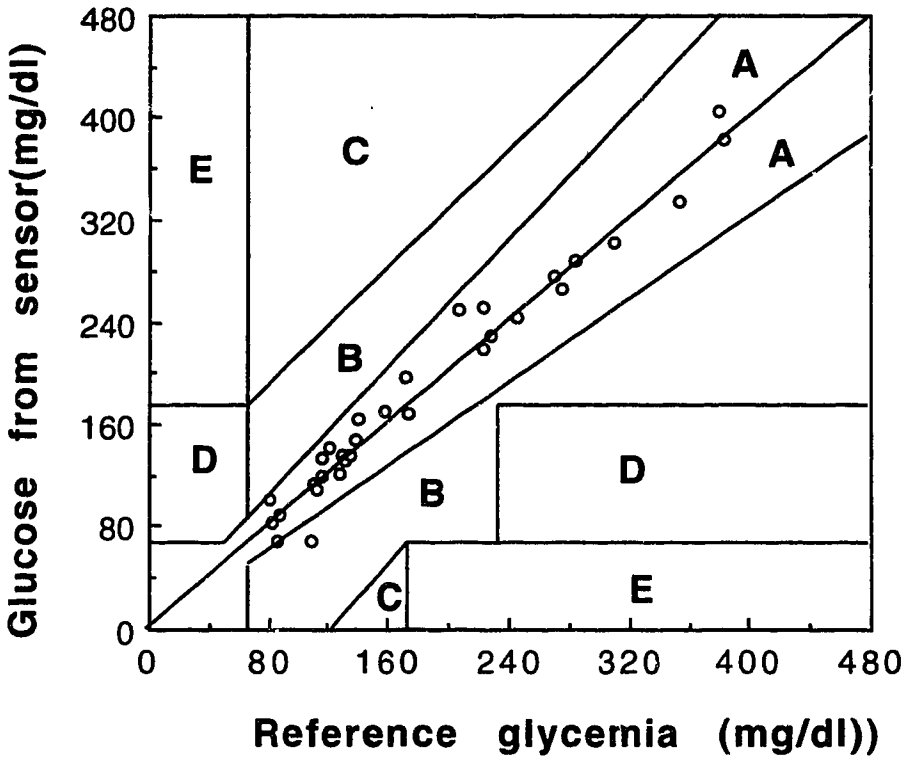


Figure 3.7 Error Grid Analysis of the output of sensors 4,7,11 and 14. The sensor output was first calibrated with Two Point Calibration method with values at time 0 and 90 minutes of the OGTT. Then the glucose levels were calculated from the calibration data.

All of the 83 values taken from the responding sensors in human tests have been put into Figure 3.4 and all of the values fell in Zones A and B with most in Zone A.

We also did this analysis excluding the sensor with the extra long lag time that is shown in Figure 3.1-4. This analysis, shown in Figure 3.5, results in all points except one lying in zone A.

To make it easier to calibrate the sensor during *in vivo* tests, the sensors were calibrated using two-point method [2]. The values at time 0 and 90 minutes were used. Figures 3.6 shows sensor responses and plasma glucose levels during the course of the OGTT for these four sensors. Similarly to the method for Figure 3.4, EGA for these four sensors is shown in Figure 4.7.

3.5 Conclusions

A needle-type subcutaneous glucose sensor based on a tri-membrane configuration employing Nafion, poly(1,3-Diamino-benzene) and immobilized glucose oxidase, which was previously tested successfully *in vitro* and in dogs, has been tested on diabetic and non-diabetic human volunteers in sixteen Oral Glucose Tolerance Tests (OGTT). The pattern of the response of the sensor was consistent with the expected OGTT response in eleven cases of the sixteen; in five cases the sensor did not respond. The majority of the sensors however, responded consistently with blood plasma glucose values acquired during the same period.

Consequently, we have demonstrated that this type of subcutaneously implanted glucose sensor can be applied to human.

The average sensitivity of the set of sensors was 5.84±3.16 nA/mM (n=11) *in vivo* and 10.45±6.39 nA/mM (n=11) *in vitro*. The *in vivo* sensitivity of the sensors was calibrated using the glucose levels measured in blood with a Hitachi Glucose Analyzer. The *in vitro* sensitivity was calibrated in phosphate buffered saline (PBS) immediately after testing in humans.

These results prove that this type of subcutaneously implanted glucose sensor can be applied to humans for short-term glucose evaluations. Currently, these sensors are being tested over longer periods in human volunteers.

3.6 References

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CHAPTER 4

CONCLUSIONS

This chapter presents a summary of the work completed during this course of the program in which the design of an implantable glucose sensor has been improved and the sensor was subsequently tested in human volunteers. Implantable glucose sensors have not yet been realized in practice because no one sensor design combines all the necessary requirements: durability, biocompatibility, stability and selectivity. Past work in this laboratory focused on developing such a sensor and this project continues this goal. Although at the conclusions of this work there are still some unsolved issues, it has been shown that the sensor, with the implemented modifications, shows good performance when used as a subcutaneously implanted glucose sensor in human subjects.

4.1 Summary of the Study on an Implantable Glucose Sensor

An implantable glucose sensor with improved sensitivity and selectivity has been developed as discussed in chapter two. Past work has shown that the Nafion outer coating gives ideal biocompatibility and stability [1], and prevents the degradation of the reference electrode [2]. The study of this work was to modify the sensor design by changing the inner polymer coating and the coating order. These changes improved the selectivity to interferents such as acetaminophen and uric acid as well as the sensitivity to glucose. Many efforts in the literature have focused on eliminating interferences to endogenous electroactive species such as acetaminophen on various different glucose sensor designs [3-8]. This

work utilized a new inner layer by electropolymerizing 1,3-diaminobenzene(1,3-DAB). The new 1,3-DAB coating resulted in better resistance to interferents, however, both the selectivity and sensitivity were decreased during the subsequent Nafion deposition steps. As a further modification, the 1,3-DAB was deposited as a final step, after enzyme and Nafion. This change resulted in much better selectivity.

The ultimate goal for an implantable glucose sensor is to be used in the human body for continuous glucose monitoring. This development is complicated by the fact that and the sensor may not respond the same *in vivo* and *in vitro*. Moreover, the biocompatibility, *in vivo* stability and durability must be tested in humans. With the permission of a local health committee, human sensor evaluations were performed. The sensors tested in humans were first sterilized by heat curing in an oven at 120°C for 120 minutes. This procedure has been found to improve the sensor characteristics by decreasing Nafion solubility, increasing the crystallinity of the polymer [9,10], decreasing the permeability to anions and neutral species [11] and protecting the Ag/AgCl reference electrode from degradation. Sixteen human trials were performed in which volunteers excused an oral glucose tolerance test (OGTT) and the sensor response was recorded and compared with measured values of blood glucose over a period of 3 hours. Out of 16 tests, 11 sensors had normal responses (i.e. sensor response tracked the actual blood glycemia) and five failed to respond to the standard OGTT. Two of the sensors failed for reasons known to be due to factors other than the sensors themselves. There is no explanation as yet for the other three failed sensors.

For the 11 working sensors, the average sensitivity was 5.84±3.16 nA/mM when calibrated from glycemia. All sensors worked in phosphate buffer saline (PBS) subsequent tests. These results suggest that the sensors could be used for longer *in vivo* testing.

4.2 Directions for Future Research

Previous experiments showed that the sensors worked well in humans for 3-hour monitoring. However, these data also showed that body movement of the human volunteers had some effect on the sensor output. To date, two long-term human trials have been performed in which the sensors are implanted over a 24-hour period. Preliminary date indicate that the sensor response is fairly stable with long-term implantation and we are investigating the reasons for the movement effect. So that it may be eliminated.

Characteristics of each coating layer is also being performed. Further determination of the 1,3-DAB structure, stability and permeability is underway in an effort to evaluate changes in the polymer with aging. In addition, Similar investigations of the aging effects at the Nafion and enzyme layers would be beneficial.

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