Design, Fabrication, and Evaluation of a Carbon Fiber Polarographic Oxygen Microelectrode

by

Tzejunn Jason Yip

Submitted to the Department of Electrical Engineering and Computer Science in Partial Fulfillment of the Requirements for the Degrees of

Bachelor of Science in Electrical Engineering and

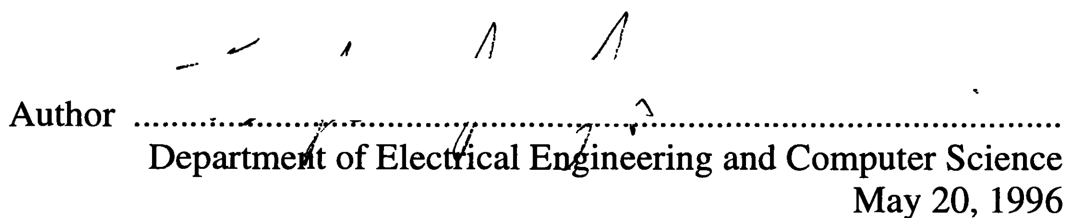
Master of Engineering in Electrical Engineering

at the

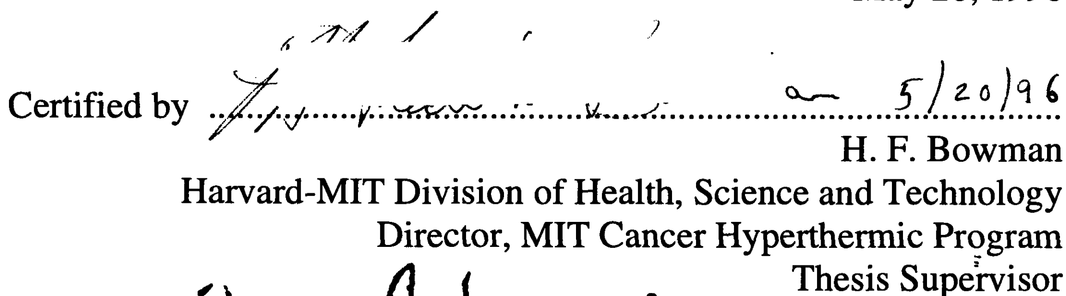
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

May 20, 1996

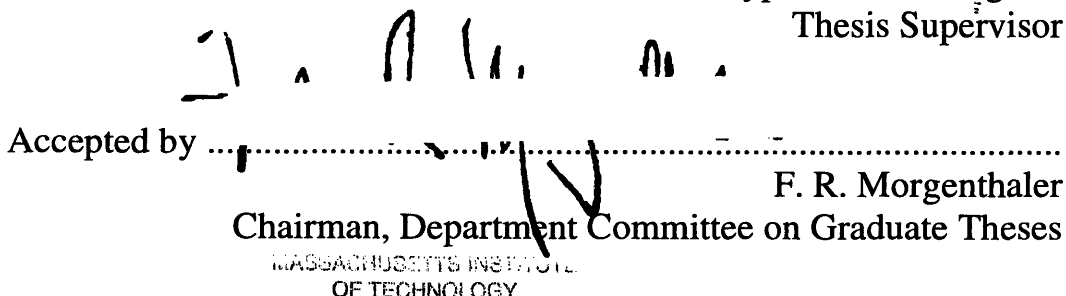
© Massachusetts Institute of Technology, 1996. All Rights Reserved.



Author ....
Department of Electrical Engineering and Computer Science
May 20, 1996



Certified by 5/20/96
H. F. Bowman
Harvard-MIT Division of Health, Science and Technology
Director, MIT Cancer Hyperthermic Program
Thesis Supervisor



Accepted by ....
F. R. Morgenthaler
Chairman, Department Committee on Graduate Theses

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

JUN 1 1 1996 Eng.

Design, Fabrication, and Evaluation of a Carbon Fiber Polarographic Oxygen Microelectrode

by

Tzejunn Jason Yip

Submitted to the Department of Electrical Engineering and Computer Science on May 20, 1996, in partial fulfillment of the requirements for the degree of Master of Engineering in Electrical Engineering

Abstract

The capability of acquiring accurate, real-time measurements of oxygen tension in tumors and surrounding tissues would represent a significant step forward in the effective management of cancer therapy. A carbon fiber polarographic oxygen microelectrode was designed and fabricated to provide an alternative method of oxygen monitoring. Current polarographic oxygen measurement technology utilizes a gold wire microelectrode as the measurement cathode. The carbon fiber microelectrode was designed, fabricated, and evaluated using existing measurement equipment. Both *in vitro* and *in vivo* studies were performed to more fully evaluate the capabilities of the microelectrodes. Results showed that the carbon fiber microelectrode worked as well as commercially available gold wire microelectrodes.

Thesis Supervisor: Dr. H. F. Bowman
Title: Senior Academic Administrator, HST
Title: Director, MIT Cancer Hyperthermia Program

Acknowledgements

First, I would like to thank my parents and sister for all of their support. A long-distance thank you to Charles Ruban and Stephan Mangin for their invaluable assistance in making this project a reality. Fred Bowman and Bill Newman were invaluable in helping me get the research done and for their constant input in finding new and innovative ways of getting through the problems that arose. Final thanks to Chipper for being around during every hour of the writing of this thing.

Table of Contents

1 Introduction 15
1.1 Project Description 15
1.2 Relevance of Project 15
Characterization of the Tumor Environment 15
Carbon Fiber Microelectrodes 16
2 Background 19
2.1 Polarographic Oxygen Measurements 19
Electrochemical Theory 19
Diffusion at the Polarographic Oxygen Sensor 23
2.2 Polarographic Oxygen Electrodes 27
Clark Electrodes 27
Needle Electrodes 28
2.3 Complications with In Vivo Polarographic Oxygen Measurements 31
3 The Carbon Fiber Microelectrode 33
3.1 Design Phase 33
Cespuglio Design 33
Ruban and Yip Design 33
Yip and Mangin Design 34
3.2 Specifications for the Final Design 35
Size 35
Conductivity of the Cathode 35
Durability 36
Reliability 36
3.3 Materials Selection 36
Carbon Junction 36
Insulation Epoxy 38
Encasing Material 40
3.4 Fabrication 41
The Gold Wire Microelectrode 41
The Carbon Fiber Microelectrode 42
The Prebundled Carbon Fiber Microelectrode 42
4 *In Vitro* and *In Vivo* Experiments 45
4.1 Objectives 45
4.2 *In Vitro* Experiments 45
Experimental Setup 45
The Long Term Experiment 47
The Short Term Experiment 49
4.3 *In Vivo* Experiments 50
Experimental Setup 50
Experiments on the Rat Liver 51
5 Results: Long Term Measurements 53
5.1 Objectives 53
5.2 Hypothesis of Current Leakage 53

5.3 Methods 55
5.4 Results......55
Reference Measurements......55
Fabricated MicroelectrodeTests......57
6 Results: Short Term Calibration Measurements......65
6.1 Objectives......65
6.2 Methods......65
6.3 Results......65
TTI Microelectrode Tests......66
Fabricated Microelectrode Tests......66
7 Results: *In Vivo* Measurements......69
7.1 Objectives......69
7.2 Methods......69
7.3 Results......69
Atmospheric Conditions......69
Breathing Pure Oxygen......71
Low Levels of Anesthesia......73
8 Discussion of Results......75
8.1 Long Term Measurements......75
Problems Observed During Experiments......75
Hypothesis......75
Discussion......75
8.2 Short Term Calibration Measurements......77
Problems Observed During Experiments......77
Hypotheses......77
Discussion......78
8.3 *In Vivo* Measurements......78
Problems Observed During Experiments......78
Hypothesis......79
Discussion......80
9 Summary and Conclusions......81
9.1 Summary of Carbon Fiber Microelectrode......81
9.2 Conclusions......83
10 Recommendations for Further Study......85
10.1 Long Term Measurements......85
10.2 Short Term Calibration Measurements......85
10.3 *In Vivo* Measurements......86
Bibliography......87
Appendix A Fabrication of the Carbon Fiber Microelectrode......89
A.1 Overview......89
A.2 Design of the Microelectrode......89
A.3 Fabrication of the Microelectrodes......91
Appendix B Protocol for *In Vitro* Experiments......103
B.1 Long Term Studies......104
B.2 Short Term Studies......105

Appendix C Protocol for *In Vivo* Rat Experiments 107
C.1 Introduction 107
C.2 Preparation of pO_2 Measurement System 107
C.3 Surgery 108
C.4 Making *In Vivo* Measurements 110

List of Figures

Figure 2.1: Polarographic oxygen measurement schematic 20
Figure 2.2: Polarogram 21
Figure 2.3: Diagram of the original Clark electrode..28
Figure 2.4: Beebe sensor in hypodermic form..29
Figure 2.5: Ballintyn procedure for platinum electrode fabrication 30
Figure 4.1: Schematic diagram of measurement system 46
Figure 4.2: Schematic diagram of ***in vitro*** experimental setup..46
Figure 4.3: Graph showing a microelectrode that has been well fabricated..48
Figure 4.4: Graph showing a microelectrode that has been poorly fabricated 48
Figure 4.5: Series of experiments leading to calibration 49
Figure 4.6: Calibration graph..50
Figure 4.7: Schematic diagram of ***in vivo*** experiment setup 51
Figure 4.8: Graph showing the effects of pure oxygen administration 52
Figure 4.9: Graph showing temporal response of rat pO_2 to low anesthesia levels 52
Figure 5.1: Graph of ***in vitro*** pO_2 reflecting hypothesized poor glass/epoxy interface..54
Figure 5.2: Graph of ***in vitro*** pO_2 reflecting hypothesized poor glass/epoxy interface..54
Figure 5.3: Graph of the 10 $M\Omega$ resistor experiment 56
Figure 5.4: Graph of the reference gold needle microelectrode experiment 57
Figure 5.5: Graph of saturation of carbon fiber probe #1 58
Figure 5.6: Probe #5: Experiment before collodion membrane applied..59
Figure 5.7: Probe #5: Experiment after collodion membrane applied..59
Figure 5.8: Bundled Probe #2: Twenty hour experiment with minimal drift..60
Figure 5.9: Bundled Probe #4: Before the membrane was applied 61
Figure 5.10: Bundled Probe #4: After the collodion membrane was applied..61
Figure 5.11: Gold Probe #3: First run showing minimal current leakage 63
Figure 5.12: Gold Probe #3: Second run showing even smaller current leak 63
Figure 5.13: Gold Probe #2: Experiment showing high steady-state current..64
Figure 5.14: Gold Probe #2: Experiment performed after tip was broken 64
Figure 6.1: Original Probe #5: Calibration experiments at 10% O_2..67
Figure 6.2: Series of nitrogen current experiments..68
Figure 6.3: Gold Probe #3: Calibration runs at 10% O_2..68
Figure 7.1: TTI Probe: Typical rat liver run 70
Figure 7.2: Bundled Probe #4: Typical rat liver run..70
Figure 7.3: Gold Probe #3: Typical rat liver run 70
Figure 7.4: Bundled Probe #5: Effects of 100% O_2 71
Figure 7.5: Gold Probe #3: Effects of 100% O_2..71
Figure 7.6: TTI Probe: Lack of response to 100% O_2..72
Figure 7.7: TTI Probe: Vasomotor response detected..73
Figure 7.8: Bundled Probe #5: Vasomotor response detected..74
Figure A.2.1: Polyurethane catheter encased carbon fiber microelectrode 90
Figure A.2.2: Glass needle encased carbon fiber microelectrode..90
Figure A.2.3: Comparison between gold wire and carbon fiber bundles 91
Figure A.3.1: Stripped copper wire 92

Figure A.3.2: Setup to connect gold and copper wires 92
Figure A.3.3: The soldered gold-copper wire junction 93
Figure A.3.4: The junction assembly inside the glass capillary tube 93
Figure A.3.5: The glass capillary tube assembly in the glass puller 94
Figure A.3.6: Syringe assembly 95
Figure A.3.7: Syringe assembly inside glass needle 95
Figure A.3.8: Connecting the polyurethane catheter and the glass needle 96
Figure A.3.9: The difference between a poor and a good epoxy seal 97
Figure A.3.10: Carbon fibers inside the polyethylene catheter 98
Figure A.3.11: The copper wire and carbon paste filled catheter assembly 98
Figure A.3.12: The copper wire and carbon fiber junction 99
Figure A.3.13: Diagram of hanging, drying bundles 101
Figure A.3.14: The two halves of glass needle after pulling gold wire microelectrode102
Figure A.3.15: Correct and incorrect methods of placing bundle inside glass needle..102
Figure C.3.1: Diagram of cuts to be made in rat to expose liver 110

List of Tables

Table 3.1: Summary of Carbon Junction Epoxies Tested 37
Table 3.2: Summary of Insulation Epoxies Tested 39
Table 4.1: Calibration Values 50
Table 5.1: Overview of the *In Vitro* Long Term Experiments 57
Table 6.1: Calibration Results 65
Table 7.1: Results from Selected Rat Liver Experiments 72
Table 9.1: Summary of Fabricated Microelectrodes 81
Table B.1: Suggested *Imon* Parameters 104

Chapter 1

Introduction

1.1 Project Description

Polarographic oxygen electrodes have been in use for several decades and have recently become increasingly important in cancer therapy settings. The capability of acquiring accurate, real-time measurements of oxygen tension in tumors and surrounding tissues would represent a significant step forward in the effective management of cancer therapy. Among gas analysis devices, the polarographic oxygen sensor is almost unique in its ability to measure directly the tension of a gas in solution. Currently, the material most widely used as the polarographic oxygen electrode cathode is a gold wire. This research investigates the fabrication of a polarographic oxygen microelectrode system that uses a bundle of carbon fibers as its cathode.

A carbon fiber oxygen microelectrode was designed and fabricated for use with existing measurement systems. This microelectrode was tested both *in vitro* and *in vivo*, and data collected was compared to results obtained with commercially available microelectrodes.

1.2 Relevance of Project

1.2.1 Characterization of the Tumor Environment

The efficacy of cancer therapy can be augmented by combined therapeutic modalities. Hyperthermia, when administered synergistically with radiotherapy and/or chemotherapy may prove to be an effective method of treatment. The early studies of Gray et al. in 1953 demonstrated that as the oxygen concentration of tumor cells was lowered, there was a corresponding decrease in the radiation response of those cells.[1] Gray further suggested that the oxygen concentration in tumor cells might be critical for the successful treatment

of human cancers by ionizing radiation because the maximum dose given is limited by damage to nearby normal tissue which is aerobic. Since the survival rate of cells decreases exponentially with increasing doses of radiation, and since production of a given degree of cell kill requires three times the radiation dose when oxygen is absent, even a very small percentage of radioresistant hypoxic cells could ultimately determine the radiocurability of tumors.[2]

Hypoxic tumor cells may be critical in determining the long term results of irradiation and drug treatment. Hyperthermia offers the cancer clinician a possible means of modifying the pO_2 levels in tumors, enhancing the effects of radiation therapy. Accurate measurements of tumor oxygenation are critical in giving the clinician a better understanding of hypoxia in healthy and unhealthy cells.

Many polarographic electrodes permit direct measurements of oxygen tension *in vivo*, when small, but lack sufficient mechanical stability for routine use in patients, and can cause tissue compression and bleeding at larger electrode sizes.[3] A reliable oxygen microelectrode fabricated to meet these criteria is much sought after. One candidate electrode system, made by Sigma-Eppendorf in Hamburg, Germany, uses needle electrodes which are mechanically stepped through the tissue to provide spatial information on tissue pO_2.

1.2.2 Carbon Fiber Microelectrodes

The research presented in this thesis examines the performance of polarographic oxygen measurements made with carbon fiber microelectrodes. This carbon fiber technology was initially developed through collaboration with the Department de Medecine Experimen-

1. Gray, L. H., Conger, A. D., Ebert, M., Hornsey, S. and Scott, O. C. A., "Concentration of oxygen dissolved in tissues at time of irradiation as a factor in radiotherapy," British Journal of Radiology, Vol. 26: pp. 638-648, 1953.
2. Ibid.
3. Kallinowski, F., Zander, R., Hoeckel, M., Nat., Rer., Vaupel, V., "Tumor tissue oxygenation as evaluated by computerized-pO_2-histography," International Journal of Radiation Oncology, Biology, and Physics Vol. 19: p. 953, 1990.

tale of the Universite Claude Bernard in Lyon, France and the Laboratorie de Physicochimie des Interfaces of the Ecole Centrale de Lyon in Ecully, France. It was thought that carbon fibers were potentially suitable candidates for polarographic cathodes, initiating this research.

Cespuglio showed that carbon fibers are an excellent material for measurements in biological tissues.[4] Gold wire electrodes were hypothesized to be unstable due to reactions with the surrounding tissue, reactions that may result from the release of ions from gold and other metals during the reduction of oxygen. The carbon fibers, composed of 95% pyrolytic graphite, are hypothesized to be more "biocompatible" and more adaptable for long term *in vivo* use.

---

4. Cespuglio, R. et al., "High sensitivity measurements of brain cathecoles and indoles in vivo using electrochemically treated carbon fiber electrodes."

Chapter 2

Background

2.1 Polarographic Oxygen Measurements

2.1.1 Electrochemical Theory

The polarographic oxygen sensor utilizes electrochemical principles to measure the oxygen tension in a medium. This sensor depends on the chemical reduction of oxygen at a surface (metal, carbon, etc.) by the electron pressure imposed on that surface by an external voltage. This process is different from electrochemical reactions where spontaneous chemical reduction and oxidation provide a flow of electrons in an external circuit.[5]

The oxygen "concentration" measured is proportional to the partial pressure of oxygen in the gas phase. The chemical activity of oxygen measured by electrochemcial sensors is not the same as the oxygen concentration measured by chemical or gasometric methods. Because oxygen is only slightly soluble in most liquids, its chemical activity is equal to its partial pressure in the gas phase in equilibrium with the liquid. The amount of oxygen dissolved in the liquid is proportional to the partial pressure of oxygen in the equilibrium gas phase, therefore, the chemical activity of oxygen measured by an electrochemical sensor is proportional to the concentration of dissolved oxygen.[6]

When a polarizing voltage is applied between the cathode and the anode, two major reactions occur. At the cathode, oxygen is reduced to hydroxyl ions by (2.1).

$$O_2 + 2\,H_2O + 4e^- \longrightarrow 4\,OH^- \qquad (2.1)$$

5. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 1.
6. Ibid.

(2.1) consumes electrons which come from the oxidation reaction at the anode. The half cell reaction of chloride ions in the solution with the metal anode produces the electrons needed to close the circuit.

$$Ag + Cl^- \longrightarrow AgCl + e^- \quad (2.2)$$

A schematic diagram of this polarographic system is shown in figure 2.1.

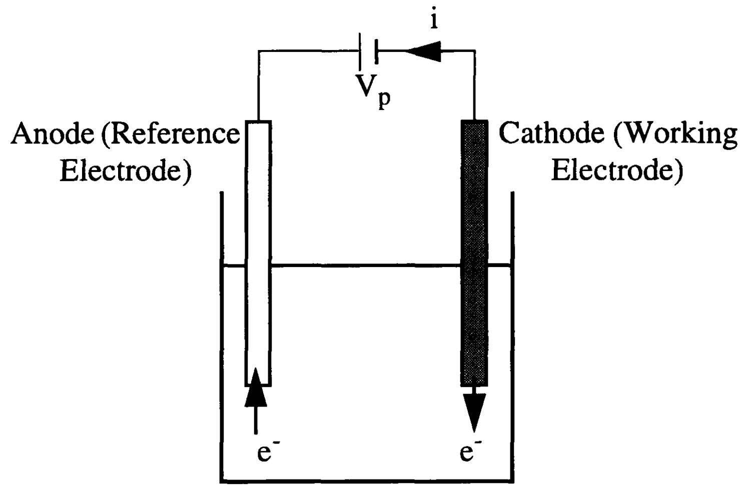


Figure 2.1: Polarographic oxygen measurement schematic

The combination of (2.1) and (2.2) produces a flow of electrons from the anode to the cathode through the electrical circuit. This current is directly proportional to both the surface concentration of oxygen at the cathode and to the reaction rate of (2.1) which is a function of electron pressure at the cathode.

The polarization voltage is the external voltage applied by the measurement apparatus across the cathode and anode. The magnitude of the polarization voltage is chosen to be positive from the anode to the cathode. A polarogram (cathodic current as a function of applied voltage) is shown in figure 2.2 for different oxygen concentrations.

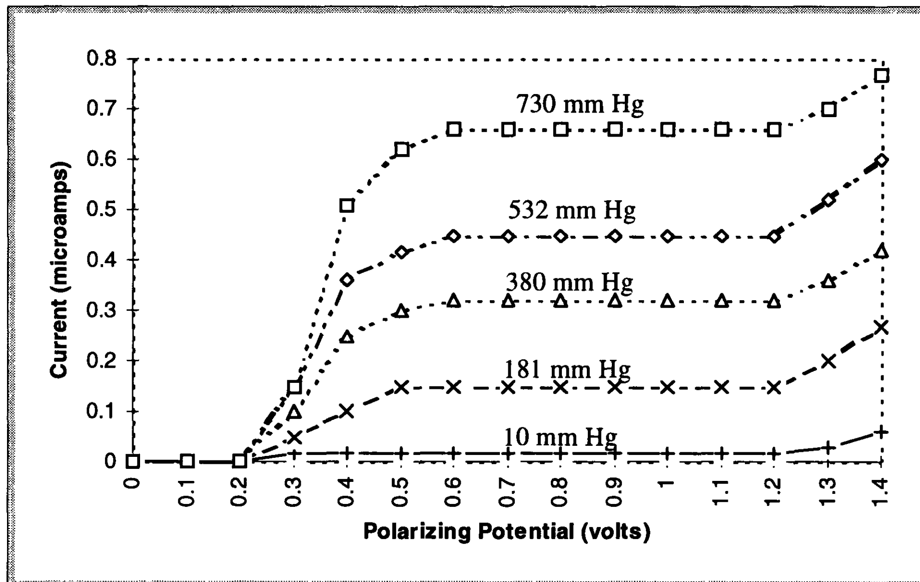


Figure 2.2: Polarogram[7]

At low polarization voltages, the current increases steeply with an increasing imposed voltage. This is due to the reduction of oxygen, (2.1), which is caused by the increase in the electron pressure. Starting at approximately 0.4 V, the rate of the increase in the current begins to slow.

- **From 0 V to 0.4 V** - The reaction rate is faster than the rate at which oxygen molecules can diffuse to the cathode. This causes the surface concentration of oxygen to decrease.
- **From 0.6 V to 1.0 V** - The current no longer increases with an increasing voltage. The electrochemical reaction rate is fast enough to deplete all oxygen molecules at the surface of the cathode. Thus, the current depends solely on how fast oxygen molecules can be transported to the cathode. The two possible mechanisms of oxygen transport are diffusion and convection. Assuming no convection, the current is limited by the rate of diffusion of oxygen toward the cathode. This reaction is said to be *diffusion-limited.*
- **Above 1.0 V** - When the polarization voltage is increased beyond 1.0 V, other

7. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 5.

cathodic reactions such as the reduction of hydrogen ions occur, resulting in a sharply increasing current.

To eliminate the effect of the applied voltage on the current, the operating polarization voltage must be chosen such that the current is diffusion limited. At a polarization voltage of -0.60 V, diffusion of oxygen to the cathode surface is shown by (2.3).

$$j = -D\frac{dc}{dx} \tag{2.3}$$

j = the oxygen flux
D = the diffusion coefficient
$\frac{dc}{dx}$ = the concentration gradient at the cathode

In summary, a polarization voltage (e.g. 0.6 V) is applied, oxygen is consumed at the cathode surface, resulting in an O_2 gradient near the surface and causing O_2 to diffuse to the surface. This O_2 is electrochemically reduced, yielding four electrons for each molecule of oxygen reduced. Because reactions at the electrode surface are very rapid, the rate of reduction becomes diffusion limited as regulated by the polarization voltage. This implies that the electrical current is proportional to the molecular flux of oxygen.

In a completely diffusion-limited reaction, the time constant for the diffusion of oxygen to the cathode surface is given by (2.4) where the time constant is proportional to the square of the distance to the cathode (δ) and inversely proportional to the diffusion coefficient (D).

$$\tau \sim \frac{\delta^2}{\pi^2 D} \tag{2.4}$$

2.1.2 Diffusion at the Polarographic Oxygen Sensor

The rate of reduction of oxygen at the cathode is controlled by diffusion from the bulk of the solution to the electrode surface. Therefore, anything that influences diffusion near the cathode will influence the measured current.[8]

The relations between oxygen tension, time, and distance from the electrode surface at any time after the electrode begins to reduce oxygen are shown in (2.5).

$$\frac{\partial P}{\partial t} = Dk\frac{\partial^2 P}{\partial x^2} \qquad (2.5)$$

P = oxygen tension
t = time
x = distance from the electrode surface
D = the diffusion coefficient for oxygen in the liquid
k = the solubility (or Henry's law constant) of oxygen in the liquid and is related to the concentration of dissolved oxygen (c from (2.3)) by Henry's Law (c = kP)

(2.3) shows that the flux of oxygen to the electrode will be proportional to the concentration gradient at the surface whose solution is found from (2.5). There are two methods by which oxygen can travel to the cathode surface, diffusion and convection. To eliminate convection as a means of oxygen flux, the cathode surface was commonly recessed from the tip of the microelectrode.

Before the polarizing potential is applied to the electrode, it is assumed there is a uniform oxygen tension of P_0 throughout the recess. When the potential is applied, it is assumed that the oxygen tension at the electrode surface, taken as x = 0, goes immediately to 0. This is only an approximation because a complex series of electrical and chemical events take place at the electrode surface immediately upon application of the potential.

8. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 9.

However, for the time scale of interest here, these events die out rapidly, and the electrode takes on a zero oxygen tension.

This "electrochemical" response time to a step change in oxygen tension is a fraction of a millisecond. This demonstrates that the much longer response times observed for oxygen sensors in stagnant or slowly moving solutions are diffusion controlled.[9]

Kolthoff and Lingane, in 1952, showed that the gradient at the electrode surface as a function of time is given by (2.6).

$$\left.\left(\frac{\partial P}{\partial x}\right)\right|_{x=0} = \frac{Po}{\sqrt{\pi D t}} \tag{2.6}$$

The electrical current, i, that flows when oxygen is reduced at the cathode is given by (2.7).

$$i = nFAf \tag{2.7}$$

n = number of faradays of electricity required per mole of electrode reaction
F = the faraday (96,500 C)
A = the electrode area
f = the flux of reducible species to the electrode

Henry's Law can now be substituted into (2.7) to give (2.8).

$$i = nFPokA\sqrt{\frac{D}{\pi t}} \tag{2.8}$$

Because this derivation assumes that the electrode current will eventually become zero, this derived electrode assembly does not yield a sensor of oxygen tension. Instead, a theoretical equation for a sensor that will yield a finite steady-state current that is linearly related to oxygen tension in the external solution must be derived.[10]

---

9. Ibid.
10. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 11.

This derivation was done by Crank in 1957, but is not reproduced here. Crank used a recessed electrode in his derivation, and he concluded that if the electrode were to be a useful sensor, the recession at the tip must be very shallow. Using Crank's model, a recess 3 cm deep would not approach its steady-state oxygen tension level in the external solution for 50 hours. However, a recess of 0.1 mm deep will give a reading that is within a few percent of its final value in about 2 seconds.[11]

Early users of polarographic oxygen sensors used bare wire or a bare exposed circular surface obtained by grinding a flat surface at right angles to a wire sealed in glass. These sensors gave high currents and a rapid response.[12]

The bare wire oxygen electrode was delicate and difficult to make, so it was only used when the sensor was inserted deep into tissue. For use in solutions or on tissue surfaces, a much more convenient electrode configuration is a disk embedded in glass. Unfortunately, the exact analysis of the time response of this system poses a formidable mathematical problem. For this reason, those studying oxygen sensors have approximated the planar disk with a hemisphere.[13]

The oxygen tension at any time after polarization of the electrode and at any point in a solution at rest surrounding the hemispherical electrode is given by (2.9).

$$P = Po\left(1 - \frac{a}{r}\right) + Po\frac{a}{r}erf\frac{(r-a)}{2\sqrt{Dt}} \tag{2.9}$$

Po = the initial uniform oxygen tension
a = the radius of the electrode
r = the distance of any point from the center of the electrode
D = the oxygen diffusion coefficient
t = time

11. Ibid.
12. Ibid.
13. Davies, P. W., and Brink, F., in *Physical Techniques in Biological Research*, Nastuk, W. E., Ed., Academic Press, New York, 1962, 137, pp. 3, 9, 12.

erf = the error function of the argument, shown in (2.10)

$$erf(x) \equiv \frac{2}{\sqrt{\pi}} \int_0^x e^{-y} dy \tag{2.10}$$

In the steady-state, as t approaches ∞, the solution becomes (2.11).

$$P = Po\left(1 - \frac{a}{r}\right) \tag{2.11}$$

(2.11) shows that the electrode reaction will reduce the oxygen tension in the system to 90% of P_o at r = 10a and to 99% of P_o at r = 100a. In other words, the sensor is sensitive to within 1% to the oxygen tension at a distance 100 times the electrode diameter.[14]

The current resulting from the reduction of oxygen at a hemispherical electrode is given by (2.12).

$$i = 2nFDkPo\pi a\left(1 + \frac{a}{\sqrt{\pi D t}}\right) \tag{2.12}$$

From (2.12), the electrode current in the steady-state ($t = \infty$) is proportional to the solution properties (Dk), the electrode radius (a), and the bulk solution oxygen tension (P_o). This electrode is a sensor of solution oxygen tension. (2.12) also shows the time to reach steady-state is dependent on electrode radius. For an aqueous solution where D is about 2 x 10^{-5} $\frac{cm^2}{seconds}$, an electrode with a 0.5 mm radius will be within 10% of its steady-state value in about 4000 seconds, too long a period to be useful in an analytic device. When the electrode radius is reduced to 5 μm, the time to reach within 10% of

14. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 12.

steady-state becomes 0.4 seconds. Polarographic oxygen sensors should ideally have cathodes on the order of 10 to 50 μm in diameter.[15]

2.2 Polarographic Oxygen Electrodes

There have been several different designs of polarographic oxygen electrodes. The evolution of the modern sensor began in the 1950's when Clark attempted to correct many problems associated with bare cathode sensors. However, Clark's sensors tended to be large and therefore applicable only for measurement in large fluid bodies. The current generation of polarographic oxygen electrodes takes the form of hypodermic needles in an attempt to minimize the invasiveness of the electrode.

2.2.1 Clark Electrodes

One of the first widely-used polarographic oxygen electrodes was the Clark electrode which contained both an anode and a cathode in a concentrated electrolyte under a gas permeable membrane such as polyethylene. This sensor is one of the most reliable polarographic electrodes available due to its insensitivity to flow and its environment and time independent calibration. However, for applications other than simple laboratory use, the Clark-type sensor lacks ruggedness, long term stability, sterilizability, ease of maintenance, and high current output.[16]

Clark discovered that many of the problems associated with the bare platinum polarographic sensor could be overcome by covering the cathode with a thin membrane of polymeric material permeable to oxygen, but impermeable to other substances normally present in aqueous solutions. The first Clark electrode had a useful current output, was not

15. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, pp. 11-2.
16. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 35.

"poisoned" by organic materials in solution, and was less sensitive to stirring effects than the bare cathode sensor.[17]

A diagram of the original Clark electrode is shown in figure 2.3.

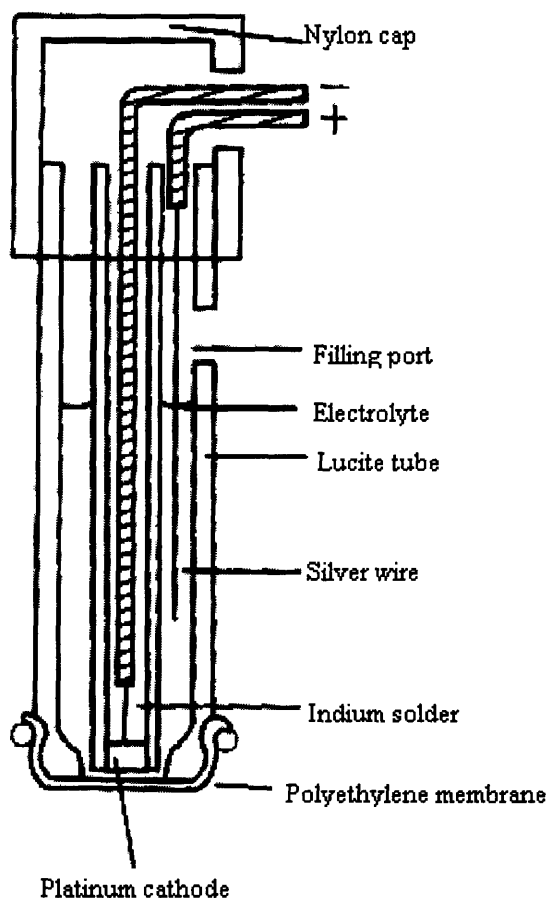


Figure 2.3: Diagram of the original Clark electrode[18]

2.2.2 Needle Electrodes

There needed to be a miniaturization of the sensor for it to be viable for use *in vivo.* Although larger cathodes had many advantages such as a high current output, negligible zero pO_2 current, durability, and ease of manufacture, they were valuable only for use in measuring oxygen tension in bulk liquids, not for *in vivo* measurements.[19] One of the first

17. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 25.
18. Ibid.

attempts to miniaturize a Clark-type sensor was made by Beebe in 1963. A sensor was created in which the anode was a silver tube, the end of which was flush with the glass-supported platinum cathode. This is shown in figure 2.4.

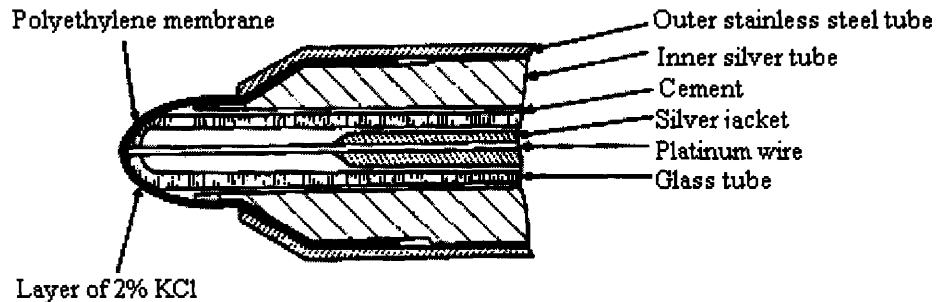


Figure 2.4: Beebe sensor in hypodermic form[20]

The Clark design, in which both the cathode and anode are close together in the same shaft, has the advantage that the electrical resistance is low and extraneous signals from the patient, experimental animal, or tissue sample are minimized. This is not the case for a polarographic oxygen sensor composed of a coated cathode and a remote anode (used as the reference electrode). The Clark electrode can only be miniaturized to a limited degree, and the Beebe design fits the Clark sensor into an 18-gauge needle is among the smallest versions.

A procedure for making a very small, sharply pointed monopolar platinum electrode is described by Ballintyn in 1961. As described above, this is a polarographic oxygen sensor where the cathode and the anode were separate. Ballintyn's procedure is shown in figure 2.5.

In figure 2.5, the upper left diagram shows a platinum wire electropolished to a point in a saturated sodium or potassium nitrite solution. An AC potential of 1 to 1.5 V is

19. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 30.
20. Ibid.

applied to the wire and a carbon rod until a point forms. The platinum wire is soldered, or preferably spot welded, to a larger copper wire. The diagram in the upper center shows a piece of glass capillary tubing sealed to the platinum wire. The hook on the glass tube holds the weight that pulls the glass upon heating by a heated wire. The lower diagrams show glass coating and tip configuration.[21]

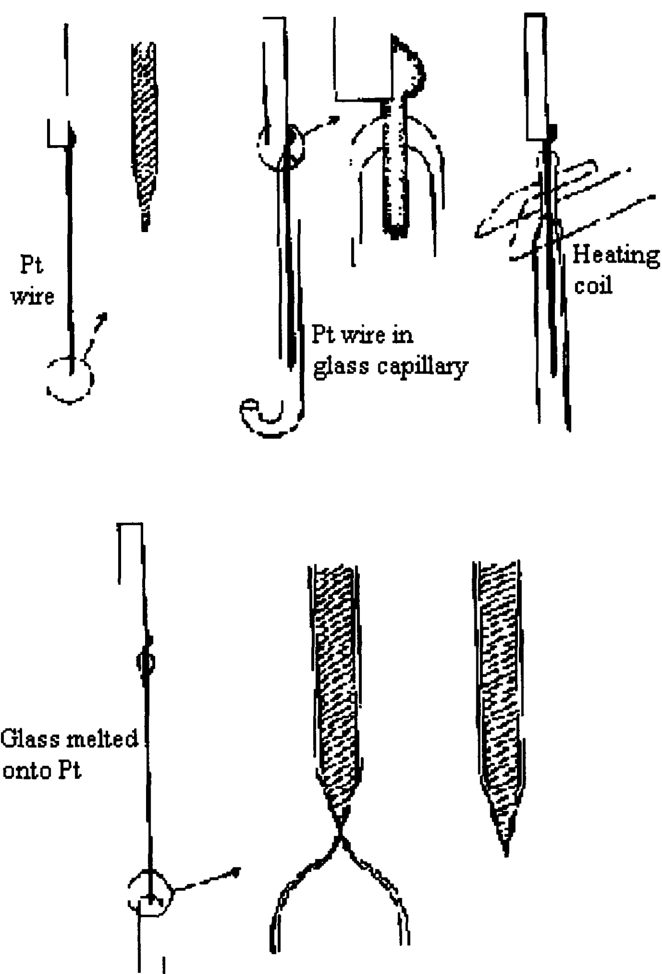


Figure 2.5: Ballintyn procedure for platinum electrode fabrication[22]

The bare needle electrode usually does not show a polarographic plateau (see figure 2.2) that is as well defined as for a covered electrode. However, since the bare needle elec-

---

21. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 64.
22. Ibid.

trode is normally used for observing relative changes in tissue oxygen tension, the absence of the polarographic plateau is not critical.[23]

Collodion was the most popular coating material in early designs. Collodion is permeable to oxygen and small ions, but impermeable to the large organic molecules that poison the electrode surface. However, collodion does not adhere very well to metallic surfaces. Today, many different coatings are used, including Teflon.

2.3 Complications with *In Vivo* Polarographic Oxygen Measurements

In vivo measurements were originally performed using dropping mercury electrodes, but they were cumbersome and inconvenient in routine clinical use[24]. Many researchers attempted to circumvent the inconveniences of the dropping mercury electrode by using an amalgamed gold wire as the polarographic cathode. The problem of protein poisoning arose when the metallic surface came into contact with blood. Inch, in 1958, observed that any metallic surface in blood or other body fluids will collect a film of proteinaceous material that can only be cleaned off with dichromate solution.[25] Leaving the film on the cathode leads to a downward drifting current and unreproducible results.

In 1951, Drenckhahn used a collodion-covered platinum cathode for polarographic measurements of oxygen in blood. The collodion coating kept protein and other deleterious materials away from the electrode, but allowed free passage of oxygen and salt ions. The passage of salt ions was essential because an electrical connection via ion conduction to the separate anode was necessary.[26]

---

23. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 63.
24. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 47.
25. Fatt, Irving, *Polarographic Oxygen Sensors*, Malabar, Florida: Robert E. Krieger Publishing Company, 1982, p. 48.
26. Ibid.

Other methods to eliminate the effects of poisoning were attempted as well. Mochizuki and Bartels, in 1955, used a bare platinum cathode in blood, but applied alternating pulses of positive and negative polarity to the cathode. The positive potential applied to the cathode was for the purpose of removing, possibly via oxidation, any organic film that might accumulate on the cathode.[27]

---

27. Ibid.

Chapter 3

The Carbon Fiber Microelectrode

3.1 Design Phase

While designing the carbon fiber microelectrode, several different designs were tested. The final design combined characteristics of the Cespuglio design with some characteristics of a Thermal Technologies, Inc. (TTI) butterfly needle microelectrode.

3.1.1 Cespuglio Design

The idea for a carbon fiber microelectrode originated in France and was introduced in the United States by Charles Ruban, a visiting French scholar. Ruban's advisor, Dr. Cespuglio, originally designed the microelectrode, not for oxygen measurements, but for measurements of other molecules in the brain.

One of Cespuglio's designs was the monofiber microelectrode which used only one carbon fiber (a cylinder 5-20 μm in diameter). This monofiber microelectrode differed from the polarographic microelectrode discussed here in two ways. First, the monofiber microelectrode used a *single* carbon fiber whereas the polarographic microelectrodes used *multiple* fibers which provided the measurement equipment with sufficient signal. Second, the monofiber microelectrode was fabricated with the carbon fiber protruding from the tip of the microelectrode, exposing not only a cross-sectional area at the tip, but an axial surface as well. In contrast, the functional cathode surface of this polarographic microelectrode was fabricated such that the carbon fibers were flush with the tip of the microelectrode, thereby exposing only the transverse cross-section.

3.1.2 Ruban and Yip Design

When the carbon fiber idea was introduced, it needed to be designed so that a viable polarographic oxygen microelectrode could be fabricated. The first step involved verify-

ing that a bundle of carbon fibers would work as a suitable cathode. The goal in this design was not to optimize the size of the microelectrode, but instead to fabricate a working device.

A polyurethane catheter approximately 700 μm in diameter was used as a housing to encase the cathode. The larger housing allowed more latitude in the design of the cathode since size was not an issue. Following cathode design optimization, the housing could be made smaller to fabricate a minimally invasive device to be used *in vivo*.

During this phase, a working cathode was fabricated inside a 700 μm catheter. The microelectrode only demonstrated sensitivity to changes in oxygen tension *in vitro*, not *in vivo*. This was most likely due to the size of the catheter which caused significant tissue compression upon insertion of the microelectrode. In *in vitro* experiments, changes in the gas concentration of the phosphate buffered solution (PBS) were mimiced by the microelectrode. However, *in vivo*, in the rat liver, presumed changes in the rat's partial pressure of oxygen brought about by administration of pure oxygen were not mimiced by the microelectrode.

3.1.3 Yip and Mangin Design

From June, 1995 until August, 1995, the glass needle microelectrode was designed and successfully tested both *in vitro* and *in vivo*. This design was a joint effort by the author and Stephan Mangin, another visiting French scholar. The framework for an effective cathode was already in place, and a more effective housing needed to be fabricated.

The glass needle design focused on minimizing the diameter of the tip of the microelectrode. By pulling glass capillary tubes, the diameter of the resulting necked glass tips was one order of magnitude smaller than that of the polyurethane catheter. The result was a tip diameter slightly larger than the diameter of the carbon fiber bundle used as the cathode.

3.2 Specifications for the Final Design

There are several specifications that must be met for a carbon fiber microelectrode to work properly both *in vitro* and *in vivo*.

3.2.1 Size

The size of the microelectrode is important for two reasons. First, the diameter of the tip must be sufficiently small to substantially minimize tissue compression effects as the microelectrode is inserted. Secondly, the body of the microelectrode must be sufficiently large to ensure that the user can manipulate it effectively. Manipulation by the user is important because a larger microelectrode body facilitates easy repositioning of the microelectrode should it become necessary. The glass needle housing for the cathode fulfills these criteria with tips on the order of 100 μm in diameter and shafts approximately 5 cm. in length.

3.2.2 Conductivity of the Cathode

The conductivity of the cathode must be within a certain range for it to work effectively with the recording equipment, the Thermal Technologies, Inc. TDP-OX. If the conductivity is too high, the current recorded by the TDP-OX will be too high and will saturate the TDP-OX's amplifier. If the conductivity is too low, the current will be undetectable by the TDP-OX.

The conductivity of the cathode is reasonably simple to optimize. Because the rate of reduction of oxygen at the cathode is dependent on the surface area of the cathode, the number of carbon fibers used in fabrication will determine the conductivity of the cathode. By using between five and forty carbon fibers, the resulting conductivity will be in the 10 $M\Omega$ range and will result in measured currents in the nA range, an acceptable range for the TDP-OX.

3.2.3 Durability

To endure repeated use, the microelectrode must be sufficiently durable to withstand heavy use. Glass is rather durable, but the tips, being very small in diameter, are not as durable and must be protected. The thin tips are easily broken when exposed to sheer forces. When in soft tissue, this does not pose much of a threat, but when tested *in vitro* in glass beakers, the threat of broken tips is greater. Although the polyurethane catheter is more durable due to its flexible nature, it has size limitations which make it impractical for *in vivo* use.

3.2.4 Reliability

The microelectrode must be fabricated such that it is reliable in repeated testing. Maximal reproducibility is a goal of this project, therefore, microelectrode reliability is of the utmost importance. In other words, the materials used in the fabrication process must be robust and able to endure the rigors of different fluids. The solution to this lies in the selection of an insulation epoxy, a process discussed in 3.3.2.

3.3 Materials Selection

There are several crucial components in a polarographic oxygen microelectrode that, unless they are fabricated properly, can lead to instability and failure of the microelectrode. It was important to ensure that the materials used in the fabrication of these components were selected carefully to ensure proper functioning of the microelectrode.

3.3.1 Carbon Junction

The function of the component termed the carbon junction is to provide a highly conductive adherent for attaching the carbon fiber bundle to the copper lead wire. This carbon junction is created by mixing a conducting epoxy with carbon powder.

There are three essential properties for this carbon junction. First, the viscosity of the conductive epoxy must be low so that when mixed with the carbon powder, the mixture

remains at a low viscosity. It is important for the viscosity be low enough so that the catheter used to hold the fibers and wire together while the epoxy mixture cures (figure A.4.1 in appendix A) can be easily filled with the epoxy-powder mixture. Second, the conductance of the carbon junction must be very high so that the junction is not the limiting resistance in the signal travelling from the cathode surface to the recording equipment. Third, the epoxy mixture must have a high adherence to both the bundle of carbon fibers and the stripped copper wire. If the adherence to either substance is not good, the result may be a decreased effective conductance.

Several epoxies were tested for mechanical strength and electrical conductivity. The results are summarized in table 3.1.

Table 3.1: Summary of Carbon Junction Epoxies Tested

Resin	Conductivity when Cured	Fragility when Cured	Viscosity (after mixing)	Mechanical Strength	Adherence to Polyethylene Catheter
Sody Escil	Very good	Not Fragile	Low	Fair	Low
Trac-Cast BB 3103	Good	Not Fragile	Low	Good	Low
Trac-Bond BA-FDA2	Good	Not Fragile	Low	Very Good	Low

Although the mechanical properties of the Trac-Cast and Trac-Bond epoxies were better, the superior electrical conductivity of the Sody-Escil epoxy made it the logical choice.

As discussed before, the carbon junction was molded in a catheter. If the catheter were left on the junction, the size of the junction would close to 1000 μm . Therefore, the catheter needed to be removed after the epoxy cured. The catheter was removed by using a scalpel to slice the side of the catheter and carefully removing it from the junction.

Because the catheter needed to be removed from the cured junction without damaging to the carbon fibers, it was important to use a catheter material that did not adhere well to the cured epoxy. Materials tested were silicone, polyurethane, and polyethylene, and it was found that the polyethylene catheter adhered least well to the cured epoxy junction. This polyethylene catheter had an inside diameter of 450 μm and an outside diameter of 700 μm.

The combination of the Sody Escil resin with a polyethylene catheter was used to fabricate the carbon junction.

3.3.2 Insulation Epoxy

The role of the insulation epoxy is to electrically protect the carbon fibers and carbon junction from the fluid or tissue being measured. This is essential for the reliability and durability aspects of the microelectrode. If fluids were to invade interstitial spaces created by degradation of the insulation epoxy, the effective surface area of the cathode may change, leading to unreliable measurements.

There are three properties desired for the insulation epoxy. First, the viscosity must be very low during the fabrication process so that the epoxy completely fills all interstitial spaces. Carbon fiber bundles have inter-fiber spaces on the order of 1 μm, and it is essential for the epoxy to be fluid enough to permeate and fill those spaces. Second, unlike the carbon junction epoxy, the insulation epoxy must be an excellent insulator. Third, the epoxy must adhere very well to both the glass capillary tube and the carbon fibers. If this adherence is weak, interstitial spaces may result.

Several different characteristics were tested. First, the color of the epoxy was a characteristic found to be important. Clear or light-colored epoxies are preferable because they allow the carbon fibers and carbon junction to be viewed. Hardness and fragility are also important because if the epoxy is fragile, movement of the microelectrode could snap the

epoxy and the carbon fibers encased within. Viscosity of the epoxy is important to ensure all interstitial gaps are filled. In table 3.2, the adherence to a polyurethane catheter is noted because this search was conducted before the glass needle design was introduced. This adherence is important so that no gaps between the carbon fibers and the catheter exist. The state of the surface after being cut refers to the catheter tip following curing of the epoxy. A sharp scalpel was used to slice across the tip of the catheter to create a sharply beveled edge. If the cut surface is filled with particles or bubbles, then the cathode surface may change over time as particles fall off of the surface.

Seven epoxies were tested, and the results are summarized in table 3.2.

Table 3.2: Summary of Insulation Epoxies Tested

Resin	Color	Hardness	Fragility	Viscosity (after mixing)	Adherence to Catheter	State of Surface after Cut
Sody Escil	Clear	Very Low	Not Fragile	Low	Good	Rough
Trac-Cast	Black	Very High	Very Fragile	Low	Poor	Very Rough
Trac-Bond	Yellow	High	Not Fragile	Low	Fair	Good - Bubbles
Epotek 301	Clear	Medium	Not Fragile	Low	Fair	Fair - Bubbles
Epotek 353	Clear	High	Fragile	Low	Fair	Fair - Particles
Eccobond 227	Clear	Medium	Not Fragile	Low	Excellent	Very Good
EP 30 FL	Clear	High	Fragile	Very Low	N/A	N/A

3.3.3 Encasing Material

The final problem was to find a suitable material to serve as the housing for the cathode. Three different materials were tested. In the initial design phase, only a polyurethane and a polyethylene catheter were tested. During the later design phases, a glass capillary tube was also tested.

An effective encasing material must have several properties. First, it must be impermeable to the fluids that are being measured. If fluids are allowed to compromise the integrity of the cathode housing, problems may result. Second, housing durability is one of the design goals. Problems associated with cracks in the microelectrode due to poor durability may manifest themselves, causing problems. Third, the encasing material must adhere very well to the insulation epoxy described in 3.3.2. This is to ensure that none of the fluid being measured can seep through the epoxy-catheter interface. If fluid travels in channels between the epoxy and the housing, the ability of the housing to act as a barrier from the measured fluid would be destroyed. Lastly, the size of the housing, in particular the tip, is very important. One of the hypothesized problems with larger microelectrodes was probe-induced tissue trauma which states that smaller tipped microelectrodes lead to a smaller degree of tissue compression at the insertion point.

The catheters were first washed clean with alcohol and filled with the Eccobond 27 epoxy. The epoxy was cured and the tips were sliced diagonally. This tip was then examined under the microscope to more closely examine any gaps between the epoxy and the catheter.

The polyurethane catheter was chosen because Eccobond 27 epoxy adhered better to it than to the polyethylene catheter. In later design phases, a pulled glass capillary tube was used due to its smaller tip size. The polyurethane catheter had an outside diameter of 900 μm, and the pulled glass capillary tube had an outside diameter of approximately 150

μm . With such a small tip, it became impossible to evaluate the adherence of the Ecco-bond 27 epoxy with the glass by examining it in the microscope. Based on *in vitro* testing, this adherence appeared to be as good as the adherence to the polyurethane catheter.

3.4 Fabrication

Three different variations of the final design were constructed to find the best design. Of these three variations, two used carbon fiber cathodes while the third used a gold wire cathode. The two carbon fiber microelectrodes were constructed in slightly different manners. A brief synopsis of the characteristics of each microelectrode is described in 3.4.1, 3.4.2, and 3.4.3, and complete fabrication instructions are shown in appendix A.

3.4.1 The Gold Wire Microelectrode

The fabrication of this design served as a template for the fabrication of the carbon fiber microelectrodes. This is because to construct the carbon fiber microelectrode, only small changes in the fabrication procedure were needed.

A thin gold wire (50 μm in diameter) was used as the cathode. The finished microelectrode was to be of the same type as other commercially available oxygen microelectrodes such as the TTI butterfly needle microelectrode. The only differences were the cathode housing (glass rather than a 27 gauge stainless steel needle) and the insulation epoxy.

The gold wire is soldered to stripped copper wire which serves as the connection wire. This wire assembly is placed into a glass capillary tube with an outside diameter of approximately 1.5 mm. The capillary tube is then necked in a glass pulling machine, resulting in a necking that forms a tight glass seal around the gold wire. Following necking, the glass breaks, and a sharp tip with the gold wire embedded within results. To

ensure the cathode is sufficiently isolated from the measured environment, the Eccobond 27 insulation epoxy is inserted into the needle using a syringe.

This glass needle assembly is then connected to a flexible polyurethane catheter to ensure flexibility of the microelectrode body. Soldering then connects the copper connection wire to a male connector for interfacing with the TDP-OX.

3.4.2 The Carbon Fiber Microelectrode

This design was the first of the two carbon fiber cathode designs fabricated. A bundle of carbon fibers was inserted into a glass capillary tube and the glass was necked around the fibers in the same manner as the gold wire microelectrode. This is different from the prebundled carbon fiber microelectrodes described in 3.4.3.

Instead of solder, a carbon junction connects the carbon fibers to the copper connection wire. This carbon junction is made using the Sody-Escil epoxy and carbon powder. After the bundle of carbon fibers is attached to the copper wire, the carbon fibers are placed into a glass capillary tube, and the glass is necked around the fibers. A syringe is used to fill the tip of the glass needle with Eccobond 27 epoxy to ensure fluid impermeability.

This glass needle assembly is then connected to a flexible polyurethane catheter to ensure flexibility of the microelectrode body. Soldering then connects the copper connection wire to a male connector for interfacing with the TDP-OX.

3.4.3 The Prebundled Carbon Fiber Microelectrode

The second carbon fiber cathode design differs only slightly from the fabrication process described in 3.4.2. Before the carbon junction is made, the bundle of carbon fibers is premixed with Eccobond 27 epoxy, hypothetically eliminating all inter-fiber spaces.

These carbon fibers are dried to form a bundle which is connected to the copper connection wire using the carbon junction. Instead of necking the glass around this bundle,

the glass capillary tube is pulled without anything inside. The resulting neck is carefully broken so that the bundle can fit through the tip. The resulting interface between the carbon fibers and the glass tip is not as tight as in the other two designs. A syringe is used to fill the glass needle with Eccobond 27 epoxy.

This glass needle assembly is then connected to a flexible polyurethane catheter to ensure flexibility of the microelectrode body. Soldering then connects the copper connection wire to a male connector for interfacing with the TDP-OX.

Chapter 4

In Vitro and *In Vivo* Experiments

4.1 Objectives

Two different types of experiments were conducted using the fabricated carbon fiber microelectrodes, the *in vitro* experiments in phosphate buffered saline solution (PBS) and *in vivo* experiments done to test the data obtained against that of commercially available microelectrodes.

4.2 *In Vitro* Experiments

Two different types of *in vitro* experiments are described in appendix B. One is the long term experiment, a set of studies that tests the fabrication and integrity of the micro-electrode. The second is the short term experiment which provides data used to calibrate the microelectrode to "directly" measure *in vivo* pO_2.

4.2.1 Experimental Setup

The equipment needed to conduct measurements with the fabricated microelectrodes included the following:

- IBM PC compatible computer with JCOX program
- TDP-OX oxygen measurement box
- TDP-100 oxygen measurement box
- Power supply for TDP boxes
- Thermoregulated water bath
- Ring stand(s)
- Phosphate-buffered saline solution (PBS)
- Flasks to hold PBS
- Gas tanks with different concentrations of O_2
- Reference electrode
- Fabricated microelectrode

A schematic diagram of the measurement system is shown in figure 4.1, and a diagram of the *in vitro* laboratory setup is shown in figure 4.2

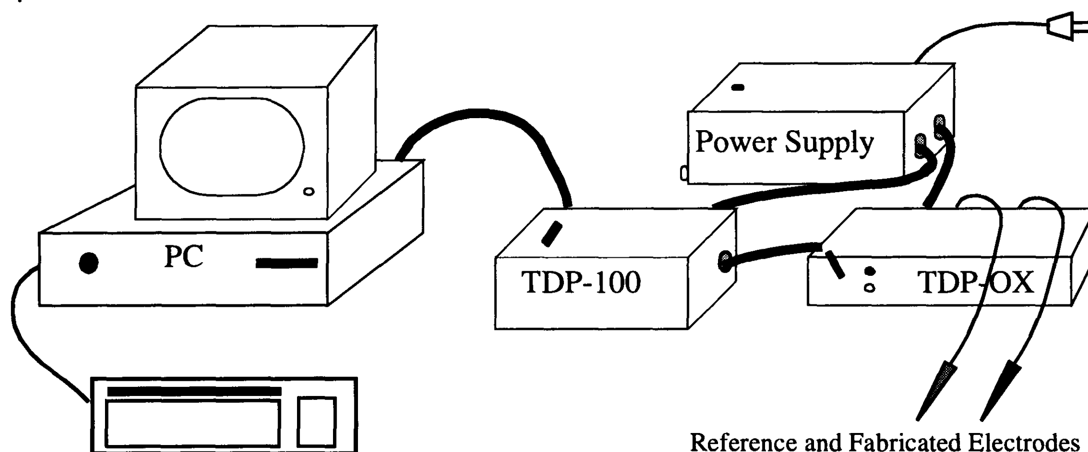


Figure 4.1: Schematic diagram of measurement system

O_2 measurements are done using two electrodes, the working electrode (the gold wire microelectrode, the carbon fiber microelectrode, etc.) and the reference electrode (Ag/AgCl). The working electrode, or cathode, is negatively polarized with respect to the reference electrode which forces reduction of oxygen at its surface. The measurement apparatus used in these experiments are controlled by an IBM PC compatible computer which also stores the acquired data.

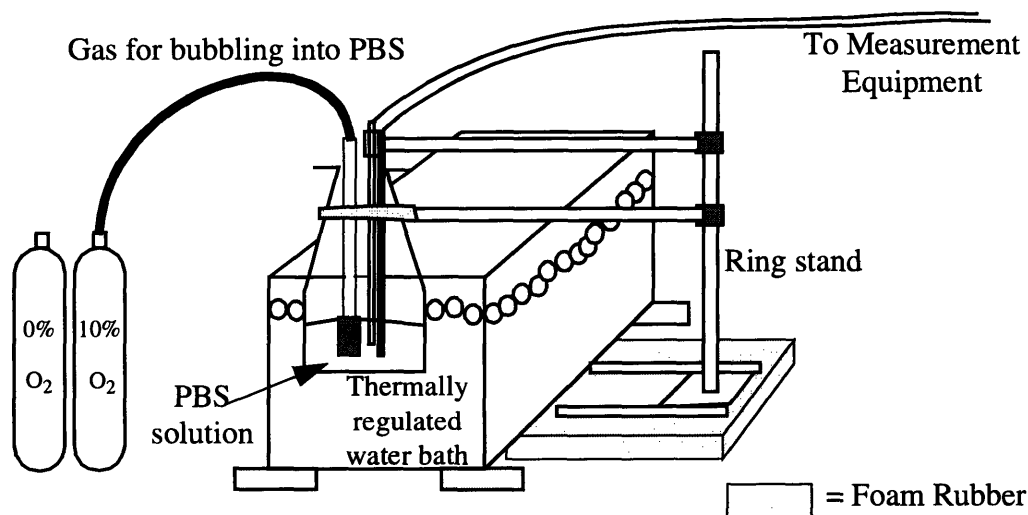


Figure 4.2: Schematic diagram of *in vitro* experimental setup

The water bath and ring stand are placed on foam rubber pads to minimize vibration which may cause convection. Convection would provide a second method through which oxygen could be transported to the cathode surface, leading to an inflated pO_2 current. The thermally regulated water bath is kept at 37 $°C$ to simulate the *in vivo* measurements temperature. The water bath has floating spheres that cover the surface for minimal heat loss. The level of the PBS in the flask must be kept lower than the water surface in the bath to ensure that all of the PBS is heated to the water bath temperature.

The calibrated gasses that are bubbled in the PBS contain various levels of oxygen. Measurements made at 0% O_2 require that calcium chloride be added to the PBS to completely consume the oxygen in the solution. To ensure the oxygen concentration in the PBS has reached equilibrium, gasses should be bubbled in the PBS for at least one hour.

4.2.2 The Long Term Experiment

In the long term experiment, the microelectrodes are tested for at least 24 hours in segments ranging from three to twenty hours in length. The purpose is to determine if the fabrication of the microelectrode created a durable and reliable cathode.

The long term experiment examined the integrity of the interfaces between the insulation epoxy and the carbon fibers and between the insulation epoxy and the glass. If the microelectrodes were well fabricated, no fluid from the experimental medium should permeate the interstices. The current recorded should be steady, remaining at the same level at the beginning as well as at the end of the 24 hour recording period. If there are interface problems, this would most likely manifest itself as a current that increases with time. A comparison of these two different scenarios is shown in figures 4.3 and 4.4.

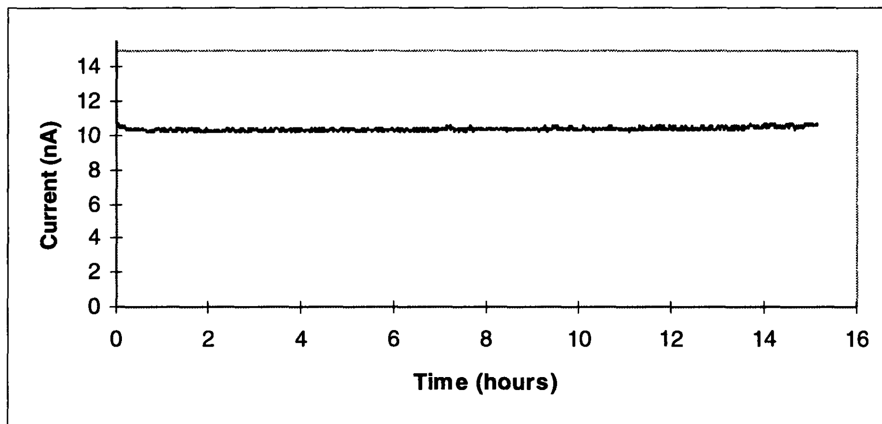


Figure 4.3: Graph showing a microelectrode that has been well fabricated

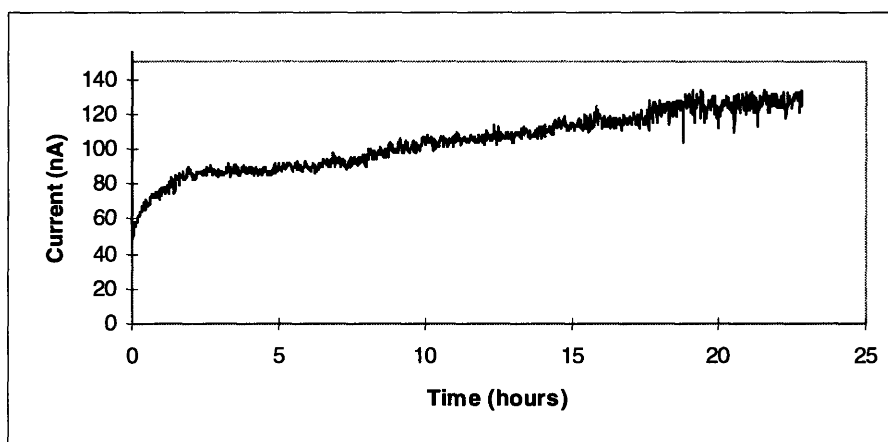


Figure 4.4: Graph showing a microelectrode that has been poorly fabricated

In figure 4.3, the curve is flat with the current unchanging over time. In contrast, figure 4.4 shows an increasing current over time, with the final current level over two times the initial level. Something is wrong with the microelectrode used in the experiment in figure 4.4, and it was hypothesized that the integrity of one of the two interfaces described above was breached, increasing the cathode surface area, increasing the current measured.

A third hypothesis to explain the increase in current was diffusion of PBS solution through the epoxy. To test this, microelectrodes were fabricated and used in PBS dyed with food coloring. If diffusion of PBS into the insulation epoxy occurred, the microelectrode tip would change color. This was not observed, and this hypothesis was disregarded.

4.2.3 The Short Term Experiment

The short term experiment is a series of five minute tests to ensure that the microelectrode would give reproducible results when tested in a manner similar to that of the *in vivo* tests. Another purpose was to calibrate the microelectrodes at various oxygen tensions. It was more costly to perform long experiments at non-atmospheric oxygen tensions because large amounts of gas would be expended. Also, it would be difficult to maintain a non-atmospheric oxygen tension without bubbling the gas through the PBS periodically, an action that would introduce convective currents.

A cycle of bubbling the gas through the PBS for five minutes, then measuring the oxygen tension for five minutes formed the short term experimental protocol. By performing multiple tests at the same oxygen tension, it was quickly apparent whether results would be reproducible for the given microelectrode. If the steady-state current was the same between runs, the microelectrode could be used in subsequent *in vivo* studies. If the steady-state current changed between runs, problems with the microelectrode may exist.

Figure 4.5 shows a series of experiments that led to calibration of that microelectrode. The steady-state currents of all three runs were essentially identical.

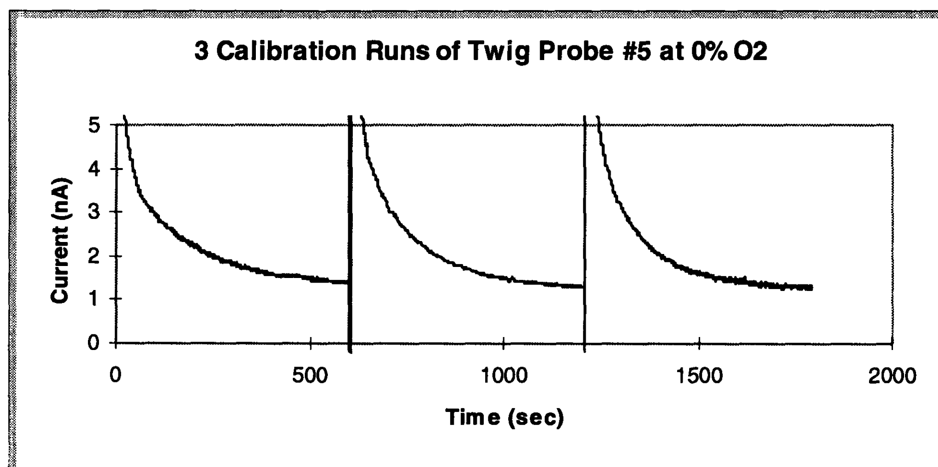


Figure 4.5: Series of experiments leading to calibration

Following a series of short term experiments at oxygen concentrations of 0%, 10%, and 21%, a calibration was performed with the results shown in table 4.1 and figure 4.6.

Table 4.1: Calibration Values

% Oxygen	Measured Current (nA)
0%	1.3 nA
10%	3.95 nA
21%	6.4 nA

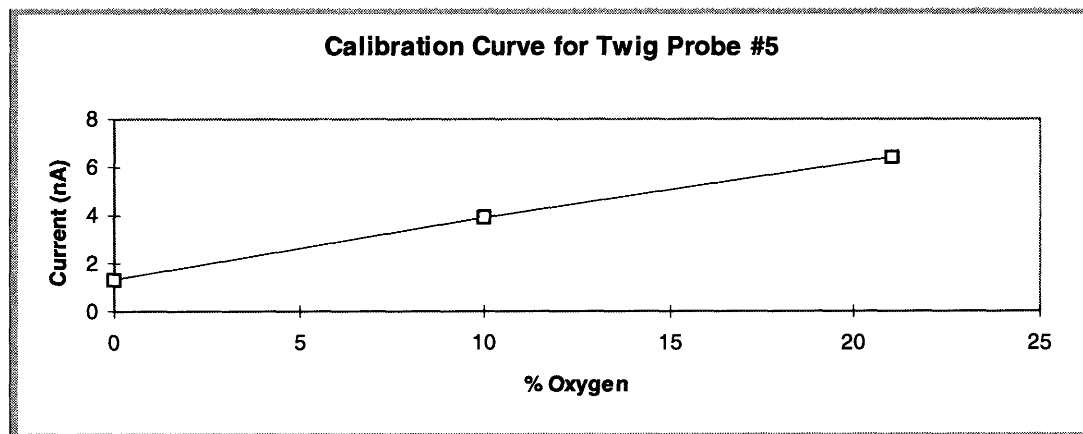


Figure 4.6: Calibration graph

4.3 *In Vivo* Experiments

4.3.1 Experimental Setup

In vivo experiments were performed in rat livers using almost all of the equipment listed in 4.2.1. The experimental protocol is described in appendix C, and a schematic diagram of the experimental setup is shown in figure 4.7.

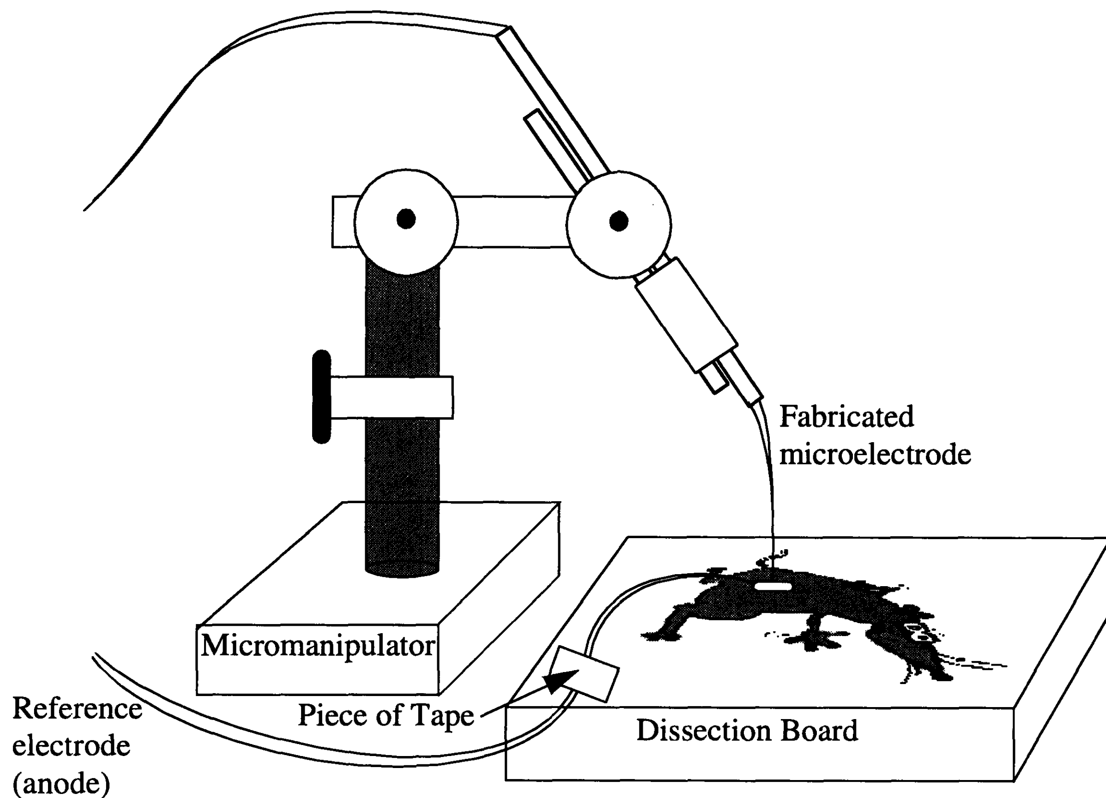


Figure 4.7: Schematic diagram of *in vivo* experiment setup

The microelectrode is attached to a micromanipulator which uses gear differentials to precisely manipulate the position of the microelectrode tip. This is so the microelectrode does not move after being placed in a particular location. Slight compression is placed on the microelectrode tip such that the probe follows the breathing motions of the rat. The reference electrode is allowed to move and is affixed to the dissection board using tape.

4.3.2 Experiments on the Rat Liver

In vivo experiments were done for two reasons. First, results obtained with fabricated microelectrodes were compared to results obtained with commercially available micro-electrodes. Second, experiments done with fabricated microelectrodes were analyzed to see if externally induced events involving rat pO_2 were recorded by the microelectrode.

Externally induced events consisted of pure oxygen gas being administered to the rat via a gas mask (modified syringe). The goal of this pure oxygen administration was to see

if there was a measured increase in the pO_2 of the liver. If, within physiological parameters, the oxygen tension in the liver did not increase following oxygen administration, either the microelectrode's sensitivity was suspect or the measurement location was not close enough to the major vascular branches to reflect pO_2 changes. Another external event was to allow the anesthesia to lighten with time, then to inject additional anesthesia to deepen the level. Vasomotor activity was detected by sensitive microelectrodes.

Results shown in figures 4.8 and 4.9 show examples where the microelectrode measured the rat's response to oxygen administration as well as to injections of anesthesia.

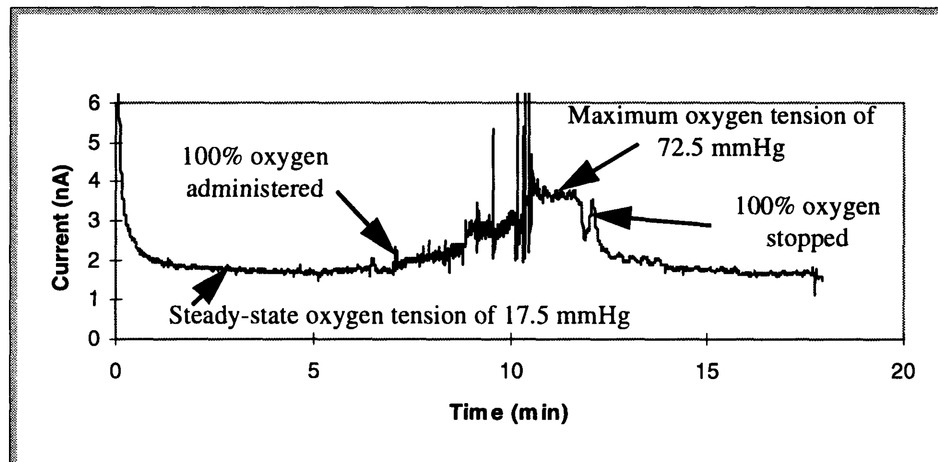


Figure 4.8: Graph showing the effects of pure oxygen administration

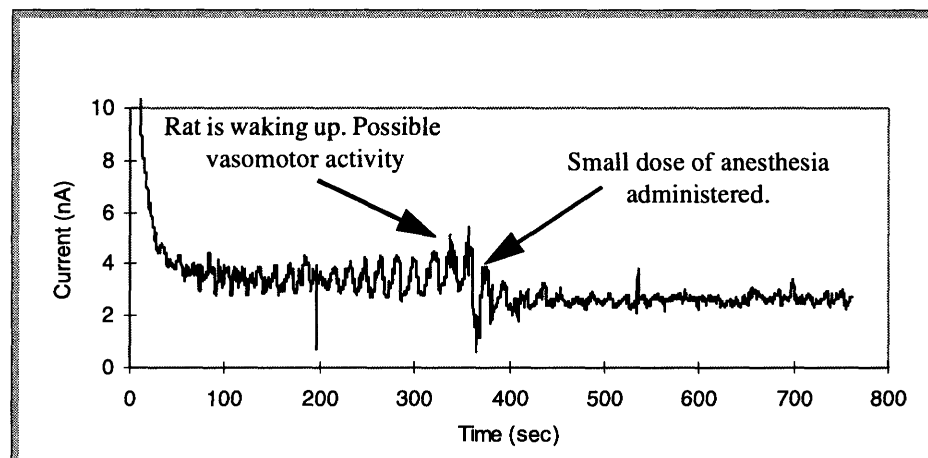


Figure 4.9: Graph showing temporal response of rat pO_2 to low anesthesia levels

Chapter 5

Results: Long Term Measurements

5.1 Objectives

The ultimate objective of this research is to fabricate stable, calibratible carbon fiber microelectrodes for use *in vivo*. Since the fabrication process is not mechanically monitored for preciseness, there may be slight differences between different microelectrodes. These differences may have no effect on the microelectrode's operation, but other differences may render the microelectrode unusable.

Stability is one of the objectives, and insulation problems can lead to instability, especially *in vivo*. If either the carbon fiber-insulation epoxy interface or the glass tube-insulation epoxy interface is not tight enough, the cathode surface exposed may change with time. This is not desirable and may lead to instability of the microelectrode.

The ability to measure *in vivo* pO_2 depends on the calibration of the microelectrode. The measured currents of each microelectrode must be within the bounds of the TDP-OX amplifier for it to be calibrated.

5.2 Hypothesis of Current Leakage

Current leakage, a steady increase in the measured current over time, was first noted in July, 1994. This leakage is indicative of an imperfect interface and prominently manifests itself during longer experiments.

The long term *in vitro* experiments shed some light on the interface problems observed. If the problem was at the interface between the insulation epoxy and the glass, the graph of the measured current would presumably appear flat for some period of time, then shoot up quickly when the PBS came in contact with the copper wire. The carbon junction was encased in insulation epoxy, therefore a poor glass-epoxy interface would

cause a short circuit only when the PBS reached the copper wire. An example of this hypothesis is shown in figure 5.1.

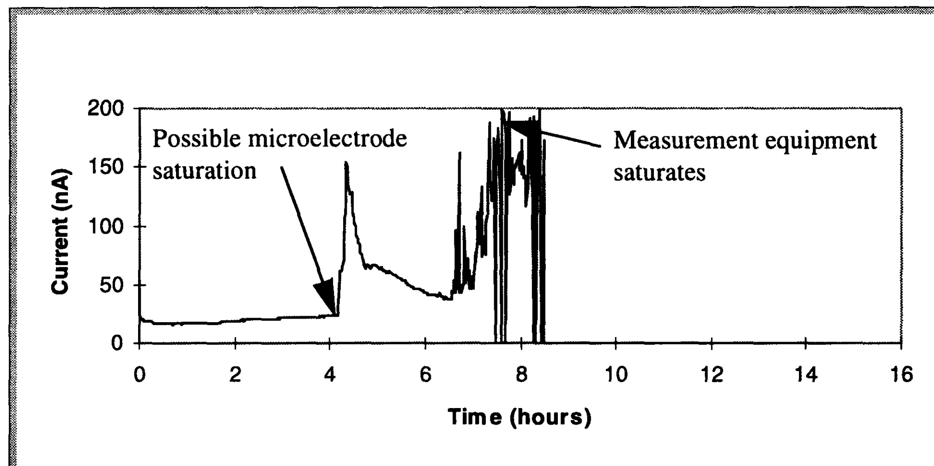


Figure 5.1: Graph of *in vitro* pO_2 reflecting hypothesized poor glass/epoxy interface

In contrast, microelectrodes that have interface problems between the insulation epoxy and carbon fibers should show a steady, gradual rise in the current level, much like that shown in figure 5.2. It was hypothesized that the cathode surface area exposed to the PBS increased with time, increasing oxygen reduction, resulting in a higher current.

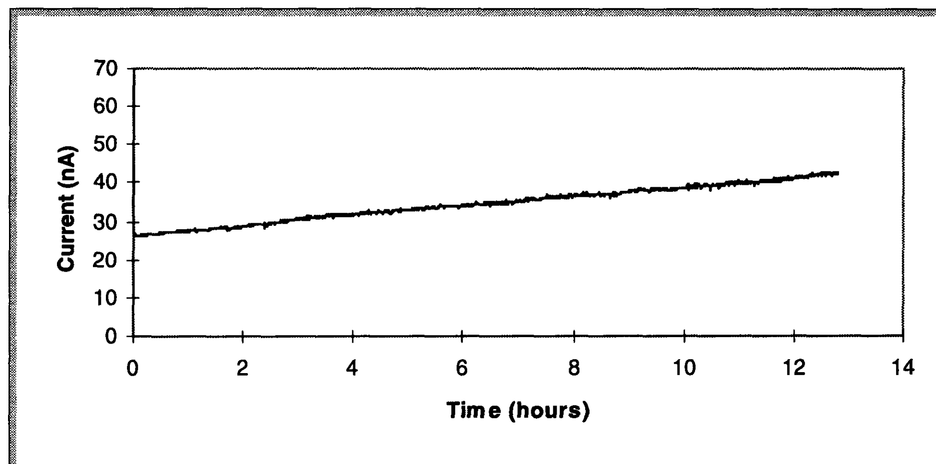


Figure 5.2: Graph of *in vitro* pO_2 reflecting hypothesized poor glass/epoxy interface

Premixing the carbon fibers and insulation epoxy would presumably eliminate all interfiber spaces, minimizing the problem illustrated in figure 5.2. In contrast, the glass-

insulation epoxy interface problem would only manifest itself once PBS had seeped past the insulation epoxy to the copper wire. Even so, the copper wire, being insulated above the carbon junction, should not conduct, and the current should not have increased.

The hypothesized origin of the current leakage is in the spaces between the individual carbon fibers. If the insulation epoxy does not completely fill those interstices, more of the cathode would be exposed, the diffusion length for O_2 would increase, and the observed current drift will result.

5.3 Methods

Experiments were performed on three different types of glass needle microelectrodes. Gold wire, carbon fiber, and bundled carbon fiber microelectrodes were used, and collodion membranes were applied to certain probes to see what effect membranes would have on the drifting current. As a reference, a 10 $M\Omega$ resistor and a TTI gold wire steel needle microelectrode were also tested to ensure the equipment was working properly.

Appendix B describes the protocol for *in vitro* experiments. Precautions were taken to minimize equipment vibration, keep the water bath temperature constant, and eliminate any oxygen consuming bacterial growth in the PBS. The reference electrode used was a silver wire polarized positively for 30 seconds versus a gold wire in a 1% NaCl solution.

5.4 Results

5.4.1 Reference Measurements

Several experiments were carried out using a 10 $M\Omega$ resistor to ensure the TDP equipment was working properly. A 10 $M\Omega$ resistor, placed across the anode and cathode terminals of the TDP-OX, should lead to a current of 62.5 nA. The polarization voltage of the TDP-OX is 0.625 V, a voltage in the diffusion-limited region of the polarogram in figure 2.2. By using the V=IR relationship, a resistor of $1 \times 10^7\ \Omega$, and a voltage of 6.25×10^{-1}

V, the resulting current should be 6.25 x 10^{-8} A or 62.5 nA. Figure 5.3 shows that the TDP equipment was functioning properly. The signal quality may not look very good, but the scale of the y-axis must be taken into account. The range of the resistor experiment is only about 0.2 nA, a minuscule spread. In figure 5.3, the spike at sixty seconds corresponds to movement of the resistor which may have briefly interrupted its contact with the TDP-OX terminals.

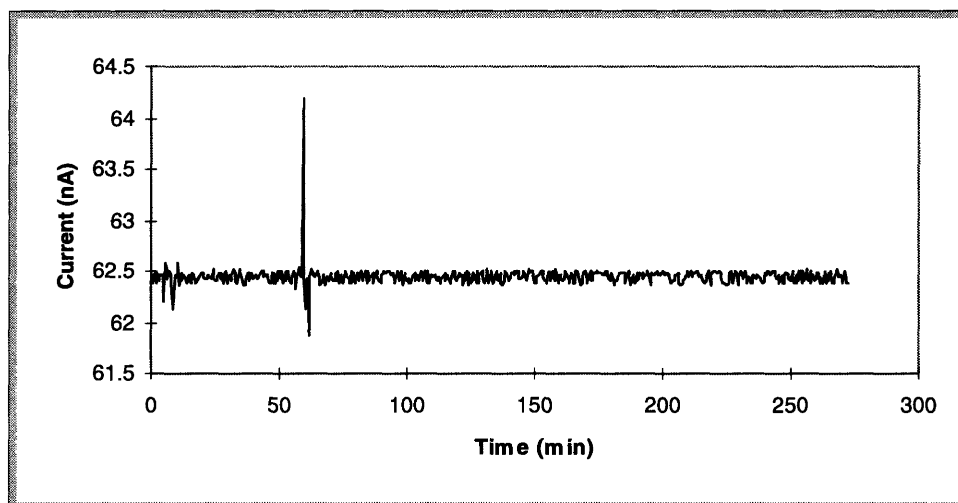


Figure 5.3: Graph of the 10 $M\Omega$ resistor experiment

The other reference measurement was done using a TTI gold wire stainless steel needle, a commercially available microelectrode. This 27-gauge needle microelectrode was placed in PBS at 37 $°C$ and at atmospheric conditions (~21% O_2 or 160 mmHg). The results from this microelectrode were encouraging, as seen in figure 5.4. The signal was clean and the steady-state current level remained flat. When a linear regression was performed on the steady-state portion of the graph, the slope of the current was 0.1 nA/hour which corresponded to a pO_2 change of approximately 0.16% per hour.

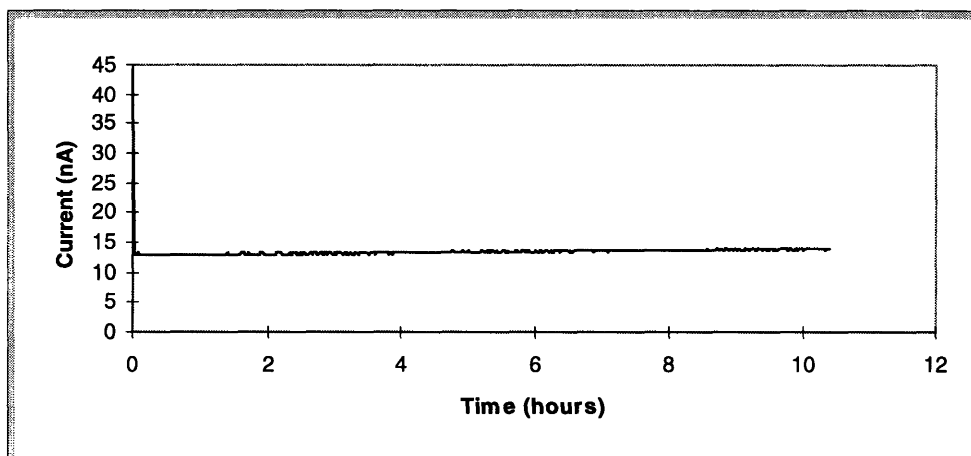


Figure 5.4: Graph of the reference gold needle microelectrode experiment

5.4.2 Fabricated Microelectrode Tests

Results from three different fabricated microelectrodes are shown in table 5.1. If the *in vitro* current measured was too low (less than 1 nA) or too high (over 200 nA), the microelectrode signal was not within the bounds of the TDP-OX amplifier. Current drifts were calculated using linear regression on the steady-state currents.

Table 5.1: Overview of the *In Vitro* Long Term Experiments

Type of Microelectrode	Number of Probes Tested	Number of Effective Probes	Names of Effective Probes	Starting Current Values (nA)	Ending Current Values (nA)	Current Drift (nA/ hour)
Original carbon fiber microelectrode	8	4	#2 #3 #4 #5	80 27 30 50	120 40 55 85	2.33 1.23 4.66 6.05
Bundled carbon fiber microelectrode	7	4	#2 #3 #4 #5	19 15 40 25	30 20 42 28	0.74 0.83 not done 0.37
Gold wire microelectrode	8	2	#2 #3	50 10	50.5 10.2	0.20 0.14

From the table, the influence of the design and fabrication process of each microelectrode on the long term stability can be seen. The bundled carbon fiber microelectrodes performed significantly better than the original unbundled carbon fiber design. However, the gold wire design appeared to be the best of the three.

5.4.2.1 Original carbon fiber microelectrode design

The original carbon fiber microelectrode design was the worst of the three tested. Its poor performance was based primarily on a high drift factor, the slope of the cathode current over time.

In figure 5.5, an example of a current drift of ~14.5 nA/hour that led to saturation of the TDP-OX amplifier is shown.

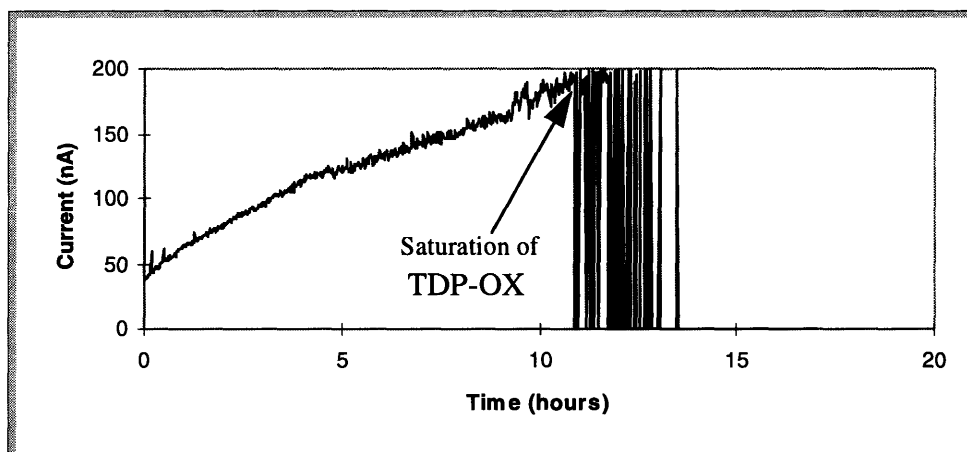


Figure 5.5: Graph of saturation of carbon fiber probe #1

Several of the microelectrodes fabricated using this design did not show as poor results as probe #1. Many showed reasonably steady currents over time and were considered possible candidates for calibration. As cumulative testing time increased, the microelectrodes began to perform more poorly. Therefore, a collodion membrane was considered for this type of microelectrode.

Collodion membranes seemed to help the microelectrodes slightly, but even with the membrane, the signal quality and current drift were not as good as those for the bundled carbon fiber microelectrode. Probe #5 is shown in figure 5.6 (current drift of 6.05 nA/hour from Table 5.1) and figure 5.7 (current drift of 1.7 nA/hour) before and after application of a collodion membrane. As noted in many microelectrodes after membrane application, the steady-state level of the oxygen current is lower after the membrane is applied. This drop in the steady-state current level is expected since the membrane is an additional resistance to O_2 movement to the cathode.

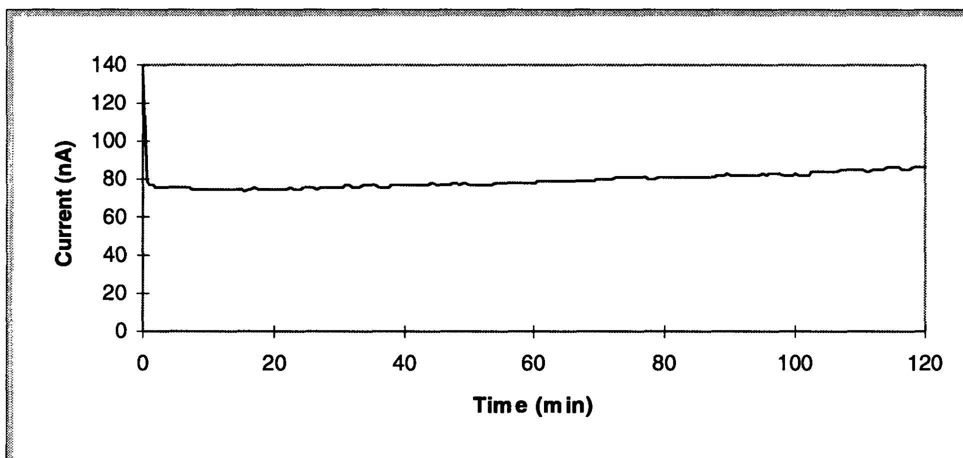


Figure 5.6: Probe #5: Experiment before collodion membrane applied

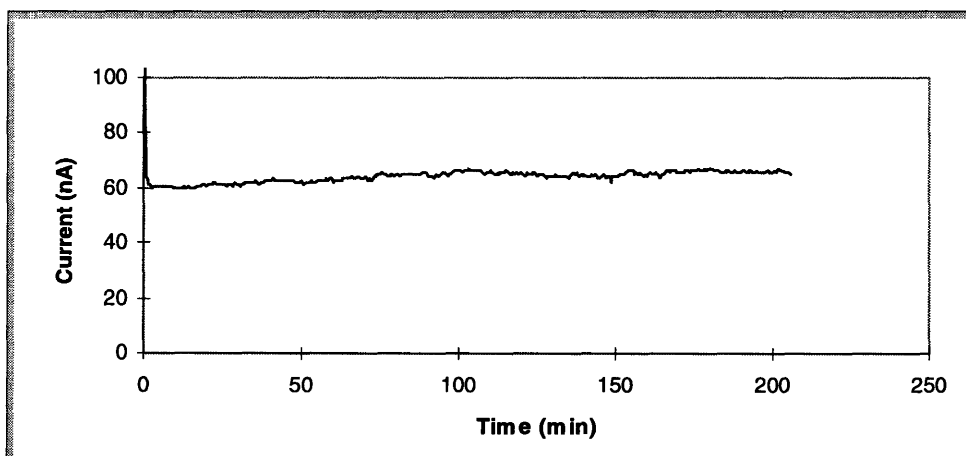


Figure 5.7: Probe #5: Experiment after collodion membrane applied

5.4.2.2 Bundled carbon fiber microelectrode design

The bundled carbon fiber microelectrodes performed much better than the original carbon fiber microelectrode design did. The bundled design was designed to address interface problems between the carbon fibers and the epoxy, and this design did result in fewer long term current leaks observed.

During the long term experiments, the signals of most of the bundled carbon fiber microelectrodes were steady. The signal quality tended to be excellent, and the drift was significantly smaller than the drift observed with the original carbon fiber microelectrodes. Figure 5.8 shows one example of a bundled carbon fiber microelectrode that had minimal current drift (probe #2), and over a twenty hour period, had a current drift of only 0.7 nA/hour.

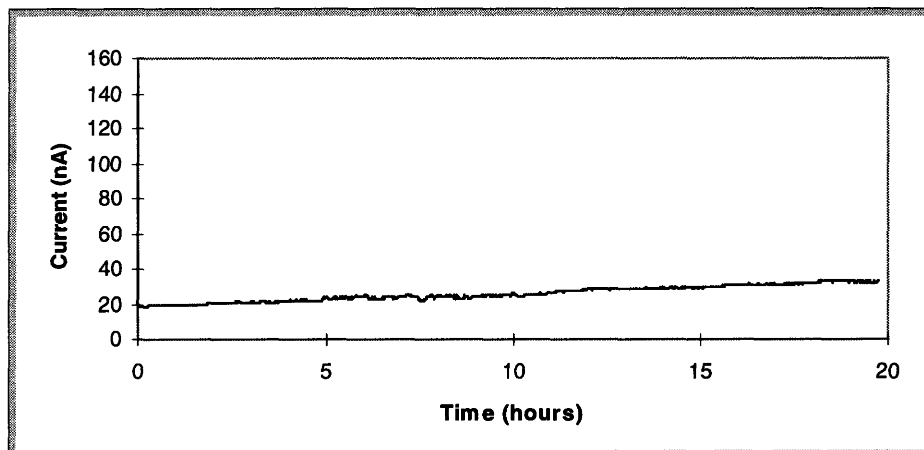


Figure 5.8: Bundled Probe #2: Twenty hour experiment with minimal drift

Collodion membranes were also tested with the bundled carbon fiber microelectrodes. These membranes seemed to help the signal quality and level of drift slightly. Figures 5.9 and 5.10 show probe #4 before and after the membrane was applied.

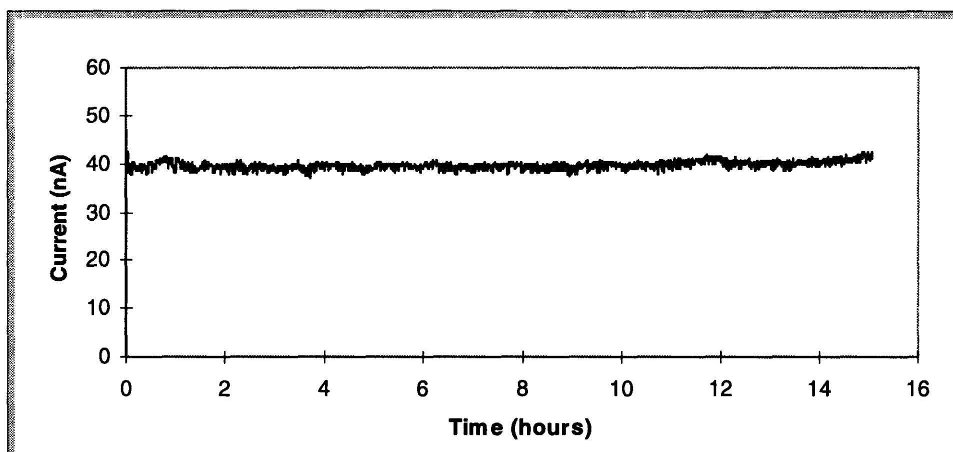


Figure 5.9: Bundled Probe #4: Before the membrane was applied

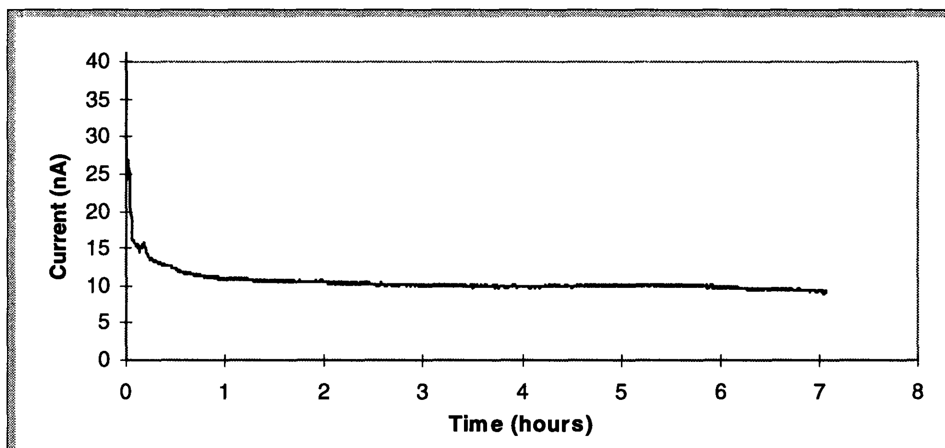


Figure 5.10: Bundled Probe #4: After the collodion membrane was applied

As seen in figures 5.9 and 5.10, the signal quality was better after the membrane was applied. The steady-state current was also lower because with a membrane, the effective diffusion of oxygen to the cathode is slowed. The current leak changed slightly after the membrane was applied, changing from 0.1 nA/hour to -0.4 nA/hour. However, the efficacy of using a collodion membrane to improve signal quality can be seen.

5.4.2.3 Gold wire microelectrode design

Of the fabricated microelectrodes, the gold wire embedded within the pulled glass needle design performed the best in terms of signal quality and current drift. These microelec-

trodes showed small current leakage as well as a very clean steady-state signal that was higher than those found in the bundled carbon fiber microelectrodes. The higher current can be attributed to the diameter of the gold wire being larger than that of the carbon fiber bundle. Due to their fragile nature, only a portion of those fabricated were suitable for use in the short term *in vitro* and *in vivo* studies.

The glass needle gold wire microelectrodes had problems with extremely fragile tips. Because the glass needle was pulled around the gold wire, the tips were rather long and sealed tightly around the gold wire. A long, thin glass tip is extremely fragile and can easily break. Therefore, extreme care was necessary when handling the glass needle gold wire microelectrodes.

Because the glass was pulled around the gold wire, the seal between the glass and the gold was generally, but not always watertight. In many cases, PBS seeped through the glass-gold wire interface, resulting in current leakage. It was extremely difficult to inject the insulation epoxy into the tip of the needle because the epoxy was not viscous enough. Therefore, unless the glass seal was perfect, small current leaks were observed.

Figures 5.11 and 5.12 show two consecutive experiments of gold probe #3. The current drift in the two experiments is small (0.14 nA/hour and 0.01 nA/hour respectively), and the signal quality was excellent in both cases.

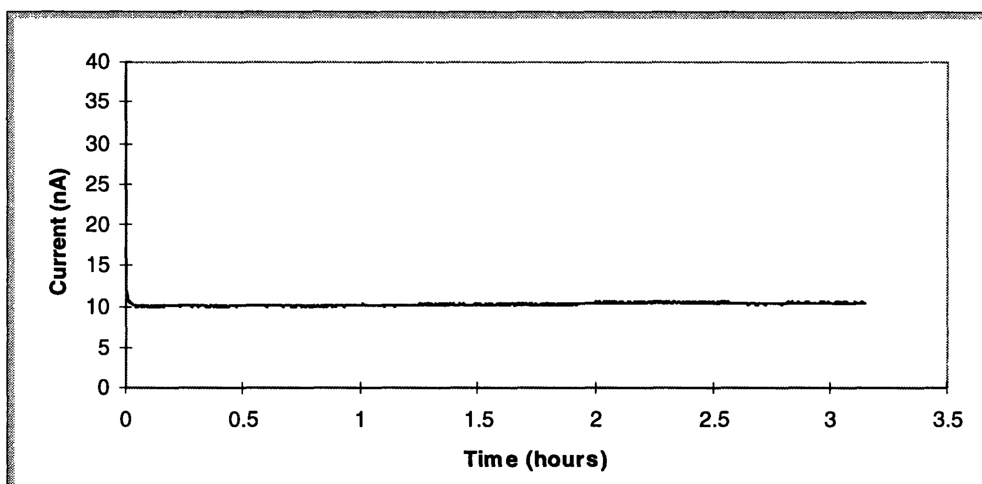


Figure 5.11: Gold Probe #3: First run showing minimal current leakage

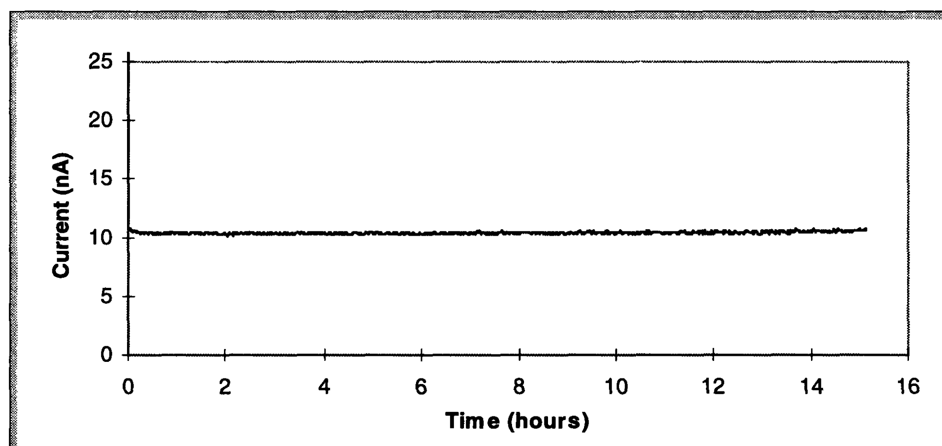


Figure 5.12: Gold Probe #3: Second run showing even smaller current leak

The low steady-state current levels of probe #3 were not the norm for this type of microelectrode. Figure 5.13 shows another glass needle gold wire microelectrode that had a much higher steady-state current and minimal current drift (0.2 nA/hour). The tip of this probe (gold probe #2) later broke, destroying the microelectrode. Figure 5.14 shows an experiment run after the tip was broken. Although the current drift is lower after the tip was broken (0.02 nA/hour), the signal quality is worse.

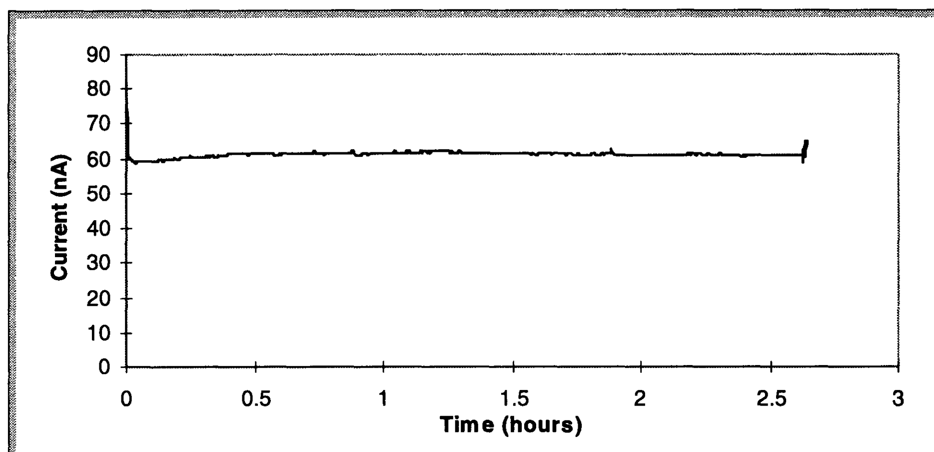


Figure 5.13: Gold Probe #2: Experiment showing high steady-state current

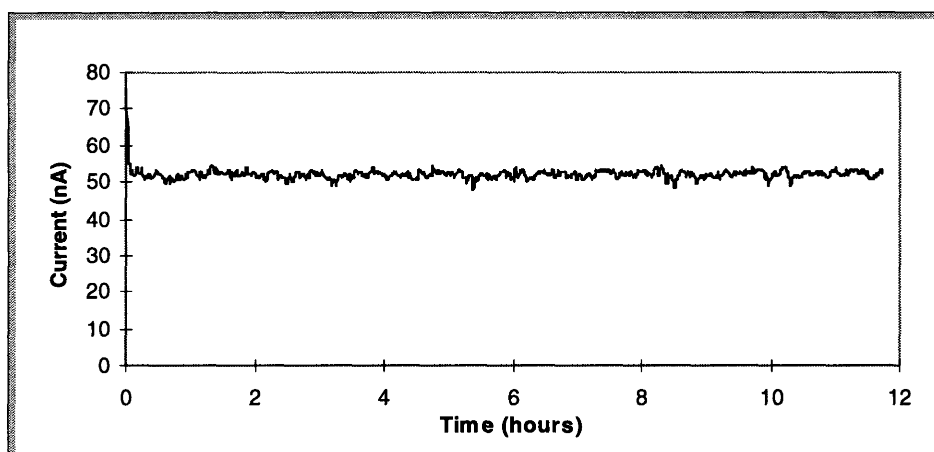


Figure 5.14: Gold Probe #2: Experiment performed after tip was broken

Chapter 6

Results: Short Term Calibration Measurements

6.1 Objectives

The short term calibration measurements were designed to evaluate the reproducibility as well as to calibrate the microelectrodes. Experiments of short duration at different oxygen concentrations were carried out in an environment similar to that *in vivo.*

6.2 Methods

Experiments were done using the gold wire, classical carbon fiber, and bundled carbon fiber microelectrodes. As a reference, the TTI gold wire steel needle microelectrode was calibrated as a baseline measurement for the *in vivo* experiments.

6.3 Results

Table 6.1 shows calibration results of several of the microelectrodes tested. Time constraints precluded calibrating all of the microelectrodes fabricated. Entries marked * denote measurements that are possibly in error.

Table 6.1: Calibration Results

Microelectrode	Reproducible Nitrogen Current Value (0% O_2)	Reproducible Current Value (10% O_2)	Reproducible Current Value (21% O_2)
TTI Probe	x	7.1 nA	14.4 nA
Original CF Probe #5	x	48 nA	105 nA
Bundled CF Probe #2	x	12.6 nA	30 nA*
Bundled CF Probe #3	x	7.3 nA	17 nA
Bundled CF Probe #4	3.55 nA	14.8 nA*	18 nA
Bundled CF Probe #5	1.3 nA	3.95 nA	6.4 nA
Gold Probe #3	10 nA	28 nA*	50 nA

6.3.1 TTI Microelectrode Tests

The stainless steel needle gold wire microelectrode was used as a reference measurement. Unfortunately, the 0% O_2 measurements (or nitrogen current) were unreproducible, but the 10% and 21% O_2 measurements were reproducible and gave enough data to calibrate the microelectrode. The nitrogen current was presumably unreproducible due to its proximity to 0 nA. The TDP-OX cannot measure currents lower than 0.43 nA, and by extrapolating the data from the 10% and 21% O_2 experiments, the value of the nitrogen current would be below 0 nA.

6.3.2 Fabricated Microelectrode Tests

Many of the fabricated microelectrodes were not successfully calibrated. Usually, the nitrogen current was unreproducible. Other times, like the TTI microelectrode, the extrapolated nitrogen current was below 0 nA, a reading that suggested that one of the other readings was in error.

Some problems may have been a direct result of experimental conditions. It was not uncommon for the probes to have been tested many times during the long term studies, deteriorating them. Other times, the PBS may not have been properly equilibrated to the desired oxygen concentration.

Another possible explanation involves a degradation of the epoxy-carbon fiber interface, leading to interfiber spaces. These interstices would lead to problems as microelectrodes that had been soaked in PBS for different lengths of time could presumably measure the same pO_2 value for different cathodic areas. Using the same PBS, there should be a pO_2 difference between a microelectrode that had PBS in all of its interfiber spaces as opposed to one without all of its interstices filled.

6.3.2.1 Original carbon fiber microelectrode design

The only original carbon fiber microelectrode calibrated was probe #5. This microelectrode did not calibrate due to a poor series of nitrogen current experiments. Figure 6.1 shows the reproducibility of the 10% O_2 experiment. The sharp impulse at approximately five minutes shows the commencement of the second experiment.

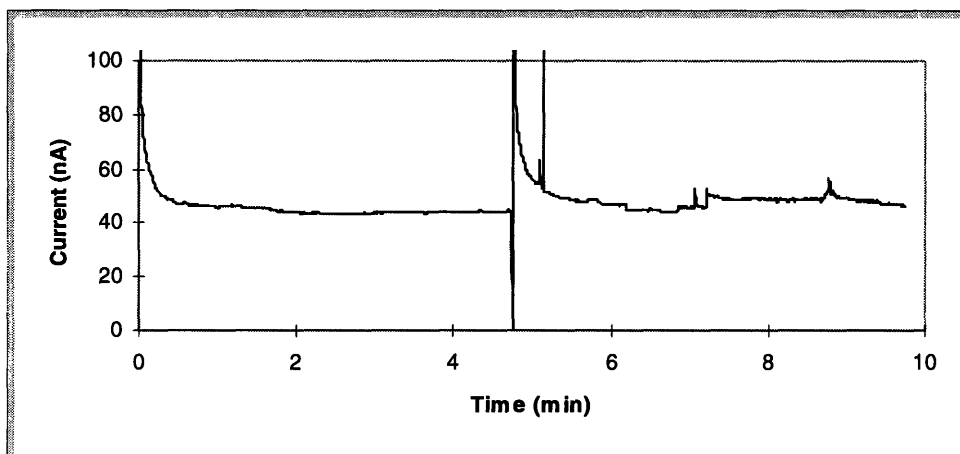


Figure 6.1: Original Probe #5: Calibration experiments at 10% O_2

The steady-state values of the two experiments are reproducible. The data for the 21% O_2 (atmospheric conditions) calibration were taken from the steady-state values of the long term experiments. In many cases, there was a slight current drift during the long-term experiments, therefore the 10% and 21% tests should have been done in close time proximity for more accurate results.

6.3.2.2 Bundled carbon fiber microelectrode design

The bundled carbon fiber microelectrodes were the most numerous in number calibrated. These microelectrodes fared better than the original carbon fiber microelectrodes.

Bundled probe #5 was one microelectrode that was calibrated, and the results are shown in figure 6.2. Table 6.1 shows other bundled carbon fiber microelectrodes that were partially calibrated.

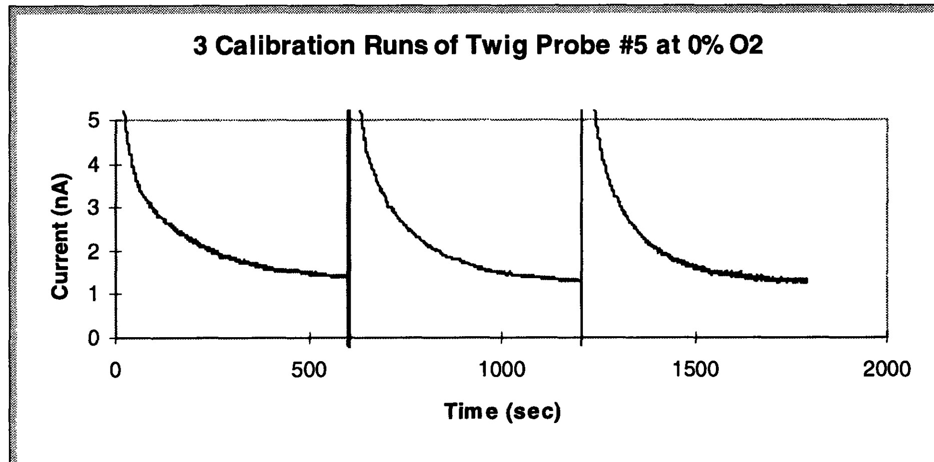


Figure 6.2: Series of nitrogen current experiments

6.3.2.3 Gold wire microelectrode design

Only one glass needle gold wire microelectrode, gold probe #3, was calibrated. Figure 6.3 shows that the 10% O_2 calibration experiments were not terribly accurate, casting suspicion on the results obtained from this calibration.

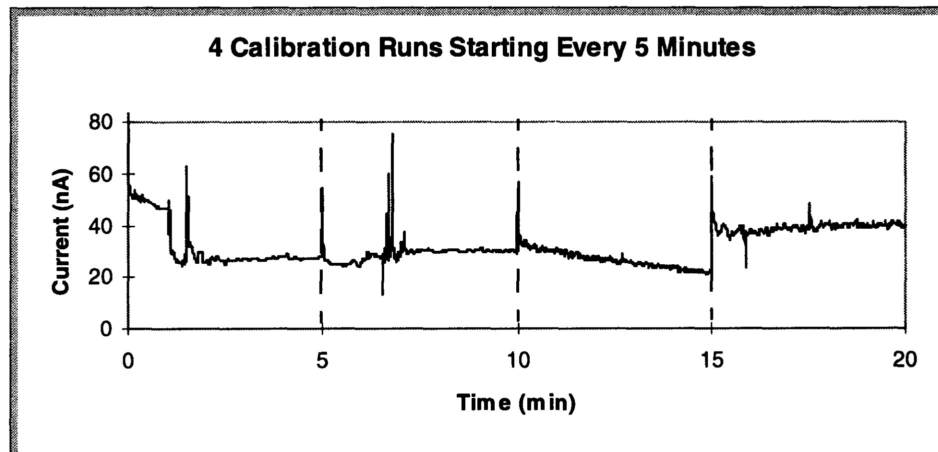


Figure 6.3: Gold Probe #3: Calibration runs at 10% O_2

Chapter 7

Results: *In Vivo* Measurements

7.1 Objectives

The final test of the fabricated microelectrodes was the *in vivo* measurements. Ideally, *in vivo* measurements would be stable over time, suggesting a lack of poisoning, and the measured liver pO_2 values would increase as the rat breathed pure oxygen, and decrease after the rat was removed from a 100% O_2 environment.

7.2 Methods

The rat liver pO_2 was measured under both atmospheric and 100% O_2 conditions. The rat was anesthetized at atmospheric conditions, perfusing all tissues with atmospheric air. Later, a portable oxygen gas tank, a rubber hose, and a large syringe that fit over the rat's snout and mouth were used to administer the 100% O_2 gas. By breathing pure oxygen for a short period of time, the tissues in the rat would presumably become hyperoxygenated.

7.3 Results

Despite not fully calibrating all of the fabricated microelectrodes, they appeared to work well *in vivo*.

7.3.1 Atmospheric Conditions

Under atmospheric conditions, the pO_2 values obtained from the rat using different measurement microelectrodes were lower than expected. Values of approximately 40 mmHg were expected, and the observed measurements were closer to 20 mmHg.

Both the TTI microelectrode and the fabricated microelectrodes performed well under atmospheric conditions. Figures 7.1, 7.2, and 7.3 show experimental runs for the TTI, bundled carbon fiber, and glass needle gold wire microelectrodes.

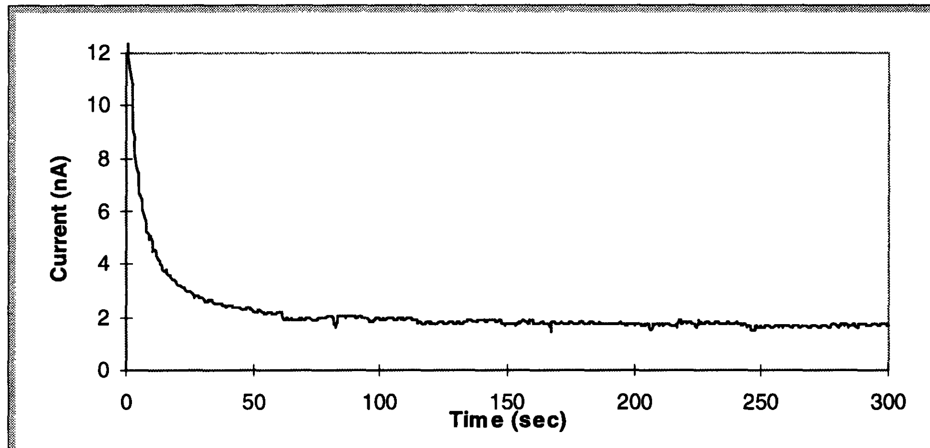


Figure 7.1: TTI Probe: Typical rat liver run

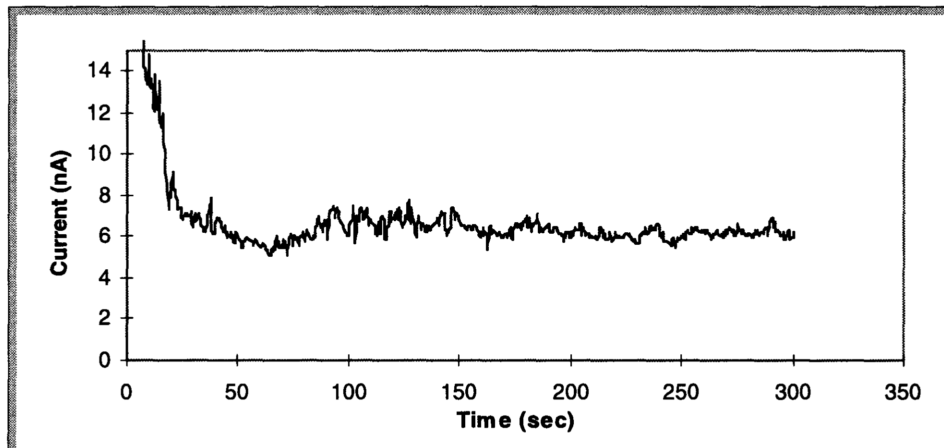


Figure 7.2: Bundled Probe #4: Typical rat liver run

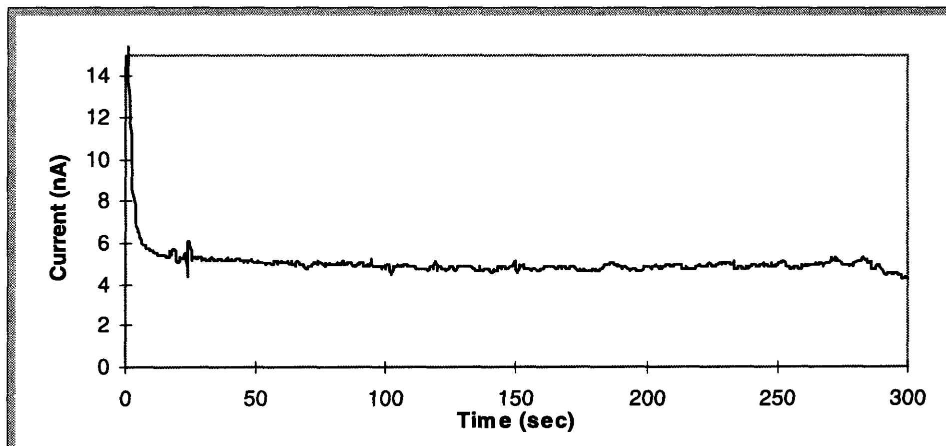


Figure 7.3: Gold Probe #3: Typical rat liver run

In each case, the microelectrode reached the steady-state current level quickly and stayed at that steady-state value throughout the experiment. Collodion membranes were applied to some microelectrodes, and their steady-state current levels dropped from pre-membrane levels.

7.3.2 Breathing Pure Oxygen

In this stage, many of the microelectrodes did not respond as expected. Occasionally, the TTI microelectrode showed no response to the administration of pure oxygen. Figures 7.4 and 7.5 show results when 100% O_2 was administered.

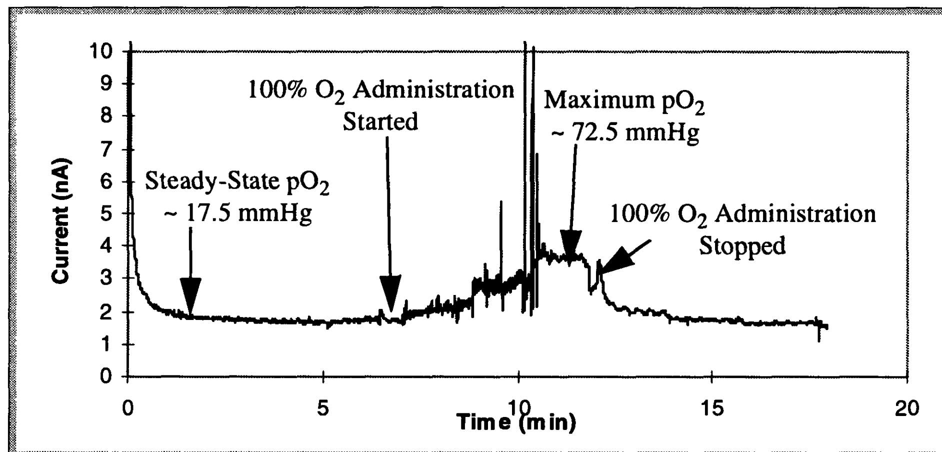


Figure 7.4: Bundled Probe #5: Effects of 100% O_2

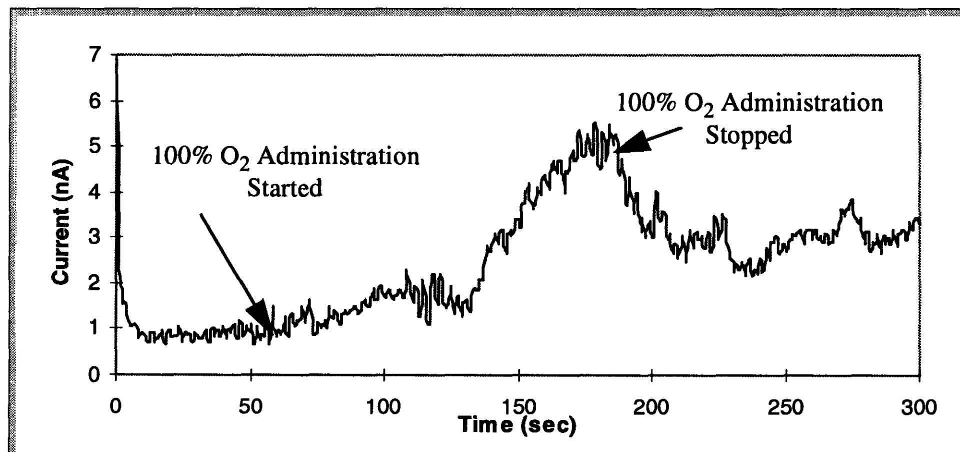


Figure 7.5: Gold Probe #3: Effects of 100% O_2

The results were all different, and figures 7.4 and 7.5 show two experiments that appeared to work as expected. Figure 7.6 shows one of the many times when there was no discernible response to the administration of pure oxygen.

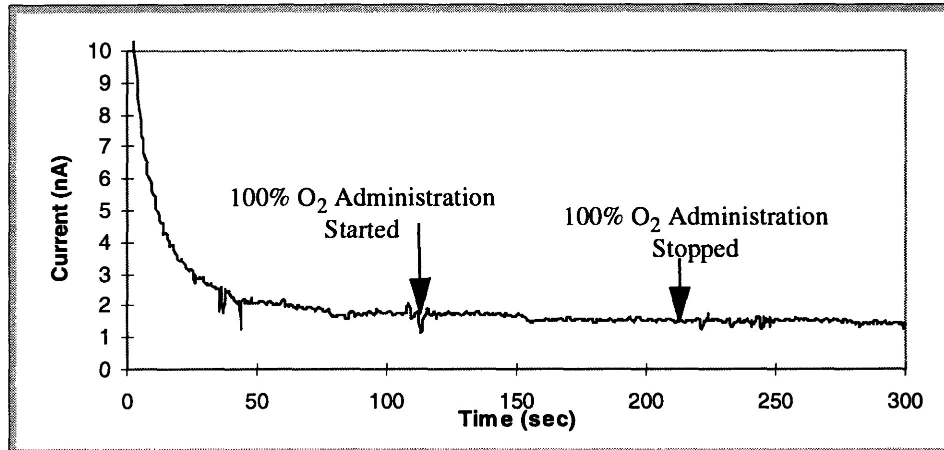


Figure 7.6: TTI Probe: Lack of response to 100% O_2

Table 7.1 summarizes the responses of the microelectrodes to the rat's breathing pure oxygen.

Table 7.1: Results from Selected Rat Liver Experiments

Microelectrode	Rat Number	Approximate Tip Diameter (μm)	Steady-State Current (room air)	Steady-State Current (100% O_2)	Increase in Current
TTI Probe	2	450	1.5 nA	1.5 nA	0%
Bundled #5	1	100	2 nA	4 nA	100%
Bundled #2	2	100	2.5 nA	5 nA	100%
Gold Probe #3	2	40	1.5 nA	5 nA	330%
Bundled #7	2	100	20 nA	20 nA	0%

7.3.3 Low Levels of Anesthesia

One interesting phenomenon observed while performing experiments on the rat liver was the signal recorded when the rat was at low levels of anesthesia. In two cases, when the rat's anesthesia level was low, an observation supported by the rat's increased movements and waking up, the microelectrodes recorded a sinusoidal signal. This sinusoid was hypothesized to be a consequence of vasomotor activity because of its oscillatory nature and frequency.

The rat was put into a state of deeper anesthesia by dripping anesthesia onto the large intestines. The recording showed a cessation of the sinusoid and a return to the previous steady-state current level. Figures 7.7 and 7.8 show the vasomotor response recorded by the TTI microelectrode and a bundled carbon fiber microelectrode.

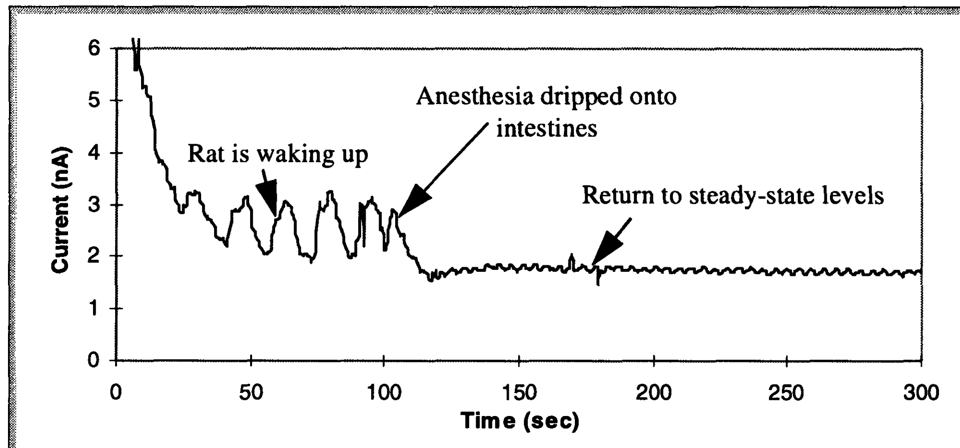


Figure 7.7: TTI Probe: Vasomotor response detected

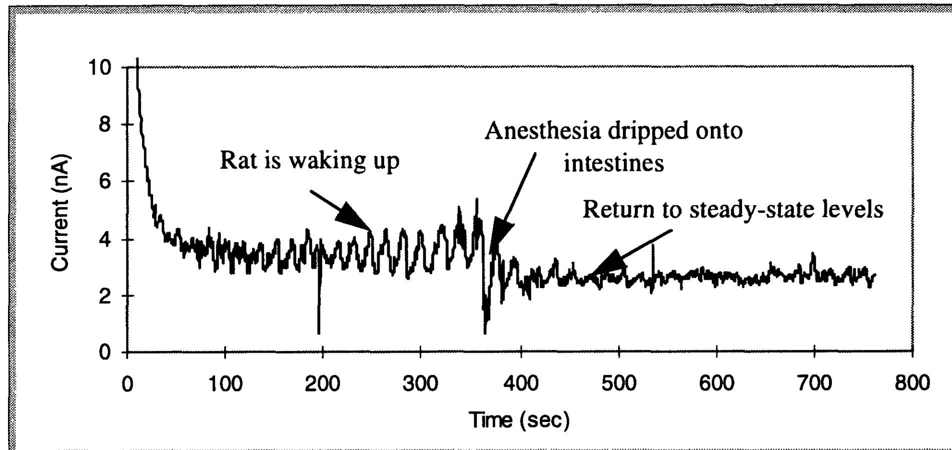


Figure 7.8: Bundled Probe #5: Vasomotor response detected

Chapter 8

Discussion of Results

8.1 Long Term Measurements

8.1.1 Problems Observed During Experiments

When the long term measurements were analyzed, it was found that the problem of current leakage had not been entirely eliminated.

8.1.2 Hypothesis

Most likely, the interface between the insulation epoxy and the carbon fibers was the source of the current leakage. The current increased steadily with time, without jumps and spikes, leading to the conclusion that the surface area of the cathode was gradually increasing with time. The interface problem that is compatible with an increasing cathode area is that of the epoxy and carbon fibers.

8.1.3 Discussion

The problem of current leakage differed for the different types of microelectrodes. As noted in table 5.1, the glass needle gold wire microelectrode had the lowest current drift and the original carbon fiber design had the highest current drift.

8.1.3.1 Original Carbon Fiber Microelectrode

This microelectrode showed the largest amount of current drift, presumably because of the fabrication process. During the fabrication process, a bundle of dry carbon fibers was placed into the glass capillary tube which was then pulled. Ideally, the interstitial spaces between the fibers would be filled with insulation epoxy, but the interfiber spaces were so small that even a highly nonviscous liquid had difficulty filling those spaces. The insulation epoxy, despite its relative low viscosity, was not able to successfully permeate each

interfiber space. When tested, PBS or other fluids could permeate the interfiber spaces, increasing the cathode surface, manifesting itself as an increasing current.

Membranes provide a possible solution to this problem. An effective membrane application process was needed to apply the collodion to the cathode surface. This would create a fluid impermeable, oxygen permeable barrier, alleviating the current leakage. The membranes applied during the long term measurement phase were not as effective as expected. There was only a decrease in the current drift observed, not an elimination of that drift.

8.1.3.2 Bundled Carbon Fiber Microelectrode

Of the carbon fiber microelectrode designs, the bundled carbon fiber microelectrode had the lowest level of current leakage. This was attributed to the fabrication method of the microelectrode. Prior to the glass being pulled, the carbon fiber bundle was premixed with the insulation epoxy, filling most of the interfiber spaces.

The premixing process did not eliminate the steadily increasing current completely. Other possible sources of current leakage included the glass-epoxy interface and a possible failure of the insulation epoxy to withstand fluids. The glass-epoxy interface could be eliminated as a source of the problem by filling the glass needle with epoxy so that the only portion of the cathode in the glass needle not coated with insulation epoxy would be the copper wire. Even if fluids travelled up the glass-epoxy interface, the cathode surface would not increase since the fluid could touch only the insulation on the wire.

If the epoxy was not fluid impermeable, then another insulation epoxy would have to be found. From simple tests done using water, food dye, and epoxy, Eccobond 27, appeared to be impermeable to fluids.

8.1.3.3 Glass Needle Gold Wire Microelectrodes

The fabrication process of the gold wire microelectrode makes it the best in keeping the measured fluid out, but also the worst in preventing against such a leak. The glass nee-

dle is pulled around the gold wire, making the seal tight. This seal may not be completely airtight, but tight enough so that insulation epoxy will not flow to the tip. The fact that the insulation epoxy cannot get to the tip of glass needle means that if the glass-gold wire seal is not perfect, then the cathode surface would increase.

Some of the current drift that occurs in gold wire microelectrodes is due to the fragility of the tips. When the tip cracks, the area of the cathode will change, shifting the baseline level of the signal. When tips crack for the first time, a scalpel is used to cut the tip and attempt to create a new, clean tip. Generally, this is difficult, and when a microelectrode tip cracks, it is often better to throw it away and start afresh with another.

8.2 Short Term Calibration Measurements

8.2.1 Problems Observed During Experiments

One major problem observed during the short term calibration phase was inconsistent measurements of the nitrogen current. It was felt that the 0% and 10% O_2 readings were the most important for calibration since most of the measurements *in vivo* were made at around 30-40 mmHg, or 4-6% O_2.

8.2.2 Hypotheses

The problem with consistently measuring the nitrogen current stems from either an inability to consume all of the oxygen in the PBS with CaCl or inadequate bubbling of the nitrogen gas in the PBS. If CaCl were the problem, then another substance must be used to consume all of the oxygen or more care must be taken to ensure that the CaCl-PBS mixture was isolated from room air. If the problem was an inadequate bubbling of the nitrogen gas, then either the gas must be bubbled for a longer period of time to drive all of the oxygen out or more care must be taken to ensure that atmospheric air does not enter the PBS beaker (i.e. cover the beaker with plastic film).

8.2.3 Discussion

These measurements differed between the different types of microelectrodes. The main reason for the poor calibration of the microelectrodes stemmed from the fact that after being tested for over 24 hours in long term experiments, the degradation of the insulation epoxy-carbon fiber interface had progressed to a level that made it impossible to accurately calibrate the microelectrode at 10% O_2. However, most of the nitrogen current problems most likely stemmed from inexact experimental conditions.

If the microelectrode displayed current leakage, recalibration at the new leakage level should be done to ensure accurate results. However, this recalibration would be transient as new measurements would further increase the leakage, and the microelectrode would never be able to be accurately calibrated.

8.3 *In Vivo* Measurements

Experiments were done in an attempt to determine whether or not the fabricated microelectrodes would work *in vivo* and to see whether problems such as protein poisoning existed with carbon fiber microelectrodes.

8.3.1 Problems Observed During Experiments

The biggest problem observed during the *in vivo* experiments was the lack of sensitivity shown by some microelectrodes to administration of 100% O_2. It was expected that the microelectrode signal would increase with the administration of pure oxygen in response to a higher level of oxygen in the lungs, leading to the perfusion of tissues with blood containing elevated oxygen levels. It is inconceivable that at atmospheric conditions, all of the hemoglobin binding sites would be saturated. Therefore, an increase in the oxygen level of the air breathed by the rat should have some effect on the oxygenation level of the blood and ultimately on the tissues and organs.

The sensitivity of the microelectrodes, including the TTI stainless steel needle microelectrode, was inconsistent. Sometimes, the microelectrodes would record changes in pO_2 levels brought on by the administration of 100% O_2. Different measurement locations in the liver were tested, but there was insufficient data to formulate conclusions.

8.3.2 Hypothesis

There are two explanations for this inconsistent sensitivity to pure oxygen, probe-induced tissue trauma and the natural heterogeneity of vasculature leading to variations of hypoxia.

The hypothesis of tissue trauma states that when a probe enters tissue, it destroys the vascular structure of the tissue. When compared to microelectrodes with smaller tips, larger microelectrodes destroy a greater amount of tissue and its associated vascular structure when inserted. This may also induce a pool of blood within the tissue to form at the tip of the microelectrode. As the tissue is destroyed upon entry, the body instinctively attempts to isolate the foreign object, the microelectrode. Also, the body reflexively tries to heal itself by constricting capillaries around the puncture and decreasing perfusion to that region in an attempt to counterbalance the regionally low blood pressure.

The body has isolated the region surrounding the puncture, and the microelectrode may measure from a small pool of blood released upon entry. Little new blood may be perfused to the puncture region, thus changes in lung pO_2 are not seen in the puncture region.

The other hypothesis to explain the lack of sensitivity is location dependence. The liver is not a homogenous organ with respect to perfusion, and depending on where the microelectrode is inserted, proximity to sinusoids, veins, and arteries will be different.

8.3.3 Discussion

It is not apparent whether the microelectrode's lack of sensitivity to the rat breathing 100% O_2 can be attributed to probe-induced tissue trauma or to location sensitivity. The microelectrodes were sensitive enough to detect vasomotor activity of the rat while its anesthesia was wearing off. Therefore, it would seem that at the same measurement location, the same microelectrode would be able to detect changes in pO_2 during 100% O_2 administration, something that changes in much larger amounts.

It is assumed that the microelectrode detected the beginning of vasomotor events, and measurements taken corresponded to instantaneous oxygen concentrations. This implies that the measured signal does not come from some constant oxygen pressure in pooled blood.

Size of the microelectrode tip does not appear to be a factor because of the limited success of the TTI microelectrode. The TTI microelectrode was larger in diameter than most of the fabricated microelectrodes, including bundled probe #5, the fabricated microelectrode that appeared to work best. The TTI microelectrode detected the vasomotor activity, but never seemed to effectively detect other pO_2 changes. Size does not appear to be the driving factor behind the lack of sensitivity, however, there may be some larger size threshold for which sensitive measurements to pO_2 changes can be measured.

Size relates directly to the hypothesis of probe-induced tissue trauma, and size not appearing to be the problem lends more weight to the location hypothesis. This is something that must be investigated further. *In vivo* measurements were performed on thirteen locations in the liver, and analysis of these results suggests that location has some effect on the signal acquired, but it was not useful in understanding why a lack of sensitivity to the administration of oxygen exists. Certain measurements may have been carried out while the rat was in a heavily sedated state, which may have led to different measurements than when the rat was in a less sedated state.

Chapter 9

Summary and Conclusions

9.1 Summary of Carbon Fiber Microelectrode

The fabricated carbon fiber microelectrodes were shown to be viable compared to commercially available microelectrodes. Despite problems such as current drift, unreproducible calibration experiments, and inconsistent *in vivo* sensitivity to pure oxygen, the microelectrodes appeared to be as effective as the TTI microelectrode. Many of the problems were not a factor of the design, but of the experimental environment instead. Table 9.1 summarizes some of the characteristics of the fabricated microelectrodes.

Table 9.1: Summary of Fabricated Microelectrodes

Design	Stability	Lowest Current Drift (nA/ hour)	Signal Quality	Long Term *In Vitro* Evaluation	Ability to Calibrate	Sensitivity *In Vivo*
Original Carbon Fiber Microelectrode	Not Very Good	1.23	Low	Poor	Poor	N/A
Bundled Carbon Fiber Microelectrode	Good	1.0	High	Very Good	Good	Good to Very Good
Glass Needle Gold Wire Microelectrode	Very Good	0.14	Very High	Excellent	Good	Good
Microelectrodes with Collodion Membranes	Variable	0.63	Very High	Unsure	Poor	N/A

Although problems were encountered, they did not detract from the research objectives. The goal was to determine whether carbon fibers could be used as a viable cathode in polarographic oxygen measurements.

Polarographic oxygen microelectrodes are increasingly being used *in vivo*, especially in the management of cancer therapy and its associated research. The efficacy of synergistically combining hyperthermia and radiation treatment in cancer is a function of pO_2 levels of the tumor and surrounding tissues. If tumor pO_2 is too low, outcome augmentation of the combined treatments are not as apparent.

In the *in vivo* studies, carbon fiber microelectrode sensitivity and stability were analyzed. Location appeared to have some effect on the measured pO_2 level, but its effect on the sensitivity of the microelectrode to the rat's response to breathing pure oxygen is unknown. Tip diameter seemed to have some effect, as smaller, more noninvasive microelectrodes appeared to more accurately detect changes in pO_2 while the rat was breathing pure oxygen. Since both the fabricated microelectrodes and the larger diameter TTI microelectrode detected the vasomotor activity, it would be logical to assume that both could detect tissue responses to high pO_2.

The long-term *in vitro* testing process was only meant to be one of several quality checkpoints for the microelectrodes. It was not assumed that all microelectrodes that worked well in long-term situations would necessarily perform well *in vivo*. Instead, those that did not perform well *in vitro* would be discarded. Similarly, the short-term *in vitro* tests were another checkpoint for the microelectrodes since these tests better simulated the pO_2 ranges encountered during *in vivo* studies.

In vivo stability was excellent for all of the microelectrodes. The signal quality was good, and there was no noticeable change in the steady-state current level over time. In an indirect way, this seems to suggest that protein poisoning was not a problem with the carbon fiber microelectrodes. The microelectrodes were usually tested for over three hours in the same rat liver. Because the measured current did not drop over time as was common in

protein poisoning, it was hypothesized that protein poisoning did not play a role in the *in vivo* measurements.

9.2 Conclusions

During the study of the carbon fiber microelectrode, more questions were raised than could be adequately answered. The research presented in this thesis fulfilled its goal, the fabrication of an experimentally viable carbon fiber microelectrode.

The carbon fiber microelectrode is a useful tool for making pO_2 measurements *in vitro* and *in vivo*. Its role in the future hinges on two factors, reliability and durability. Since reliability is directly linked to durability, durability must be the focal point. Making the microelectrodes more durable would enhance their already reliable qualities and provide the cancer clinician with a valuable tool in enhancing cancer therapy.

The research presented in this thesis provides a solid basis for further research. With the emergence of the Sigma-Eppendorf system for measuring pO_2 levels in tumors, the need for a durable and reliable polarographic oxygen measurement system is strengthened. The carbon fiber microelectrode might possibly better resist protein poisoning, a problem with conventional polarographic oxygen microelectrodes. The research presented here may lead to the next generation in polarographic oxygen microelectrodes.

Chapter 10

Recommendations for Further Study

10.1 Long Term Measurements

Long term measurements were necessary to determine whether or not the microelectrodes were well fabricated. To complete a fully exhaustive analysis of the carbon fiber microelectrode's long term stability, the following could be done.

- Experimental conditions must be more isolated from electrical noise, vibration, etc.
- New materials must be available at every step of the fabrication process. Materials should never be reused.
- A more exhaustive materials search for a water impermeable insulation epoxy should be conducted.
- Other commercially available polarographic oxygen microelectrodes should be available for comparison.

This study gave excellent comparative results on the *in vitro* stability for different types of fabrications and designs. In particular, the long term experiments showed the importance of the bonding strength between the insulation epoxy and the carbon fibers or gold wire. Further research can be done to eliminate interface problems between the insulation epoxy and the cathode.

It would be useful to better understand the structure of the microelectrode tip. Its fragility poses practical problems, and the addition of membranes or post-fabrication tip polishing could be useful in enhancing the microelectrode durability.

Membranes appear to be the key to solving the interface problem, but the application process for membranes is not reliable now. Improvements can be made once more is learned about the structure of the tip of the pulled glass. With a properly applied membrane, the lifetime and reliability of the microelectrode can be greatly enhanced.

10.2 Short Term Calibration Measurements

For the short term calibration measurements, reliability of the PBS is the greatest concern.

Methods to ensure the oxygen concentration of the PBS is accurate are needed. Setting up three different PBS beakers, one for 0% O_2, one for 10% O_2, and one for 21% O_2 in the heated water bath would help to reduce problems of changing the oxygen concentration of the PBS between runs. The beakers must be as airtight as possible to stop atmospheric air from leaking into the 0% and 10% beakers.

For the calibration experiments, instead of using a PBS solution, other media which have properties better simulating biological tissues, such as gels with a lower diffusivity, higher viscosity, and different oxygen solubilities should be tested. This would help to ensure that the *in vitro* calibration graphs have more applicability to *in vivo* studies.

10.3 *In Vivo* Measurements

The *in vivo* experiments were useful in showing the physiological response of the rat liver to external stimuli such as pure oxygen or low levels of anesthesia. However, applicability of the microelectrode to other organs or muscles in the body should also be investigated.

Location dependence must be systematically examined. For some of the reported studies, thirteen locations on the liver surface were tested, but insufficient data was obtained. Because microelectrodes such as the TTI microelectrode detected vasomotor activity, but did not always detect pO_2 changes subsequent to the rat breathing pure oxygen, location of the measurement in a heterogeneous perfusion volume may be a factor. The liver is not homogenous, and cathode proximity to the intricate network of sinusoids, veins, and arteries can affect the signals measured.

A histological investigation of the measurement location would reveal the microelectrode tip's proximity to veins, arteries, and sinusoids. Theoretical calculations can be done to understand how the oxygen profile within tissues changes with proximity to sinusoids, and may shed more light on the location dependence of the microelectrodes.

Bibliography

1. Cespuglio, R. et al., "High sensitivity measurements of brain cathecoles and indoles in vivo using electrochemically treated carbon fiber electrodes."
2. Davies, P. W., and Brink, F., in Physical Techniques in Biological Research, Nastuk, W. E., Ed., Academic Press, New York, 1962, 137.
3. Fatt, Irving, Polarographic Oxygen Sensors, Malabar, Florida: Robert E. Krieger Publishing Company, 1982.
4. Gray, L. H., Conger, A. D., Ebert, M., Hornsey, S. and Scott, O. C. A., "Concentration of oxygen dissolved in tissues at time of irradiation as a factor in radiotherapy," British Journal of Radiology, Vol. 26, 1953.
5. Kallinowski, F., Zander, R., Hoeckel, M., Nat., Rer., Vaupel, V., "Tumor tissue oxygenation as evaluated by computerized-pO_2-histography," International Journal of Radiation Oncology, Biology, and Physics Vol. 19, 1990.

Appendix A

Fabrication of the Carbon Fiber Microelectrode

A.1 Overview

The design and fabrication of the carbon fiber microelectrode is a process that must be carefully done to construct reliable, durable microelectrodes. Two phases are involved, the design and fabrication of the microelectrode.

A.1.1 Design

The final design is a modification of that first designed by Charles Ruban and Jason Yip. The first design called for a carbon fiber cathode encased in a polyurethane catheter of approximately 1 mm in diameter. The glass needle microelectrode was then created, its tip measuring approximately 0.1 mm in diameter. The current design is a modification on the glass needle microelectrode. Access to a capillary tube puller helped to make the current probe design more effective.

A.1.2 Fabrication

Steps for the fabrication of the carbon fiber microelectrode were not very different from those performed by Ruban and Yip.

A.2 Design of the Microelectrode

Figure A.2.1 shows the original design for the carbon fiber microelectrode, a carbon fiber cathode encased in a polyurethane catheter and sealed with epoxy.

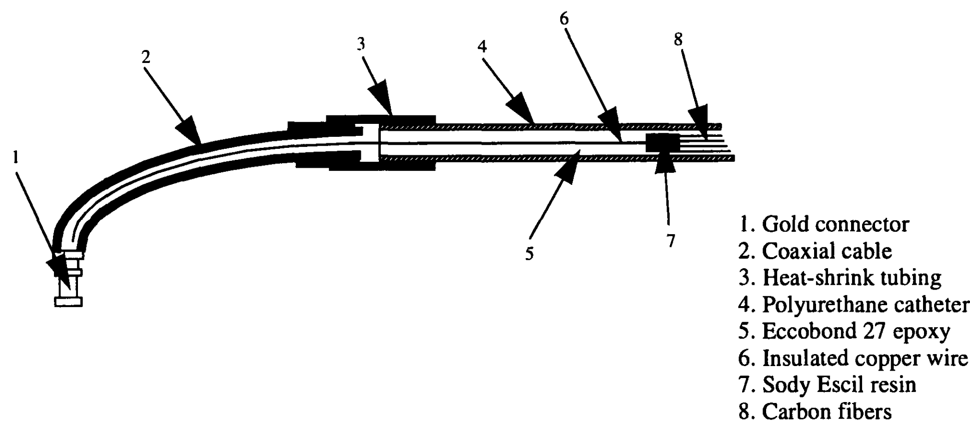


Figure A.2.1: Polyurethane catheter encased carbon fiber microelectrode

The design for the glass needle carbon fiber microelectrode shown in figure A.2.2 is slightly different, using a pulled glass capillary tube as the cathode housing instead of the polyurethane catheter. Each individual carbon fiber is between 5-10 μm in diameter, and approximately 10 carbon fibers were used. After the glass was pulled tightly around the carbon fiber bundle, the tip diameter was on the order of 100 μm in diameter.

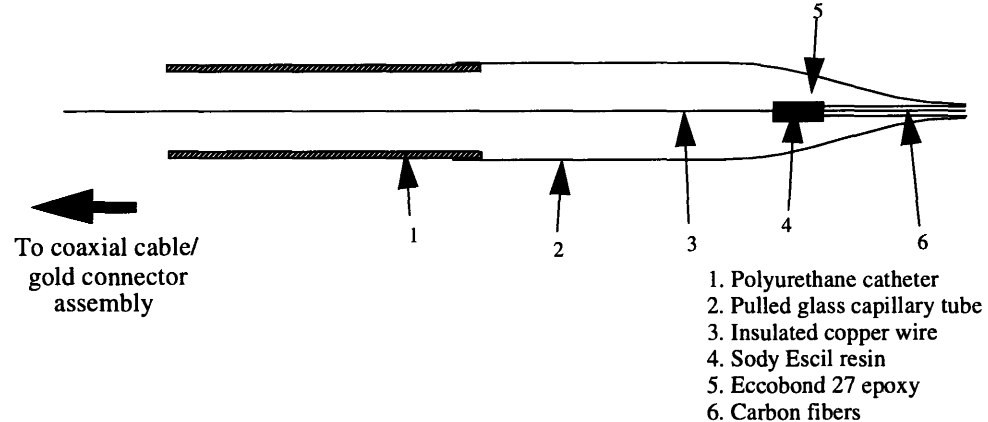


Figure A.2.2: Glass needle encased carbon fiber microelectrode

The probe was designed to have the glass capillary tube pulled with the carbon fibers inside to ensure a tight outer seal between the glass and carbon fibers. The difficulty in constructing a watertight seal at the tip of the microelectrode comes from the inter-fiber spaces. As shown in figure A.2.3, a more traditional gold wire microelectrode does not have the same problems as a multifibered carbon fiber microelectrode. The spaces

between individual carbon fibers need to be filled, and the insulating epoxy must be of low enough viscosity to seal all inter-fiber gaps.

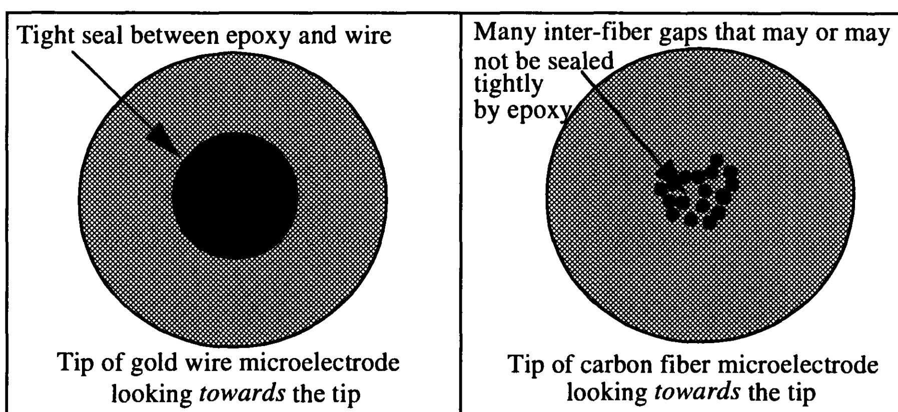


Figure A.2.3: Comparison between gold wire and carbon fiber bundles

It can be argued that a gold wire probe would be more effective because of the ease of probe fabrication. The main advantage of using a carbon fiber bundle is that carbon is hypothesized to have a lower degree of protein poisoning than gold and other metals.

To circumvent the interfiber gap problem, carbon fiber bundles were premixed with insulation epoxy. These bundles would be created *before* the copper wire-carbon fiber junction was created. The bundle would ideally have significantly fewer interfiber gaps.

To create the premixed cathodes, carbon fibers would be dipped into the insulating epoxy to thoroughly saturate them with epoxy. The carbon fibers would dry as bundles. One end of the bundle was left uncoated with the epoxy to connect it to the copper wire.

Both types of probes were constructed and current leakage data was compared. Collodion membranes were added to the microelectrodes, and their performance was evaluated.

A.3 Fabrication of the Microelectrodes

Two different cathode materials will be used to construct oxygen microelectrodes, gold wire and carbon fibers. This will allow comparisons to be made between two different cathodes using microelectrodes created in the same manner.

A.3.1 Gold Wire Microelectrodes

Unlike gold wire microelectrodes such as the TTI gold wire microelectrode, the microelectrodes fabricated will be inside pulled glass capillary tubes. The fabrication process can be broken down into four stages. The first stage is the fabrication of the junction. This is followed by the pulling of the glass capillary tube around the gold wire-copper wire assembly. The third stage involves placing insulating epoxy (Eccobond 27) at the tip of the pulled glass capillary to prevent liquid from leaking into the glass needle. The final stage involves connecting the glass needle assembly to coaxial cable.

A.3.1.1 Junction Fabrication

The junction is constructed by soldering the gold wire to the copper wire. Some preparation must take place before the actual soldering occurs.

First, the 8", insulated copper wire must be scraped from one end using a scalpel. No more than 1.5 cm should be scraped away since the junction itself will not be longer than 1 cm. After the copper wire is stripped and inspected, it is dipped in alcohol to ensure that the surface is clean. A schematic of a stripped copper wire is shown in figure A.3.1.

Figure A.3.1: Stripped copper wire

The copper and gold wire (about 4" in length) are affixed to a clamp with tape (figure A.3.2) to facilitate tinning of the ends of the wires before they are connected.

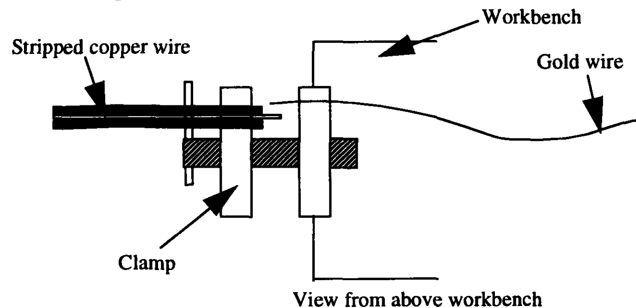


Figure A.3.2: Setup to connect gold and copper wires

After the ends of both wires are tinned (a thin layer of solder placed on the last 1 cm of the wire), the clamp is closed and the two wires are soldered together. Care must be taken in regulating the heat of the soldering iron. Gold melts very easily, and if the soldering iron is allowed to heat above ~500 degrees Fahrenheit, the gold may melt. A suggested tip heat is 450 degrees Fahrenheit. A finished junction is shown in figure A.3.3.

Figure A.3.3: The soldered gold-copper wire junction

A.3.1.2 Glass Pulling

As shown in figure A.3.4, the gold wire-copper wire assembly is placed into a glass capillary tube so that the solder junction is at least 2" from the copper wire end of the tube.

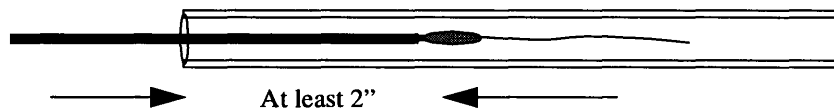


Figure A.3.4: The junction assembly inside the glass capillary tube

The glass capillary tube is placed into the glass puller. The glass puller used is old, but is sufficient to produce high-quality tips. Settings used for the gold wire were:

Heater: 70

Solenoid: 40

The glass tube was placed so that the heating coil on the glass puller was at least 2" from the junction towards the gold wire side. This is shown in figure A.3.5.

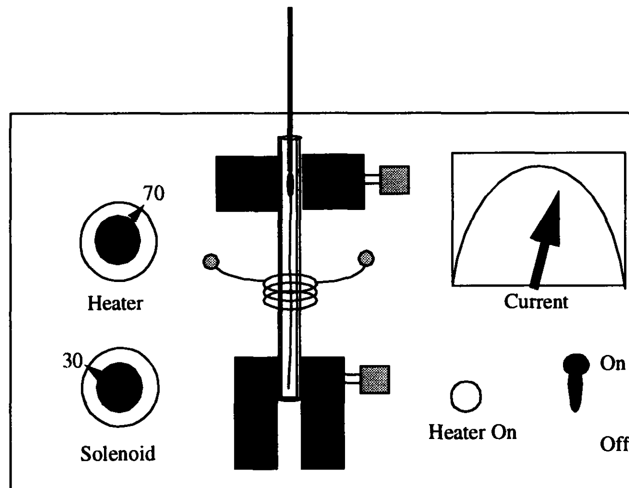


Figure A.3.5: The glass capillary tube assembly in the glass puller

The soldered junction should be situated so that it is at least 4 cm from the heating coil or the solder may melt and the gold wire and the copper wire may separate.

After the glass is pulled, it is allowed to cool before being removed from the glass puller. Care must be taken when handling the pulled glass needle since the tip is very fragile and the gold wire may or may not be completely sealed in the pulled tip. Pulling on the gold wire may pull the wire out of the tip. The other half of the glass capillary tube (the half connected to the bottom of the puller) can be saved and used as the housing for the prebundled carbon fiber microelectrodes.

A.3.1.3 Sealing the Tip with Epoxy

The next step is to seal the tip of the needle with epoxy to ensure that liquid cannot enter the needle. To do this, Eccobond 27 must be mixed (in a 10:3 ratio by *weight* with parts A and B respectively) and used immediately after mixing to ensure proper viscosity. After it is mixed, the epoxy is pulled into a small syringe, inserted into the glass needle and expelled. The syringe assembly is shown in figure A.3.6, and figure A.3.7 shows the syringe assembly in the glass needle

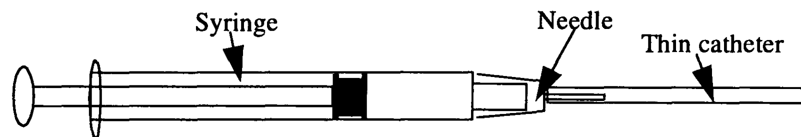


Figure A.3.6: Syringe assembly

Figure A.3.7: Syringe assembly inside glass needle

The epoxy is pushed through the syringe into the glass needle until the tip of the needle is visibly filled with epoxy. An oven set to ~65 $°C$ may be used to help the epoxy cure faster. However, a special orientation of the probe must be used while curing to ensure the epoxy does not improperly cure.

If the epoxy is allowed to cure so that the epoxy gravitates to one side of the needle, this may lead to a poor glass-epoxy interface. Epoxy also should not be allowed to drip out of the tip of the needle. With gold wire microelectrodes, the seal between the gold wire and the glass is usually tight enough that epoxy will not leak from the tip. Therefore, the epoxy can be cured with the tip of the glass needle pointing downwards. The epoxy will not flow to the tip of the glass needle very well if the seal between the gold wire and the glass catheter is very tight.

The final step in this stage of fabrication is to affix the polyurethane catheter to the glass needle. To do this, a small amount of epoxy (Eccobond 27 or 5-minute epoxy) should be applied to the open end of the glass needle. Enough epoxy should be placed so that the copper wire is securely sealed. A polyurethane catheter measuring approximately 6” in length is slipped over the copper wire and about 0.5 cm inserted into the open end of the glass catheter. This process is shown in figure A.3.8.

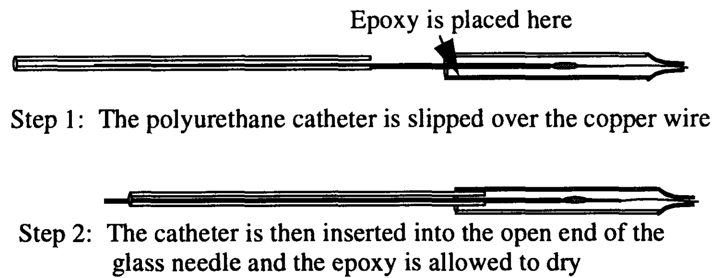


Figure A.3.8: Connecting the polyurethane catheter and the glass needle

A.3.1.4 Connection to the Coaxial Cable

The final step in constructing the gold wire microelectrode is to connect it to the coaxial cable, assumed to already be connected to the gold connector.

First, the excess copper wire protruding from the end of the polyurethane catheter is cut approximately 1.5 cm from the end of the catheter. This end of the copper wire is now stripped with a scalpel in exactly the same manner as the copper wire was first stripped to connect it with the gold wire. This should be done only after the epoxy is dry. The scraped end is cleaned in alcohol.

Two pieces of heat shrink tubing should be placed onto the copper wire and the coaxial cable. A piece of tubing of larger diameter measuring about 1" in length are put over the end of the coaxial cable. A smaller diameter piece of tubing also about 1" in length should be placed over the copper wire and polyurethane catheter. It is important to do this step now since if the two wires are soldered together, sliding the tubing over the extremely fragile glass tip is the only way to get it onto the wires.

As with creating the gold and copper wire junction, the two wires, the coaxial cable and the copper wire, are then taped to the clamp where both ends are tinned and connected. The connection should be made as small in diameter as possible to fit comfortably inside the heat shrink tubing.

Five-minute epoxy should then be mixed and a small amount of epoxy placed on top of the newly soldered junction. This serves to strengthen the soldered connection and provide a small amount of insulation for the connection. While drying, the epoxy must not be allowed to collect on either side of the connection. The probe must be twisted and turned periodically to keep the epoxy as uniform over the connection as possible. Figure A.3.9 shows a good epoxy seal as opposed to a poor one.

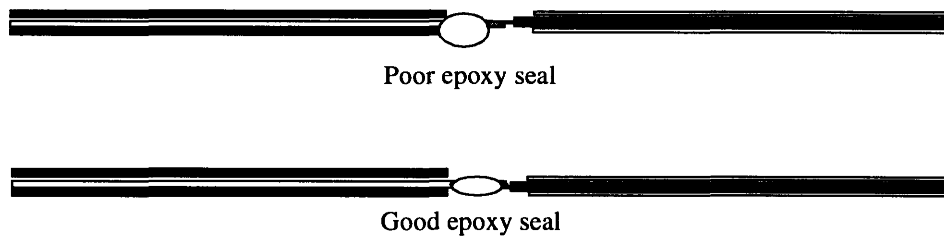


Figure A.3.9: The difference between a poor and a good epoxy seal

After the epoxy has dried, the heat shrink tubing can be slid over the connection and blown dry. Care must be taken when using the heat gun, as the polyurethane catheter will melt if too much heat is blown onto it.

A.3.2 The Carbon Fiber Microelectrode

The fabrication of the carbon fiber microelectrodes parallels the fabrication of the gold wire microelectrodes in many ways. The largest difference is in creating the junction between the copper wire and the carbon fibers, a method that uses a carbon paste made from Sody Escil epoxy and carbon powder. There are four steps to the fabrication of the probe. First, the junction between the carbon fibers and the copper wire must be made. The next three steps are exactly the same as those for the gold wire with only minor changes.

A.3.2.1 Junction Fabrication

The junction between the carbon fibers and the copper wire is created using a carbon paste, a mixture of Sody Escil resin and carbon powder. The resin is mixed 10:1 by vol-

ume, the catalyst added being $\frac{1}{10}$ the volume of the resin added. The carbon powder is mixed in to saturate the resin and create a black paste.

Before the carbon paste is mixed, the copper wire is stripped and cleaned in alcohol. A group of carbon fibers (between one and thirty, depending on the number desired) is cut to a length of approximately 10 cm. A small piece of a polyethylene catheter is used to hold the carbon fibers together. This is shown in figure A.4.1.

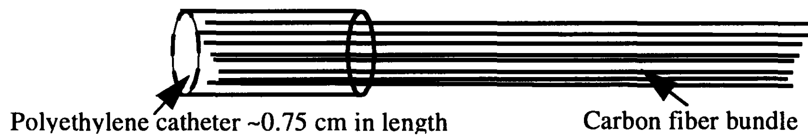


Figure A.3.10: Carbon fibers inside the polyethylene catheter

To create the junction, the stripped end of the copper wire is dipped into the carbon paste, then placed into the polyethylene catheter. Although this will not completely fill the catheter, repeatedly dipping the copper wire into the carbon paste and putting it into the catheter will soon fill the catheter with carbon paste. When it appears that there is a sufficient amount of carbon paste in the catheter, the copper wire (still the stripped end) is the placed into the catheter and allowed to dry. This is shown in figure A.4.2.

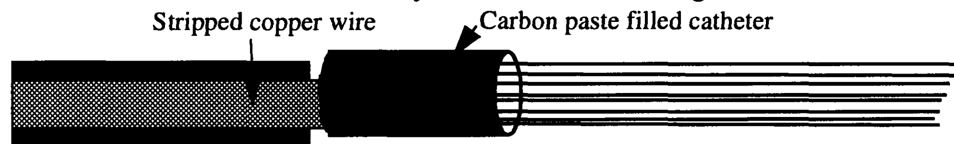


Figure A.3.11: The copper wire and carbon paste filled catheter assembly

After the carbon paste has dried (6 hours at room temperature is usually sufficient), the polyethylene catheter can be cut off carefully with a sharp scalpel. The easiest way to remove the catheter is to hold the assembly with a pair of tweezers and to carefully slice the catheter lengthwise. Special care must be taken not to slice too deeply and cut the junc-

tion. Once the catheter is sliced, it can be peeled off using a pair of tweezers. The final product is shown in figure A.4.3.

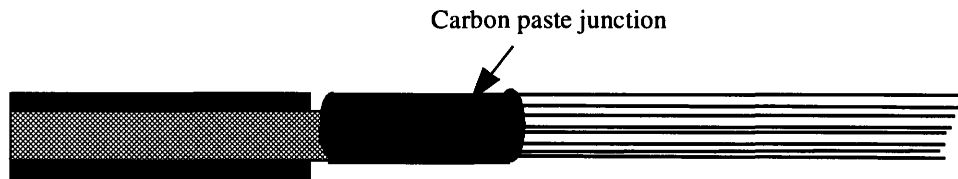


Figure A.3.12: The copper wire and carbon fiber junction

A.3.2.2 Pulling the Glass Capillary Tube

This stage is exactly the same as for the gold wire microelectrodes. Instead of a solder junction, the carbon paste junction is used. As with the gold wire probes, the junction should be at least 2" from the copper wire end of the capillary tube. The only difference in the pulling is the heater and solenoid settings. For carbon fibers, the settings should be:

Heater: 60

Solenoid: 20

Generally, the glass will pull well around the carbon fibers, but the seal will not be as tight as that of the gold wire microelectrodes. Therefore, the epoxy stage is much more important for the carbon fiber probes.

A.3.2.3 Sealing the Tip with Epoxy

The carbon paste junction is generally larger than the solder junction in the gold wire probe. Therefore, the syringe/catheter tool used to push epoxy into the tip of the needle will mostly likely go no further than the carbon paste junction.

The epoxy should flow easily to the tip since unlike the gold wire probes, the seal at the tip is not quite as tight. The carbon fiber microelectrodes should be allowed to dry tip down as well. If a bubble of epoxy forms at the tip, it can be carefully cut off.

As with the gold wire probes, the polyurethane catheters should be attached to the large end of the glass needle using either Eccobond 27 or 5-minute epoxy.

A.3.2.4 Connecting the Coaxial Cable

This stage is exactly the same as for the gold wire microelectrodes.

A.3.3 The Prebundled Carbon Fiber Microelectrodes

In many ways, the prebundled carbon fiber microelectrodes are the same as the carbon fiber probes described in A.3.2. The carbon fibers are bundled using the Eccobond 27 insulating epoxy. This bundle is then connected to the copper wire with carbon paste. Instead of pulling the glass needles with the bundle inside them, the glass is pulled first, then the carbon fiber assembly is placed inside the needle. The finishing steps are exactly the same as those for the other carbon fiber microelectrode.

A.3.3.1 Making the bundle

The bundle is formed from carbon fibers, cut at least 10 cm in length. This is because the ends of the bundle may need to be trimmed later in the process.

This bundle is stirred in a container of Eccobond 27 epoxy (directions on how to mix are described in A.3.1.1) until the carbon fibers are thoroughly saturated with the epoxy. A small section at one end (the end that is held) should remain dry and without epoxy. Generally, it is easiest when the epoxy is allowed to cure slightly. This way, the epoxy is tackier and won't run through the fiber bundle as quickly.

Using latex gloves, the fiber bundle should be held between the fingers and the excess epoxy pulled out. This can be done by grasping the dry end of the fiber bundle in one hand, and running the gloved fingers down the bundle from top to bottom. The excess epoxy should drip from the bottom. To dry the bundles, the dry section can be taped to a ledge and allowed to dry for at least twelve hours. The bundles should not be allowed to dry too near each other since any air movement may cause the bundles to contact each other and dry as one. Usually, 5 cm is enough space to place between bundles.

While the epoxy is still not dried, the gloves can be used to squeeze bubbles out of the drying, hanging fiber bundles. If air bubbles collect at the bottom of the bundle, that portion of the bundle can be cut off. Figure A.5.1 shows the hanging bundles.

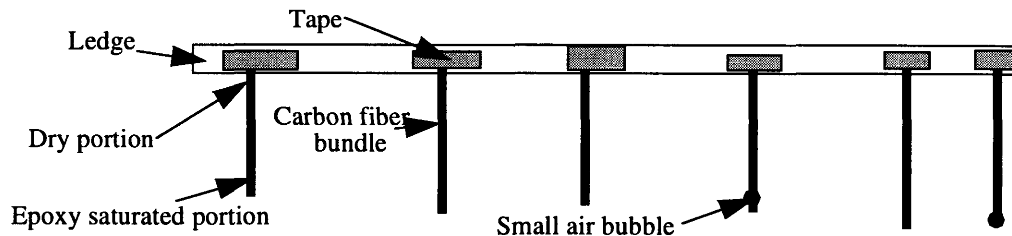


Figure A.3.13: Diagram of hanging, drying bundles

A.3.3.2 Creating the Bundle-Copper Wire Junction

Once the bundles are sufficiently dry, they can be removed carefully and the bundle cut just below the location of the tape. Attempting to remove the tape by peeling it from the dry portion of the bundle may result in ripped carbon fibers. The dry end of the bundle can now be placed into a polyethylene catheter and the junction made in the same manner as the other carbon fiber microelectrode.

A.3.3.3 Placing the Bundle into the Glass Needle

The difference between the fabrication of prebundled microelectrodes and others is that the glass is not pulled around the bundle. If the prebundled assembly were placed into the glass puller, the heating coil would melt the Eccobond 27 and damage the bundle.

The easiest method of finding already pulled glass needles is to use those from other pulled probes. The glass puller creates two pulled ends since it melts the glass, then pulls the two ends apart. The only end used thus far is the top half, the part with the copper wire and either gold wire or carbon fiber assemblies. The bottom half is a pulled glass needle as well, and the carbon fibers or gold that are embedded in the tip after the carbon fiber or gold wire probes are pulled can be removed easily. By using a sharp scalpel, the tip of the

needle can be enlarged. By breaking a hole slightly larger than that of the cathode, the prebundled assembly can then be inserted into the glass needle. Figure A.5.2 shows the two halves of the glass needle after pulling a gold wire microelectrode.

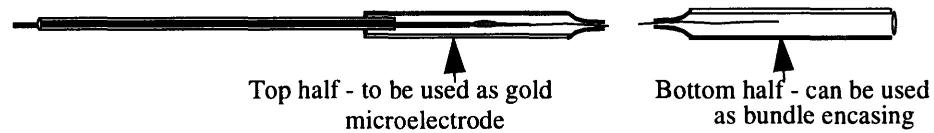


Figure A.3.14: The two halves of glass needle after pulling gold wire microelectrode

The bundle, trimmed with a scalpel to remove any sections with air bubbles, can now be placed into the glass needle.

A.3.3.4 Finishing the Prebundled Microelectrode Fabrication

The remainder of the bundle microelectrode is basically the same as that of the other carbon fiber probe. Epoxy (Eccobond 27) must be squeezed into the tip of the glass needle. When the insulating epoxy is squeezed in, the bundle should extend about 0.75 cm from the end of the glass needle tip. This is so the epoxy, when it drips through the hole at the end of the needle, will collect at the bundle tip and not interfere with the glass itself as shown in figure A.5.3.

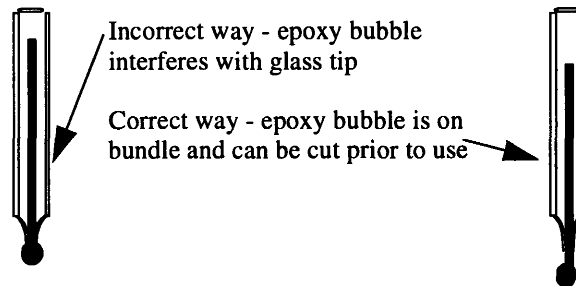


Figure A.3.15: Correct and incorrect methods of placing bundle inside glass needle

Other than this, the connections for finishing the prebundled carbon fiber microelectrode are the same as those for the carbon fiber microelectrode described in A.3.2.4.

Appendix B

Protocol for *In Vitro* Experiments

There were two different types of *in vitro* studies conducted, long term and short term (calibration) experiments. There are several precautions that must be taken when setting up the experimental system. Refer to figure 4.2 for the experimental setup.

- The cathode and anode (microelectrode and reference electrode) were attached to a holder to ensure that they did not move relative to the test medium during the experiments.
- Foam rubber pads were placed under the thermoregulated water bath and the ring stand to reduce baseline vibration.
- The bubbling of the water bath, the air conditioner, and other electrical devices were checked to ensure that they did not interfere with the signal being recorded. If they did, they were switched off prior to beginning the experiments.
- The thermoregulated water bath was turned on at least one hour before the first experiment.
- The TDP recording equipment was turned on thirty minutes before the first experiment.
- The temperature of the water bath was 37 $°C$, and the floating spheres on the water surface were evenly distributed to minimize heat loss, providing a higher thermal stability.
- The vibration of the experimental area was held at a minimum.

Both long and short term *in vitro* experiments took place in phosphate buffered saline solution (PBS), made as described below.

- The PBS is made using tablets (SIGMA P-4417), sterilized beakers, and deionized water.
- The PBS is mixed with sodium azide (NaN_3), 0.2% by weight, to eliminate microbial growth.
- The PBS is *not* mixed with glucose.
- Each day, the PBS is changed to ensure that it is not contaminated.
- At least 150 ml of PBS must be present in a 200 ml beaker.
- The level of the PBS in the beaker must be lower than the water level in the thermoregulated water bath.

B.1 Long Term Studies

B.1.1 Purpose

There are two purposes of long term studies. First, tests regarding the durability and reliability of the microelectrode can be conducted when the cathodes are subjected to experiments of over three hours duration. Second, the long term studies are very useful in pinpointing problems in the fabrication process.

B.1.2 Experimental Protocol

Long term studies involved the microelectrode being continuously tested for over 24 hours. These 24 hours were broken up into several segments, ranging from three to twenty hours in length. All of the long term studies were conducted with the PBS equilibrated to room air, containing an oxygen concentration of approximately 21%.

- The experimental devices (water bath, TDP boxes, etc.) were turned on and warmed up for at least thirty minutes.
- The PBS was warmed up to the temperature of the water bath (37 $°C$) by leaving it in the water bath for at least one hour.
- The PBS was exposed to room air for at least one hour.
- The cathode (fabricated microelectrode) was affixed to a metal rod which was attached to the ring stand.
- The reference electrode was completely submerged in the PBS.
- The computer program, *jcox*, was run on the recording PC.
- At the jcox prompt, the command, *init*, was typed, followed by the TDP-100 box number.
- The commands *oxcal* followed by *imon* were typed to begin measurements.
- For the proper *imon* parameters, the following table was used.

Table B.1: Suggested *Imon* Parameters

Length of Experiment	*Imon* Length	*Imon* Logfile	*Imon* Save Interval
3 hours	720	<date>.<run>	30
5 hours	600	<date>.<run>	60
10 hours	600	<date>.<run>	120
20 hours	600	<date>.<run>	240

- At the *plot* prompt, type 'y' and the measurements will commence immediately.

The logfile in table B.1 should be named <date>.<run> so that the sequential order of the experiments is readily apparent. For example, an experiment taken on January 31, 1996 would have the filename, "013196.001."

B.2 Short Term Studies

B.2.1 Purpose

The purpose of the short term, or calibration, studies is twofold. First, polarograms which relate the microelectrode's recorded current at a given polarization to the corresponding oxygen tension must be obtained to properly calibrate the microelectrode. Second, the short term stability and reproducibility of the microelectrodes can be analyzed from these experiments.

B.2.2 Experimental Protocol

In this set of *in vitro* studies, the length of the individual experiments ranged from approximately three to five minutes in length. These experiments are performed in PBS equilibrated at different levels of oxygen tension to get data necessary for probe calibration. For these studies, gasses containing 0% and 10% O_2 were used with room air which was assumed to be 21% O_2.

- The experimental apparatus (water bath, TDP boxes, etc.) were turned on and warmed up for at least thirty minutes.
- The PBS was warmed up to the temperature of the water bath (37 $^{\circ}C$) by leaving it in the water bath for at least one hour.
- Calcium chloride (CaCl) was added to the PBS solution to consume some of the oxygen. For the 0% experiments, 0% O_2 gas was still bubbled through the PBS to drive any atmospheric air out of the PBS.
- The desired gas (0% or 10%) was bubbled through the PBS solution for at least 1 hour.
- The experiments were performed in cycles, with five minutes devoted to the bubbling of the O_2 gas into the PBS and five minutes to the subsequent O_2 measurement process.

- During the five minute measurements, the gas dispersion tube was lifted and held so that the end of the tube was at the narrow portion of the flask. This was so room air (21% O2) could not easily enter the flask and contaminate the PBS, altering the pO_2 level.
- The cathode (fabricated microelectrode) was affixed to a metal rod which was attached to the ring stand.
- The reference electrode was completely submerged in the PBS.
- The computer program, *jcox*, was run on the recording PC.
- At the jcox prompt, the command, *init*, was typed, followed by the TDP-100 box number.
- For the *imon* parameters, the *length* was set to 301, the *filename* to <date>.<run>, and the *save interval* to 2.
- Following each five minute experiment, the gas dispersion rod was lowered back into the PBS and bubbled through the solution for five minutes.
- The pressure of the gas was not so high as to cause the PBS to bubble out of the flask.
- The presence of the gas dispersion tube at the bottom of the flask was positioned so that it did not damage the tip of the microelectrode.
- Experiments were not run while the gas dispersion tube was bubbling gas directly into the PBS.
- Calibration points were taken by pressing 'c' during the *imon* run and entering the requested parameters. The thermometer attached to the water bath console was used to monitor the temperature of the water bath. The tag attached to the gas tank provided the exact oxygen composition of the gas.

Appendix C

Protocol for *In Vivo* Rat Experiments

C.1 Introduction

This protocol, adapted from one designed by Charles Ruban, Matthew Newman, and Sherriff Ibrahim, details a technique for making *in vivo* pO_2 measurements in the rat liver and leg muscle. These measurements will take place in the muscle tissue of the upper thigh as well as in the unexcised liver of an anesthesized rat. This protocol tests an invasive probe to determine if probe-induced trauma may alter the tissue's physiological status, or whether improved probe designs are indeed suitable for routine use in living tissues.

C.2 Preparation of pO_2 Measurement System

C.2.1 Materials needed to run TDP system

- 1 TDP-OX box
- 1 TDP-100 box
- 1 power supply
- At least 3 pO_2 probes (in case one does not work properly)
  - Carbon fiber glass needle microelectrodes
  - Gold wire glass needle microelectrodes
  - TTI butterfly needle microelectrodes
- 1 Ag/AgCl reference electrode
- Necessary cables for electrodes and power supply
- 1 IBM PC compatible computer with JCOX measurement software

C.2.2 Preparation

- The probe(s) to be used in the *in vivo* monitoring of the rat pO_2 levels should be subjected to *in vitro* monitoring for at least 1 hour before *in vivo* testing commences.
- The measurement equipment should be turned on for at least 1 hour before *in vivo* monitoring commences.
- The program, *jcox*, should be run from the TDP100 directory.
- The initialization command, *init*, followed by the TDP100 serial number should be typed at the *jcox* prompt.
- The oxygen calibration command, *oxcal*, should be used to calibrate the voltage from the TDP-OX box.
- The first probe to be used should be defined by using the command, *oxyprobe*, followed by the pO_2 probe name and the expected measurement temperature.

C.3 Surgery

C.3.1 Materials needed to perform surgery

- Surgical scissors (several sizes)
- Microsurgical scissors
- Tweezers (both blunt and fine tipped)
- Semi-curved tweezers
- Gauze pads
- Forceps
- Xylazine and ketamine (anesthetics)
- Heparin (anti-coagulant)
- Syringes (1, 2, and 3 cc.)
- 2 25 gauge, 5/8 inch long needles
- Electric shaver
- Nair (hair removal cream)

C.3.2 Preparation of the surgical table

- Cover the surgical table with a "chux diaper," the absorbent side facing up so that and fluids during surgery can be absorbed
- Connect twine or wire to the four clips on the table to immobilize the rat limbs during surgery
- Lay out all surgical supplies on the table

C.3.3 Administration of anesthesia

- Place the rat into the glass gas chamber and allow 100% CO_2 to flow into the chamber for approximately 8-10 seconds. Wait for the rat to become unconscious, but ensure that it is still breathing.
- Weigh the immobilized rat.
- Prepare a 1 cc. syringe with a combination of Xylazine (13 mg/kg) and Ketamine (87 mg/kg) using the following conversion:

$$\text{ml Xylazine} = 0.65 \text{ ml/kg} \times \text{weight}$$

$$\text{ml Ketamine} = 0.87 \text{ ml/kg} \times \text{weight}$$

The weight of the rat is taken in kilograms

- Inject the anesthesia using a 25 gauge needle into the peritoneal cavity of the rat by carefully lifting the skin surface with tweezers. Care should be taken to avoid damaging organs below the surface of the skin. When the needle is first inserted, the plunger should be pulled back slightly to ensure that no blood enters the syringe. If

there is blood in the syringe, it must be repositioned, for the needle is in a vessel. Anesthesia injected directly into the bloodstream will cause death.

- For safety in case the rat regains consciousness, place the rat back into the CO_2 chamber until it is once again unconscious.
- If pO_2 measurements are to be made on the leg muscle, one of its hind legs must be shaved with an electric razor. After the surface hair is shaved by the razor, vacuum the hair and apply Nair to the shaved area. After 5-10 minutes, wipe the Nair away.
- Place approximately 1 inch worth of gauze pads on the surgical table for the rat to lie on.
- Secure the rat on the surgical table by tying each of its limbs to a piece of twine or wire. If leg measurements are to be made, the leg that was shaved should not be tied down. The rat should be tied such that the ventral side is exposed.
- Test whether the rat is unconscious by pinching the web of the feet with a pair of forceps. If there is no reflex, the rat is sufficiently under and surgery may commence.

C.3.4 Surgery

- If pO_2 measurements are to be made on the leg, it is advisable to begin with the leg.
- Using tweezers, hold up the skin of the rat's leg and using a scalpel, cut an incision 1.5 cm. in length parallel to the axis which runs from the rat's foot to the joint of the leg and the body. This should be made in several "cuts," progressively cutting deeper into the tissue. This is done to prevent accidentally cutting the muscle at this time.
- Make two more incisions, each about 0.75 cm. in length starting from each end of the first incision, cutting perpendicular to the original cut.
- Place the reference electrode under the skin flap which has just been cut.
- Oxygen measurements may now be taken.

At this point, pO_2 measurements can be made on the muscle, between the muscle and the skin, or with a small incision, directly in the muscle. Proceed to section C.4 for details on making in vivo pO_2 measurements. Following satisfactory completion of these measurements, continue with the surgery.

- Using tweezers to hold and lift the skin, use a large pair of scissors and cut from the lower abdomen to each side of the rat, making a V-line incision from the abdomen (near the belly button area) up to the diaphragm (at the base of the rib cage). Be very careful not to nick the liver. This is shown in figure C.3.1.
- Fold the skin over the rib cage.
- Place the reference electrode into the peritoneal cavity, under the intestines. As long as the reference electrode resides in the cavity, the exact location is not crucial.

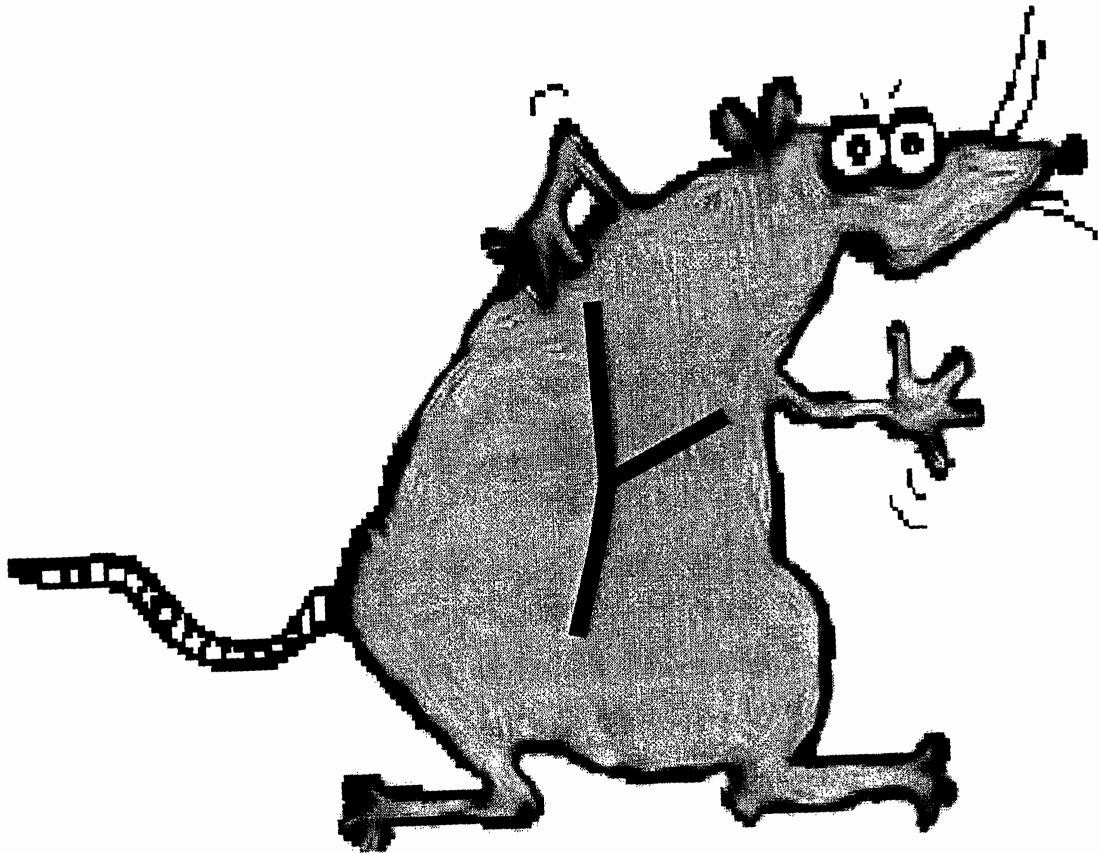


Figure C.3.1: Diagram of cuts to be made in rat to expose liver

C.4 Making *In Vivo* Measurements

- Place the oxygen electrode under the skin or into the lobe of the liver. Typically, several positions must be tried to find a position that shows sensitivity. In the liver, the probe must be pushed into the lobe. With the glass needle microelectrodes, no incision needs to be made prior to insertion.
- From the *jcox* prompt, the command, *oxmon*, should be typed to begin measuring the pO_2 levels. Instead of the pO_2 levels, the current can be measured with the *imon* command. Parameters for the monitoring command should be as follows:

  length: Press enter to accept the default value of *forever* to record until the user stops the recording sequence.

  logfile name: Type the name of the file that the experiment data should be stored in.

  save interval: Press enter to accept the default value of *1*.

  plot: Type *y* to display plots on the monitor.

  The recording will immediately begin following the input to the *plot* query.

- Wait for the steady-state conditions to be met and record the resting, baseline pO_2 value.
- 100% O_2 gas may be administered to the rat by placing the head into a large syringe connected to an oxygen tank via tubing. If there is no noticeable change in the recorded pO_2 level or current level after two minutes, move the oxygen electrode to a different location and start another *imon* or *oxmon* command to being a new measurement sequence. Before the second recording sequence is started, allow the rat to breathe room air for at least two minutes.
- Experiments that can be performed include:

  Effects of breathing different oxygen concentrations on measured results.

  Changing probe placement.

  Differences between measurements obtained at the beginning and end of the rat's anesthesized life.

  Differences between measurements taken at the liver tissue and leg muscle.

  Differences in the direction of probe placement into the liver or muscle (parallel or perpendicular.)

  Test the effects of incisions on probe responsiveness to pure O_2.

  Test the difference in probe placement in, on, or between the muscle and the skin flap.