The Development of a Minimally Invasive Glucose Sensing System

by

Lisa Ellen Sambol

B.A., Physics (1997)

B.S., Mechanical Engineering (1997)

Columbia University

Submitted to the Department of Mechanical Engineering
in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Mechanical Engineering

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

February 2000

Author.....
Department of Mechanical Engineering
January 14, 2000

Certified by .....
Lynette Jones
Principal Research Scientist of Mechanical Engineering
Thesis Supervisor

Accepted by .....
Ain A. Sonin
Chairman, Department Committee on Graduate Students

The Development of a Minimally Invasive Glucose Sensing System

by

Lisa Ellen Sambol

Submitted to the Department of Mechanical Engineering
in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Mechanical Engineering

Abstract

The overall goal of this research is to develop a minimally invasive glucose sensing system for diabetic patients. The device will consist of an array of micro-needles that penetrates only the stratum corneum layer of the skin and tests glucose levels in the interstitial fluid (IF). This device does not reach the depth of the nerve endings, and so provides a painless means of glucose measurement. Glucose will be detected amperometrically using a two step chemical process in which glucose is broken down into hydrogen peroxide which is then oxidized to provide a current. By coating the outer surface of the micro-needle array with layers of rhodium, cellulose acetate, and glucose acetate using electrochemical deposition, the needles can be sensitized to the presence of glucose.

Successful work has been completed in which needle surfaces were sensitized to glucose by layering the reaction catalyst (glucose oxidase) and cellulose acetate onto rhodium coated 0.51 mm diameter stainless steel wires. This was achieved through a combination of galvanostatic depositions and dip coatings. These sensors were able to detect various concentrations of glucose within the biological range (0-20 mM). Micro-needles arrays have been fabricated from stainless steel and coated with rhodium in order to sensitize the surface to hydrogen peroxide. The array design increases the probability that the micro-needles will reach the IF rather than become embedded in the surrounding epithelial cells. The array design also increases the sensor surface area, thus yielding higher amperometric response signals. Other benefits of microelectrodes such as faster response times, higher signal to noise ratios, and lower sensitivity to convection are amplified when micro-needles are used in an array format. Future work on this device will be to integrate the glucose sensor with the array design to create a microneedle array that can effectively measure glucose levels in IF.

Thesis Supervisor: Lynette Jones

Title: Principle Research Scientist

Acknowledgements

I would like to thank Dr. Lynette Jones and Professor Ian Hunter for giving me the opportunity and resources to work in the Bioinstrumentation Laboratory. Thanks as well to Dr. Tanya Kanigan who helped guide me through the project and who introduced me to many of the basic chemistry concepts and resources.

Thanks to John Madden, Peter Madden, Luke Sosnowski, and Bryan Crane for their help and patience in teaching me the use of much of the lab equipment as well as walking me through many of the difficult theoretical concepts.

Thanks to Tim Alvey whose creativity and hard work helped the array design progress much faster than I could have done on my own. Thanks to Shirley Mihardja for assisting me in the initial phases of my reintroduction to chemistry. Thanks to Keith Wai-Leung for help with the glucose sensor patent searches.

Thanks to James Tangorra who not only patiently helped me through some of the engineering challenges, but who also helped preserve my sanity throughout the graduate school process.

Thanks to my parents, Lawrence and Shirley Loewenthal, who have continually loved, supported, and encouraged me in everything I have done.

I don't know that I can ever give enough thanks to my husband, Moshe Sambol, who has listened to me every day, who has guided me, supported me, and who has always been on my side. He has never stopped showing how proud he is of me, and if not for his love, I would not have made it this far.

Finally, the financial support of the National Science Foundation Graduate Research Fellowship is greatly appreciated.

Table of Contents

List of Figures

List of Tables

Chapter One: Introduction

The overall goal of this research is to develop a minimally invasive miniature device for diabetic patients that controls the delivery of insulin using feedback from an integrated glucose sensor. This system will incorporate a micro-needle array to measure glucose levels in the interstitial fluid (IF) rather than in the blood, because IF, which lies below the outer layer of the skin, can be sampled relatively painlessly and directly from the dermis. The device must be able to measure glucose levels accurately and dispense the appropriate amount of insulin, using the glucose measurement as feedback in the control loop. In its final form, the device must also be easily miniaturizable and able to be mass-produced.

Although many commercial entities are working towards the development of similar technology, no one as yet has been able to create a closed-loop system that can adequately measure the dynamic human response to glucose levels following the injection of insulin. All devices on the market currently are open-loop systems which either record only current glucose levels or deliver bolus amounts of insulin at pre-programmed times. The most notable recent development in this area came on June 15, 1999 when the U.S. Food and Drug Administration (FDA) approved the application for MiniMed's continuous glucose monitoring system [MiniMed, 1999]. This system is able to monitor continuously glucose for up to three days using an electrode inserted under the patient's skin. However, patients are still required to perform conventional fingerprick blood glucose tests at least four times a day for calibration purposes.

Although the MiniMed sensor is indeed progress towards the goal of a closed-loop monitoring system, it has not yet solved many of the issues involved in glucose measurement, such as the elimination of invasive and painful blood glucose testing, the integration of insulin delivery with glucose sensing, and a characterization of the dynamic human response to glucose and insulin fluctuations. The ultimate goal of the present research is to address all of these issues. The array of glucose-sensitive micro-needles is designed to measure glucose from the IF without contacting nerve fibers and so creates a minimally invasive and painless method of glucose testing. By using system identification techniques, the dynamic human response to insulin injections can be characterized. Knowledge of the diabetic impulse response function will allow application of a predictive model to insulin delivery. The glucose sensor/insulin delivery system will anticipate glucose level increases much as the human pancreas does in non-diabetic patients. System identification techniques will also be applied to determine the sensor response to glucose injections in a diffusive medium. When combined with an integrated pump that delivers glucose proximal to the sensor, this information may be used to calibrate the sensor in situ.

This thesis describes the initial work that has been done on the development of a minimally invasive glucose sensing system. It also discusses current products and research in the field of minimally invasive closed-loop glucose detection and insulin delivery.

Chapter Two: Diabetes Overview

2.1 Diabetes Defined

Diabetes mellitus, often referred to simply as "diabetes," is a disease which affects people of all ages and backgrounds. It may cause severe disability and even death to those who do not receive adequate treatment. It is estimated that 15.7 million people in the United States today are diabetic with approximately 800,000 new cases diagnosed each year [American Diabetes Association (ADA), 1999a]. It is also estimated that 5.4 million of these people are unaware that they have the disease. Diabetes mellitus is listed as the seventh leading cause of death in the United States, and there is no cure. Complications that can arise from diabetes mellitus include retinopathy, kidney disease, neuropathy, heart disease, and stroke. Total healthcare costs have been estimated at over $98 billion per year [ADA, 1999a]. Approximately 20% of diabetics are completely dependent on insulin therapy to control their diabetes. The other 80% are able to control their diabetes through a program that includes dietary control and exercise. These patients may have little or no need for supplementary insulin therapy [Rifkin and Bernstein, 1988].

Diabetes mellitus is difficult to define as it is a combination of symptoms which together make up what is seen as a disease. Basically, diabetes mellitus is a disorder of carbohydrate metabolism characterized by hyperglycemia (high blood sugar) and glycosuria (discharge of glucose in the urine) which results from inadequate production or utilization of insulin. An operational definition was developed in 1979 by the National Diabetes Data Group and accepted by the World Health Authority in 1980. They define

diabetes mellitus as a condition where the venous plasma glucose level is greater than or equal to 8 mM after fasting for at least 8 hours and/or greater than or equal to 11 mM two hours after an ingestion of 75 g of glucose. In healthy people, the hormone insulin aids in the absorption of glucose by the body's cells. Diabetics, however, lack normal insulin activity, and thus are unable to regulate this flow of glucose into cells properly [Crabbe, 1987]. Diabetes mellitus occurs in various forms; 90% of the cases of diabetes mellitus are classified as "spontaneous diabetes" and are further divided into two categories: Type 1 and Type 2.

2.1.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is an autoimmune disease characterized by damage to the pancreas and the destruction of pancreatic B cells. People with Type 1 diabetes mellitus are unable to synthesize or secrete any insulin and must therefore take daily insulin injections in order to stay alive. This type of diabetes is found most often in children and young adults and accounts for 5-10 % of diabetics. It is often referred to as insulin-dependent diabetes mellitus (IDDM). Current evidence suggests that IDDM may be a genetically determined disorder as patients with IDDM show an increased frequency of some histocompatiability antigens [Espinal, 1989]. These antigens are glycoproteins found in the cell surface of all cells and are responsible for non-self recognition. IDDM may also involve other autoimmune processes, as certain autoimmune disorders such as Graves' disease, myasthenia gravis, pernicious anemia, and Addison's disease have long been associated with IDDM patients. Environmental factors such as viruses may also be a cause for IDDM. Congenital rubella occurs with a high frequency in patients who later

develop IDDM. Other viruses such as Coxsackie virus B4 have also been reported to induce IDDM in humans and mice [Espinal, 1989].

2.1.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is a metabolic disorder that decreases the body's ability to produce enough insulin or to use the insulin that is produced properly. This type of diabetes accounts for 90-95 % of spontaneous diabetics and is often found in the obese and the elderly. People with Type 2 diabetes mellitus are sometimes able to control their condition through diet and exercise; however, these people often have to resort to oral medications or insulin injections to keep their glucose levels within an acceptable range [Rifkin and Bernstein, 1988]. Type 2 diabetes is also referred to as non-insulin-dependent diabetes mellitus (NIDDM); 90% of NIDDM patients have relatives with the disease, however a genetic component of NIDDM is not as evident as with IDDM. NIDDM is not associated with the histocompatiability antigens identified in people with IDDM. There is, however, a much stronger association of the disease with environmental factors such as age, diet, exercise, and psychosocial stress. NIDDM patients appear to be insulin resistant. The cause for this resistance could include a defect or mutation in the insulin gene, excess production of proinsulin instead of insulin, the presence of antibodies to insulin and its receptor, and a defect in the mechanism of insulin action on its target cells [Espinal, 1989]. Recent progress in the Human Genome Project has suggested that a major gene for NIDDM may lie on Chromosome 20, indicating a possible DNA mutation related to this disease [NHGRI, 1999].

2.1.3 Other Forms of Diabetes Mellitus

There are various other forms of diabetes mellitus. The main categories of these are secondary diabetes, impaired glucose tolerance, and gestational diabetes. Secondary diabetes is defined as a disease that is caused by an insult to the pancreas, drug treatment, excess counter-regulatory hormones, or genetic hyperglycemia. Patients with impaired glucose tolerance have normal fasting plasma glucose levels but very high glucose levels following glucose ingestion. Gestational diabetes is a temporary form of diabetes mellitus found in some pregnant women. This condition often reverses itself after the woman has given birth, but it does put these women at a higher risk for developing Type 2 diabetes mellitus later in life [Espinal, 1989].

2.2 The Clinical Perspective

Two major studies have been carried out to address how diabetics can best control their glucose and insulin levels so that they remain within a normal range. One such study was carried out in the United Kingdom (UK) beginning in 1977. It is referred to as the UK Prospective Diabetes Study (UKPDS) and was designed to determine whether Type 2 diabetics would have a reduced risk of macrovascular and microvascular complications, including stroke, heart failure, angina, renal failure, amputation, and death, if they maintained intensive control of their blood glucose levels. The UKPDS followed 5,102 patients over the course of 10 years. These patients were divided into two groups, one using conventional diabetes therapy and the other using a more intensive approach. Conventional therapy was defined as one or two insulin injections per day (Figure 2.1), while the intensive therapy regimen attempted to keep patient blood glucose

values as close to the normal range as possible through the use of three or more daily insulin injections.

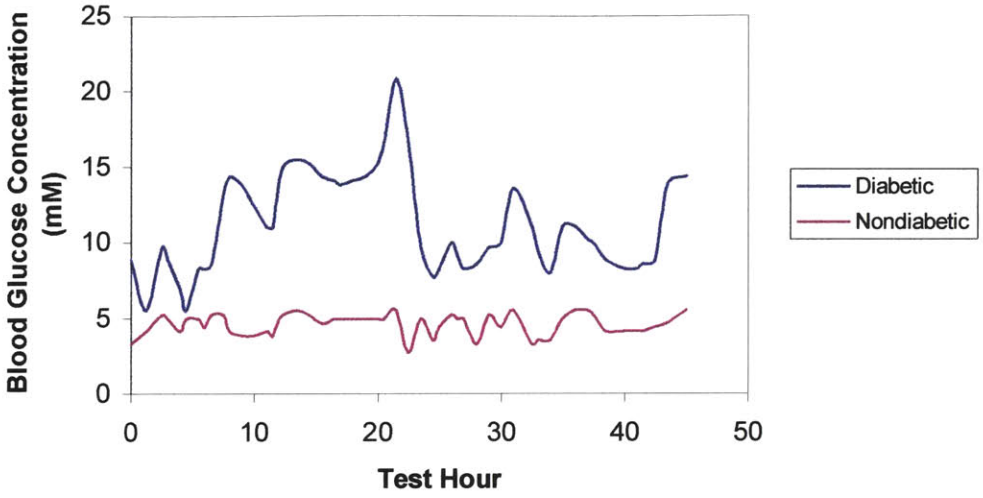


Figure 2.1: Comparison of average nondiabetic blood glucose levels with those of a diabetic patient under conventional insulin injection therapy over the course of a day. Insulin injections were given to the diabetic patient at hours 19 and 42 [Data from Weinless, 1986].

The conventional group also used methods of dietary control where necessary to maintain glucose levels below 15 mM. The intensive group was encouraged to do regular home-glucose monitoring in order to maintain glucose levels below 6 mM. The UKPDS showed that there was a decrease in the frequency of many clinical complications in Type 2 diabetics who followed an intensive blood glucose control regimen. However, it also found that intensive control tended to lead to an increased risk of hypoglycemic episodes (low blood sugar caused by too large an insulin dosage) and greater weight gain [UKPDS, 1998].

The second major study, the Diabetes Control and Complications Trial (DCCT), was started in 1983 in the United States. This study followed 1,441 patients with IDDM over an average span of 6.5 years [DCCT, 1993]. It was designed as a multicenter, randomized clinical trial whose purpose was to compare conventional diabetes mellitus therapy with a more intensive approach. Patients using intensive therapy adjusted their insulin dosages according to the results of blood glucose tests performed a minimum of four times a day. These patients also accounted for dietary intake and anticipated exercise in their dosages. The development of diabetic retinopathy in patients was used as the central measure in the study, however the development of renal, neurological, cardiovascular, and neuropsychological complications was also recorded, along with other adverse effects directly related to the treatment regimens [DCCT, 1993].

The results of this study showed that intensive therapy delayed the onset and slowed the progression of retinopathy by 35-70%. Intensive therapy was also shown to reduce the risk of other microvascular and neurologic complications. Although, intensive therapy did increase the risk of severe hypoglycemia, most patients were able to deal with the hypoglycemic episodes themselves through sugar pills or other sugar intakes, and the overall health benefits seemed to outweigh this risk. It was also suggested that tight control of intensive therapy should help to reduce the occurrence of such episodes [DCCT, 1993].

Based on the results of these two studies, most physicians now agree that both Type 1 and Type 2 diabetics should maintain tighter control of their blood glucose levels

through a more aggressive monitoring program. The goal of diabetics should be to maintain their blood glucose levels as close to the normal levels as possible throughout the day. This means maintaining preprandial (before meal) levels between 4.40-6.66 mM (80 and 120 mg/dl) and bedtime levels between 5.55-7.77 mM (100 and 140 mg/dl) [ADA, 1999b]. In order to accomplish this, it is suggested that patients test their glucose levels a minimum of four times per day and adjust their daily routines, through diet, exercise, and/or insulin injections, based on these readings.

2.3 Insulin Therapy

Insulin is a hormone and protein produced by the beta cells in the pancreas. It is essential for the proper metabolizing of blood sugar (glucose) and for the maintenance of proper blood sugar levels. For people who do not produce enough insulin or do not use the insulin they do produce properly, additional insulin must be added to their systems. Since insulin is a protein that breaks down during digestion, it cannot be ingested in the form of a pill or oral medication. As such, insulin must be injected in order to properly work its way into the blood. Using various methods, including a scintillation camera, to monitor the distribution of injected insulin throughout the body, it has been shown that insulin enters the blood stream at different rates when injected at different body sites. Areas around joints and large nerve clusters have been found to be less effective in delivering the insulin, whereas fast delivery is achieved when insulin is injected into the abdomen. The upper arms, thighs, and buttocks are also acceptable injection areas [ADA, 1997; Galloway and deShanzo, 1990; Schwartz, 1989].

There are approximately thirty different types of insulin currently sold commercially in the United States. These come from the pancreas of pigs and cows or may be synthetically produced. Synthetic processes include the use of recombinant DNA technology involving E. coli or the conversion of porcine to human insulin [Heine, 1988]. Each of these insulin types can be made in a variety of forms, characterized by how long it takes for the insulin to begin working (onset), when the insulin is working the most effectively (peak time), and how long the insulin remains in the body (duration). The insulin types are generally classified as rapid-acting (Lispro), short-acting (Regular), intermediate-acting (Lente), and long-acting (Ultralente). Rapid-acting insulin reaches the bloodstream in about fifteen minutes, peaks one hour after injection, and remains in the blood for two to four hours [Galloway and deShanzo, 1990; Strowig and Raskin, 1990]. Short-acting insulin can reach the blood within thirty minutes from the time of injection, peaks two to three hours later, and stays in the blood for three to six hours. Intermediate-acting insulin has an onset of two to four hours after injection, peaks four to twelve hours later, and remains in the blood for ten to eighteen hours. Long-acting insulin has an onset of six to ten hours from injection time, has a small peak (if any) fourteen to twenty hours after injection, and remains in the blood for eighteen to twenty hours [Galloway and deShanzo, 1990; Strowig and Raskin, 1990].

Lispro is the newest insulin analog on the market. Due to its fast onset and peak times, it offers a more convenient and flexible approach to insulin therapy for patients. Lispro can be administered immediately before meals, and patients do not need to wait a specified time before eating or schedule specific meals around the injection time. Insulin

therapy varies from patient to patient, and many people mix these different types of insulin at different times during the day [ADA, 1998; Galloway and deShanzo, 1990; Strowig and Raskin, 1990].

The site of injection also plays a role in insulin absorption. Insulin enters the blood fastest when delivered in the abdomen, and more slowly from areas such as the upper arms, thighs, and buttocks. However, repeated injections into the same area can lead to tissue scarring which then impedes insulin absorption. It is therefore recommended to rotate the site for injections around the region of choice. Conventional insulin therapy is defined as taking the same volume of insulin injections at the same time each day, usually twice a day. Intensive therapy entails three to four injections daily or the use of an insulin pump. The timing of injections is usually based upon the results of self monitoring of blood glucose levels to discover how long after an injection the insulin begins to lower glucose levels. The goal is to time injections such that the insulin begins to work at the same time the glucose from food begins to enter the bloodstream [DCCT, 1993; Schwartz, 1989].

The amount of insulin needed to maintain normal glucose levels is on the order of 0.02 to 0.03 mg per kilogram of body weight each day [Schwartz, 1989]. It is common to refer to insulin in "units" where a single unit of insulin is defined as 0.04167 mg [Diem, 1962] and the most commonly used insulin is U-100 which contains 100 units of insulin per milliliter of fluid [ADA, 1997]. Insulin injections are then adjusted based on the results of glucose tests during the beginning stages of therapy by approximately one unit

of insulin for each 500 mg/l over the desired glucose range [Schwartz, 1989]. The amount of insulin needed to maintain these levels may decrease if a more continuous means of drug delivery were adopted. It should also be noted that more dilute concentrations of insulin (0.42 g/l (10 U/ml) vs. 20.80 g/l (500 U/ml)) are more rapidly absorbed than higher concentrations, and deeper injections into the tissue have a faster onset and a higher peak time. An increase in ambient temperature also aids in the absorption of subcutaneously injected insulin [Galloway, 1990]. These aspects of insulin absorption may affect future iterations of implantable insulin pumps.

2.4 Models of the Human Glucose-Insulin Reaction

Current treatment for diabetes mellitus is, as described above, based on an open-loop strategy requiring that patients themselves close the loop. Patients are required to measure their glucose levels and determine insulin dosages. The development of a completely closed-loop approach would not only free the patient from the burden of determining dosage levels, but it would also provide a better method for tight and continuous control of blood glucose levels. To this end, there has been some research into the development of a model of the human glucose-insulin reaction, which could lead to the development of a closed-loop system.

Many initial forays into glucose-insulin modeling produced systems that were general, not designed for individual patient simulations, and based mainly on non-physiological mathematical functions. Lehmann and Deutsch (1992) developed a more advanced system that incorporated basic information from some of these earlier systems

and also included a model of glucose pharmacodynamics based on experimental data. They claimed that their model had the ability to adapt to individual patients and that it could predict the blood glucose profile expected from an adjustment in diet and from insulin injections. The anatomical basis of this model is that of a patient completely lacking endogenous insulin secretion. The model allowed different parameters to be applied for specific patients. The goal of this model was to aid physicians in determining an appropriate regimen for specific patients using a minimum amount of insulin, with possible future applications in automated insulin dosage systems [Lehmann and Deutsch, 1992].

The Lehmann and Deutsch (1992) model was based on four differential equations and twelve auxiliary relations, along with the experimental data from Guyton et al. (1978). The first of the four differential equations computes the change in the plasma insulin concentration:

$$\frac{dI}{dt} = \frac{I_{abs}}{V_i} - k_e I \quad ,$$

where I is the plasma insulin concentration, I_{abs} is the rate of insulin absorption, V_i is the volume of insulin distribution, and k_e is the first-order rate constant of insulin elimination. The second equation deals with the build-up and deactivation of the active insulin pool, I_a, and is assumed to obey first-order kinetics:

$$\frac{dI_a}{dt} = k_1 I - k_2 I_a \quad ,$$

where k_1 and k_2 are first-order rate constants describing the delay in insulin action. The change in glucose concentration with time is given by the third differential equation:

$$\frac{dG}{dt} = \frac{G_{in}(t) + NHGB(t) - G_{out}(t) - G_{ren}(t)}{V_G} ,$$

where G is the plasma glucose level, G_{in} is the systemic appearance of glucose as a result of glucose absorption from the gut, G_{out} is the overall rate of peripheral and insulin-independent glucose utilization, NHGB is the net hepatic glucose balance, G_{ren} is the rate of renal glucose excretion, and V_G is the volume of distribution for glucose. The amount of glucose in the gut, G_{gut}, following the ingestion of a meal containing carbohydrates is defined in the final equation:

$$\frac{d(G_{gut})}{dt} = G_{empt} - k_{gabs} G_{gut} ,$$

where G_{empt} is the rate of gastric emptying and k_{gabs} is the rate constant of glucose absorption from the gut into the systemic circulation. The auxiliary equations are used to describe such model elements as the rate of insulin absorption, the steady-state insulin profile, and the duration of the period during which gastric emptying is constant and maximal [Lehmann and Deutsch, 1992].

The results of Lehmann and Deutsch's (1992) model show fairly accurate results, but there are some inherent problems with the model. The model itself was kept intentionally simple, making certain broad assumptions such as that insulin absorption and elimination depends solely on body weight. Other issues such as the role of ketones and the change in renal threshold of glucose for elderly patients were not included in the model. The authors state that this model cannot be applied to all patients under all conditions [Lehmann and Deutsch, 1992], and as such, it would need further modification before it could be used reliably in a commercial product.

Another model was developed by Parker, Doyle, and Peppas (1999) which built on Lehmann and Deutsch's model. This model combines empirical approaches which capture system behavior from input-data output (such as insulin injections and related glucose excursions), together with additional physiological details such as insulin dynamic behavior and kinetics. This model is based on a linear step-response which estimates future output based on a series of past inputs. A linear approximation of the output can therefore be calculated when a past input profile is given:

$$y'(k) = \sum_{i=1}^{M} s(i)\,\Delta\,u(k-i) + s(M)\,u(k-M-1) \quad ,$$

where y'(k) is the predicted output, s(i) is the step-response coefficient, u is the past input, and M is the number of sample times. In this equation, the first term represents the response of the model to the input change, and the second term represents the steady state of the process prior to the input change. The step-response coefficients can be calculated from an identified impulse-response model of the system:

$$y(k) = \sum_{i=1}^{M} h(i)\,u(k-i) \quad \text{and}$$

$$s(k) = \sum_{i=1}^{k} h(i) \quad ,$$

where h(i) are the impulse response coefficients [Parker et al., 1999].

The Parker et al. (1999) model shows a strong correlation between predicted glucose levels and those achieved using a nonlinear diabetic patient model, however no data are shown to compare these predicted levels with actual patient measurements. The

authors are also working on adding parameters to the model related to food intake and exercise as well as other factors that may affect glucose levels [Parker et al., 1999]. Although this model shows much potential, it has yet to be proven that a purely mathematical approach can be applied to the physical realities of diabetes mellitus treatment.

Many other models of the human glucose-insulin response have been proposed such as linear, non-linear, probabilistic, compartmental, non-compartmental, and parametric models [Bremer and Gough, 1999]. Many of these approaches have proved useful in research environments, however their scope has been very limited in clinical applications. Most of these methods rely on continuous or frequently updated data, and continuous sensors which are able to provide these data are still only in the research realm. However, it has been shown that even a simple linear model can accurately predict glycemic excursions in humans for up to ten minutes with very high accuracy [Bremer and Gough, 1999]. Future models will also have to take into account the fact that normal insulin secretion is signaled by neural and hormonal messengers before glucose levels actually begin to rise. In this way, insulin is immediately available when blood glucose levels begin to increase rather than allowing the glucose levels to rise dramatically before there is enough insulin to start the regulation process. This type of neural and hormonal sensitivity needs to be pre-programmed into automatic closed-loop controllers to ensure proper glucose regulation [Reboldi et al., 1991]. By further studying this phenomenon, gathering more patient data, and increasing efforts into the

development and commercialization of continuous glucose sensors, it is possible that a closed-loop system may someday provide better glucose control to diabetic patients.

Chapter Three: Current Technology for Glucose Sensing and Insulin Delivery

3.1 Devices Currently Available for Glucose Testing

The goal of current approaches to the control of diabetes mellitus is to keep blood glucose levels as close to normal as possible. Currently, the best way to achieve this is through frequent monitoring via self-administered glucose tests coupled with insulin injections and dietary adjustments. Many types of glucose tests currently exist, but few of these have been shown to be truly reliable under all circumstances. Some of the tests, including those derived from samples of urine, sweat, and saliva, reveal average values for glucose levels during the past few hours, but do not indicate what the present levels are. These tests are also patient dependent, as the relation between the level of glucose in the blood and in these other fluids varies for each person.

By testing their blood, patients can get an accurate reading of the current glucose levels in their bodies. This type of testing is done by pricking the finger with some type of lancet to obtain a drop of blood, which is then placed on a special test strip. In one technique for testing, the test strip has been chemically treated and will change color according to detected glucose levels. The strip is then compared to a color chart to determine the current glucose range. In another more common technique, the test strip with the impregnated blood is placed in a meter which detects the glucose levels using electrochemical sensors. This method gives a more precise value of the glucose level.

Some of these meters are able to store previous test results for future analysis [Ginsberg, 1992; Tamborlane and Amiel, 1987].

Glucose meters, lancets, and test strips come in many different shapes and sizes. Scanning through any magazine for diabetics, such as ADA's "Diabetes Forecast," shows that many different companies are marketing these types of devices. However, this technique has many drawbacks. Although it has been shown to be the most accurate method for glucose detection available, it is far from flawless. An evaluation of six popular blood glucose meters in 1996 showed that there were substantial differences between the readings recorded by different meters during hypoglycemia. Using 119 different glucose concentrations, 663 different readings were recorded. The correlation coefficients between the meter readings and the glucose reference values of the tested meters ranged from 0.78 to 0.93, and as many as 7% of the readings fell outside 40% of the reference values [Trajanoski et al., 1996]. Another evaluation of six blood glucose meters in 1998 also found that the performance of home blood glucose meters varied significantly within expected glycemic ranges [Brunner et al., 1998].

In addition to issues of meter accuracy, there are other factors that affect the reliability of patient use of glucose meters. Many sources of error in patient glucose meter readings relate to how the patient uses the meter and include the use of inadequate quantities of blood, faulty timing, and improper calibration of the meter [Tamborlane and Amiel, 1987]. Another issue for diabetics is the fact that insurance companies do not usually cover the cost of the glucose meters or the test strips which must be thrown out

after a single use. Finally, one of the most significant issues is the fact that this technique involves drawing blood. As doctors are recommending a minimum of four glucose test each day, this means at least four painful finger pricks each day with little time for old wounds to heal as well as an increased risk of exposure of the general population to infection and blood transferred diseases such as HIV and hepatitis.

The pain associated with lancing and often a phobia of the needles themselves discourage many diabetics from testing often enough or even from testing all together. Without these vital glucose tests, patients run an extremely high risk for many diabetic complications. There are a few new devices such as that offered by Chronimed [D'Arrigo, 1999], that try to eliminate the use of actual needles by using a laser lancing device to puncture a hole in the skin. Chronimed's device uses an Erbium YAG 2940 nm laser and penetrates the finger 0.6 – 2.4 mm. The entire device size is 250 mm × 100 mm × 50 mm. While this device is still somewhat painful, the discomfort is supposed to subside faster than the pain associated with traditional lancing. This device does take some training to use and is prohibitively expensive at $1000 - $2000 for the unit and costs about 15 cents for the single-use disposable shield used for each test. However, for those with extreme needle phobias, it does offers a needle-free approach to glucose testing [D'Arrigo, 1999].

Much research is currently being done to try to develop a painless glucose sensor for diabetic patients. The most recent developments have come from MiniMed [MiniMed, 1999] with FDA approval of their continuous subcutaneous glucose monitoring system, which MiniMed eventually hopes to combine with an insulin pump. The sensor in this

system is designed to be inserted into subcutaneous tissue and worn for three days using a system of insertion directly beneath the skin, similar to that used in MiniMed's insulin pumps [MiniMed, 1999]. A similar type of insertion system was patented in 1994 by Eli Lilly and Company [Mastrototaro, 1994]. The sensor is attached with a wire to a pager-sized glucose monitor which records the sensor readings during the three day period. The monitor measures approximately 9 mm x 7 mm x 2 mm and weighs 100 gm. The sensor records electrical signals proportional to glucose levels in the subcutaneous tissue and takes readings every ten seconds, storing only 5 minute averaged values. While this is a major step in the direction of continuous sensing, it is still not designed for regular patient use. The current sensor simply stores values until the physician downloads the information to a personal computer for analysis. It does not give feedback to the patient during use, and the stored information is only available after 72 hours. According to the FDA approval, these systems are only to be used as a supplement to, and not a replacement for, standard glucose testing. The information generated is intended as a guide for future management and not as a means to adjust immediate insulin dosages [MiniMed, 1999]. The sensor also still requires several finger-prick tests a day for calibration purposes and to check sensor accuracy [Jacobs, 1999].

Another device is the GlucoWatch being developed by Cygnus. This device is still pending FDA approval, but Cygnus hopes to obtain approval within the first quarter of 2000. The GlucoWatch operates by sending a low amplitude electrical current into the skin to open the pores and then extracts interstitial fluid which resides below the outer layer of the skin and contains information on body glucose levels. The extracted fluid

contacts an electrochemical sensor which can detect current glucose levels, and the reading is presented digitally on a watch display. The GlucoWatch aims to be a painless device that can take frequent readings and so lead to better diabetes control. Clinical research on the GlucoWatch has shown that variations in physiological factors and environmental factors, such as temperature, as well as common drugs do not affect the accuracy of the device. Pricing estimates have placed the watch itself at $175-$200 and the consumable sensor at $2.75 a piece. The sensor needs to be replaced every twelve hours [Cygnus, 1998].

Although there is current work being done towards the development of a painless glucose sensor, patients are still forced to rely on more traditional methods for verification of the newer devices' readings according to both FDA requirements and manufacturers' specifications. These methods involve painful finger pricks that are aversive for many diabetics. In addition, none of these methods combine glucose sensing with insulin delivery. As such, there is a large opportunity in the field for the development of an accurate system that can replace these traditional methods.

3.2 Devices Currently Available for Insulin Delivery

The two primary methods for insulin delivery currently available to diabetics are manual injections of insulin and insulin pumps.

3.2.1 Insulin Manual Injection

The most traditional method of insulin delivery is through insulin injections. Patients are advised by their physicians as to the amount and type of insulin that should be administered and at what times during the day. Dosages are adjusted according to individual lifestyles. Conventional approaches tend to advocate only one or two injections a day, whereas tighter control calls for up to four injections a day to try to mimic more closely normal human insulin production [DCCT, 1993]. This method of delivery entails the use of syringes. Patients must be trained how to use the syringe as well as how to dispose of the needle.

Some companies are working to eliminate the use of syringes for insulin delivery which are often perceived as daunting by prospective users. One option currently on the market is the jet injector marketed by Medi-Ject. This type of injector uses a high-speed stream of insulin itself to penetrate the skin. The stream has a diameter of about 150 μm, which is about half the diameter of a standard needle. While not all patients find this method effective and it can be painful, it does eliminate the need for actual syringes. The cost of these devices can be in the order of $400, and some insurance plans do cover jet injectors. While this is not a perfect solution, many patients prefer these devices to conventional syringes [Medi-Ject, 1999].

Another alternative to the bolus injection method may be through pulmonary drug delivery. Phase III clinical trials are currently being conducted by Pfizer, Inc. on the

delivery of insulin through an inhaler-type device. Previous studies have shown that inhaled insulin achieves the same blood glucose control as that obtained with traditional injections in both Type 1 and Type 2 diabetics [Patton, 1998; Walsh, 1998]. While this is not yet a commercially available option, there is definitely promise in this type of delivery system.

3.2.2 Insulin Pumps

The second major category of insulin delivery is the insulin pump. An insulin pump is a pager-size device containing a reservoir of insulin. Connected to this reservoir is a catheter with a needle at the end which is inserted into subcutaneous tissues, usually in the abdomen. The catheter needs to be replaced every 2-3 days. The pump is then programmed to deliver a basal, or continuous, infusion of insulin usually in the range of 0.35 to 1.38 μg/s (0.5 to 2.0 units/h). Premeal boluses are then administered automatically according to the therapy regimen about thirty minutes before mealtime. Approximately 40-50% of the patient's total daily insulin dose is delivered by the basal rate infusions during a 24 hour period. The remaining insulin is then divided between the bolus injections. The program can be adjusted for varying activities and meals, accommodating to the lifestyles of individual patients. The insulin used is regular insulin, making insulin absorption by the body more predictable [Strowig and Raskin, 1990]. Many current pumps are being designed with communications capabilities with the hope of interfacing these pumps with glucose sensors for better feedback control in the future. Approximately 6 to 8% of Type 1 diabetics currently use insulin pumps. However, 60% of the medical community who are also diabetic (including physicians, endocrinologists,

nurses, and diabetes educators) use insulin pumps to control their insulin levels due to the more regular rates of insulin infusion [Noonan, 1999].

While insulin pumps have many advantages, such as the potential for a more flexible lifestyle, and offer a more normal schedule of insulin infusion to the body, there are some risks associated with their use. Interruption of insulin delivery can lead to a rapid deterioration of diabetic control. Since the insulin used is short acting and there is no build up of insulin in the body, an interruption of regular pump operation can lead to hyperglycemia in a matter of hours. As insulin pumps also operate while patients are sleeping, a problem may not be readily detected, and patients could deteriorate considerably while they are asleep. Patients must be made aware of these types of issues before beginning treatment with a pump [Strowig and Raskin, 1990]. Another disadvantage is that the pump must be worn at all times, including during bathing, swimming, sleeping, and intimacy, although short periods of pump disconnection can be tolerated [Koivisto, 1988].

3.3 Glucose Sensor Design Methods in Research

The original approach to improving the control of diabetes mellitus was to create an entire artificial pancreas consisting of a glucose sensor and insulin pump. In 1974 one such device was tested on human subjects. This system was shown to provide very good control of blood sugar for diabetic patients using less than half of the daily insulin requirements of the traditional insulin delivery mechanisms [Albisser et al., 1974]. However, further work in this area was hampered by the technology of the time. The

need for large computers, sensors, and pumps meant that this was not an implantable system and that it could not be used outside of a clinical environment. Work continued into the 1980s [Shichiri et al., 1984], but none of these devices ever achieved real clinical success due to the issues listed above. Current technological advances now allow for the miniaturization of such systems and there is hope that a true artificial pancreas will soon be a reality. To this end, much work is being done to develop miniature glucose sensors using a variety of different methods.

3.3.1 Optical Techniques

One method of glucose detection, which has been investigated since the 1970s, is to shine a light into the patient's eye, measure the reflected radiation, and compute the spectral absorption of the aqueous humor which is claimed to be proportional to the blood glucose level. Patents have been issued for this type of process from 1975 through 1998, however, none of these devices has been shown to be accurate for large numbers of patients. While the method is noninvasive, the device itself is not easily used without assistance as it entails shining lights at specified positions into the eye [Hattori and Ushizawa, 1998; March, 1976, 1977; Miyagawa and Toida, 1998; Stark, 1995].

A related method radiates the patient's skin with a light source, such as infrared illumination, and measures the glucose levels via light absorption, polarization of the light, or characteristic wavelength shifts using Raman spectroscopy. Again, many patents for these types of procedures have been issued, but no such devices have yet been approved for clinical use in the United States for several reasons [Ariizumi and Higashio, 1996;

Buchert, 1995; Clarke and Wang, 1993; Elmerick and Peters, 1999; Kim and Yang, 1993; Steffes and Tarr, 1993]. Many of these methods suffer from low sensitivity, low accuracy, poor selectivity of glucose signals, and dependence on personal characteristics of the patient at the measurement site [Wilkins and Atanasov, 1996]. These devices also tend to be large and expensive. Work continues on these methodologies with the hope that such a completely noninvasive method for sensing can one day be used.

3.3.2 Chemical Techniques

The most common method of glucose sensing uses an electrochemical sensor with the immobilized catalyst glucose oxidase (GOx) used to spur the reaction that detects glucose (see Chapter Four for a discussion of this reaction). This type of sensor has been shown to be accurate and reliable for the majority of patients, and is currently used in both standard glucose meters and in implantable sensors. There are numerous patents for glucose sensors using this type of method, and they are available in a variety of shapes (disks, needles, etc.) and sizes (macro-scale to miniature sensors). GOx sensors have been fabricated that can sense glucose both internal and external to the human body. Sensing blood glucose levels in this way has also been approved by the FDA in a variety of applications. Some of the major difficulties with these types of sensors have been size, stability, and interference with the measurement from other bodily substances. Research is currently being done to investigate better methods of electrochemical sensor fabrication and enzyme immobilization. Table 3.1 lists a few of these methods indicating areas of major differences. Many other methods exist, but most seem to employ similar techniques to those listed.

Authors	Working Electrode	Immobilization material for GOx	Working Potential vs. Ag/AgCl (volts)	Response Time (seconds)	Maximum current achieved (10^{-5}A/M glucose)	Sensor Stability (months)
Yao, 1983	Platinum	BSA and glutaraldehyde	+0.7 (vs.SCE)	10		1
Gunasingham, et al. 1989	Platinum	BSA		60		
Moussey, et al. 1993	Platinum	BSA and glutaraldehyde	+0.7	20-40	2	0.5
Steinkuhl, et al. 1996	Platinum	Gelatin	+0.6	65-104	1.5	
Li, et al. 1998	Platinum	Pyrrole	+0.2 (vs.SCE)	30	60	6
Lukachova, et al. 1998	Platinum	DMF			35	
Bindra, et al. 1991	Platinum -Iridium	CA and glutaraldehyde	+0.6		0.4	1
Wang, et al. 1992	Rhodium	Rhodium	+0.3		9	
Yang, et al. 1998	Rhodium	BSA and glutaraldehyde	+0.25	10	5	3
Furbee, et al. 1998	Carbon	Pyrrole	+0.7	14	0.2	2
Milardovic, et al. 1997	Nickel	BSA and glutaraldehyde	-0.2		0.05	

Table 3.1 Various methods of electrochemical glucose sensor fabrication
BSA = Bovine Serum Albumin, SCE = Standard Calomel Electrode,
DMF = Dimethylferrocene, CA = Cellulose Acetate

Possible interference from other bodily substances is a major concern in the fabrication of electrochemical sensors. The main interferents for the glucose sensor are uric acid, acetaminophen, and ascorbic acid. In order to avoid these types of interferents, additional coatings are added to the sensor surface which act as semipermeable membranes. One popular coating of this kind is Nafion (developed by DuPont). An undesired side effect of Nafion is that it reduces the amperometric response of the sensor dramatically, necessitating the use of a more sensitive meter for recording currents [Yang et al., 1998]. One must also be sure that if the sensor is implantable, the coatings used are

biocompatible. Other types of coatings such as polyvinyl chloride (PVC) are also under investigation [Christie et al., 1992].

3.4 Problems in Painless Monitor Development

One of the major problems encountered by physicians in treating diabetics is that of convincing the patients to monitor their glucose levels closely. The pain and inconvenience of monitoring are often sufficient discouragements to dissuade people from performing these tests regularly. New research is trying to address this problem by creating painless (or at least less painful) ways to test glucose. However, these methods are still required to meet the standards that are currently achieved by traditional testing methods. These include a fast response, accurate readings, and stability so sensor readings can be appropriately analyzed [Gough and Armour, 1995].

In the search for an implantable alternative to the glucose sensor, many researchers have tried to develop short-term subcutaneous implantable sensors [Johnson et al., 1992; Pickup et al., 1993; Poitout et al., 1993; Shichiri et al., 1982; Shichiri et al., 1986]. These sensors present serious difficulties due to problems such as signal stability and sensor lifetime. They are typically implanted for a period of several days, and results have shown that they produce a reliable response to blood glucose under ideal conditions. Although these results have looked promising, there are still a number of issues which need to be addressed, in particular the decay in sensitivity over time. In many of the published experiments, the reported results are based on signals that were recalibrated after the experiment [Gough and Armour, 1995]. This technique does not allow for analysis of the

decay in the actual signal. To overcome this, a more sophisticated method of calibration must be determined. The other problem with these implantable sensors is that improper insertion may lead to tissue inflammation and blood pooling which can interfere with the sensor signal [Gough and Armour, 1995]. Studies need be conducted which look at the effects of sensor insertion and the reliability of signals from the sensors. To understand fully how these sensors act in vivo, a valid model of glucose transport in living tissue must be determined. This is an area that is severely lacking in glucose sensor research conducted to date [Gough and Armour, 1995].

3.4.1 Continuous Monitoring

There are two main approaches to developing a continuous glucose sensor. One is a percutaneous sensor such as a needle placed in the skin with a wire outside the body connected to a readout device. The second approach is an entirely implantable device that would communicate to an external receiver using radio telemetry. Both of these methods need to deal with the body's response to a foreign substance. The body's natural response is to encapsulate the foreign element in order to protect the rest of the body. The hope is that percutaneous sensors will be able to obtain signals before this response takes place. A fully implanted sensor would need to have some way of dealing with this issue on a long term basis. A minimally invasive means of implanting the sensor would need to be developed. The fully implanted sensors also need to be larger than the percutaneous ones in order to survive in the body for long periods without requiring replacement (current designs require a battery of approximately 5 mm height and 20 mm diameter). The advantage of such a sensor would be that patients would not need to monitor their glucose

levels on a daily basis. Percutaneous sensors, on the other hand, do not involve any surgery and can be placed on the body by the patients themselves. There may, however, be a risk of infection at the insertion site of such sensors [Henry, 1998].

There are many hurdles that exist in the search for an effective sensor. As previously mentioned, biocompatibility remains a very important issue. Other issues are the need for recalibrating the sensor, the lack of understanding of the relationship between blood glucose levels and those found in other tissues, interferents from other bodily substances, and safety and stability issues. All of the existing sensors require daily recalibration with traditional fingerstick sensors [Henry, 1998].

3.5 Glucose Levels through Interstitial Fluid Sampling

One of the more recent approaches to painless glucose monitoring is to determine what other substances in the body can give accurate glucose measurements. One such substance that has been closely investigated is the interstitial fluid (IF) which resides in the outer layer of skin. The skin itself is the largest organ of the human body (approximately 2 square meters) [Sherrick and Cholewiak, 1986], and thus access to IF can be obtained at a variety of sites. The skin consists of two main layers, the epidermis and the dermis (Figure 3.1).

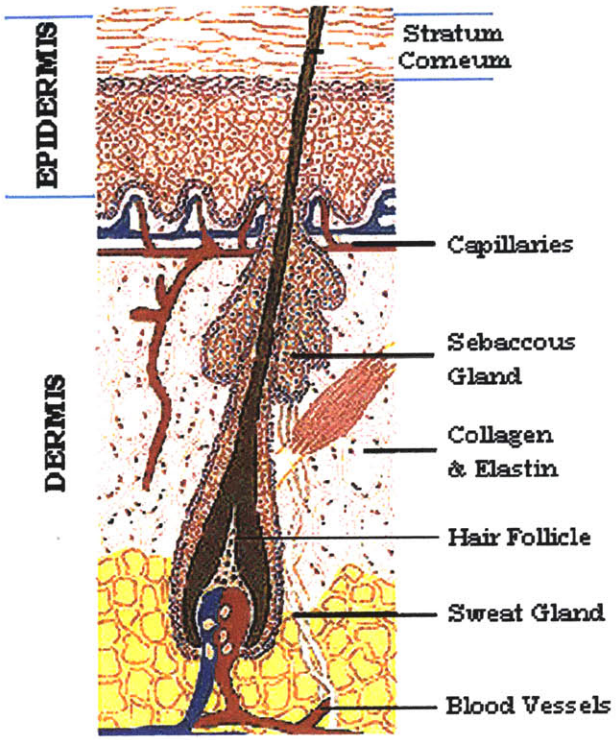


Figure 3.1: Diagram of human skin [Adapted from Repechage, 1999].

The epidermis is the outer layer of the skin and is approximately 100 μm thick. The epidermis itself is comprised of various layers. The outermost layer is the stratum corneum, made by the layering of thin cells, and is typically about 10 μm thick [Sherrick and Cholewiak, 1986]. Below this are layers of epithelial cells [Repechage, 1999], and the IF flows between these cells. Since the epithelial cells are found above the nerve endings and capillaries, accessing the IF to measure glucose could be a painless and bloodless process if done correctly.

Since IF is a bodily fluid that could be accessed without pain, it is necessary to determine whether it contains information such as glucose levels in the body which would lead to more options for glucose sensing. Many such studies have been carried out and have shown that there is a positive correlation between IF glucose levels and blood glucose levels [Bantle and Thomas, 1997; Rebrin et al., 1999; Tamada et al., 1995]. Experiments have also shown that the IF glucose profile is slightly shifted in time as compared to the blood glucose levels, indicating that a lag exists between these two fluids. In one study where IF was extracted from the body, a process which took 20 minutes per extraction, the lag time was no more than one test interval [Tamada et al., 1995]. Other groups have shown the delay to be between 3 – 14 minutes. This delay can be compensated for by the use of a simple digital filter [Rebrin et al., 1999].

The Tamada et al. (1995) study also showed that 95% of glucose measurements taken using IF match conventional blood glucose measurements. This level of accuracy is similar to that obtained with commercially available blood glucose monitors, which shows that IF measurements are reproducible and reliable [Tamada et al., 1995]. Other studies have shown a high correlation between IF, capillary, and plasma glucose levels in diabetic subjects. A correlation coefficient of 0.95 was obtained when plasma glucose was compared with IF glucose, and a correlation coefficient of 0.87 was obtained when plasma glucose was compared with capillary glucose in a group of 17 diabetic subjects. Capillary blood glucose levels, therefore, were shown to have no higher correlation with plasma glucose levels than the values obtained using IF [Bantle and Thomas, 1997]. Although it has been argued that IF glucose concentrations are significantly different from plasma

values and that IF levels may lead or lag the plasma values by as much as 45 minutes, many of these latter studies have technical variations in methodology including different implantation sites, device operation, and sensitivity. All of these factors have influenced the results achieved. When more careful and consistent measurements were done, it appears that IF does in fact reflect accurately the glucose levels in both capillaries and plasma [Rebrin et al., 1999].

Many different methods of testing IF have been proposed. The majority of these require the extraction of a small amount of IF for external testing. One popular method is to form a small erosion on the outer layer of skin using a mild suctioning technique to expose the IF for collection. The advantage of this approach is that after testing, the outer layers of the epidermis regenerate rapidly. Svedman and Svedman (1998) used this method to study both diabetic and non-diabetic subjects, in which a skin erosion was formed in 15-70 minutes. Another suction cup was then placed over the erosion in order to extract the IF. This process took another 20 minutes and extracted 5 μl of IF. The glucose level in this small volume of IF was measured, and results were compared with capillary and plasma glucose levels to verify their accuracy. It was found that this method caused minimal discomfort consisting of a light tingling sensation during the formation of the erosion. As the same site was used for IF extraction over a 6 day period, it was found that on the final day less than 5 μl could be extracted from some subjects. There was a slight pigmentation of the skin after testing which the subjects considered trivial [Svedman and Svedman, 1998]. Other studies using this technique showed similar extraction rates

and confirmed a 10 to 20 minute delay in IF glucose levels as compared to blood glucose levels [Kayashima et al., 1992; Kimura, 1993].

Another method of accessing IF is by applying a small current across the skin, creating a perm-selective membrane which allows certain ions to flow through the skin. The current used in this method is 2.5 A/m^2 applied for 60 minutes. It was found that periods of less than 15 minutes yielded contaminated samples which skewed test results. The results indicated the average blood glucose level during the sampling interval and not the current blood glucose level. Patients experienced mild tingling sensations from the application of current to the skin surface, but this generally lasted no longer than 30 minutes [Rao et al., 1995].

Although these methods are promising, they are still not ideal. They do not allow for long-term continuous monitoring of glucose and IF collection and testing time is long (15-20 minutes). Many companies are working to find more viable methods for a commercial product. The current leaders in this field are MiniMed, Cygnus, Integ, TCPI, and SpectRx. SpectRx uses a laser to create micropores in the skin to provide access to IF. Integ's technology creates small holes in the dermis through which IF samples are collected. Current MiniMed and Cygnus technologies were discussed in Section 3.1. TCPI uses a transdermal patch which is able to draw out the IF [Mendosa, 1997]. While many of these products look promising, none has yet received FDA approval. In addition, high production costs have hampered the development of these new devices.

Chapter Four: Theory of Sensor Fabrication and Measurement

4.1 Enzyme Kinetics

One of the most successful types of sensors developed for glucose detection uses an enzyme to catalyze an electrochemical reaction (see Section 4.2). It is therefore necessary to understand the chemical kinetics of such a reaction.

A catalyst is defined as a substance that speeds up the rate of a reaction and is regenerated to its original state after the reactant has been converted to products. All catalyzed reactions lower the required activation energy of the reaction. Enzyme catalysts are also able to catalyze certain reactions selectively and discriminate against others. The enzyme itself is a protein with one or more active sites where the reaction takes place. The active site is thought to have a rigid structure while the substrate molecule has a complementary structure, a feature that may account for the selective abilities of the enzyme [Chang, 1981].

An important aspect of enzyme kinetics is the initial rate (v_o) of a reaction. This quantity is important for several reasons:

1) The rate of the reverse reaction needs to be minimized. This rate increases with the product concentration.
2) During the reaction, there may be significant heat or pH changes that may change the reaction rate.
3) The product may bind to the enzyme and inhibit its ability to function.

4) The initial rate corresponds to a known fixed concentration of substrate which will decrease with time.

It can be shown that at low concentrations of the substrate [S], the reaction rate increases rapidly and then gradually levels off at higher concentrations. The relationship between rate and concentration can be defined as:

$$v_o = \frac{a\,[S]}{b + [S]} \quad ,$$

where a and b are constants [Chang, 1981].

Enzyme reactions using a single substrate can be expressed as:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \ ,$$

where the substrate S combines with the enzyme E, forming an enzyme-substrate complex. This complex subsequently breaks down into the products P and the freed enzyme. k_1, k_{-1}, and k_2 are kinetic constants of the particular reactions [1/s]. A short time after the enzyme and substrate are mixed, the concentration of the enzyme-substrate complex reaches a steady-state value. Using this steady-state approximation and the rate equation above, the Michaelis-Menten equation can be derived:

$$v_o = \frac{V_m\,[S]}{K_m + [S]} \quad ,$$

where V_m is the maximum rate of the reaction and K_m is the Michaelis-Menten constant. K_m is defined as:

$$K_m = \frac{k_{-1} + k_2}{k_1} \ .$$

When the initial rate is half of the maximum rate, the value of K_m is equal to the substrate concentration [Chang, 1981].

It is also important to note that the activity of many enzymes varies with pH. These enzymes have a characteristic pH at which they have a maximum activity level. Varying the pH in either direction from this optimal point will decrease the enzyme activity. Enzymes that are active within cells generally have an optimal pH close to the range of the cells' normal functioning pH [Chang, 1981].

4.2 Amperometric Sensor for Glucose Detection

The most common type of sensor used for glucose detection is an electrochemical sensor that uses the enzyme glucose oxidase (GOx) to catalyze a reaction with glucose. Glucose can be detected amperometrically by a two-step chemical process. GOx catalyses the oxidation of β-D-glucose, in which the glucose breaks down into hydrogen peroxide and gluconic acid. Electrons are then extracted from the hydrogen peroxide through a subsequent oxidation step (Figure 4.1) [Wilkins, 1996].

$$\text{GLUCOSE} + O_2 \xrightarrow{\text{GLUCOSE OXIDASE}} \text{GLUCONIC ACID} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{OXIDIZED by ELECTRODE}} 2H^+ + O_2 + 2e^-$$

Figure 4.1: Chemical reactions involved in glucose detection.

A circuit can now be set up to measure the resulting current produced from this reaction. The current measured in this circuit will be linearly proportional to the amount of glucose present in a fluid such as the IF (Figure 4.2).

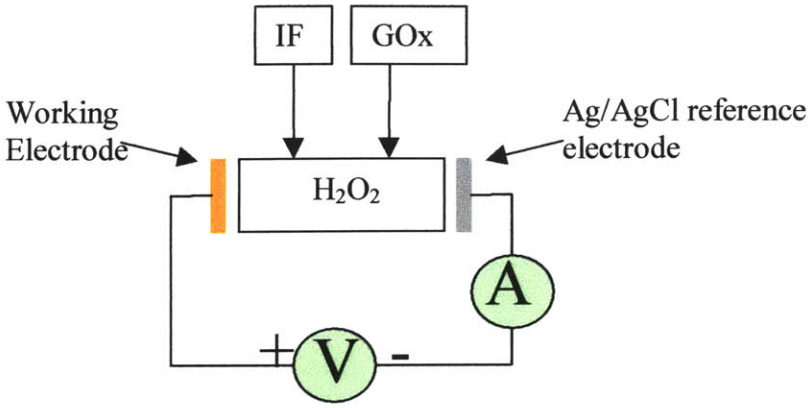


Figure 4.2: Circuit to detect amount of glucose in IF.
Ag/AgCl = Silver/Silver Chloride.

This is the type of method used by the researchers cited in Chapter 3. With different reference electrodes, different activating potentials are required, as shown in Table 3.1.

4.3 Using Microelectrodes for Chemical Sensing

The use of microelectrodes for chemical sensing has opened up a new realm of possibilities in sensor development. The term "microelectrode" is generally used for electrodes having at least one dimension not greater than 25 μm. With the ability to miniaturize electrodes, the potential now exists to explore certain microscopic domains, measure local concentration profiles, detect microflow, and analyze very small sample volumes. Studies have expanded to single cell recordings in the brain and high resolution characterization of surfaces [Wang, 1994].

Microelectrodes have many characteristics that are lacking in standard electrodes. Among these are a relative immunity from certain resistance effects, high rates of mass transfer which yield higher sensitivity, and high signal-to-noise ratios [Glass et al., 1990]. The improvement in signal-to-noise ratios for microelectrodes is due to the fact that the electrical signal is proportional to the total geometric area, whereas the noise is proportional to the active element area only [Penner and Martin, 1987]. Microelectrodes can also be used for measurements in unconventional environments without needing additional supportive electrolytes. Many of these advantages become even more apparent when microelectrodes are used in arrays [Glass et al., 1990].

Currents at these types of electrodes are very small, and therefore the microelectrodes are essentially nondestructive to the item under observation. The small current also allows microelectrodes to be used in solutions with very high resistance which were previously inaccessible to conventional electrodes [Wightman, 1981]. The double-layer capacitance of microelectrodes is significantly smaller than that of conventional electrodes, and so they form electrochemical cells with small RC time constants. This feature allows for very high speed voltammetric experiments, at scan rates higher than 10^6 V/s. This permits the study of kinetics of high speed electron transfer and associated chemical reactions. In addition, the mass transport rate to and from an electrode increases as electrode size decreases. Not only does this enhance the signal-to-noise ratio, but it also means that steady-state currents are obtained with very short settling times [Wang, 1994].

Microelectrodes can be fabricated in a variety of shapes. The common feature of all styles is that the electrode diameter is significantly smaller than the diffusion layer at the electrode surface. Cylindrically shaped microelectrodes can be several millimeters long and yield a large current while maintaining the other benefits of a microelectrode [Wang, 1994].

Since the benefits of microelectrodes become amplified when they are used in an array format, which includes a proportional increase in the current detected due to the increased number of electrodes, a large potential exists for the use of such electrode arrays in conjunction with drug delivery techniques. Specifically, since the IF lies on average about 50 μm from the skin surface, use of this type of micro-array to access the IF could lead to the development of a potentially painless glucose sensor and insulin delivery system. Only a few groups have tried micro-array formats for drug delivery. A group at the Georgia Institute of Technology has started to publicize success with drug delivery using micro-needle arrays [Henry et al., 1999]. The goal of their work was to puncture the stratum corneum with a micro-needle array to increase the permeability of the skin. The array was then removed and the required drug (calcein) was applied to the punctured skin surface. The array was fabricated in silicon using a reactive ion etching technique. The micro-needles formed in this manner were 180 μm in height and 80 μm in diameter. It was found that these needles could pierce the skin with a pushing force estimated at 10 N. When these needles were inserted and left imbedded in the skin, permeability was seen to increase by a factor of 1000 over non-penetrated skin. When the array was removed after 10s, permeability increased by a factor of 10,000, and when

the array was removed after 60 minutes, permeability increased by a factor of 25,000. None of the subjects experienced any pain associated with the needle insertion [Henry et al., 1999]. This work shows that micro-needle arrays can be used successfully to access IF and also that use of these arrays could provide a viable means for transdermal drug delivery.

4.4 Cyclic Voltammetry as an Analysis Tool

Cyclic voltammetry is one of the most popular tools for qualitatively analyzing electrochemical reactions. It is able to provide information on the thermodynamics of the redox process, the kinetics of the electron-transfer reactions, and on other coupled chemical reactions and adsorption processes. It can also locate the reaction's redox potential quickly. Cyclic voltammetry is done by scanning the potential of a stationary working electrode with a specified potential waveform. Most commonly, this waveform is triangular, although cyclic voltammetry can be performed with other wave shapes, including sinusoids. A potentiostat is then used to measure the current during the potential sweep. The resultant plot of current versus potential for the entire cycle (or for multiple cycles) is called a cyclic voltammogram. Peaks occur in the voltammogram when the redox potential is reached. These peaks are caused by the formation of a diffusion surface near the electrode surface. The peaks are characteristic of the particular reaction taking place, and they may also shift or change shape in solutions of varying concentrations [Wang, 1994]. Cyclic voltammetry was done with a H_2O_2 sensor to illustrate this peak shift (Figure 4.3).

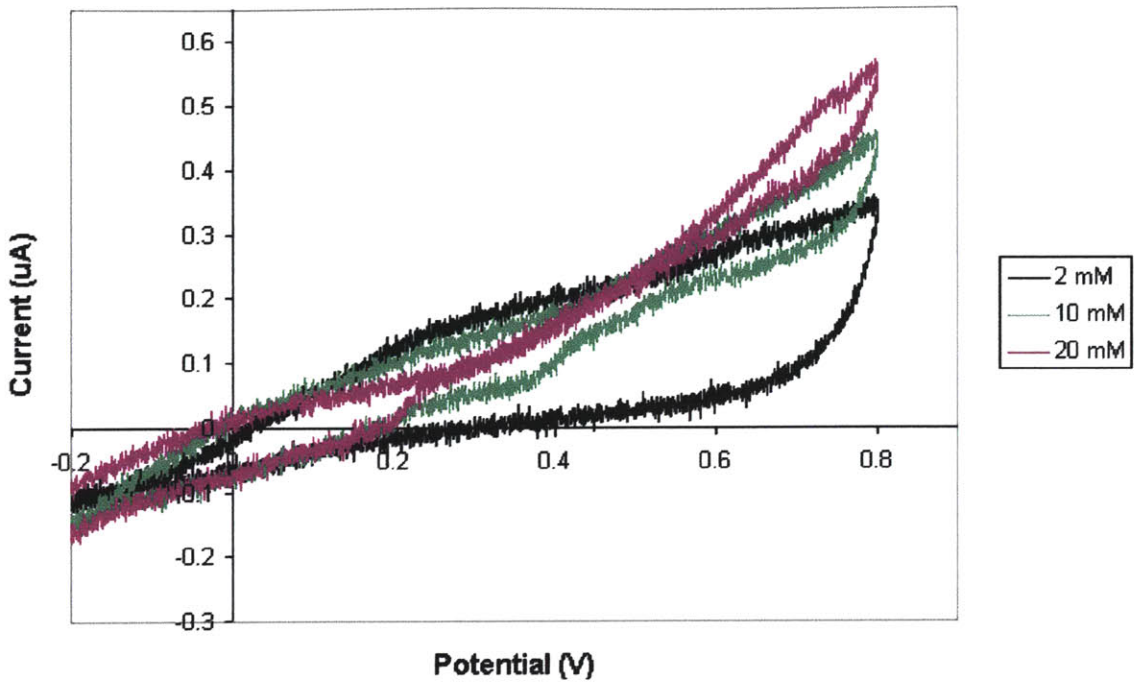


Figure 4.3: Cyclic voltammogram of a hydrogen peroxide sensitive electrode as the concentration of H_2O_2 is increased.

Several parameters characterize the voltammogram. At 25°C, the peak current for a reversible reaction is provided by the Randles-Sevcik equation:

$$i_p = (2.96 \times 10^5)\, n^{3/2}\, A\, C\, D^{1/2}\, \nu^{1/2} \quad ,$$

where n is the number of electrons transferred, A is the electrode area (m^2), C is the concentration (M), D is the diffusion coefficient (cm^2/s), and ν is the scan rate (V/s). Another characterizing feature is the separation between peak potentials which can be used to determine the number of electrons that have been transferred. This separation is given by:

$$\Delta E = \frac{0.059}{n}\ \text{V}.$$

A fast one-electron process will have a ΔE of about 59 mV. These peak potentials are independent of the scan rate used for the experiment. In reversible processes that consist

of multielectron transfers, the cyclic voltammograms show several distinct peaks that depend on the separation of the potentials for the individual steps. For an irreversible process, the individual peaks will have smaller amplitudes and be widely spaced. A completely irreversible system will display shifts in the peak potentials as the scan rate changes [Wang 1994].

Cyclic voltammetry is very useful for the qualitative diagnosis of chemical reactions. Changes in the shapes of the voltammogram can be used to identify the reaction pathways and indicate the presence of reactive intermediates. Information on the rates of reactions can also be obtained by adjusting the scan rate used. Common scan rates are usually between 0.02 and 200 V/s [Wang, 1994]. Faster scan rates of up to 10^6 V/s can be used when microelectrodes are used instead of more conventionally sized electrodes. This ultrafast cyclic voltammetry has three major applications. The first of these is the ability to detect short-lived species generated by the reaction (with life times as short as 25 ns). Subsequent chemical reactions due to these species can then be investigated. Therefore, by using high speed cyclic voltammetry a picture of the transient species produced by a chemical reaction is obtained, which is information that would be lost with the slower scan rates of conventional electrodes. The second application is the measurement of rate constants for fast interfacial electron transfer. This is achieved by using fast scan rates that do not allow the diffusion layer to form completely, and so currents that reflect planar diffusion at the electrode surface can be obtained. The third application is the determination of the formal potential for reactive products of electron transfer [Penner et al., 1991].

Cyclic voltammetry can be coupled with microelectrodes to characterize in-vivo chemical reactions such as the detection of glucose. The resulting voltammograms can be compared to determine the presence of various reactants in order to deduce the actual reaction that is taking place. In in-vivo applications, these differences in voltammograms can be used to help identify possible signal interferents that may affect measurements of levels of fluid such as glucose.

Chapter Five: Sensor Fabrication Experimental Results

5.1 Sensor Fabrication Experiments

Since amperometric glucose sensing has been shown to be the most accurate method available to detect glucose, it was used for the sensors of this research. Initially, glucose sensor fabrication followed an electrochemical deposition method described by Yang et al. (1998) (see Table 3.1). In this method, the outer surface of a needle is made into an amperometric glucose sensor by coating it with various layers. The coatings are formed using a series of potentiostatic (constant potential) and galvanostatic (constant current) chemical depositions. This particular method was chosen because the resulting sensor was stated to have a quick response time to glucose (10 seconds), a low operating potential (+0.25 V), sensor stability lasting three months, immunity to chemical interferents, a straightforward fabrication method, and the potential for miniaturization [Yang et al., 1998]. Using this method, 1.02 mm diameter (18-gauge) stainless steel needles were consistently sensitized to hydrogen peroxide, the intermediary reaction product, as seen in the data collected from one multi-layered needle (Figure 5.1). The results show a distinct and constant rise in current as the hydrogen peroxide concentration increases which follows a second-order model:

$$i = -0.54\ C^2 + 22.18\ C + 6.92\ ,$$

where i is current in μA and C is concentration in mM. This increase in current was evident throughout the expected biological range of hydrogen peroxide concentrations (0 – 20 mM) and the rise time of the response was less than 5 s. It is expected that this response will reach a saturation point as the concentration greatly exceeds 20 mM due to the limited amount of sensor area and accessibility of rhodium.

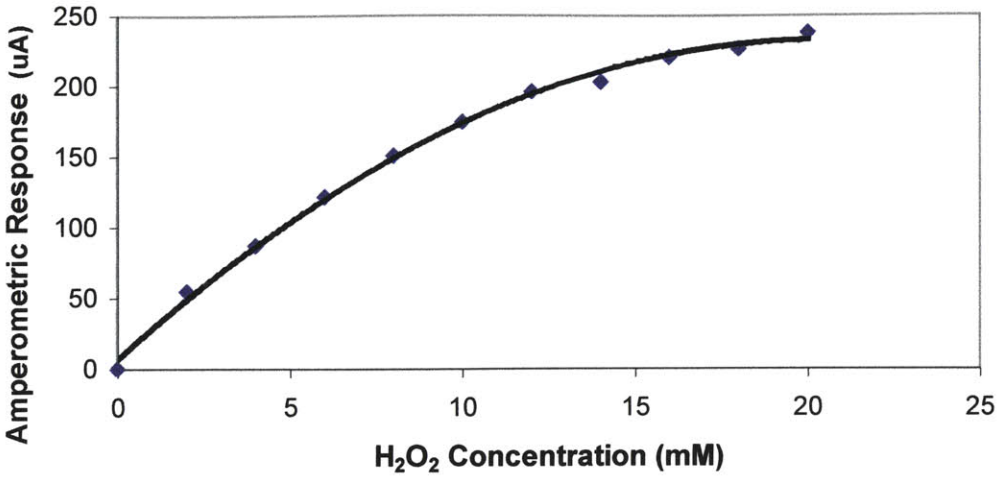


Figure 5.1: Sensor response to hydrogen peroxide.

The H_2O_2 sensor was coated with GOx in a bovine serum albumin solution in order to sensitize it to glucose. Despite the sensor's performance when exposed to H_2O_2, only weak and inconsistent responses to glucose were elicited using this procedure. Over the physiological range of glucose (0 – 20 mM) the maximum response shown by this sensor was an increase of less than 3 μA (Figure 5.2). The expected response over this range according to the data reported by Yang et al. (1998) was approximately 12 μA.

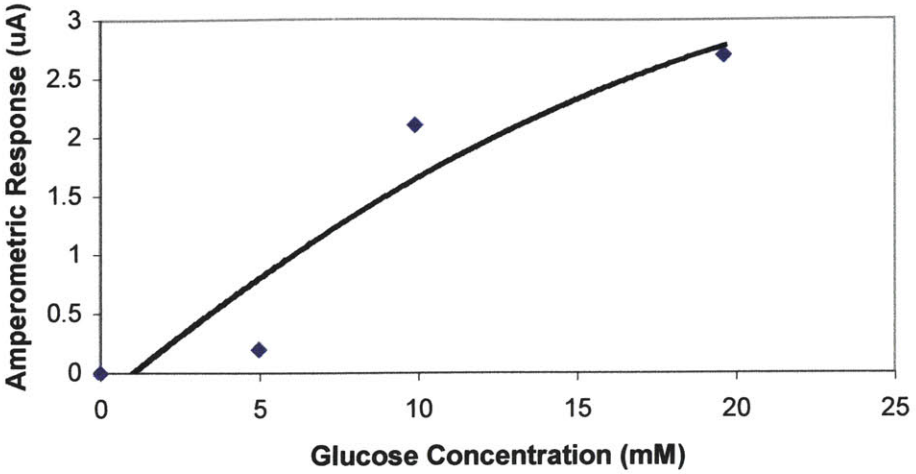


Figure 5.2: Sensor response to increasing glucose concentrations. Current readings at 0 mM glucose concentration were considered as background noise and subtracted from all subsequent values in that trial.

By trying many variations of the fabrication procedure, such as covering the sensor with Teflon shrink wrap, increasing the applied current, using a higher activity level of GOx, and making solutions of GOx and 1,3 diaminobenezene, the sensor could be made to respond to various glucose concentrations. The responses, though, showed no consistency during subsequent trials with a single needle. These results indicated that either an insufficient amount of glucose oxidase was immobilized on the needle surface yielding a weak amperometric response or that the coating was not uniform along the needle surface. It is also possible that the inner surface area of the hollow needle influenced the surface area calculations to a greater extent than expected. The procedure was tried again using a more active strain of the glucose oxidase as well as sealing the hole in the needle and reducing the total surface area by coating part of the needle with Teflon. None of the sensors fabricated in this way showed any sensitivity to glucose.

At this point, alternative methods were sought that would still retain the desired characteristics of a quick response time, low operating potential, sensor stability, immunity to chemical interferents, easy fabrication, and potential for miniaturization. Some of the resulting sensors did respond to glucose, but their responses were again very small and there was no consistency across trials with each sensor. In some tests, the sensor response seemed to decrease with subsequent trials, and questions arose as to the long-term stability of these sensors (Figure 5.3).

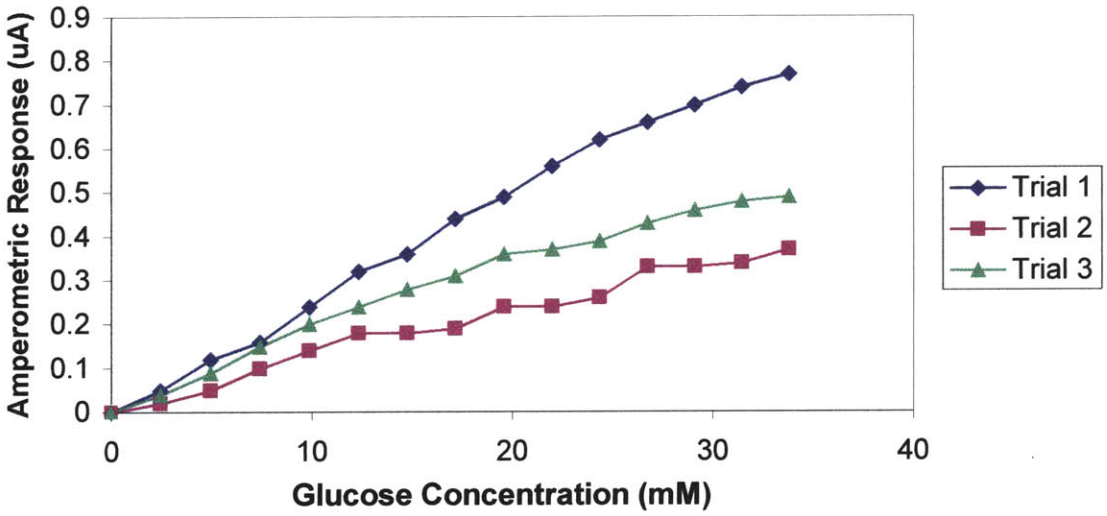


Figure 5.3: Sensor response to increasing glucose concentrations obtained from a sensor fabricated using the method of Wang et al. (1992). Current readings at 0 mM glucose concentration were considered as background noise and subtracted from all subsequent values in that trial.

One explanation for this decreased response is that excess glucose oxidase was on the needle surface immediately after fabrication and was then washed away during the trials. Reliable sensors were eventually fabricated using a method based on one proposed by Bindra et al. (1991). The fabrication procedure for these sensors will be described in the next section.

5.2 Final Sensor Fabrication Procedure

5.2.1 Materials

Materials used for the final sensor fabrication were nitric acid (see Section 5.2.2 for solution concentrations), hydrochloric acid 37%, rhodium atomic absorption standard solution, cellulose acetate (39.8 wt. % acetyl content), type VII-S glucose oxidase from aspergillus niger (E.C. 1.1.3.4, 200,000 units/g), 0.1 M phosphate-buffered saline (PBS) pH 7.4, glutaric dialdehyde 25 wt. % solution in water, Nafion trimethylsilylated, hydrogen peroxide 30%, and glucose (Sigma-Aldrich, St. Louis, MO). Silver/silver chloride (Ag/AgCl) 0.5 mm diameter reference electrodes (part #EPO5, World Precision Instruments, Inc., Sarasota, FL) were used. All water used to prepare solutions was deionized. PBS was prepared at least 24 hours in advance to allow for mutarotation. When glucose is made into solution, it breaks up into α-D-glucose and β-D-glucose. Initially, the concentrations of these two types of glucose are not stable. Since the glucose detection method used detects only β-D-glucose, the glucose solutions were prepared 48 hours in advance to allow for stability in the β-D-glucose concentration, and they were replaced every 7 days.

5.2.2 Fabrication Procedure

A 0.51 mm diameter stainless steel wire was cleaned by washing in acetone and soaking in 110 g/l nitric acid solution for a minimum of 30 minutes. The wire was then rinsed in stirred distilled water for 4 hours, and finally rinsed in stirred PBS for at least 2

hours. After this it was submerged in a 10 ml beaker of 10% HCl for cathodic pretreatment. A rhodium wire was used as the counter electrode, and a current density of -50 A/m^2 was applied for 2 minutes. Rhodium was galvanostatically deposited onto the needle surface immediately following the HCl soak by submerging the wire and rhodium electrode into a 10 ml beaker of stirred 0.35 mM rhodium absorption solution for 3 minutes under a current density of -50 A/m^2.

Before any testing was done, the Ag/AgCl electrode was tested for proper stability. The electrode was shorted to an identical electrode and submerged in 0.1 M KCl for approximately 30 minutes, after which both electrodes were rinsed in distilled water. The two electrodes were submerged in a beaker of PBS and the voltage between them was recorded. The electrode was considered experimentally viable if the voltage difference was less than 3 mV.

After testing the Ag/AgCl electrode for stability, the needle was tested for hydrogen peroxide sensitivity to confirm deposition of rhodium on the needle surface. The needle was immersed in 16 ml of stirred PBS using the Ag/AgCl electrode as a counter electrode. A potential of +0.25 V was applied to the needle. Steady state currents were recorded after every 2 mM addition of H_2O_2 (3.6 µl increments). An acceptable rhodium coating would exhibit a rise in current similar to that shown in Figure 5.1 over the biological range of 0-20 mM H_2O_2 concentrations.

After the H_2O_2 test, the needle was dipped into a 5% cellulose acetate solution (CA) in 50% acetone – 50% ethanol for 10 s, exposed to the vapor above the CA for 5 s, and dipped again into the CA for another 10 s. The CA coated needle was dried in air at room temperature for approximately 2 minutes. The needle was then placed in a soak of deionized water for at least 6 hours to permit displacement of entrapped solvent in the membrane pores. The water in the soak was replaced once during the 6 hour period. After this, 20 mg of GOx was added to 1 ml of PBS in a plastic container and stirred gently so as to retain the activity level of the GOx. The needle was dipped into this GOx solution and allowed to dry for 30 minutes at room temperature while it was held horizontally. The needle was then placed in a 2% aqueous glutaraldehyde solution for 1 hour, after which it was rinsed in deionized water. It was then dried in air for 1 hour before any testing. The final sensor was stored in phosphate buffered saline at 0° C.

An Ag/AgCl electrode was again used as the reference electrode to determine the sensor's ability to detect glucose. The sensor and electrode were placed in 20 ml of stirred PBS, a +0.25 V potential was applied to the sensor, and 2.5 mM increments of glucose were added. The steady state currents achieved with each glucose addition were recorded.

A Nafion coating was added to the external surface of the glucose sensor to enhance sensor sensitivity to glucose by creating a membrane impenetrable to other chemical interferents and to enhance long-term sensor stability. Solutions of 0.5%, 2.5%, and 5% were made using 1:1 isopropyl alcohol and deionized water as the solvent. The

sensor was dipped in the 0.5%, 2.5%, and 5% solutions sequentially, with 15 minutes to dry at room temperature between each dip. The sensor was then left to dry at room temperature overnight.

5.3 Experimental Fabrication Results

During the fabrication procedure, the various layers of coatings can be seen visually on the needle surface. While the initial uncoated wire is silver colored and shiny, the rhodium coating is a matted black color. The sensor then has a smooth white surface after the CA and GOx layers have been deposited (Figure 5.4).

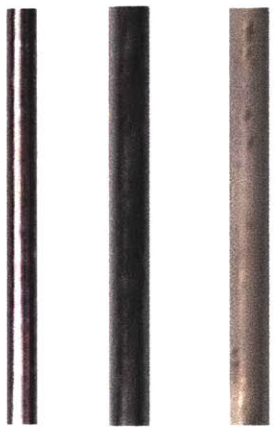


Figure 5.4: Sensor surface at various stages of coating. The sensor on the left has no coatings, the one in the middle is coated with rhodium, and the one on the right it coated with GOx and CA.

By combining elements of the process described by Bindra et al. (1991) with the hydrogen peroxide sensor successfully made using the method of Yang et al. (1998), sensors were formed which showed potential for use as a stable glucose sensor with a large amperometric response. These glucose sensors displayed a response to increasing

glucose concentrations in the microamp range with good response consistency over a two day period (Figure 5.5).

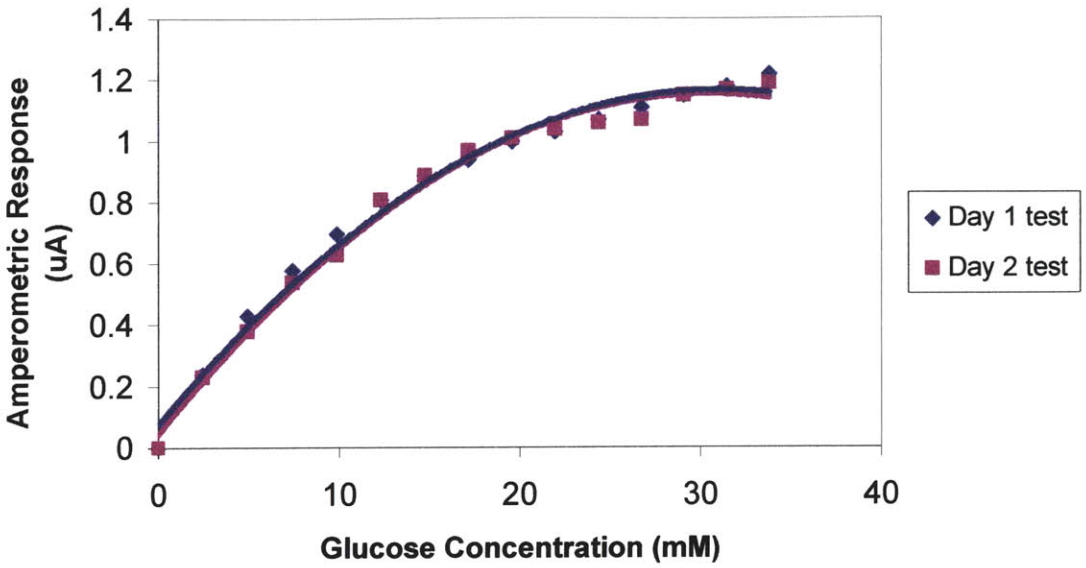


Figure 5.5: Sensor response to increasing glucose concentrations. Current readings at 0 mM glucose concentration were considered as background noise and subtracted from all subsequent values in that trial.

A second-order model was fitted to these data using a least squares fit routine:

$$\text{Day 1: } i = -0.0011\ C^2 + 0.0705\ C + 0.0742,$$

$$\text{Day 2: } i = -0.0012\ C^2 + 0.0728\ C + 0.0434,$$

where i is current in μA and C is concentration in mM. Due to the close similarities of these two models, it is possible to derive an average model to describe the general system:

$$i = -0.00115\ C^2 + 0.0717\ C + 0.0588.$$

The currents recorded from various sensors showed some variation from sensor to sensor as indicated in Figure 5.6, although most sensors seem to respond within 10% of the mean averaged across all fabricated sensors.

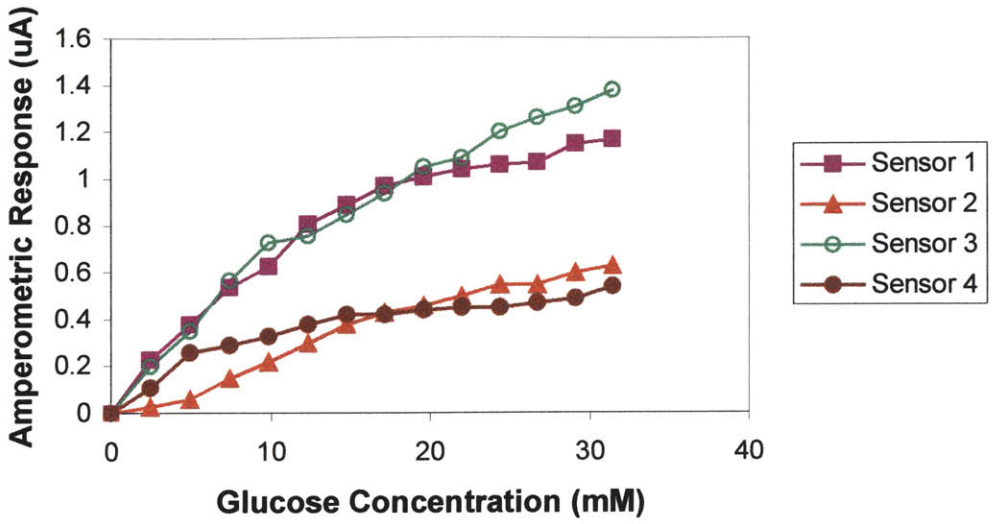


Figure 5.6: Sensor response to increasing glucose concentrations for various sensors. Current readings at 0 mM glucose concentration were considered as background noise and subtracted from all subsequent values in that trial.

As expected, after the Nafion coating was deposited on the sensor surface there was a dramatic decrease in the amperometric response of the sensor (see Figure 5.7).

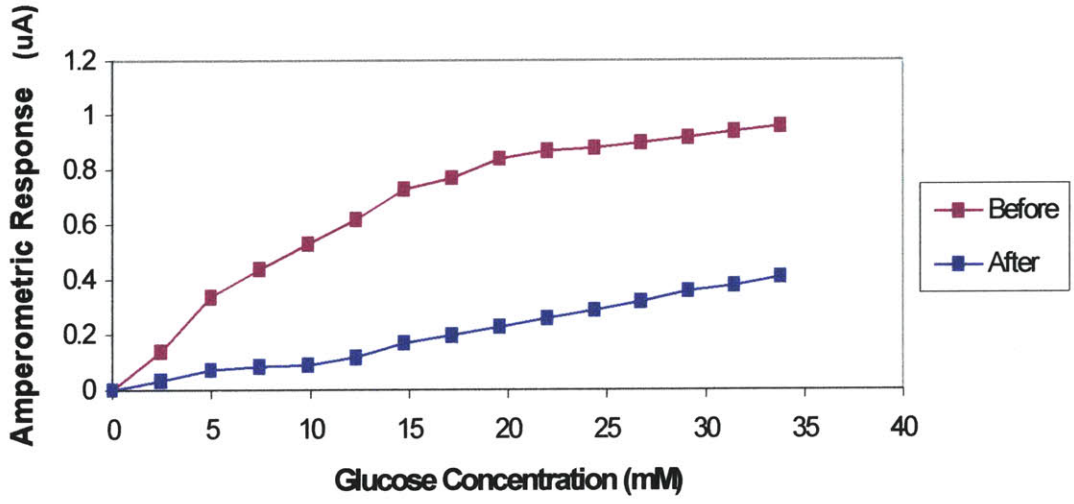


Figure 5.7: Sensor response to increasing glucose concentrations before and after application of the Nafion coating. Current readings at 0 mM glucose concentration were considered as background noise and subtracted from all subsequent values in that trial.

Unfortunately the coating did not prolong the lifetime of the sensor as intended. The next day the sensor showed a very slight and unstable response to increasing glucose concentrations. Yang et al. (1998) had reported that sensors with Nafion coatings remained stable for up to 3 months.

5.4 Cyclic Voltammetry Analysis of Sensors

Two of the major interferents to electrochemical glucose sensors are ascorbic acid and acetaminophen. It would be useful, therefore, to find a way to eliminate the signal due to these interferents so that an accurate reading of glucose levels can be obtained even in their presence. Since cyclic voltammograms are specific to each species, it is possible to use cyclic voltammetry to compare the signals of specific tests and determine which signals are due to the interferents and which are the desired signals.

Cyclic voltammetry was performed on hydrogen peroxide sensors to determine whether this method was viable for this application (Figure 5.8).

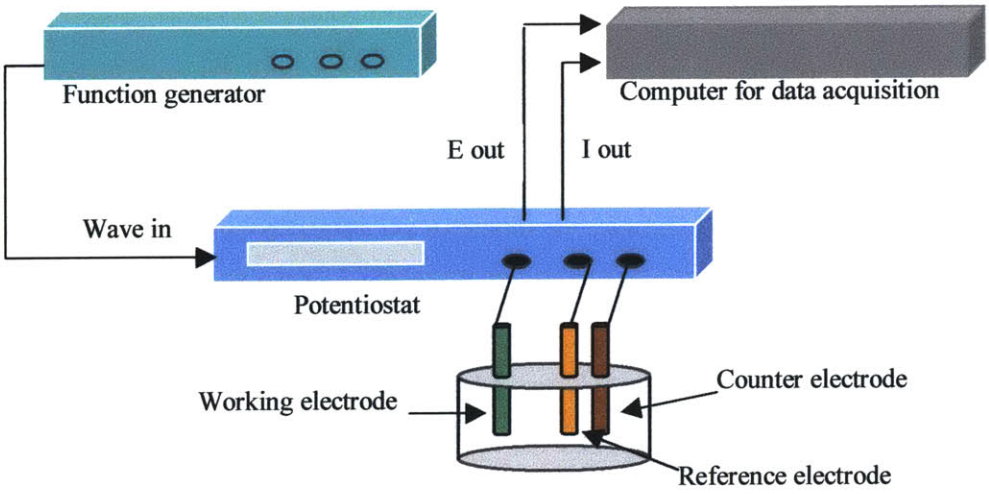


Figure 5.8: Diagram of cyclic voltammetry apparatus.

A platinum-iridium wire was used as the counter electrode, and the Ag/AgCl electrode described above was used as the reference electrode. The input signal was a sine wave with an amplitude of 0.8 V and a frequency of 1 Hz. Data were sampled at a frequency of 1000 Hz. The electrodes were placed in a stirred bath of PBS and the concentrations for H_2O_2, ascorbic acid, and acetaminophen were increased in separate trials by adding small amounts of these substances using a pipette in order to simulate concentrations expected in a human subject (Table 5.1).

Biological Concentrations of Various Species

Species	**Max. Concentration**
H_2O_2	20 mM
Ascorbic Acid	100 μM [Kallner, 1981]
Acetaminophen	2.65 mM [McNeil, 1999]

Table 5.1: Maximum concentrations of H_2O_2, ascorbic acid, and acetaminophen used for cyclic voltammetry tests.

The results from these tests are shown in Figures 5.9, 5.10, and 5.11.

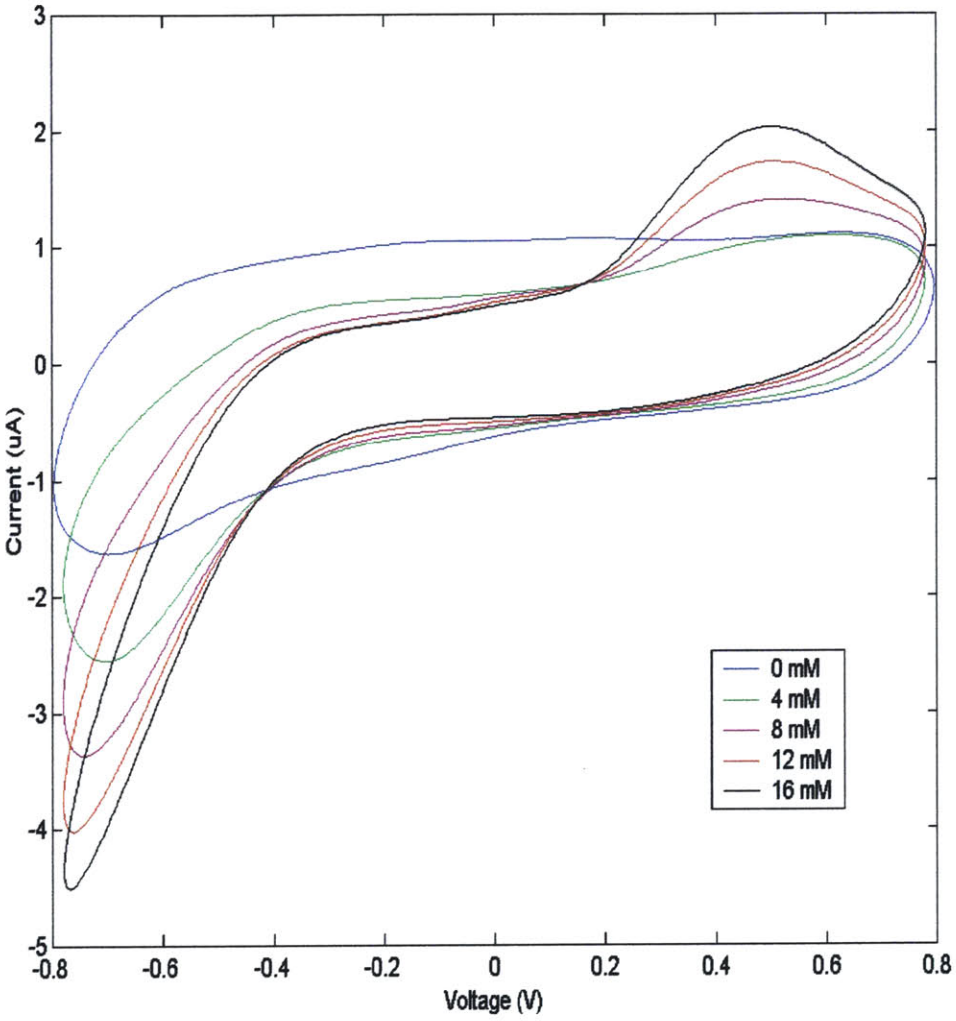


Figure 5.9: Voltammogram for increasing concentrations of H_2O_2 in a PBS bath.

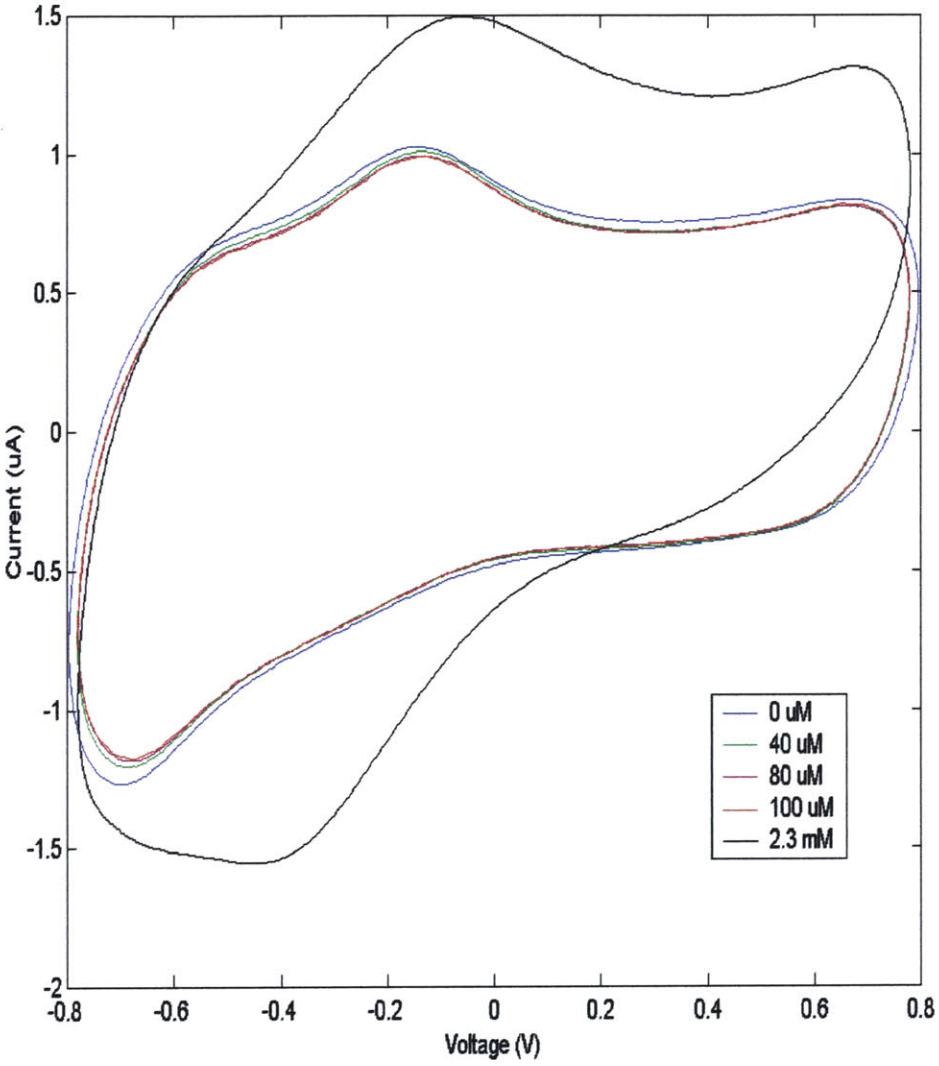


Figure 5.10: Voltammogram for increasing concentrations of ascorbic acid in a PBS bath.

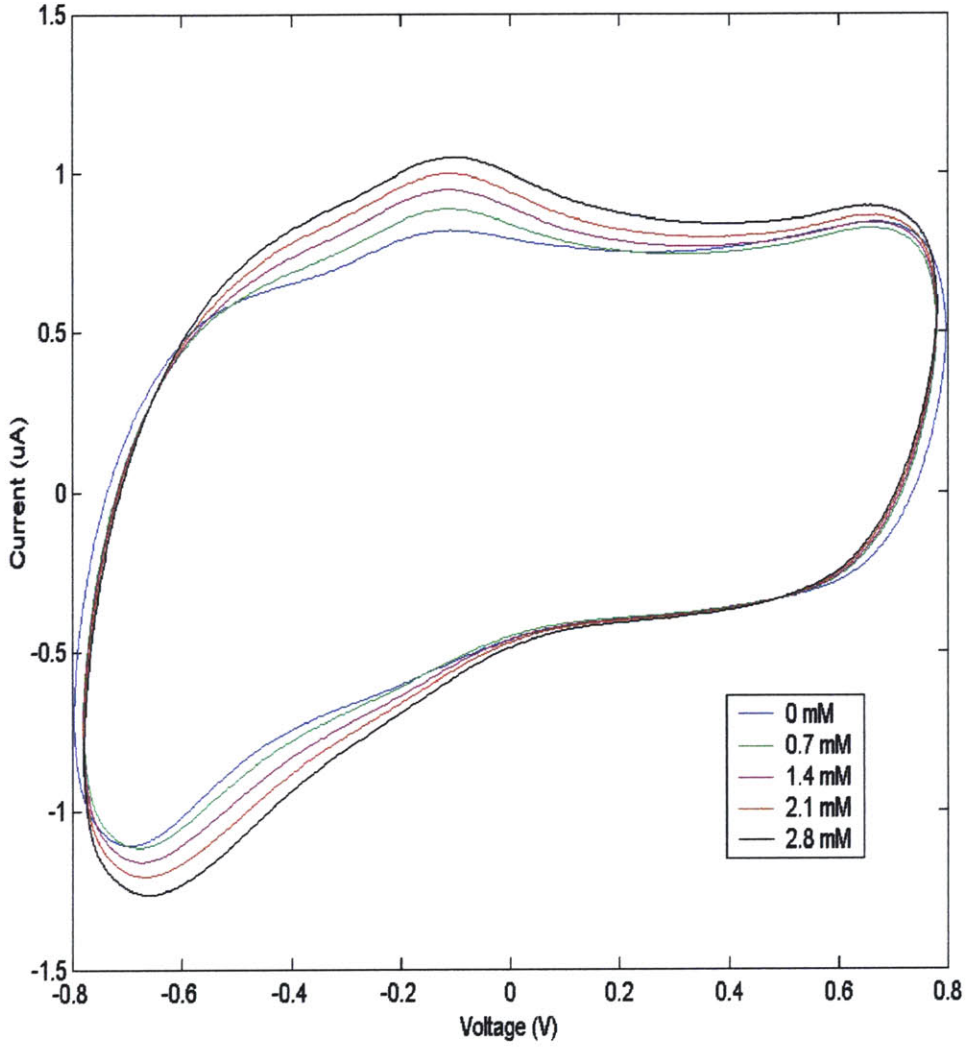


Figure 5.11: Voltammogram for increasing concentrations of acetaminophen in a PBS bath.

A comparison of these three voltammograms indicates that there are clear differences between them. First, increased concentrations of H_2O_2 cause the peaks around 0.5 V and –0.8 V to change dramatically in amplitude as the central area of the curve flattens. The magnitude of this change in the peaks is not evident in either of the other two voltammograms. Secondly, increased concentrations of ascorbic acid do not

seem to have a large influence on the voltammogram until the concentration has reached a level well outside the biological range of 100 μM. Even when the voltammogram is affected by the increased concentration of ascorbic acid, it appears that ascorbic acid magnifies the entire curve without greatly affecting any particular segment. Finally, the voltammogram for acetaminophen shows an increase at the peak found at about –0.2 V as concentration is increased. This increase is not seen in the H_2O_2 curves at all.

5.5 Conclusions

Using a method for sensor fabrication based on the work of Bindra et al. (1991) and Yang et al. (1998), needles were consistently sensitized to the addition of glucose. The variation in the amperometric response from sensor to sensor indicates that a calibration method will need to be used for each sensor. These sensors showed stability over at least a 48 hour period. They also retained the desired characteristics of a fast response time (less than 10 seconds), a low operating potential (+0.25 V), and a process that can be miniaturized. As such, it appears that this is an appropriate method for sensor fabrication. Further research is needed to determine an appropriate coating technique for these sensors to enhance their stability and to block other interferents from the amperometric response.

The analysis of the voltammograms obtained from the various interferents indicates that the three substances show very different voltammograms with increased concentrations. As such, it seems possible to separate out signals from interferents in order to obtain a pure signal from the H_2O_2 reaction (and subsequently the response to

glucose concentration) using such cyclic voltammograms. By doing Fourier transforms on these data, it may also be possible to find peaks which could be used to differentiate further between the various signals.

Chapter Six: Micro-needle Array Fabrication

6.1 Justifications for Array Design

The main objective in the glucose sensor design is to create a sensor that measures glucose levels in such a manner as to eliminate or greatly minimize the pain and discomfort experienced by the patient. The IF lies directly under the outer layer of skin above the nerve bundles and reflects the body's glucose levels, as discussed in Chapter 3. By using a micro-needle to access this fluid without penetrating to the depth of the nerve endings, glucose measurements can be obtained painlessly.

The epithelial region, however, also contains many large cells. With a single micro-needle sensor, there is a high probability that the needle will be imbedded in the epithelial cells rather than the IF. In order to avoid this problem, an array of micro-needles was designed. In this case, many needles will be in contact with the IF, and a glucose reading can be taken by summing the signals from the entire array. The array design increases the sensor surface area (as compared to that of a single micro-needle) that can be embedded in the IF and thus yields a higher amperometric response.

There are other advantages associated with choosing an array of many micro-needles over a single "macro-needle." As electrode dimensions decrease, so do the associated current and double layer thickness, whereas the rate at which chemical species diffuse towards the electrode increases. This means that microelectrodes respond faster, with higher signal to noise ratios, less sensitivity to convection, and in more highly resistive solutions as compared to macroscale electrodes (such as our current single needle

sensors) [Andrews and Harris, 1998; Wightman, 1988]. Many of these benefits are amplified when the micro-needle is used in an array format.

6.2 Initial Array Design

Considerable effort has been devoted to developing a low cost method of manufacturing the micro-needle array. The initial approach consisted of forming a microhole array in a base plate of Delrin and then placing and securing the micro-needles into these holes (Figure 6.1).

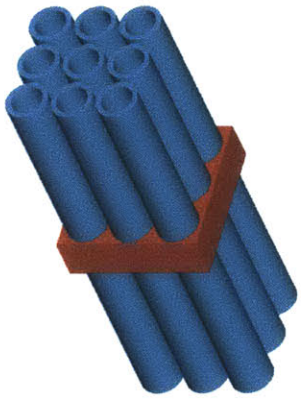


Figure 6.1: Initial micro-needle array with Delrin baseplate.

Each of the 200 holes has a diameter of 250 μm with a 500 μm center-to-center spacing. Delrin was chosen for two reasons: 1) its insulating characteristics allow for each needle to be isolated electrically which will be necessary with a two electrode sensing technique, and 2) it is a soft material in which small holes can be machined at easily attainable speeds. Parameters for drilling the holes of this array were based on the work described by Nielsen (1998).

Machining of the micro-hole array was done on a computer numeric controlled (CNC) HAAS machine (model VF-OE). Problems arose in the fabrication of the micro-hole array due to melting of the Delrin surface and significant burring in the holes, which would have made needle insertion difficult. Attempts at milling the array surface to remove the burrs resulted in closing the holes. It was found that much better holes could be obtained with significantly less burring if a spindle speed of 24,000 rpm was used with a feed rate of 100 mm/min. A minimum of 250 μm wall thickness was also specified for each hole to prevent the holes from joining due to wall melting. The use of a peck drilling routine helped to eliminate some of the burring (Figures 6.2 and 6.3).

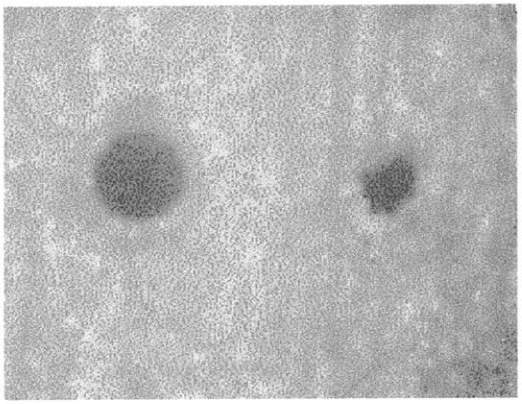


Figure 6.2: Holes with a 250 μm diameter drilled in Delrin. The hole on the right shows how burring affects the hole opening.

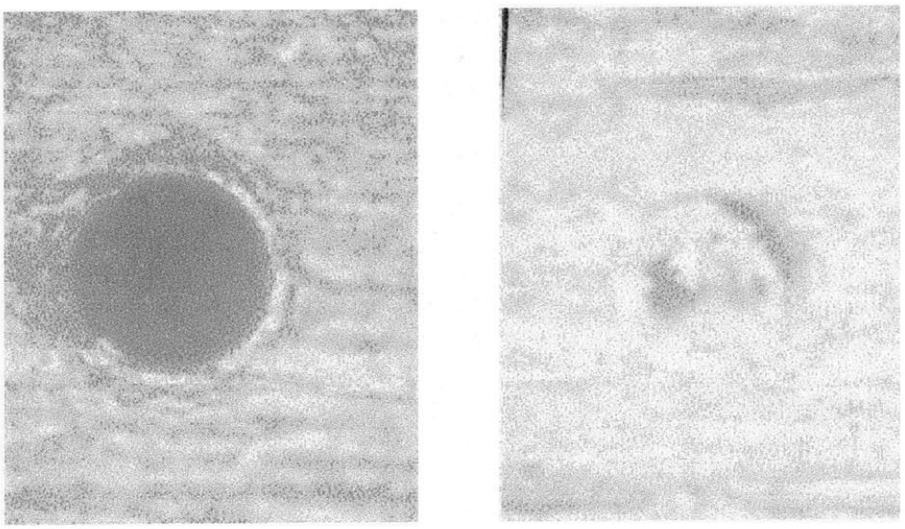


Figure 6.3: Close up view of a cleared (left) and a burr-filled (right) 250 μm diameter hole.

Standard hypodermic tubing with an outer diameter of 200 μm was obtained. Approximately 25 mm length sections of the tubing were tightly packed into a larger tube and then placed in the wire EDM (Electrostatic Discharge Machining) (Charmilles Technologies ROBOFIL 1020SI) vice. The wire EDM was used to cut the needles to the desired length of 3 mm using an angled cut, thereby forming a pointed tip on each needle for easier penetration of the stratum corneum of the skin (Figure 6.4).

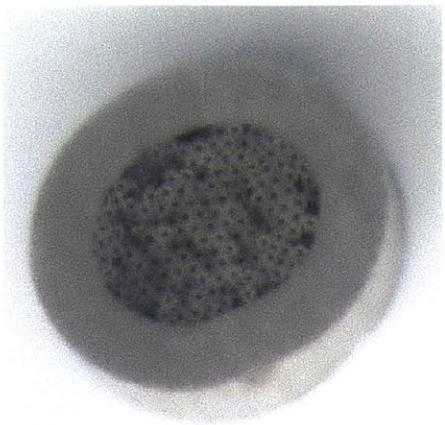


Figure 6.4: Results of needle cutting using wire EDM
after packing needles in larger tubing.

The EDM machine was used since it imparts almost no force onto the objects it cuts. This eliminated the potential problem of closing the needle openings during cutting (Figure 6.5).

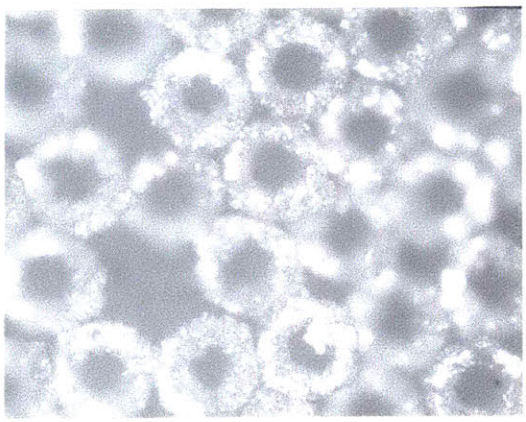


Figure 6.5: Close up of needle openings after EDM machining.
Outer diameter of each needle is 200 μm.

The needles were inserted into the baseplate manually and positioned such that only 50 μm protruded from the array. The needles were then secured in place using epoxy. This manually constructed array was used to show that a viable array can be

constructed by combining a baseplate hole array with individual hollow needles (Figure 6.6).

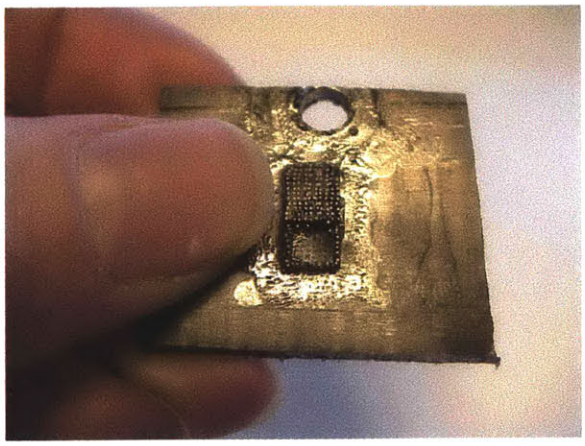


Figure 6.6: Example of a partially filled array with manually inserted needles.

Due to the tedium of manually inserting needles into the array, alternative insertion methods were investigated. A magnetic shaker system was designed specifically for the insertion task (Figure 6.7).

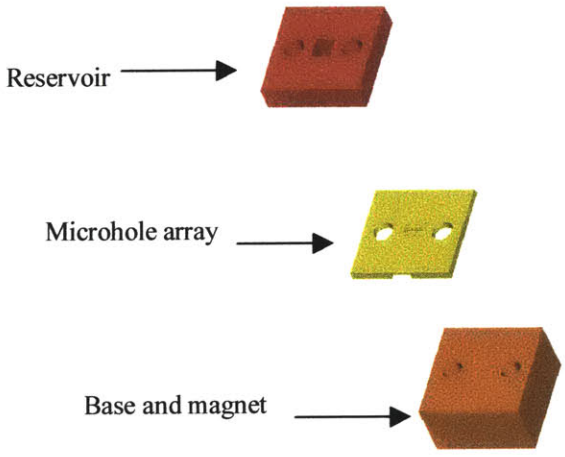


Figure 6.7: Exploded view of magnetic shaker design.

The needles were placed in a small reservoir positioned directly over the hole array. A magnet was placed beneath the array in such a manner as to align the needles perpendicular to the array and to hold the needles in position once they were inserted. A cap was placed over the reservoir and the entire unit was shaken vigorously until the needles were inserted (Figure 6.8).

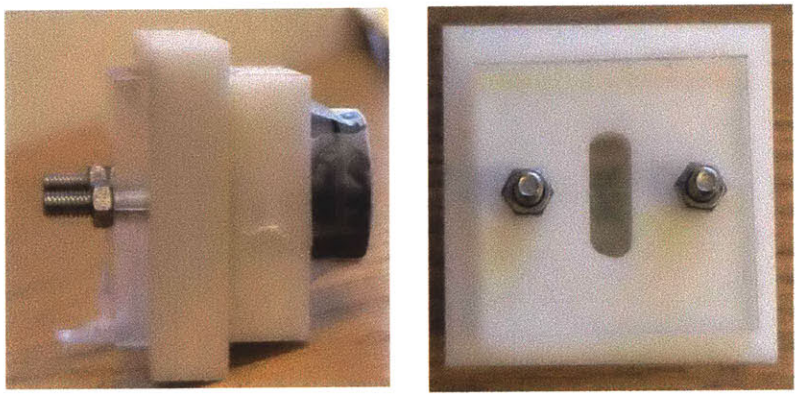


Figure 6.8: Side and top view of actual magnetic shaker.

Although many configurations were tried using this method, only limited success was achieved. Approximately 80 needles out of 200 were inserted into the array before too much magnetic interference between the inserted needles and the free needles became an issue. After insertion, the needles were positioned such that only 50 μm protruded from the array and fixed in place using epoxy.

6.3 EDM Array Design

Due to some of the problems with the original design of the array, work has also been done to determine whether the EDM offers a more appropriate method of array fabrication. Multi-needle arrays have already been fabricated using the EDM, however these arrays contain solid needles rather than hollow ones. One possibility is to use solid

needles for the glucose detection (Figure 6.9) and have a hollow needle array used solely for drug delivery.

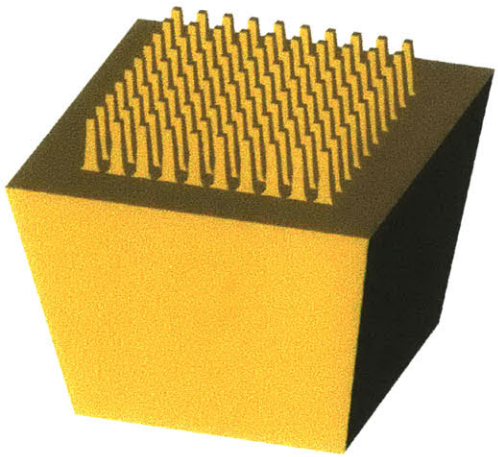


Figure 6.9: EDM fabricated micro-needle array.
[Diagram printed with permission of Luke Sosnowski.]

Arrays have been fabricated using stainless steel, tungsten-carbide, and copper, with as many as ten thousand needles in a single array. These needles have been made with a base cross-sectional area as small as 100 μm × 100 μm having both uniform cross-sections and tapered points (Figures 6.10, 6.11).

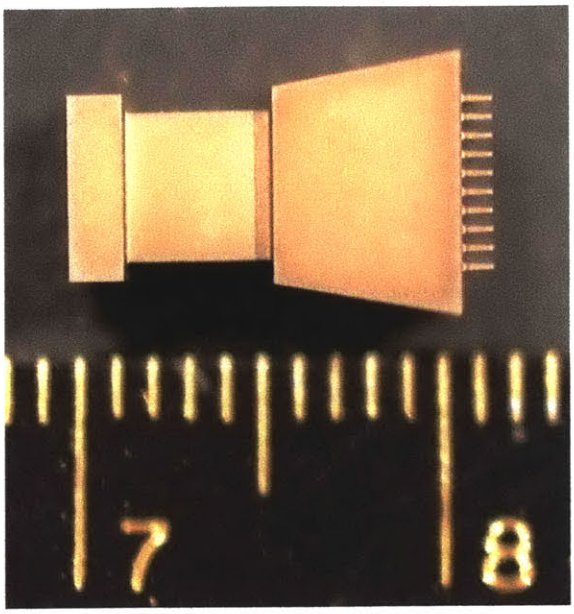


Figure 6.10: Micro-needle array formed by microwire EDM.
[Array designed and fabricated by Luke Sosnowski and Tanya Kanigan.]

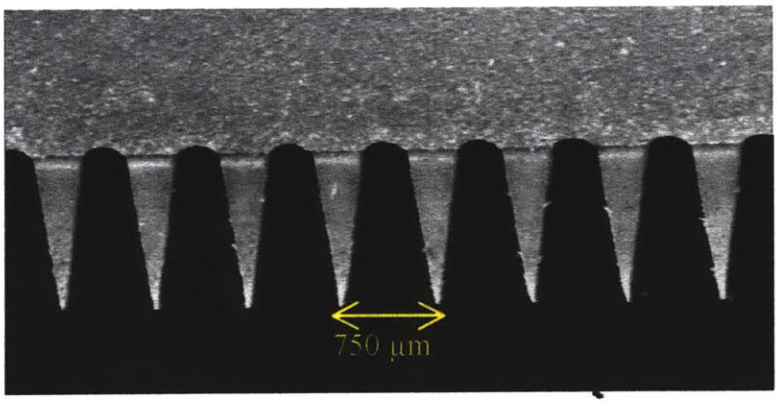


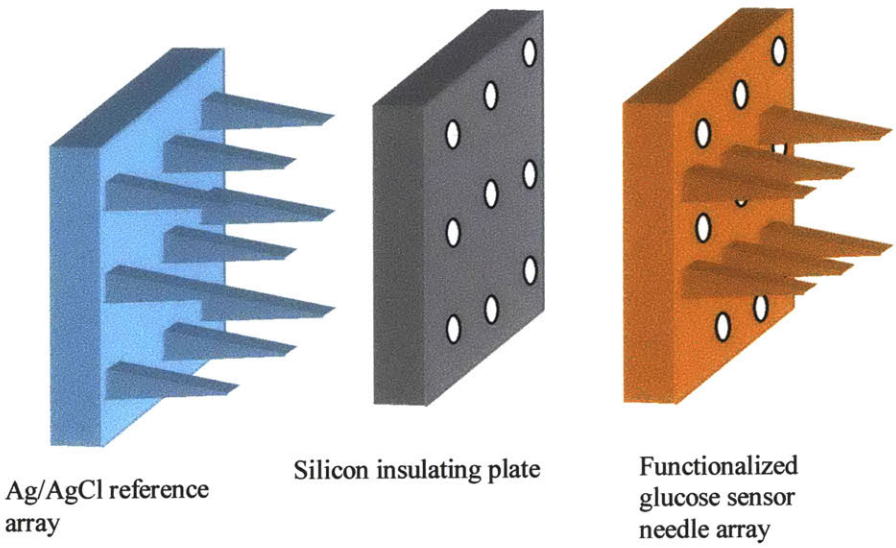
Figure 6.11: Tapered tips of a micro-needle array formed by EDM.
[Array designed and fabricated by Luke Sosnowski and Tanya Kanigan.]

For the glucose sensor array, an array of tapered needles will be fabricated to facilitate entry into the skin. Assuming an average needle tip cross-section of 25 µm and

penetration depth of 50 μm, the sensor surface area per needle is estimated at $5x10^{-9}$ m^2. Based upon the $0.1A/m^2$ response obtained with the current sensor (after coating with Nafion), a 10,000 needle array would be necessary to produce an amperometric response in the microamp range. However, since currents in the nA range are routinely measured, an array of only 100 needles should produce acceptable signal levels.

The challenge for the glucose sensor array design is that the sensor consists of a reference and working electrode that must be electrically insulated from one another. Recent investigations in the Bioinstrumentation lab at MIT have shown that silicon can be machined using the EDM technology. This leads to the possibility of using passivated silicon as the array baseplate in order to electrically insulate the working and counter electrodes. A silicon wafer could be used to fabricate an array of matching through holes. The surface of this wafer could be thermally oxidized in order to form an electrically insulating layer, and the resulting oxidized silicon plate could be used as an insulating sheath between an array of glucose sensors and an array of the Ag/AgCl reference electrodes. The process of fabricating this device consists of functionalizing the needles of an EDM array as the working electrodes, inserting this array into a silicon baseplate predrilled with a matching hole array, and then inserting them through a second needle array whose needles are functionalized as glucose sensors (Figure 6.12).

A)



B)

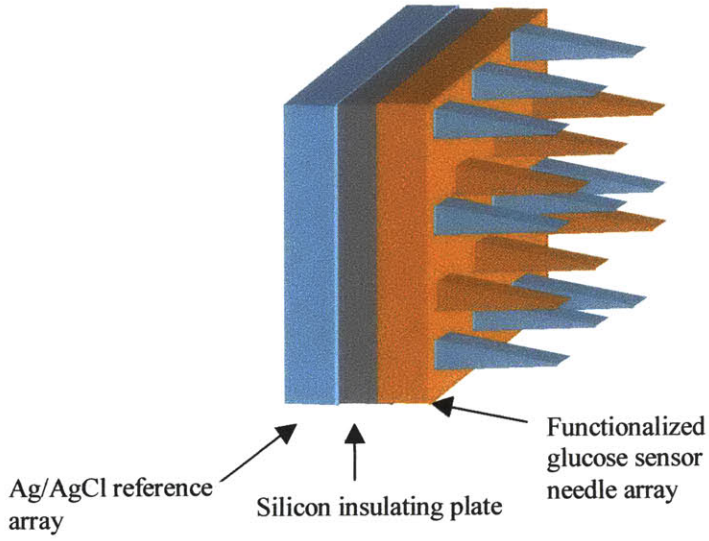


Figure 6.12: Design of EDM fabricated array.

A) Exploded view of individual components.

B) Assembled view of final array.

These arrays will be attached using an adhesive coating. The insides of the holes in the glucose sensor array need to be coated with a non-conducting polymer film to prevent electrical contact between the base of the glucose sensor array and the pins of the Ag/AgCl reference array (Figure 6.13).

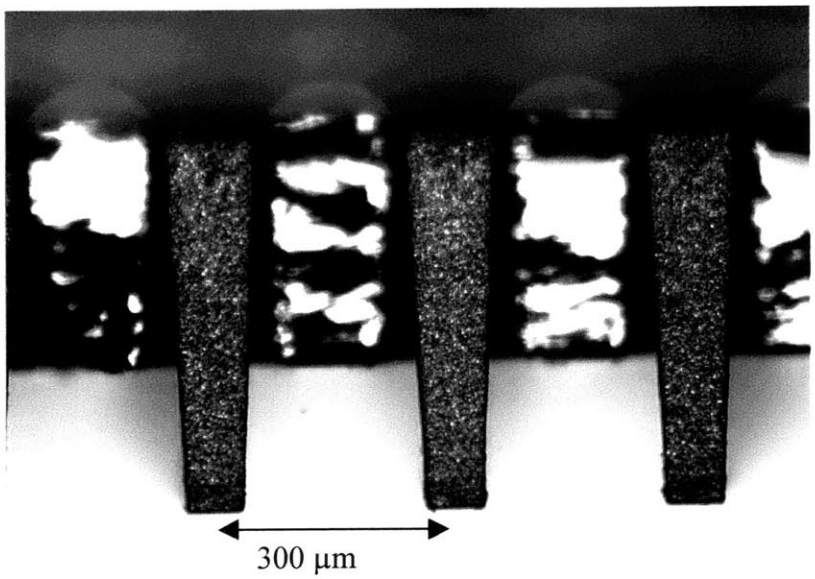


Figure 6.13: Cross-section of stainless steel micro-needle array inserted through a 500 μm thick silicon baseplate. [Baseplate fabricated by Tanya Kanigan.]

Although many multi-needle arrays have been fabricated with the EDM, they have not yet been successfully functionalized as glucose sensors. Initial attempts to sensitize a 100 needle array to hydrogen peroxide have been successful, showing an increase in current with increasing concentrations of hydrogen peroxide over the biological range of 0 – 20 mM (Figure 6.14). These measurements were made by placing the needles of the array into PBS and adding small increments of hydrogen peroxide through a syringe. The reference electrode used was Ag/AgCl, and the setup of this experiment was the same as that for the single needle sensor.

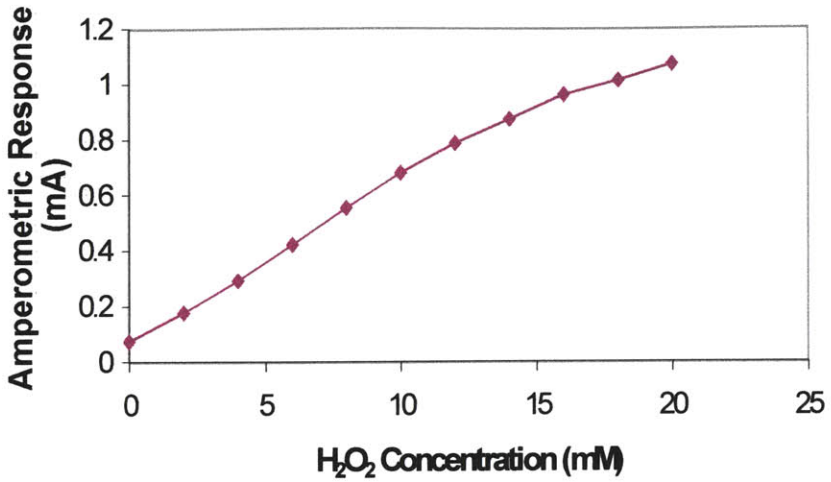


Figure 6.14: Array response to hydrogen peroxide.

Due to the increase in surface area provided by the use of an array of micro-needles, the current response is now in the milliamp range as compared to the microamp range measured on a single 1.02 mm diameter (18-gauge) needle. Further attempts to sensitize the arrays to glucose indicated that some modifications to the sensor fabrication procedure may be necessary. During these attempts, the cellulose acetate tended to solidify between the micro-needles, thereby closing up the spacing between them. A more diluted CA solution may solve this problem, or the CA may need to be replaced entirely.

Chapter Seven: Future Work

7.1 System Identification Techniques

Most glucose sensor research to date has assumed that a simple statistical correlation exists between the sensor signal and the glucose concentration. However, the glucose signal in vivo is greatly affected by the dynamic characteristics of the environment. Thus, there exists a great need to develop a dynamic model of the mechanisms of glucose transport in living tissue, which could then be used for predictive measures [Gough and Armour, 1995]. To accomplish this, system identification techniques could be used to characterize the glucose response in tissue which would provide vital information to the field of sensor design. An extensive literature search did identify a few papers in this area, but all of these studies have been done using mathematical models of the human glucose response to insulin injections (see Chapter 2). Clearly such analyses should be performed with actual human glucose response data to determine the human impulse response to insulin. These analyses would then enable the design of devices for diabetics that could predict glucose fluctuations as does the normally functioning human pancreas.

One problem with the amperometric needle glucose sensors is that the sensitivity (i.e., the magnitude of the current produced for some concentration of glucose) varies significantly from one sensor to the next, and for a single sensor over time. System ID techniques can be used as a calibration method for the glucose sensor. By using a stochastic input of glucose and measuring the sensor's amperometric response, the impulse response of the sensor can be determined. Such a calibration technique would allow the sensor to be calibrated easily in vivo.

In order to show the applicability of system ID techniques to sensor calibration, a simple setup has been designed that can be used to test the dynamic behavior of glucose and sensor interaction (Figure 7.1).

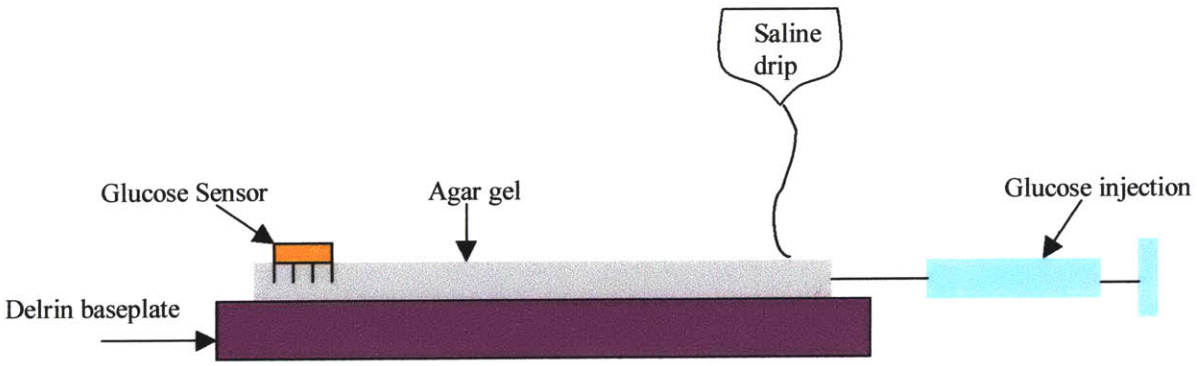


Figure 7.1: System ID test setup

A physiological saline solution will be fed via a gravity feed through a slab of agar gel. The saline will provide a constant flush to the system. The glucose sensor will be inserted into the far end of the gel, and glucose will be periodically injected at the opposite end using a micro-stepper-driven syringe. By using standard system identification techniques to analyze the sensor response, the dynamic response of the system can be characterized.

A simple version of this setup has been tried using an array sensitive to H_2O_2. This was done as a proof of concept showing that the array did respond when H_2O_2 was injected into the agar gel. A more complete system needs to be built to provide a

stochastic input of glucose to the system and thereby measure and characterize the dynamic response.

7.2 Conclusions

This thesis documents the development of the minimally invasive glucose sensor. A method has been developed for glucose sensor fabrication, and with minor modifications to this method, it may be possible to functionalize the outer surface of a micro-needle array. Various micro-needle arrays have been fabricated by different group members and positive responses have been achieved when these needles are sensitized to hydrogen peroxide. It has also been shown that cyclic voltammetry can be a powerful tool in the signal detection process.

Future work includes the fabrication of a prototype micro-needle array that can effectively measure glucose levels in IF. Techniques to increase the longevity of the glucose sensors and appropriate means of fabricating the micro-needle array must be investigated further. System identification techniques need to be used to identify the dynamic characteristics of the system as well as to analyze the human insulin response, which is a vital link in diabetes management. Fourier transform analysis can also be employed as a powerful tool in further identifying the glucose signal. The sensor and array designs must be miniaturized in order to create a prototype that can sample IF in a painless manner.

By creating a biocompatible glucose sensor, glucose measurements can take place in vivo, eliminating the need to remove IF from the patient. Further work must be done to determine the best way to couple this sensor with an automated insulin delivery system. In this manner, a completely closed-loop system could be designed that would use measured glucose levels as feedback to the insulin delivery device. This technique could then be used to maintain optimum glucose levels in the patient throughout the day, alleviating much of the stress and pain currently endured by diabetics.

References

Albisser, A.M., B.S. Leibel, T.G. Ewart, Z. Davidovac, C.K. Botz, W. Zingg, H. Schipper, and R. Gander. 1974. Clinical control of diabetes by the artificial pancreas, *Diabetes*, **23(5)**, 397-404.

American Diabetes Association. 1997. All about insulin, *American Diabetes Association Inc. Pamphlet.*

American Diabetes Association. 1998. First things first...., *American Diabetes Association, Inc. Pamphlet*

American Diabetes Association. 1999a. Diabetes facts and figures, *http://www.diabetes.org/ada/facts.asp*

American Diabetes Association. 1999b. Glycemic control for people with diabetes, *http://www.diabetes.org/DiabetesCare/Supplement198/suplescans/PGS24T1.gif*

Andrews, M.K. and P.D. Harris. 1998. Fabrication and sensing applications of microelectrodes on silicon substrates, *Electroanalysis,* **10**, 1112-1118.

Ariizumi, M. and K. Higashio. 1996. Apparatus for measuring a glucose concentration; medical equipment for diabetic patients comprising two light sources projecting radiation wavelength, a photosensor receiving radiation transmitted through the sample and a calculator. United States Patent 5,529,755. Minolta Camera.

Bantle, J.P. and W. Thomas. 1997. Glucose measurement in patients with diabetes mellitus with dermal interstitial fluid, *Journal of Laboratory and Clinical Medicine*, **130**, 436-441. http://www.integonline.com/integ/espec/htm/Bantle.htm

Bindra, Dilbir S., Y. Zhang, G.S. Wilson, R. Sternberg, D.R. Thevenot, D. Moatti, and G. Reach. 1991. Design and in vitro studies of a needle-type glucose sensor for subcutaneous monitoring, *Analytical Chemistry*, **63**, 1692-1696.

Bremer, T. and D.A. Gough. 1999. Is blood glucose predictable from previous values? A solicitation for data, *Diabetes*, **48**, 445-451.

Brunner, G.A., M. Ellmerer, G. Sendlhofer, A. Wutte, Z. Trajanoski, L. Schaupp, F. Quehenberger, P. Wach, G.J. Krejs, and T.R. Pieber. 1998. Validation of home blood glucose meters with respect to clinical and analytical approaches, *Diabetes Care*, **21(4)**, 585.

Buchert, J. 1995. Method, apparatus, and procedure for non-invasive monitoring of blood glucose by measuring the polarization ratio of blood luminescence. United States Patent 5,383,452.

Chang, R. 1981. Physical Chemistry with Applications to Biological Systems, 2nd ed. Macmillan Publishing Co., Inc., NY.

Christie, I.M., P.H. Treloar, and P. Vadgama. 1992. Plasticized poly(vinyl chloride) as a permselective barrier membrane for high-selectivity amperometric sensors and biosensors, *Analytica Chimica Acta*, **269**, 65-73.

Clarke, R.H. and Q. Wang. 1993. Infrared glucose sensor. United States Patent 5,222,496. Angiomedics II Inc.

CNN. 1999. Device may mean the end of painful pricks for diabetics, June 22, *http://www.cnn.com/HEALTH/9906/22/diabetes.treatment*

Crabbe, M.J.C. 1987. The complications of diabetes. *In* Diabetic Complications: Scientific and Clinical Aspects. M.J.C. Crabbe, ed. Churchill Livingstone, Edinburgh. 1-23.

Cygnus. 1998. GlucoWatch, *http://www.cygn.com/glucowatch.html*

D'Arrigo, T. 1999. Zap! Laser lancing device now available for home use, *Diabetes Forecast*, April, 35-36.

Diabetes Control and Complications Trial Research Group (DCCT). 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus, *The New England Journal of Medicine*, **329(14)**, 977-986.

Diem, K., ed. 1962. Documenta Geigy Scientific Tables, 6th ed. Geigy Pharmaceuticals.

Elmerick, D. and Peters, R.K. 1999. Non-invasive glucose measuring device and method for measuring blood glucose. United States Patent 5,910,109. Emerging Tech Systems.

Espinal, J. 1989. Understanding Insulin Action: Principles and Molecular Mechanisms. Ellis Horwood Limited, Chichester.

Furbee, J.W. Jr., T. Kuwana, and R.S. Kelly. 1994. Fractured carbon fiber-based biosensor for glucose, *Analytical Chemistry*, **66(9)**, 1575-1577.

Galloway, J.A. and R.D. deShanzo. 1990. Insulin chemistry and pharmacology; insulin allergy, resistance, and lipodystrophy. *In* Ellenberg and Rifkin's Diabetes Mellitus: Theory and Practice, 4th ed. H. Rifkin and D. Porte, Jr., ed. Elsevier, NY. 497-513.

Ginsberg, B.H. 1992. An overview of minimally invasive technologies, *Clinical Chemistry*, **38(9)**, 1596-1600.

Glass, R.S., S.P. Perone, and D.R. Ciarlo. 1990. Application of information theory to electroanalytical measurements using a multielement, microelectrode array, *Analytical Chemistry*, **62(18)**, 1914-1918.

Gough, D.A. and J.C. Armour. 1995. Development of the implantable glucose sensor: what are the prospects and why is it taking so long? *Diabetes*, **44**, 1005-1009.

Gunasingham, H. and C.B. Tan. 1989. Platinum-dispersed Nafion film modified glassy carbon as an electrocatalytic surface for an amperometric glucose enzyme electrode, *The Analyst*, **114**, 695-698.

Guyton, J.R., R.O. Foster, J.S. Soeldner, M.H. Tan, C.B. Kahn, L. Koncz, and R.E. Gleason. 1978. A model of glucose-insulin homeostasis in man that incorporates the heterogenous fast pool theory of pancreatic insulin release, *Diabetes*, **27**, 1027-1042.

Hattori, T. and N. Ushizawa. 1998. Blood glucose measurement apparatus. United States Patent 5,820,557. Terum Corp.

Heine, R.J. 1998. The insulin dilemma: which one to use? *In* World Book of Diabetes in Practice, vol.3. L.P. Krall, ed. Elsevier, Amsterdam. 150-159.

Henry, C. 1998. Getting under the skin: Implantable electrochemical glucose sensors are moving closer to commercialization, *Analytical Chemistry News & Features*, September 1, 594A-598A. http://pubs.acs.org/hotartcl/ac/98/sep/skin.html

Henry, S., D.V. McAllister, M.G. Allen, and M.R. Prausnitz. 1999. Micromachined needles for the transdermal delivery of drugs, *Georgia Institute of Technology Working Paper*.

Jacobs, P. 1999. Success, one step at a time: MiniMed hopes to keep lead in diabetes devices, *Los Angeles Times*, August 15, C1.

Johnson, K.W., J.J. Mastrototaro, D.C. Howey, R.L. Brunelle, P.L. Burden-Brady, N.A. Bryan, C.C. Andrew, H.M. Rowe, D.J. Allen, B.W. Noffke, W.C. McMahan, R.J. Morff, D. Lipson, and R.S. Nevin. 1992. In vivo evaluation of an electroenzymatic glucose sensor implanted in subcutaneous tissue, *Biosensors and Bioelectronics*, **7**, 709-714.

Kallner, A. 1981. Vitamin C – man's requirement. *In* Vitamin C (Ascorbic Acid). J.N. Counsell and D.H. Hornig ed. Applied Science Publishers, London. 63-73.

Kayashima, S., T. Arai, M. Kikuchi, N. Nagata, N. Ito, T. Kuriyama, and J. Kimura. 1992. Suction effusion fluid from skin and constituent analysis: new candidate for interstitial fluid, *American Journal of Physiology*, **263**, H1623-H1627.

Kim, Y. and W.S. Yang. 1993. Non-invasive method and apparatus for measuring blood glucose concentration. United States Patent 5,267,152.

Kimura, J. 1993. Noninvasive blood glucose concentration monitoring method with suction effusion fluid by ISFET Biosensor, *Applied Biochemistry and Biotechnology*, **41**, 55-58.

Koivisto, V.A. 1998. Open-loop pumps: update. *In* World Book of Diabetes in Practice, **3**. L.P. Krall, ed. Elsevier, Amsterdam. 171-175.

Lehmann, E.D. and T. Deutsch. 1992. A physiological model of glucose-insulin interaction in type 1 diabetes mellitus, *Journal of Biomedical Engineering*, **14**, 235-242.

Li, Q.S., B.C. Yi, B.X. Liu, J.J. Zhong. 1999. Enhancement of the sensitivity and selectivity of oxidation of H_2O_2 on platinum wire at low working potential by platinization and covering of heteropolypyrrole film for amperometric micro-biosensor construction, *Fresenius Journal of Analytical Chemistry*, **363**, 246-250.

Lukachova, L.V., A.A. Karyakin, Y.N. Ivanova, E.E. Karyakina, and S.D. Varfolomeyev. 1998. Non-aqueous enzymology approach for improvement of reagentless mediator-based glucose biosensor, *The Analyst*, **123**, 1981-1985.

March, W.F. 1976. Non-invasive automatic glucose sensor system. United States Patent 3,958,560.

March, W.F. 1977. Non-invasive glucose sensor system. United States Patent 4,014,321.

Mastrototaro, J.J. 1994. Apparatus and method for implantation of sensors. United States Patent 5,299,571. Eli Lilly and Company.

McNeil Consumer Healthcare. 1999. Guidelines for the management of acute acetaminophen overdose, *McNeil Consumer Healthcare brochure.*

Medi-Ject Corporation. 1999. Medi-Jector choice: needle-free insulin delivery system, *Medi-Ject Corporation Pamphlet.*

Mendosa, R. 1997. The race for a painless monitor, *Diabetes Wellness Letter*, November, 1-3. http://www.mendosa.com/painless.htm

Milardovic, S., I. Kruhak, D. Ivekovic, V. Rumenjak, M. Tkalcec, and B.S. Grabaric. 1997. Glucose determination in blood samples using flow injection analysis and an amperometric biosensor based on glucose oxidase immobilized on hexacyanoferrate modified nickel electrode, *Analytica Chimica Acta*, **350**, 91-96.

MiniMed. 1999. Insulin infusion pump: glucose monitor: fact sheet: subcutaneous glucose monitoring system, *http://www.minimed.com/files/snsr_fac.htm*

Miyagawa, I. and M. Toida. 1998. Glucose concentration measuring method and apparatus with short coherence source and heterodyne interferometer. United States Patent 5,835,215. Fuji Photo Film Co. Ltd.

Moussy, F., D.J. Harrison, D.W. O'Brien, and R.V. Rajotte. 1993. Performance of subcutaneously implanted needle-type glucose sensors employing a novel trilayer coating, *Analytical Chemistry*, **65**, 2072-2077.

Nielsen, W. 1998. Micro-well array fabrication. B.S., Mechanical Engineering Thesis, Massachusetts Institute of Technology.

National Human Genome Research Institute (NHGRI). 1999. Division of Intramural Research – Investigators and Advisors.
http://www.nhgri.nih.gov/Intramural_research/People/collins.html

Noonan, B. 1999. MiniMed customer representative. Personal communication.

Parker, R.S., F.J. Doyle, III, and N.A. Peppas. 1999. A model-based algorithm for blood glucose control in Type I diabetic patients, *IEEE Transactions on Biomedical Engineering*, **46(2)**, 148-156.

Patton, J.S. 1998. Deep-lung delivery of proteins, *Modern Drug Discovery*, September/October, pg.19-28.

Penner, R.M. and C.R. Martin. 1987. Preparation and electrochemical characterization of ultramicroelectrode ensembles, *Analytical Chemistry*, **59(21)**, 2625-2630.

Penner, R.M. and N.S. Lewis. 1991. Invisible electrodes with amazing powers, *Chemistry and Industry*, November, 788-791.

Pickup, J.C., D.J. Claremont, G.W. Shaw. 1993. Responses and calibration of amperometric glucose sensors implanted in the subcutaneous tissue of man, *Acta Diabetologica*, **30**, 143-148.

Poitout, V., D. Moatti-Sirat, G. Reach, Y. Zhang, G.S. Wilson, F. Lemonnier, and J.C. Klein. 1993. A glucose monitoring system for on line estimation in man of blood glucose concentration using a miniaturized glucose sensor implanted in the subcutaneous tissue and a wearable control unit, *Diabetologia*, **36**, 658-663.

Rao, G., R.H. Guy, P. Glikfeld, W.R. LaCourse, L. Leung, J. Tamada, R.O. Potts, and N. Azimi. 1995. Reverse iontophoresis: noninvasive glucose monitoring in vivo in humans, *Pharmaceutical Research*, **12(12)**, 1869-1873.

Reboldi, G.P., P.D. Home, G. Calabrese, P.G. Fabietti, P. Brunetti, and M. Massi Benedetti. 1991. Time delay compensation for closed-loop insulin delivery systems: a simulation study, *The International Journal of Artificial Organs*, **14(6)**, 350-358.

Rebrin, K., G.M. Steil, W.P. Van Antwerp, and J.J. Mastrototaro. 1999. Subcutaneous glucose predicts plasma glucose independent of insulin: implications for continuous monitoring, *American Journal of Physiology*, **277**, E561-E571.

Repechage. 1999. The skin, *http://www.repechage.com/skin.html*

Rifkin, H. and G. Bernstein. 1998. The treatment of Type II diabetes mellitus. *In* World Book of Diabetes in Practice, vol.3. L.P. Krall, ed. Elsevier, Amsterdam. 164-166.

Schwartz, S.L. 1989. Management of Diabetes Mellitus. Essential Medical Information Systems, Inc., Dallas, TX.

Sherrick, C.E. and R.W. Cholewiak. 1986. Cutaneous sensitivity. *In* Handbook of Perception and Human Performance: Sensory Processes and Perception, vol.1. K.R. Boff, L. Kaufman, and J.P. Thomas, ed. John Wiley and Sons, New York. 12/1-12/58.

Shichiri, M., R. Kawamori, Y. Yamasaki, N. Hakui, and H. Abe. 1982. Wearable-type artificial pancreas with needle-type glucose sensor, *Lancet*, **2**, 1129-1131.

Shichiri, M., R. Kawamori, N. Hakui, Y. Yamasaki, and H. Abe. 1984. Closed-loop glycemic control with a wearable artificial endocrine pancreas: variations in daily insulin requirements to glycemic response, *Diabetes*, **33**, 1200-1202.

Shichiri, M., N. Asakawa, Y. Yamasaki, R. Kawamori, and H. Abe. 1986. Telemetry glucose monitoring device with needle-type glucose sensor: a useful tool for blood glucose monitoring in diabetic individuals, *Diabetes Care*, **9**, 298-301.

Stark, E.W. 1995. Non-invasive glucose measurement method and apparatus. United States Patent 5,433,197.

Steffes, P.G. and R.V. Tarr. 1993. Non-invasive blood glucose measurement system and method using stimulated Raman spectroscopy. United States Patent 5,243,983. Georgia Tech Research Corp.

Steinkuhl, R., C. Dumschat, C. Sundermeier, H. Hinkers, R. Renneberg, K. Cammann, and M. Knoll. 1996. Micromachined glucose sensor, *Biosensors & Bioelectronics*, **11(1/2)**, 187-190.

Strowig, S. and P. Raskin. 1990. Insulin treatment and patient management. *In* Ellenberg and Rifkin's Diabetes Mellitus: Theory and Practice, 4th ed. H. Rifkin and D. Porte, Jr. ed. Elsevier, NY. 514-525.

Svedman, P. and C. Svedman. 1998. Skin mini-erosion sampling technique: feasibility study with regard to serial glucose measurement, *Pharmaceutical Research*, **15(6)**, 883-888.

Tamada, J.A., N.J.V. Bohannon, and R.O. Potts. 1995. Measurement of glucose in diabetic subjects using noninvasive transdermal extraction, *Nature Medicine*, **1(11)**, 1198-1201.

Tamborlane, W.V. and S.A. Amiel. 1987. Insulin therapy, metabolic control and diabetic complications. *In* Diabetic Complications: Scientific and Clinical Aspects. M.J.C. Crabbe, ed. Churchill Livingstone, Edinburgh. 24-40.

Trajanoski, Z., G.A. Brunner, R.J. Gfrerer, P. Wach, and T.R. Pieber. 1996. Accuracy of home blood glucose meters during hypoglycemia, *Diabetes Care*, **19(12)**, 1412.

UK Prospective Diabetes Study (UKPDS) Group. 1998. Intensive blood-glucose control with sulphonylurea or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33), *The Lancet*, **352**, 837-853.

Walsh, J. 1998. Inhaled insulin: will it really take your breath away? *http://diabetesnet.com/inhale_ins.html*

Wang, J. 1994. Analytical Electrochemistry. Wiley-VCH, New York.

Wang, J. and L. Angnes. 1992. Miniaturized glucose sensors based on electrochemical codeposition of rhodium and glucose oxidase onto carbon-fiber electrodes, *Analytical Chemistry*, **64**, 456-459.

Weinless, N.L. 1986. A Theoretical Model for Insulin Secretory Dynamics in a Hybrid Artificial Pancreas. M.S., Chemical Engineering Thesis, Massachusetts Institute of Technology.

Wightman, R.M. 1981. Microvoltammetric electrodes, *Analytical Chemistry*, **53(9)**, 1125A-1134A.

Wightman, R.M. 1988. Voltammetry with microscopic electrodes in new domains, *Science,* **240**, 415-420.

Wilkins, E. and P. Atanasov. 1996. Glucose monitoring: state of the art and future possibilities, *Medical Engineering and Physics*, **18**, 273-288.

Yang, Q., P. Atanasov, and E. Wilkins. 1998. Development of needle-type glucose sensor with high selectivity, *Sensors and Actuators B*, **46**, 249-256.

Yao, T. 1983. A chemically-modified enzyme membrane electrode as an amperometric glucose sensor, *Analytica Chimica Acta*, **148,** 27-33.