Design of a fiber optic oxygen sensor based on immobilized hemoglobin

Mendu Govind Raj
New Jersey Institute of Technology

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Design of a fiber optic oxygen sensor based on immobilized hemoglobin.

by
Mendu Govind Raj

Thesis submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Master of Science in Electrical Engineering.
1988
Title of Thesis: Design of a fiber optic oxygen sensor based on immobilized hemoglobin.

Name of Candidate: Mendu Govind Raj.
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<th>Degree</th>
<th>Date of Degree</th>
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<td>Sri Venkateswara University</td>
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<td>B.Tech</td>
<td>May 1982</td>
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ABSTRACT

Title of Thesis: Design of a fiber optic oxygen sensor based on immobilized hemoglobin.

Mendu Govind Raj, Master of Science, 1988

Thesis Directed By: Dr. Sam S. Sofer, Professor.
Sponsored Chair in Biotechnology.

The fiber optic Oxygen sensor consists of a thin layer of immobilized deoxyhemoglobin at the common end of a bifurcated fiber. Immobilization is done using the alginate gel procedure. The immobilized reagent is separated from the standard oxygen sample by the gel with or without semi-permeable membrane, and the entire configuration is enclosed in an oxygen free sample cell. Light from a tungsten-halogen lamp, filtered through a 405nm filter is passed through one arm of a bifurcated fiber to the sample cell. The reflected intensity (a highly polished aluminum foil or a plate is placed at the bottom of the sample cell) from the other arm of the bifurcated fiber is measured using a photomultiplier tube and a power meter.
The above procedure is repeated for a 435 nm filter. The ratio of the reflected intensities at 435nm and 405nm gives an indication of the concentration of oxygen in the standard samples. Since this is a ratio measurement, it is insensitive to slow loss of reagent and drift.

The reflected intensities decrease with increase in the amount of deoxyhemoglobin and with increase in the thickness of the immobilized layer. Precautions should be taken to prevent the oxidation of hemoglobin to methemoglobin.

Response time is an important factor in determining the time required for equilibrium to be attained. It takes about 3 minutes to reach equilibrium for a 0.5mm thick layer of immobilized deoxyhemoglobin, coated with the membrane and 2 minutes without the membrane.
I would like to express my sincere and grateful thanks to my supervising adviser, Dr. Sam S. Sofer, for his friendship, invaluable guidance and moral support throughout my period of research. Without his chemical expertise and jovial manner, the chemical aspects of the project would not have been accomplished.

I am also grateful to Dr. Niver and Dr. Carr who are serving on the panel of advisers for their able guidance on the electro-optics aspect of the research work. Dr. Niver's course on integrated fiber optics gave me a welter of information on the various practical aspects of the project.

Sincere thanks are due to Dr. Farhad Ansari who, with his experience on lasers and other optical equipment, was of immense help in solving the problems of shielding and proper alignment of the light beam.

Special thanks to all my advisers for the stimulating discussions and constructive criticism offered during the work.
Thanks also to my friends Ioannis, Ema and Doug whose moral boosting was of tremendous help in keeping me motivated. Their help in setting up the apparatus for the measurement of standard oxygen samples is also appreciated.

And most of all thanks to the Lord.
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CHAPTER I

INTRODUCTION

Optical fibers can be used in a surprisingly wide range of applications other than transmission of data. In the past few years, the potential for analytical applications of fiber optics in sensors has become more and more widely recognized. Fiber optics is valuable whenever one needs to do real time monitoring as opposed to isolated sampling. The devices considered here involve a reagent on the end of a fiber. In operation, interaction with analyte leads to a change in optical properties of the reagent which is probed and detected through fiber optics. Depending on the sensor the optical property measured can be absorbance, reflectance or luminescence [5] [4] [1] [6].

The term OPTRODE [15] is used to describe these devices. These optical sensors are very similar to electrodes. However in operating principle they are quite different, offering new possibilities compared to conventional electrodes.

Attractive features of optical sensors:

(1) Immunity to interference due to electric and magnetic fields.
(2) No need for the reference electrode.

(3) The reagent does not have to be physically in contact with the fiber. It is convenient to change reagent. In fact, it is possible that in some context it will be practical to use the reagent on a disposable basis. Also the reagent can be in a different environment from the fiber. For example, the reagent could be in a high pressure reactor and be probed through a window in the reactor.

(4) Optical sensors can offer significant cost advantages over electrodes, particularly if a single spectrometer is used with several sensors.

(5) Use of multiwavelength and temporal information. For example one can envision sensors that respond simultaneously to two or more analytes distinguished by measurements at two or more detection wavelength combinations.

(6) Optical sensors can be developed to respond to analytes for which electrodes are not available.

Optical sensors also are subject to certain limitations like:

(1) Ambient light will interfere with optical sensors. They must be either used in dark environment, or the optical signal must be encoded (by modulation).

(2) Long term stability is likely to be a problem for reagent systems used in optical sensors, although to some extent
this can be compensated for by multiple-wavelength detection and by the ease of changing reagent phases.

(3)Because the reagent and analyte are in different phases, there is a mass transfer step before constant response is reached. This in turn limits response time of an optical sensor.

(4)For several types of optical measurements, observed intensities are proportional to the amount of reagent phase. If the amount of reagent phase is small, detected intensities can be increased by using more intense probe radiation, however, this will accelerate any reagent photodegradation processes. As a consequence, optical sensors involve a three way tradeoff between amount of reagent phase, intensity of probe radiation and stability [13].

(5)In general optical sensors will have limited dynamic ranges compared to electrodes.

Optical sensors can be classified as reversible and irreversible. A sensor is reversible if the reagent phase is not consumed by its interaction with analyte. If the reagent phase is consumed then the sensor is non-reversible. For such a device to be useful for sensing, the relative consumption of reagent phase must be small.

The role of oxygen sensors in the present advanced research has become so important that various new types of sensors are being designed. Optical sensing seems to be one promising field,
especially in view of the outstanding advantages it offers over conventional electrodes.

We, at the NJIT Biotechnology Laboratory are doing research in four different areas, leadby the detox group, blood group, bioproducts group and food processing group. All these fields depend heavily on accurate sensing of oxygen. For example, the detox group requires oxygen sensors for the study of reaction rates for decontamination of water, the blood group to detect oxygen in patient blood, the food technology group for the study of viability of yeast, and the bioproducts group to monitor liver enzyme reactions.

In view of this increasing demand for oxygen sensors, a fiber-optic oxygen sensor based on immobilized hemoglobin is designed. This sensor is based on the shift in Soret absorption band when oxygen comes in contact with hemoglobin. The detected parameter is the reflectance observed at two different wavelengths (which correspond to the peaks in the absorption spectra of hemoglobin and deoxyhemoglobin.) The ratio of reflectance values gives an indication of the concentration of oxygen.

Reflectance-based sensors are just one type of reversible sensors that include the following varieties:

(1) Competitive binding sensors [5],
(2) Absorbance based sensors [5],
(3) Fluorescence based sensors [7],
(4) Fluorescence quenching sensors [7], and
(5) Competitive binding sensors based of fluorescence [5].

Sensors can also be non-reversible type, where the reagent is consumed. The use of tetrakis to measure oxygen [26] is one such example. A detailed description of these sensors is given in Appendices B and C.
CHAPTER II

THEORY OF OPTICAL FIBERS

Ever since the idea of transmitting light in glass medium was conceived, there has been a quantum leap in the information transfer technology and today optical fiber technology has found applications in various diverse disciplines. Among the many applications particularly in the field of medicine and biotechnology[14] [16] optical fiber sensors has shown outstanding performance. The potential for analytical applications has been incredible especially when one needs to do real time monitoring as opposed to manual sampling. These devices involve a reagent at the tip of a bifurcated optical fiber. Upon association with an analyte the optical properties of the reagent change which are probed and detected. In a typical experimental set-up light from an optical source is guided through one leg of a
bifurcated fiber to the reagent and the reflected intensity is sent via the other leg to the detector. Depending on the device the optical parameter measured can be reflectance, absorbance, or luminescence.

2.1 METHODS OF PREPARATION:

The usual basic starting material for the optical fibers is silica. The methods used to prepare highly transparent materials for optical fibers may be divided into two broad groups: crucible or liquid phase methods, and vapor deposition methods. In both cases to achieve low attenuation requires most stringent control over the purity of the starting material and the avoidance of contamination during the whole manufacturing process.

In general crucible processes may be used to produce fibers from glasses with low melting points. The highly purified, powdered components are heated together in a platinum or silica crucible. An electric furnace can be used where the components are heated by radiation from the furnace walls which have to be maintained free of contaminants. Alternately rf induction heating is sometimes used. With a metal crucible, the rf coupling may be made to the crucible and the heat is transferred by conduction. In order to use a silica crucible, direct coupling to the melt has to be obtained, so the powdered glass components have to be
pre-heated in a high rf field. The melt is then at a higher temperature than the crucible and so is less susceptible to contamination from it. Silica crucibles cannot be normally used more than once, since they do not withstand thermal cycling.

With the double crucible arrangement[31], the starting materials may be fed in either the powder form or by means of high purity preformed rods. The double crucible is mounted inside a vertical, silica lined muffle furnace capable of raising the melt to 1000-1200°C. An inert gas atmosphere is maintained inside the furnace. When a dopant such as thallium with a relatively high rate of diffusion in silica is used to create the refractive index difference between core and the cladding, some index grading at the interface occurs during the pulling process. The index difference is obtained by varying the concentrations of the components SiO₂, B₂O₃, CaO. The diffusion across the core-cladding boundary within the melt gives sufficient grading to reduce multipath dispersion to between 1 and 5 ns/km.

The melting temperature of glasses with high silica content is too high for crucible methods, so vapor deposition methods have to be used. Some of these use flame hydrolysis to synthesize fine particles of glass from halide vapors of the constituents, in reactions such as:
And in the technique that first yielded fibers with attenuation lower than 20dB/km, a glass 'soot' was deposited this way onto an aluminum rod. The halide vapours were introduced into a methane-oxygen flame directed onto the rod. Many layers were built up, their composition being varied so as to produce either a step or graded variation of refractive index. The rod is then removed, leaving a porous glass preform. This was sintered to form a clear glass and this in turn was drawn into fiber. Here the main difficulty is the removal of excess water vapour left after the hydrolysis.

Another hydrolysis method that also enables large preforms to be made is the Vapour Axial Deposition (VAD) technique. Core and the cladding glasses are deposited simultaneously onto the end of a seed rod which is rotated to ensure azimuthal homogeneity and is drawn up in an electrical furnace at about 2.5mm per minute. There it is heated up to about 1500°C in an atmosphere of oxygen and thionyl chloride vapor. Here two conditions are fulfilled:

(1) Any water is removed by chemical reaction, and

\[ \text{SiCl}_4 + 2\text{H}_2\text{O} = \text{SiO}_2 + 2\text{H}_2 + 2\text{Cl}_2, \]
\[ \text{GeCl}_4 + 2\text{H}_2\text{O} = \text{GeO}_2 + 2\text{H}_2 + 2\text{Cl}_2, \text{ and} \]
\[ 2\text{POCl}_3 + 3\text{H}_2\text{O} = \text{P}_2\text{O}_5 + 3\text{H}_2 + 3\text{Cl}_2. \]
At the same time the rod, which is porous and initially may be about 60mm in diameter and and 200mm long, is consolidated into a transparent glassy preform of some 20mm diameter.

Another technique that is sometimes referred to as modified chemical vapor deposition (MCVD) [31] is also used. It differs from the VAD process in that the need to maintain control over the deposition processes limits the size of the MCVD preform to one that would normally pull into 3-5 km of graded-index fiber. The refractive index of the VAD fibers tend to be smoother than those of MCVD fibers.

2.2 PROPAGATION IN OPTICAL FIBERS:

In an optical fiber a layer of higher refractive index is sandwiched between layers of slightly lower refractive index. The higher refractive layer called the core is responsible for the propagation of the light and the outer layers help in confining the light only to the core region. Here, the propagation of light is due to total internal reflection.

Refraction at an interface between uniform media is governed by Snell's law which is demonstrated in Figure 1 [31].
Figure 1. Refraction and total reflection at a dielectric interface.

(a) Ray AA' is refracted according to Snell's law.
(b) Ray BB' is the critical ray.
(c) Ray CC' is totally reflected at the interface.

This figure is from "Optical communications systems" by John Gower.
A ray of light is shown, passing from a medium of higher refractive index, $n_1$ into a medium of lower refractive index, $n_2$. For $0 < \theta < \theta_c$ and $0 < \theta' < \pi/2$,

$$n_1 \sin \theta = n_2 \sin \theta'$$

where $\theta$ and $\theta'$ are the angles of incidence and refraction respectively and $\theta = \theta_c$ is the critical angle at which $\theta' = \pi/2$. Hence

$$n_1 \sin \theta_c = n_2.$$

For $\theta > \theta_c$ total internal reflection takes place with no losses at the boundary.

Consider an optical fiber with core refractive index $n_1$ and outer cladding refractive index $n_2$ as shown in Figure 2. The beam will propagate unattenuated along the fiber by means of multiple internal reflections provided that the angle of incidence onto the core-cladding interface is greater than the critical angle, $\theta_c$. This requires that the angle of obliqueness to the fiber axis, $\phi = \pi/2 - \theta$, be less than $\phi_c = \pi/2 - \theta_c$, and that the angle of incidence, $\alpha$, of the incoming beam onto the end face of the fiber be less than a certain value, $\alpha_m$. 
Figure 2. Propagation in optical fiber.

This figure is taken from "Optical communication systems" by John Gower.

AA' is the axial ray.

BB' is the critical ray for total internal reflection at the interface. CC' enters too obliquely for reflection at the interface and passes out into the cladding.
In order to calculate $a_m$ and $\varphi_m$ we assume that $n_a = 1$ and apply Snell's law:

$$\sin a = n_1 \sin \varphi = n_1 \cos \theta$$

For the critical rays,

$$\sin a_m = n_1 \sin \varphi_m = n_1 \cos \varphi_c$$

But we know

$$n_1 \sin \theta_c = n_2$$

Hence,

$$\cos \theta_c = \frac{(n_1^2 - n_2^2)^{1/2}}{n_1}$$

$$\sin a_m = \frac{(n_1^2 - n_2^2)^{1/2}}{n_1}$$

if we let

$$N = n_1 - n_2$$

and

$$n = \frac{n_1 + n_2}{2},$$

then

$$\sin a_m = \left(2nN\right)^{1/2}.$$

The greater the value of $a_m$, the greater is the percentage of the light incident on the end face that can be collected by the fiber and be propagated by total internal reflection. By analogy with the term used to define the light gathering power of microscopic objectives, $n_a \sin a_m$ is known as the numerical aperture (NA) of the fiber. Thus putting $n_a = 1$, $(NA) = \sin a_m = (2nN)^{1/2}$.

In the case when light is emitted by a small diffuse source situated on the fiber-axis near to the end face, only a fraction $(NA)^2$ can be collected and propagated by the fiber.
The amount of the power that can be coupled into the fiber in case of a diffuse light source is of immense practical importance. A simple formula can be used to know the amount of coupled power and is as follows.

Consider a small diffuse light source such as the isotropic (Lambertian) radiator shown in the Figure 3.
Figure 3. Diffuse light source.

The elementary annular ring whose radius is $r$ subtends an angle $\omega$. It also subtends a solid angle when its width $dr$ subtends at the source.

This is taken from "Optical communication systems," by John Gower.
The power radiated per unit solid angle in a direction at an angle $\theta$ normal to the surface is given by

$$I(\theta) = I_0 \cos \theta.$$ 

The total power $\mathcal{E}_0$ emitted by such a source is obtained by integrating $I(\theta)$ over all forward directions

$$\mathcal{E}_0 = \int_{-\pi}^{\pi} (I_0 \cos \theta)(2\pi \sin \theta) \, d\theta$$

$$= -2\pi I_0 [\cos \theta]_0^{\pi/2} = I_0 \frac{\pi}{2}$$

$$= \pi I_0 \frac{\pi}{2}$$

The power that can be collected from such a source by an adjacent fiber whose core diameter is greater than the diameter of the source is given by $\mathcal{E}$ where

$$\mathcal{E} = \int_{-\pi}^{\pi} (I_0 \cos \theta)(2\pi \sin \theta) \, d\theta$$

$$= -2\pi I_0 \cos \theta |_{0}^{\pi/2}$$

$$= I_0 \pi \sin^2 \frac{\pi}{2} = I_0 \pi = \mathcal{E}_0 (NA)^2.$$ 

Hence

$$\mathcal{E}/\mathcal{E}_0 = (NA)^2 = 2nN.$$ 

Clearly, in order to collect as much light as possible it is necessary to make $n$ and $N$ as large as possible.

Depending on the variation of refractive index in the core and cladding regions the fibers can be classified as:

1) Step-index fibers.

2) Graded index fibers.
3) W-profile fibers, and so on.

Depending on the mode of propagation the fibers can be classified as single mode fibers and multi mode fibers. The problems of dispersion and time delay of the arriving rays can be overcome by the use of single mode fibers where only one mode is propagating.

2.3 ATTENUATION IN FIBERS:

A very important parameter to be considered for light propagation in optical fibers is the attenuation. This may not be that important for sensor applications but certainly very important for communications applications. The attenuation has come down from 20dB/km to a mere 0.2dB/km during the period of last 20 years [31].

The mechanisms of attenuation may be classified into two principal groups:

2.3.1 ABSORPTION:

Basically a material property and it takes place when electronic transitions, the resonances are excited within the material and are followed by non-radiative relaxation processes. As a result there is an increase in thermal energy in the material.
2.3.2 SCATTERING:

Scattering is a partly material property and may also be due to the imperfections in the material.

Attenuation does not depend only on the quality of the core material. The cladding material may also be significant. In the process of the total internal reflection, the electromagnetic fields penetrate the core-cladding interface and extend into the cladding. Thus a small fraction of the total optical power propagates in the cladding. If this is of poor quality or is highly absorbing it will contribute to the overall fiber attenuation. The fibers required to have minimal attenuation are made with cladding material as high in quality and as carefully controlled in the core. It is necessary to ensure that any light scattered into the cladding does not propagate and enter the detector because this is likely to increase the range of propagation velocities and make the dispersion worse. Two steps are taken to avoid this.

The outer layers of the cladding can be made absorbing so that the scattered rays are attenuated there but propagating light is unaffected, and

The cladding itself can be surrounded by a protecting layer of polymer of higher refractive index into which the scattered rays pass and are absorbed.
The geometry of the fiber also contributes a lot to the losses in the fiber. Bends and imperfections that occur lead to the guided rays being scattered into rays which are transmitted away from the interface and large imperfections inside the fiber core each give rise to a large local scattering loss.

Tight bends similarly cause some of the light not to be internally reflected but to propagate into the cladding and be lost. In theory the optical power scattered out of a fiber at a major bend depends exponentially on the bend radius, R. The bending loss is then proportional to \( \exp \left( -\frac{R}{R_c} \right) \) where the critical radius \( R_c = \frac{a}{(NA)^2} \), a being the radius of the fiber core. At a bend of radius \( R_c \), the loss would be very considerable, but because of the form of the exponential function, the losses decrease rapidly for less tight bends.

In practice it is the mechanical properties of the fiber rather than the bending losses that determine the minimum allowable bending radius. If the fiber is bent so tightly that the surface strain exceeds about 0.2% then significant stress cracking is likely to develop in the fiber. This is generally prevented by enclosing the fiber in a suitably rigid cable.

Although the losses from gross bends may be unimportant, a continuous succession of the very small bends may cause a very significant rise in fiber attenuation. The effect is known as microbending loss, and is particularly noticeable when an un-
sheathed fiber is wound in tension on to a drum. The microbends arise as the fiber is distorted by imperfections on the drum surface. A similar effect can easily be caused by the pressures exerted on a fiber by adjacent members inside a cable. Continuous but small variations inside the core diameter, which can easily arise during manufacture, may give rise to a similar scattering mechanism and cause waveguide losses.
3.1 EXPERIMENTAL SET-UP:

Figure 4 depicts the experimental set-up which consists of:

3.1.1 THE SOURCE- TUNGSTEN-HALOGEN LAMP:

This is a 250 watt, 24V, 10,000 lumens filament lamp. The spectrum of this lamp resembles of the black body. The color temperature depends on the operating condition and is approximately 3750°C. It is operated by a DC stabilized power supply.

3.2.2. COOLING AND SHIELDING CONSIDERATIONS:

In view of the high power dissipation of the lamp, cooling was provided using a high speed blower. How to maximize the light that can be coupled into the fiber and how to incorporate an interference filter between the lamp and the fiber, were the main aspects that were considered when the shielding was designed.
Figure 4. Block diagram representation of the set-up.

1--The source—Tungsten-halogen lamp
2--Interference filter
3--Bifurcated optical fiber
4--The Detector—PM tube
5--Electronic amplifier
6--The oscilloscope
7--Immobilized deoxyhemoglobin layer
8--Converging lens
9--Semi permeable membrane
10--Photometer or Power meter
3.1.3 INTERFERENCE FILTERS:

Interference filters operate through a complex process of constructive and destructive interferences to select precisely an often very narrow range of wavelengths with a minimum transmission efficiency. They consist of a suitable base substrate on to which carefully controlled, varying thicknesses and type of chemical layers are vacuum deposited in such a fashion that the transmission of certain wavelengths is enhanced while other wavelengths are either reflected or absorbed. For the experiment two interference filters at 405nm and 435nm which work on the same principle were used. The principle of operation of interference filters is given in Appendix A.

3.2 IMMOBILIZATION OF HEMOGLOBIN:

3.2.1 BRIEF SYNOPSIS OF IMMOBILIZATION:

Immobilization is a technique of confining a biological system (enzymes or microorganisms, etc;) into some type of non-toxic water-soluble matrix by means of chemical or physical methods. In this case, entrapment of deoxyhemoglobin within a water insoluble matrix (calcium alginate gel) is used.
3.2.2 WHAT ARE THE BENEFITS?

(1) Enhances the resistance of an enzyme's properties to certain environmental changes (i.e. thermal stability, high activity, extended half life.)

(2) Offers considerable operational advantages over freely mobile systems.

(3) Serves as models for natural invivo membrane bound systems.

3.2.3 ADVANTAGES:

Some of the operational advantages are:

(1) Reusability.

(2) Possibility of batch or continuous modes.

(3) Rapid termination of reactions.

(4) Controlled product formation.

(5) Greater variety of engineering designs for continuous processes.

(6) Possible greater efficiency in consecutive multistep reactions.

As mentioned above immobilization has many advantages and all these are exploited in the design of an optical oxygen sensor based on immobilized hemoglobin, like the immobilized layer at the tip of the bifurcated layer can be reused after several washes with calcium chloride solution.
As a first step towards this measurement oxy and deoxy hemoglobins were immobilized and their absorption spectra were plotted using a spectrophotometer.

3.3 PREPARATION OF 2% ALGINATE GEL:

The steps involved in the preparation of the gel are as follows.

(1) Dissolve 1.87 gms of sodium chloride in 100ml of 0.05 molar phosphate buffer-pH 7.4.

(2) Dissolve 2 gms of sodium alginate in 100 ml of buffered sodium chloride solution prepared as in step(1)

(3) Heat the mixture while continuously stirring until all the alginate gel is dissolved.

(4) Sterilize the gel in autoclave for about 30 minutes at 120°C.

3.4 PREPARATION OF IMMOBILIZED HEMOGLOBIN BEADS:

The steps involved in preparation of calcium chloride solution are as follows.

(1) Dissolve 14.7 gms of calcium chloride in 1000ml of water to get 0.1M solution.

(2) Mix about 0.25 gms of hemoglobin with 10ml of alginate gel.
(3) With the help of a syringe, extrude the gel dropwise into calcium chloride solution. The beads solidify upon association with calcium chloride solution.

(4) The immobilized hemoglobin is stored in refrigerator when not used.

To make immobilized deoxyhemoglobin, the procedure is as follows:

(1) Add 0.05 gms of sodium dithionite to 0.25 gms of hemoglobin. This proportion was achieved after trying various ratios and examining their absorption spectra. The above combination gave a peak for immobilized deoxyhemoglobin which was very close to the theoretical value at 435nm.

(2) Repeat steps (2) to (4).

3.5 ABSORPTION SPECTRA:

The absorption spectra for immobilized hemoglobin and immobilized deoxyhemoglobin were obtained using a uv spectrophotometer. 0.1 Molar calcium chloride was taken as the reference and suspensions of oxyhemoglobin and deoxyhemoglobin were taken as sample 1 and sample 2. The absorption maxima for immobilized oxyhemoglobin and deoxyhemoglobin were found to be 405nm and 430nm respectively. Figure 5 shows the absorption spectra.
Wave length (nanometers)

A-Absorption peak for hemoglobin at 405 nm.
B-Absorption peak for deoxyhemoglobin at 435 nm.

0.1 Molar calcium chloride solution is taken as reference.
3.6 IMMOBILIZATION OF DEOXYHEMOGLOBIN ON THE COMMON END OF THE BIFURCATED FIBER:

For immobilization of deoxyhemoglobin on the common end of the bifurcated fiber, the steps involved are:

(1) About 0.5 gms of hemoglobin is mixed with 0.1 gms of sodium dithionite and the mixture dissolved in 10ml of alginate gel.

(2) The common end of the bifurcated fiber is immersed into this mixture and quickly transferred into calcium chloride solution.

(3) The thickness of the immobilized layer is measured using a micrometer.

(4) The immobilized layer at the common end is enclosed in the air equilibrated environment of the chamber.

(5) About 1ml of concentrated deoxyhemoglobin gel is diluted with 1ml, 2ml, 5ml, 10ml, 15ml, .. 30ml of clear gel successively, then immobilized and the reflected intensities obtained.

(6) Next the amount of dilution of deoxyhemoglobin with alginate gel is maintained constant (1:10) and the thickness of the immobilized layer of deoxyhemoglobin varied by repeated coating of the gel followed by fixing in calcium chloride solution and the experiment repeated.
3.7 THE DETECTOR—THE PHOTOMULTIPLIER TUBE:

The photomultiplier, one of the most common optical detectors, is used to measure radiation in the near ultraviolet, visible, and near infrared regions of the spectrum. Because of its inherent high current amplification and low noise, the photomultiplier is one of the most sensitive instruments devised—which involves long integration time, cooling of the photocathode, and pulse height discrimination—and has been used to detect power levels as low as $10^{-19}$ watts [35]. A detailed description of the photomultiplier tube and its principle of operation is given in Appendix D.
CHAPTER IV

RESULTS AND DISCUSSION

The sensor developed in this thesis consists of a thin layer of immobilized deoxyhemoglobin on the common end of the bifurcated cable. An O$_2$ permeable membrane (High sense from Yellow Springs Instruments, Ohio) separates the immobilized layer from the sample. The entire setup is enclosed in an oxygen free environment. The output from a tungsten-halogen lamp (150W) is passed through an interference filter, with peak transmittance at 405nm, and guided through one arm of the bifurcated fiber to the immobilized layer of Deoxyhemoglobin. Upon association with O$_2$, the deoxyhemoglobin gets converted to oxyhemoglobin and yields reflected intensity which is measured using a photomultiplier tube (Oriel Co.) and an optical power meter. The above procedure is repeated for 435nm filter and the reflected intensity is measured. The ratio of the reflected intensities $I_{435} / I_{405}$ gives a measure of the concentration of the sample. Temperature, pressure, thickness of the layer and all other parameters are maintained constant. The entire experi-
mental setup is covered with a black cloth to prevent the interference of any ambient light with that of the lamp.

The optical fiber used is a single mode glass fiber (Oriel) with NA=.55, bundle diameter=4.5mm and l=915mm. Non-magnetic interlocking stainless sheathing is used as a shield for the cable.

There are certain precautions to be observed carefully for proper recording of the data. They are:

(1) The bifurcated fiber end should be stored in an oxygen free environment.

(2) The samples should be introduced with the help of a syringe otherwise the cell is sealed to prevent any ambient oxygen from interfering with the sample.

(3) The useful lifetime of the immobilized deoxyhemoglobin is very short unless stored in a refrigerator in which case it can be used for a week.

(4) The response time to reach steady state is a function of the temperature. So all the readings should be taken at a constant temperature.

(5) The bifurcated fiber should not be bent, otherwise there will be a loss in the cable.

(6) The experiment should be done on a vibrationless table.

(7) The high-voltage power supply used to operate the PMT should be connected very carefully.
(8) The interference of the ambient light should be minimized to the maximum extent.

(9) There should be a very effective cooling system for the tungsten-halogen lamp.

4.1 REFLECTED INTENSITIES AT 435 AND 405NM FOR DIFFERENT OXYGEN CONCENTRATIONS:

As mentioned above, with all the precautions in mind the experiment was performed using a 435nm filter for 0.5mm thick immobilized deoxyhemoglobin layer. A recirculation system was used to make standard oxygen samples. The values are tabulated in Tables I, II, and III.
Table I: Reflected intensity vs oxygen concentration at 435nm with $O_2$ permeable membrane for 0.5 mm thick immobilized layer.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Oxygen concentration in percent</th>
<th>Reflected intensity in terms of power (nw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>8.6</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>8.7</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>8.9</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>9.0</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>9.2</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>9.4</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>9.5</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>9.8</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>10.1</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>10.6</td>
</tr>
</tbody>
</table>
Table II: Reflected intensity vs oxygen concentrations at 405nm with O₂ permeable membrane for 0.5 mm thick immobilized layer.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Concentration of oxygen in percentage</th>
<th>Reflected intensity in terms of power(nw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>5.9</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>5.3</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>5.1</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>5.0</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>4.8</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>4.6</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>4.5</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>4.2</td>
</tr>
</tbody>
</table>
Table III: Ratio of reflected intensities vs oxygen concentration with \( o_2 \) permeable membrane for 0.5 mm thick immobilized layer.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Oxygen concentration in percentage</th>
<th>Reflected intensity in terms of power (nw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1.45</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>1.52</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>1.61</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>1.69</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>1.73</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>1.88</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>1.97</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>2.13</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>2.24</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>2.52</td>
</tr>
</tbody>
</table>

The graph showing the concentration vs the ratio of reflected intensity is depicted in Figure 6.
Figure 6. Ratio of reflected intensities vs. oxygen concentration.

Reflected intensity at 435nm.
Reflected intensity at 405nm.

Ratio of intensities.

Concentration of oxygen (percentage).

Reflected intensity in terms of power (mw).
The electronic circuits that were used to convert the output from the photomultiplier tube are shown in Figures 7 and 8.

In the photon counting circuit that was built, the circuit begins with an inverting input amplifier (Transistors 6,9) with charge feedback via capacitor 2 and resistor,4. The input follower presents a low driving input impedance to transistor7,(which provides the voltage gain) to reduce the effects of feedback capacitance. The follower at the output of the gain block, transistor 9, provides a low output impedance while allowing transistor 7, to have a reasonable amount of gain. The signal at this point is a small positive pulse corresponding to the negative charge input from the PM tube. A DC feedback stabilizes transistor 9's output at about 2 \( V_{be} \). Transistor 15 is biased as a class A emitter follower, giving a low impedance monitor output of the amplified photomultiplier pulses before discrimination.

Differential amplifier pair (20) forms the discriminator. The threshold is set by Resistor (26) referenced to a voltage that tracks the input amplifier's 2 \( V_{be} \) quiescent point. This diode-drop (Transistor 25 operating as an adjustable diode.) tracking occurs because the transistors 9-25 are in a monolithic transistor array and are all at the same temperature. Transistor
29 forms a cascode with transistor 20 both for high speed and level shifting. Two stages of follower, arranged with opposite polarity transistors to cancel $V_{be}$, offsets complete the circuit.
Table IV. Component values for the amplifier circuit in figure 7.

<table>
<thead>
<tr>
<th>Number</th>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1N5711—Diode</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Capacitor</td>
<td>1pF</td>
</tr>
<tr>
<td>3</td>
<td>NPN Transistor</td>
<td>1.2k</td>
</tr>
<tr>
<td>4</td>
<td>Resistor</td>
<td>10k</td>
</tr>
<tr>
<td>5</td>
<td>NPN Transistor</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Resistor</td>
<td>0.027k</td>
</tr>
<tr>
<td>7</td>
<td>Resistor</td>
<td>0.051k</td>
</tr>
<tr>
<td>8</td>
<td>Load</td>
<td>0.1k</td>
</tr>
<tr>
<td>9</td>
<td>Load</td>
<td>0.050k</td>
</tr>
<tr>
<td>10</td>
<td>Capacitor</td>
<td>0.01µF</td>
</tr>
<tr>
<td>11</td>
<td>Resistor</td>
<td>0.022k</td>
</tr>
<tr>
<td>12</td>
<td>Resistor</td>
<td>0.036k</td>
</tr>
<tr>
<td>13</td>
<td>Resistor</td>
<td>0.033k</td>
</tr>
<tr>
<td>14</td>
<td>Resistor</td>
<td>0.56k</td>
</tr>
<tr>
<td>15</td>
<td>2N2369A—NPN Transistor</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Resistor</td>
<td>0.12k</td>
</tr>
<tr>
<td>17</td>
<td>Resistor</td>
<td>0.43k</td>
</tr>
<tr>
<td>18</td>
<td>Signal monitor</td>
<td>BNC</td>
</tr>
<tr>
<td>19</td>
<td>Resistor</td>
<td>0.51k</td>
</tr>
<tr>
<td>20</td>
<td>Differential pair</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>NPN Transistor</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Resistor</td>
<td>0.27k</td>
</tr>
<tr>
<td>23</td>
<td>Resistor</td>
<td>0.22k</td>
</tr>
<tr>
<td>24</td>
<td>NPN Transistor</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Capacitor</td>
<td>0.01µF</td>
</tr>
<tr>
<td>26</td>
<td>Resistor</td>
<td>0.1k</td>
</tr>
<tr>
<td>27</td>
<td>Resistor</td>
<td>0.13k</td>
</tr>
<tr>
<td>28</td>
<td>Resistor</td>
<td>0.33k</td>
</tr>
<tr>
<td>29</td>
<td>PNP Transistor</td>
<td>2N4313</td>
</tr>
<tr>
<td>30</td>
<td>Resistor</td>
<td>0.12k</td>
</tr>
<tr>
<td>31</td>
<td>Resistor</td>
<td>0.16k</td>
</tr>
<tr>
<td>32</td>
<td>Capacitors</td>
<td>0.01µF</td>
</tr>
<tr>
<td>33</td>
<td>R</td>
<td>0.2k</td>
</tr>
<tr>
<td>34</td>
<td>NPN Transistor</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>PNP Transistor</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Resistor</td>
<td>1k</td>
</tr>
<tr>
<td>37</td>
<td>R</td>
<td>0.027k</td>
</tr>
<tr>
<td>38</td>
<td>R</td>
<td>0.051k</td>
</tr>
<tr>
<td>39</td>
<td>Load</td>
<td>0.1k</td>
</tr>
<tr>
<td>40</td>
<td>Load</td>
<td>0.050k</td>
</tr>
<tr>
<td>41</td>
<td>C</td>
<td>0.01µF</td>
</tr>
<tr>
<td><strong>PM Input</strong></td>
<td><strong>7.5-15v</strong></td>
<td></td>
</tr>
</tbody>
</table>
Figure 7. High speed charge sensitive amplifier for PM tube.
Some interesting facts about this circuit are:

(1) The transistor quiescent currents are set rather high to get better high speed performance.

(2) The cascode base (29) is bypassed to $V_+$ and not to ground as its input signal is referenced via resistor (31).

(3) The comparator's current source is a current mirror, convenient since transistor(25) is already used for threshold reference.

(4) Diodes are used to improve overload performance.
Figure 8. Electronic interfacing circuit for PM tube.

\[ R=100k \]
\[ C=1 \text{ microF} \]

* From PM tube.
4.2 EFFECT OF DEOXYHEMOGLOBIN LOADING ON THE REFLECTED INTENSITY:

The above mentioned immobilization procedure was repeated for immobilization of deoxyhemoglobin.

About 1 ml of the deoxyhemoglobin-alginate gel mixture was mixed with 1 ml of alginate gel and the combination was immobilized onto the common end of the bifurcated cable. After several washes with calcium chloride solution, the reflected intensity is measured at atmospheric conditions.

The same procedure is repeated for various quantities of alginate gel. Results for these measurements are presented in Table V and Figure 9.
Table V: Effect of deoxyhemoglobin loading on reflected intensity with $O_2$ permeable membrane.

<table>
<thead>
<tr>
<th>Sample.no</th>
<th>Amount of gel for dilution</th>
<th>Reflected intensity in microamperes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1 ml</td>
<td>0.06</td>
</tr>
<tr>
<td>2.</td>
<td>2 ml</td>
<td>0.14</td>
</tr>
<tr>
<td>3.</td>
<td>5 ml</td>
<td>0.31</td>
</tr>
<tr>
<td>4.</td>
<td>10 ml</td>
<td>0.62</td>
</tr>
<tr>
<td>5.</td>
<td>15 ml</td>
<td>0.95</td>
</tr>
<tr>
<td>6.</td>
<td>20 ml</td>
<td>1.24</td>
</tr>
<tr>
<td>7.</td>
<td>25 ml</td>
<td>1.56</td>
</tr>
<tr>
<td>8.</td>
<td>30 ml</td>
<td>1.84</td>
</tr>
</tbody>
</table>
Figure 9. Effect of hemoglobin loading on the
Reflected Intensity (atm oxygen pressure)

Thickness of the layer = 0.5mm.

Reflectance in terms of photocurrent (micro amps)

Amount of deoxyhemoglobin (ml) for 5ml of Gel.
It is seen that the reflected intensity increases as the amount of deoxyhemoglobin decreases at atmospheric conditions for a given thickness of the immobilized layer.

4.3 EFFECT OF THICKNESS OF THE IMMobilIZED DEOXYHEMOGLOBIN LAYER ON THE REFLECTED INTENSITY:

The above mentioned immobilization procedure was repeated for immobilization of deoxyhemoglobin. About 10 ml of alginate gel was added to 1 ml of the above mixture, and this constant dilution was maintained.

The thickness of the immobilized layer was varied at atmospheric conditions, and the reflected intensity measured in terms of a photocurrent.

A micrometer was used to measure precisely the thickness of the immobilized layer. The values are tabulated in Table V.
Table V: Effect of thickness on reflected intensity with the oxygen permeable membrane.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Thickness of the layer</th>
<th>Reflected intensity in terms of microamperes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.3mm</td>
<td>0.94</td>
</tr>
<tr>
<td>2.</td>
<td>0.6mm</td>
<td>0.72</td>
</tr>
<tr>
<td>3.</td>
<td>0.9mm</td>
<td>0.52</td>
</tr>
<tr>
<td>4.</td>
<td>1.2mm</td>
<td>0.36</td>
</tr>
<tr>
<td>5.</td>
<td>1.5mm</td>
<td>0.14</td>
</tr>
<tr>
<td>6.</td>
<td>1.8mm</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The graph showing the variation of the reflected intensity with the thickness of the immobilized layer is depicted in Figure 10.
Figure 10. Effect of thickness of the immobilized layer on the reflected intensity under atm. oxygen conditions.

Loading of deoxyhemoglobin is maintained constant.
Ratio of deoxyhemoglobin to alginate gel = 1:1

Thickness of the immobilized layer in mm.
It is seen that the reflected intensity decreased as the thickness of the immobilized layer increased.

4.4 EFFECT OF THICKNESS OF THE IMMOBILIZED LAYER ON THE RESPONSE TIME:

Response time is an important parameter which is proportional to the time required for attainment of equilibrium when oxygen comes in contact with deoxyhemoglobin. There is a mass transfer step involved for the reaction between oxygen and deoxyhemoglobin. That is, it takes time for oxygen to diffuse into the and through the gel, and to react with deoxyhemoglobin.

The above mentioned procedure was repeated to immobilize a layer of deoxyhemoglobin on to the common end of the bifurcated fiber. The thickness of the immobilized layer is measured using a micrometer.

Response time was recorded for different values of layer thickness at room temperature and atmospheric pressure. The values are tabulated in Table VII.
Table VII: Effect of thickness of the immobilized layer on the response time with the oxygen membrane.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Thickness of the layer</th>
<th>Response time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3 mm</td>
<td>1 min 30 sec</td>
</tr>
<tr>
<td>2</td>
<td>0.6 mm</td>
<td>3 min 48 sec</td>
</tr>
<tr>
<td>3</td>
<td>0.9 mm</td>
<td>5 min 44 sec</td>
</tr>
<tr>
<td>4</td>
<td>1.2 mm</td>
<td>7 min 28 sec</td>
</tr>
<tr>
<td>5</td>
<td>1.5 mm</td>
<td>9 min 41 sec</td>
</tr>
<tr>
<td>6</td>
<td>1.8 mm</td>
<td>11 min 3 sec</td>
</tr>
</tbody>
</table>

Figure 11 shows the effect of thickness on the response time.
Figure 11. Effect of thickness of the immobilized layer on the response time.

Loading of deoxyhemoglobin is maintained constant at room temperature and atmospheric oxygen conditions. Ratio of deoxyhemoglobin to gel = 1:10.
4.5 RECIRCULATION SYSTEM:

This technique is used to make standard oxygen samples with different concentrations. Figure 12 depicts the configuration of the system. Oxygen is constantly bubbled into the container(1) that has 100 ml of water. This is circulated to chambers (2,3). Chamber 2 has a Clarke oxygen electrode that is connected to a strip chart recorder and the common end of the bifurcated fiber (with a layer of immobilized hemoglobin) is inserted into chamber 3. Recirculation is maintained using a peristaltic pump. Oxygen is bubbled until saturation occurs and then the supply of oxygen is cut off. The saturation point is taken as maximum concentration. The intensity values are recorded at saturation point and at subsequent values of oxygen concentrations. The same procedure is repeated for both filters.
Figure 12. Recirculation calibration system.

1, 2, 3—chambers.
4—Recorder
5—Clark electrode
6—Bifurcated fiber
7—oxygen supply.
4.6 SOLUBILITY OF OXYGEN IN WATER:

In order to calculate the amount of pure oxygen dissolved in 100 c.c of water at room temperature, a graph is plotted showing the solubility of pure oxygen at different temperatures based on theoretical data. Figure 13 depicts the graph. The amount of oxygen that dissolved in water at room temperature was calculated to be 1071 nmoles / ml. The data is taken from Chemical Engineering Handbook by Perry and Chilton.

Treating 1071 nmoles/ml as the saturation value corresponding to mole fraction of 1, the solubility of oxygen at different mole fractions is computed. Table VIII gives the values and Figure 14 depicts the solubility curve.
Figure 13. Solubility of oxygen at different temperatures.

200 nmoles of oxygen /ml of air or 1071 nmoles of oxygen /ml of pure oxygen at room temperature.
TABLE VIII: Solubility of oxygen at different mole fractions.

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Mole fraction</th>
<th>Solubility nmoles/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>107.1</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>214.2</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>321.3</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>428.4</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>535.5</td>
</tr>
<tr>
<td>6</td>
<td>0.6</td>
<td>642.6</td>
</tr>
<tr>
<td>7</td>
<td>0.7</td>
<td>749.7</td>
</tr>
<tr>
<td>8</td>
<td>0.8</td>
<td>856.8</td>
</tr>
<tr>
<td>9</td>
<td>0.9</td>
<td>963.9</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>1071.0</td>
</tr>
</tbody>
</table>

4.8 SOLUBILITY OF OXYGEN AT DIFFERENT OXYGEN CONCENTRATIONS:

The reflected intensities for 405nm and 435nm filters are obtained for oxygen samples whose concentration is expressed as percentage. Due to the nonlinear characteristics of the oxygen
electrode, it is best to express the oxygen concentration in terms of nmoles/ml.

The solubility of oxygen at different oxygen concentrations (expressed as percentage) is obtained using hydrogen peroxide method. This system contains catalase, which converts hydrogen peroxide to oxygen and water.

Step 1. Bubble in nitrogen to remove any oxygen from the sample. In this case 100ml of water was taken.

Step 2. Then add 5 ml of 3% hydrogen peroxide and observe the increase in the slope of the graph on the strip chart recorder.

Step 3. Note the time taken for oxygen to be consumed (slope is almost zero).

Step 4. Once again add 5ml of peroxide and repeat step 3.

Step 5. Repeat steps 2 and 3 until there is no change in the slope of the graph for any further addition of peroxide.

It is seen from the chart recorder graph that the curve is non-linear. So all the peaks and their corresponding concentration values are plotted to get a best fit equation that can be used to get the solubility values at different concentration values like 10%, 20%, 30% and so on.

The X-axis of chart recorder from 20 to 80 corresponds to 0-100 percent concentration.
The Y-axis from 0 to 871 nmoles/ml corresponds to solubility.

Table IX gives the peak values and their corresponding concentrations

Table IX: Concentration vs solubility values:

<table>
<thead>
<tr>
<th>S.no</th>
<th>Concentration in percent</th>
<th>Solubility peak nmoles/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>174</td>
</tr>
<tr>
<td>4</td>
<td>44.3</td>
<td>261</td>
</tr>
<tr>
<td>5</td>
<td>55.9</td>
<td>348</td>
</tr>
<tr>
<td>6</td>
<td>68.2</td>
<td>435</td>
</tr>
<tr>
<td>7</td>
<td>72.3</td>
<td>522</td>
</tr>
<tr>
<td>8</td>
<td>75.2</td>
<td>609</td>
</tr>
<tr>
<td>9</td>
<td>81.3</td>
<td>696</td>
</tr>
<tr>
<td>10</td>
<td>87</td>
<td>783</td>
</tr>
</tbody>
</table>

Figure 15 depicts the graph. The best fit equation was obtained to be $Y = (X^{**1.11}) \times 4.3185$. 
The solubility of oxygen at various concentrations (10%, 20%, 30% and so on) is computed and the values are tabulated. Table IX gives the oxygen concentration values and their corresponding solubility values.
This plot is based on theoretical data.
The solubility of oxygen at mole fraction of 1 is 1071 nmoles/ml.
Figure 15. Solubility of oxygen at different concentrations.
(The peak values corresponding to points where peroxide was added are considered.)

The linear plot corresponds to theoretical values.
The non linear plot corresponds peroxide method.

The best fit equation for the plot is found to be $Y = (X^{1.11}) \times 4.3185$
Table X: Solubility vs concentration.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Concentration in percent</th>
<th>Solubility in nmoles/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>55.6</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>120.08</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>189.2</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>259.2</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>332.03</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>406.5</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>482.3</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>559.45</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>637.9</td>
</tr>
</tbody>
</table>

Now the reflected intensity values that were plotted for oxygen concentrations expressed in percentage can be related to oxygen concentrations expressed in mole fraction values using graphs 15 and 6.
4.9 EFFECT OF THICKNESS ON THE RESPONSE TIME WITHOUT THE SEMI-PERMEABLE MEMBRANE:

In Section 4.4 response times were recorded for different values of thickness of the immobilized layer. A layer of semi-permeable membrane was separating the immobilized layer from the standard oxygen sample. Here the same experiment is repeated without the membrane, as the immobilized deoxyhemoglobin-gel layer can itself act as a permeable membrane.

TABLE XI: Effect of thickness on the response time without the membrane:

<table>
<thead>
<tr>
<th>S.no</th>
<th>Thickness of the layer</th>
<th>Response time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
<td>56 sec</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>2 min 12sec</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>3 min 53sec</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>5 min 41sec</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
<td>7 min 22sec</td>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>9 min 6sec</td>
</tr>
</tbody>
</table>
The effect of thickness on the response time is shown in Figure 16. It is seen that the response time is less for the same thickness values without the membrane.
Figure 16. Effect of thickness of the layer on Response time (without membrane).

Loading of deoxyhemoglobin is maintained constant at room temperature and atmospheric oxygen conditions. Ratio of deoxyhemoglobin to gel 1:10. The gel-deoxyhemoglobin layer acts as membrane.
CONCLUSIONS AND RECOMMENDATIONS

The developed oxygen sensor has been found to be effective as long as the immobilized deoxyhemoglobin is active. Generally hemoglobin gets converted to methemoglobin and renders itself unstable. The stability of hemoglobin is the main drawback, with good precaution the immobilized hemoglobin can last for a week if stored in a refrigerator.

Other studies [3] have shown that immobilization on a cation exchanger resulted in a shift in the Soret absorption band towards a longer wavelength where in the peaks for immobilized hemoglobin and deoxyhemoglobin were found to be less than 405 nm and 435 nm respectively. The alginate-gel technique circumvents this problem. Being a very simple but effective technique, this method can also help in continuous use of the sensor.
Other methods of immobilization can be tried for longer lifetime. Crosslinking the hemoglobin with butyrylaldehyde is also recommended.

From Table V it can be seen that it takes about 4 min to reach equilibrium with 0.6mm thick layer of immobilized hemoglobin with membrane at room temperature. This long duration is due to the mass transfer of oxygen into the immobilized hemoglobin phase. Fig 9 gives the effect of thickness on the response time and it is seen that the response time is inversely proportional to the thickness of the immobilized layer. Effect of temperature on sensing should also be studied.

It is seen that the reflected intensity increases with the concentration of oxygen in the sample for 435nm filter and decreased for 405nm filter. The plot of the ratio of the reflected intensities to the concentration is shown in Figure 6. Because it is a ratio measurement, it is insensitive to source fluctuations, drift and slow loss of reagent.

The circuits tried for converting the output of PM tube are adequate but a pre-amp version is highly recommended.

For making the standard samples, the recirculation technique is used. Here, the standard oxygen electrode was used to
measure the oxygen concentration. Proper calibration has to be done as the response of the probe is non-linear. But a better way would be to use a gas proportioner to get these standard oxygen samples.

One of the best advantages of this sensor is that it is based on a spectral shift. Because both hemoglobin and deoxyhemoglobin have distinct spectra, there is more information available when compared to other sensors.

The effect of other gases interfering with the properties of hemoglobin would be worth observing. From the properties of hemoglobin it can be stated that all the gases that interfere in oxidation would be potential danger to the sensitivity of the sensor, like carbon monoxide.

The accuracy of the sensor mainly depends on the life of the immobilized layer. Precautions should be taken to prevent the conversion of hemoglobin to methemoglobin. Temperature also plays a very important role in determining the accuracy. All the measurements were recorded at room temperature.

The sensor can be used repeatedly as long as the immobilized layer is alive. The layer is washed with calcium chloride solution before reuse. Also, the thickness of the layer has to be the same.

The entire experimental set-up is bulky with the transformer, (for the lamp) high voltage power supply and other com-
ponents. With miniaturization, production of the sensor won a commercial scale would be easier.
APPENDIX A

PRINCIPLE OF OPERATION OF INTERFERENCE FILTERS:

Interference filters operate through a complex process of constructive and destructive interference to select precisely an often very narrow range of wavelengths with a minimum transmission efficiency. They consist of a suitable base substrate on to which carefully controlled, varying thicknesses and type of chemical layers are vacuum deposited in such a fashion that the transmission of certain wavelengths is enhanced while other wavelengths are either reflected or absorbed.

In general two basic types exist:

(1) Band pass filters which transmit light only within a defined spectral band ranging from less than one to many nanometers wide.

(2) Edge filters which transmit only above or below a certain "cut-on" or "cut-off" wavelength, and continue to transmit efficiently throughout that range until reaching the transmission limits of the substrate material. Some of the terms involved are defined as follows.
Peak wavelength-- The wavelength of maximum transmission.
Band width------- The spectral width of the transmission band at half power points.
Blocking-------- The degree of rejection at wavelengths outside the band pass.

In its simplest form, a multi layer band pass filter can be best described as a Fabry-Perot cavity consisting of two partially reflective metallic layers separated by a transparent dielectric space layer.

The thickness of the dielectric determines the peak transmission wavelength for the filter. Whenever the spacer layer thickness equals an integer multiple of one half the wavelength of light in the beam, constructive interference between the multiple order reflections from the two metallic layers allows that wavelength to transmit with high efficiency. All other wavelengths present interfere destructively with the cavity and are severely attenuated.
APPENDIX B

TYPES OF REVERSIBLE SENSORS:

Most reversible sensors involve an equilibrium between analyte and immobilized reagent. If the stoichiometry of a reaction is 1:1, then the reaction may be represented by

\[ A + R = AR \]

where \( A \) is the analyte, \( R \) is the reagent and \( AR \) is the combined analyte-reagent. The equilibrium constant \( k \) may be represented by \( k = AR/R[A] \), where \([A]\) is analyte concentration and \( R \) and \( AR \) are the number of free and combined reagent molecules in the immobilized phase, respectively. It is assumed that activity effects in the immobilized phase are equivalent for \( R \) and \( AR \) and thus cancel. \( AR \) and \( R \) vary with analyte concentration as follows:

\[ AR = K[A]C(r)/(1+K[A]) \]
\[ R = C(r)/(1+k[A]) \]

where \( C(r) \) is the sum of free and combined reagent molecules. Here, there are two cases in which the measured optical parameter is proportional to \( AR \) and proportional to \( R \). In these cases it is assumed that the amount of analyte combining with reagent is small relative to the amount of analyte in the sample, a condition that can be achieved by keeping the amount of reagent small.
If this condition is not achieved, the analyte will be depleted in the sample. The response time has to be revised to account for this, and the device is not a true sensor since it is perturbing the sample. The importance of the equilibrium constant in determining the sensor response is stated above. The equilibrium constant is involved in the response function whether one measures R, AR or AR/R ratio.

The conditions for optical sensing thus differ from the common analytical situation where large equilibrium constants and reagent excesses are desired to drive an analytical reaction to completion. If that were the case with a sensor, essentially all the analyte would be extracted into the reagent until the point where the reagent phase was saturated. The device would not function as a reversible sensor.

Because the response depends on the equilibrium constant, optical sensors will measure only the concentrations of analytes in a form available to interact with reagent. For example an optical metal ion sensor based on immobilized ligand will measure free metal ion rather than the total metal.

COMPETITIVE-BINDING SENSORS:

A competitive-binding-based sensor includes an immobilized reagent R, that specifically binds the analyte A. The reagent phase also includes a ligand L, which competes with A for binding sites on R. The reactions and associated equilibria are
assuming both L and R are in solution. This can be achieved if L and R are larger molecules than A such that R and L can be confined by a dialysis membrane that allows analyte A to transfer freely between phases. An essential feature of L is that its optical properties change in a measurable way upon binding to R. The detected parameter is based on L, either [L] or [LR].

In the case of the sensor being designed the immobilized reagent is the layer of deoxyhemoglobin and the analyte is oxygen gas which upon association with deoxyhemoglobin forms hemoglobin and changes the reflected parameter.

Competitive-binding-based sensors make it possible to use analytical reactions that don't directly produce an optical change. The use of antibodies as the specific reagent would allow more possible sensors.

**ABSORBANCE-BASED SENSORS:**

The first reversible optical sensor described was a pH sensor based on the absorption of phenol red covalently bound to polyacrylamide microspheres. Radiation from a tungsten-halogen lamp is directed onto the reagent through one fiber. Radiation scattered back is detected through the other fiber using a filter.
for wavelength selection. The plug at the end of the fiber keeps incident radiation from interacting with the sample [16].

In the usual absorbance measurement, the reference intensity is measured at the same wavelength as the intensity transmitted by the sample. However, in the sensor, it is necessary to measure a reference intensity at a different wavelength where neither the acid nor the base form of the indicator is absorbing. This intensity is proportional to the reference intensity at the absorption wavelength. The proportionality constant must be accounted for in the calibration procedure. The resulting probe is small enough to be used for in vivo measurements. The response time for optical sensors is limited by diffusion of analyte into the reagent.

**FLUORESCENCE SENSORS:**

Fluorescence is particularly well suited for optical sensing. It is a single optic measurement because the detected radiation can be distinguished from probe radiation by a change in-wavelength. In addition fluorescence is an inherently sensitive technique capable of measuring low analyte concentrations. At low fluorophor levels response to analyte is linear. However, in sensors it may often prove more practical to work at reagent levels where probe radiation is absorbed to a significant extent in the reagent phase. If the absorbance changes with analyte
concentration, the response may be affected by the inner filter effect. The geometry of a fluorescence-based sensor corresponds to front surface detection.

The simplest type of fluorescence sensor involves measuring fluorescence at a single wavelength [2][7]. An example of this would be a pH sensor based on fluoresceinamine covalently coupled to cellulose. Here pH is related to an increase in intensity as the acid form of immobilized dye is converted to base. This also illustrates one of the difficulties that can be encountered in preparing fluorescence sensors. Concentration quenching occurs because the immobilized molecules are too close together, causing the intensity from the immobilized reagent to be relatively weak.

**TWO-WAVELENGTH FLUORESCENCE MEASUREMENTS:**

A pH sensor based on fluorescence illustrating two wavelength measurements has been designed. The system involves the trisodium salt of 8-hydroxypyrene-1,3,6 trisulfonic acid (HOPSA) immobilized electrostatically on an anion exchange membrane. Electronically excited HOPSA undergoes rapid excited-state deprotonation so that HOPSA⁻ fluorescence is observed between pH 1 and 7 even though HOPSA is the dominant ground state species. The measured parameter in this sensor is the ratio of fluorescence intensity emitted at 510nm and excited at 470nm (specific for base) to intensity excited at 405nm (selective for acid). The
ratio measurement is insensitive to source fluctuations, drift, temperature, quenching, ionic strength and slow loss of reagent. The same ratio measurement technique is used in the sensor design to offset the effects of source fluctuations, loss of reagent, etc. Other approaches to ratio measurements are used in pH sensors based on fluorescence.

**FLUORESCENCE QUENCHING:**

Sensors also can be based on a decrease in reagent phase fluorescence upon association with analyte. This is inherently less desirable than systems involving increases in intensity with analyte concentration, it allows access to analytes that could not otherwise be sensed by fluorescence.

An example of a sensor based on quenching is the oxygen probe developed by Peterson et al, [19][9]. Here the reagent phase is a dye, perylene dibutyrate, adsorbed on a polymeric support, and the membrane containing the immobilized reagent is porous polypropylene, which is hydrophobic and highly permeable to oxygen. Fluorescence is excited through one fiber and observed through the other. Filters are used to measure separately the green fluorescence of perylene dibutyrate and scattered blue excitation radiation. The intensity of fluorescence ratio to the intensity of scattered radiation will compensate for changes in source intensity. The sensor is particularly attractive because
it is reversible. Unlike the widely used electrode, it does not require continuous mass transfer of oxygen to maintain a constant signal.

The oxygen sensor is based on dynamic quenching, i.e., an excited-state interaction with the fluorophor. One of the reasons pyrene dibutyrate was selected as the dye for the oxygen sensor was that its quenching constant has the right magnitude to yield sensitive response to the range of oxygen partial pressures of physiological interest. In addition to the oxygen sensors, sensors based on transition metal quenching of ligand fluorescence and iodine quenching of rubrene fluorescence were proposed.

COMPETITIVE-BINDING SENSORS BASED ON FLUORESCENCE:

A fluorescence-based, competitive binding sensor for glucose has been reported [17]. The specific glucose-binding reagent concanavalin A immobilized on sepharose. The competing ligand is dextran labeled with fluorescein.

The optical fiber fits in a hollow cylinder with a plug on the end. Glucose can diffuse freely through the hollow fiber but dextran cannot. The immobilized concavalin A diffuses on to the walls of the fiber and thus out of the volume illuminated by the fiber-optic. Increasing the glucose concentration displaces the labeled dextran from the concavalin A, causing it to be free to
diffuse into the illuminated solution volume. This in turn leads to an increase in fluorescence which is related to glucose concentration.

The sensor is shown in Figure 17.
Figure 17. Competitive binding fluorescence sensor for glucose.

- **P**—Probe radiation.
- **D**—Detected radiation.

**Legend:**
- **F**—Bifurcated cable.
- **C**—Cladding of fiber-optic bundle.
- **L**—Competing ligand (dextran labeled with fluorescein.)
- **R**—Reagent.
- **A**—Concavalin.
  (immobilized on sepharose.)
- **M**—Walls of the hollow fiber.
REFLECTANCE-BASED SENSORS:

When dealing with solid phases, it becomes difficult to measure transmitted light satisfactorily. In these cases the intensity of reflected light may be used as a measure of the color of an immobilized reagent phase. In the context of a sensor, a true reference intensity cannot be measured at the analytical wavelength. Instead, an intensity proportional to the reference intensity must be measured at a wavelength other than the analytical wavelength. This wavelength should be so chosen that it is not absorbed either by reagent or reagent combined with analyte.

Because it is difficult to prepare a sensor that truly measures absorbance, reflectance is likely to be more widely used technique for sensors based on color changes. An attractive feature of reflectance is that it can be used to measure the ratio of free and combined reagent if the analytical reaction is accompanied by a change in color. The functional relationship between the reflectance and concentration will depend on the nature of the reagent, phase, and optical arrangement.

In the optical sensor that is designed at the NJIT biotechnology laboratory, the reflected intensity for deoxyhemoglobin is measured at 435nm and 405nm where 435nm corresponds to the peak observed in the absorption spectrum for deoxyhemoglobin and 405nm
corresponds to the peak in the absorption spectrum for hemoglobin.

Another example of a reflectance-based sensor is an ammonia detector [27]. In this particular device a layer of oxazine dye is coated on the outside of a fiber. Interaction with ammonia changes the color of the dye, changing its reflectance and modifying the intensity of light transmitted to the detector.
NON-REVERSIBLE SENSORS:

It is possible to design sensors based on analytical reactions that consume reagent. Although such sensors necessarily have finite lifetimes, these lifetimes can be very long if the rate of reagent consumption is small relative to the total amount of reagent available. Another disadvantage of nonreversible sensors is that they require a steady-state mass transfer to get a constant signal. Any processes that perturb mass transfer are potential sources of error.

The difference between reversible and non-reversible optical sensors is similar to the difference between potentiometric and amperometric electrodes. Amperometric electrodes, like non-reversible optical sensors, involve a reaction and require mass transfer to the electrode surface.

The use of tetrakis(dimethylaminoethylene) (TMEA) to measure oxygen is an example of nonreversible sensor. The device is illustrated in Figure 18.
Figure 18. Non reversible oxygen sensor.

M--Teflon membrane.
R--Reagent phase.
D--Detection system.

The oxygen diffuses through Teflon membrane into the reagent phase where it reacts to yield chemiluminescence measured by detection system.
Oxygen in the sample diffuses through an oxygen permeable membrane into a reservoir of TMAE. There it reacts with TMAE yielding chemiluminescence which is measured by a light detection system. (No probe radiation is required for this device). In the present case of oxygen sensor designed at NJIT Biotechnology lab, a light source is required because the detected parameter measured is reflectance and not chemiluminescence. Also oxygen diffuses through an oxygen permeable membrane into a small reservoir where it interacts with the immobilized layer of deoxyhemoglobin (at the tip of the bifurcated fiber.)

INSTRUMENTATION:

The instrumentation for fiber optic based sensor can be simple or complex depending on the particular application. Laser excitation may be used for long distance transmission of excitation or to get a useful signal from a small amount of reagent. However, it is also possible to construct devices with incandescent sources that use filters for wavelength selection and phototubes to measure optical response.

Devices may involve either bifurcated or single fiber bundles as shown in Figure 19. A 36 inch long glass bifurcated fiber optic cable with a numerical aperture of 0.55 was used.
Figure 19. Fiber optic bundles.

P—Probe radiation
D—Detected radiation
R—Reagent phase

(a) represents a bifurcated cable where probe and detected radiations are passed through different legs of the cable.

In (c) R (outside) changes the transmission characteristics of fiber
A single fiber or a bundle requires that the detected radiation be distinguished from probe radiation either temporally or by a wavelength to avoid serious background problems due to scattered probe radiation. Alternately, the reagent can be coated on the outside of a single fiber to modify its transmission characteristics. In bifurcated devices, separate fibers transmit probe and detected radiation. These devices observe only the zones of reagent that fall within both the cone of emitted probe radiation and the cone of accepted detected radiation.

The overall diameter of the fiber can be of the order of the wavelength of the transmitted light. In practice sensors in the millimeter range are also being developed.
THE PHOTOMULTIPLIER TUBE

It consists of a photocathode (C) and a series of electrodes called the dynodes, which are labelled 1 through 8. The dynodes are kept at progressively higher potential with respect to the cathode, with a typical potential difference between adjacent dynodes of 100 volts. The last electrode A is used to collect the electrons. The whole assembly is contained within a vacuum envelope in order to reduce the possibility of electronic collisions with the gas molecules.

The photocathode is the most crucial part of the photomultiplier since it converts the incident optical radiation to electronic current and thus determines the wavelength response characteristics of the detector and its limiting sensitivity. The photocathode consists of materials with low work function. Compounds involving Ag-O-Cs and Sb-Cs are often used. These compounds possess work functions as low as 1.5ev as compared to 4.5ev in typical metals.
The quantum efficiency is defined as the number of electrons released per incident photon. The electrons that are emitted from the photo cathode are focussed electrostatically and accelerated towards the first dynode arriving with a kinetic energy of about 100 ev. Secondary emission from the dynode surface causes a multiplication of the initial current. This process repeats itself at each dynode until the initial current emitted by the photocathode is amplified by a very large factor. If the average secondary emission multiplication at each dynode is \( d \) (that is secondary electrons for each incident one) and the number of the dynodes is \( N \), the total current multiplication between the cathode and the anode is

\[
G = d^N.
\]

**NOISE MECHANISMS IN PHOTOMULTIPLIERS:**

The random fluctuations observed in the photomultiplier output\[41\] \[36\] are due to

(1) Cathode shot noise given according to the equation

\[
(i_{\text{N1}}^2) = G^2 \varepsilon (i_c + i_d)^2
\]

where \( i_c \) is the average current emitted by the photocathode due to the signal power which is incident on it. The dark current \( i_d \) is due to the random thermal excitation of electrons from the surface as well as excitation by cosmic rays and radioactive bombardment.
(2) Dynode shot noise is due to random nature of the secondary emission process at the dynodes. Since current originating at a dynode does not exercise the gain of the tube, the contribution of all the dynodes to the total shot noise output is smaller by a factor of $\gamma^{-1}$ than that of the cathode. It generally amounts to a small correction and can be ignored.

(3) Johnson noise is the thermal noise associated with the output resistance $R$ connected across the anode. Its magnitude is given by

$$ \langle i_n^2 \rangle = 4kT \frac{v}{R}. $$

**MINIMUM DETECTABLE POWER IN A PHOTOMULTIPLIER:**

Photomultipliers are used primarily in one of the two ways. In the first the optical wave to be detected is modulated at some low frequency $w_m$ before impinging on the photocathode. The signal consists of an output current oscillating at $w_m$, which has an amplitude proportional to the optical intensity. This mode of operation is known as video or straight detection.

In the second mode of operation, the signal to be detected whose optical frequency is $w_s$ is combined at the photocathode with a much stronger optical wave of frequency $w_s + w$. The output signal is then a current at the offset frequency $w$. This scheme is known as the heterodyne detection.

The optical signal in the case of video detection may be taken as
where the factor \((1 + m \cos w_m t)\) represents the amplitude modulation of the carrier.

The photocurrent then is given by

\[
i_C(t) = [E_s(1+m \cos w_m t)]^2
\]

\[
= E_s^2[(1+m^2/2) + 2m \cos w_m t + m^2/2 \cos 2w_m t]
\]

To determine the proportionality constant, consider the case of \(m=0\). The average photocathode current due to the signal is then

\[
i_C = \frac{Pn}{hv_S}
\]

where \(v_S = w_S/2\), \(P\) is the average optical power, and \(n\), the quantum efficiency, is the average number of electrons emitted from the photocathode per incident photon. This number depends on the photon frequency, the photocathode surface and in practice found to approach 0.3.

\[
i_C(t) = \frac{Pn}{hv_S}[(1+m^2/2) + 2m \cos w_m t + m^2/2 \cos 2w_m t]
\]

The signal output current at \(w_m\) is

\[
i_s = G \frac{Pn}{hv_S} (2m \cos w_m t)
\]

If the output of the detector is limited by filtering to a bandwidth \(v\) centered on \(w_m\), it contains a shot noise current which accordingly has a mean squared amplitude

\[
(i_{n1}^2) = 2G^2E (i_c + i_d) v
\]

where \(i_c\) is the signal average current and \(i_d\) is the dark current.
The signal to noise ratio at the output is thus

\[ \frac{S}{N} = \frac{i_s^2}{(i_{N1}^2) + (i_{N2}^2)} = \frac{2 (Pe_n/hv_s)^2 g^2}{2G^2} (i_c + id) \Delta v + \left(4kT_e \Delta v / R \right) . \]

Due to the large current gain, the first term in the denominator of the above formula which represents amplified cathode noise is much larger than the thermal and amplifier noise term \(4kT_e \Delta v / R\). Neglecting the term \(4kT_e \Delta v / R\), assuming \(id >> ic\) and setting \(S/N = 1\), the minimum detectable power can be obtained as

\[ P_{\text{min}} = hv_s (id \Delta v)^{1/2}/ne^{1/2} . \]
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