The Use Of Fluorescence Quenching To Measure Oxygen Concentration

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ABSTRACT

The method of fluorescence quenching is used to measure the concentration of molecular oxygen. The method is rapid, reversible, and does not consume oxygen. The method may provide the basis for a unique biomedical sensor. The key to developing such a device lies in the choice of a fluorophor/polymer composite matrix having the desired optical and transport properties. Experimental results will be presented for certain parameters essential for assessing device development. The properties of interest include the kinetics of fluorescence quenching, the biomolecular rate constants, the temperature dependence of oxygen solubility and diffusivity in the composite matrix, and the oxygen diffusion coefficient.

Poly(dimethyl siloxane) [PDMS] was chosen as the polymer host because it is biocompatible, hydrophobic, has a high diffusivity for the simple gases, and is easily bonded to fused silica. 9,10-diphenyl anthracene [9,10-D] was selected since it is readily soluble in a number of organic solvents, has an excitation spectrum in the near UV, an emission spectrum in the visible, a long fluorescence lifetime, and a high quantum yield. When incorporated into PDMS, the optical spectra of 9,10-D does not alter appreciably.

The response time of the device is determined by the solution/diffusion kinetics of oxygen in PDMS. The solubility of oxygen in PDMS decreases with increasing temperature and an enthalpy of solution of

$$\Delta H = -3.0 \text{ kcal/mole.} \tag{1}$$

The diffusion of oxygen in PDMS is found to obey an Arrhenius relation over the temperature range of 5°C to 45°C with

$$D = D_o \exp(-E_D/RT) \tag{2}$$

where

$$D_o = 0.115 \text{ cm}^2/\text{s} \tag{3}$$

and

$$E_D = 4.77 \text{ kcal/mole.} \tag{4}$$

Results of these studies indicate that an appropriate device, based on a fluorophor/polymer composite, for the measurement of oxygen concentration should be sensitive over those ranges which are important for physiological monitoring.

INTRODUCTION

Investigations of physical and chemical sensors for the measurement of analytes is currently a very active area of research. Optical techniques are expected to be a very useful approach, especially when the sensor is to be used in a hostile environment, such as the human body. The research reported in this paper was undertaken to develop a material which could be used as an optical sensor for measuring molecular oxygen for biomedical and other applications. One key objective of this study was to characterize important material properties so that selected device parameters could be estimated. The use of fluorescence quenching effects to measure the solubility and diffusivity of oxygen in silicone formed the basis of this work.

Methods for measuring the diffusion coefficient of a solute in a polymer host are most commonly based on independent measurements of permeability and solubility. The diffusion coefficient is then calculated as a ratio of these two quantities. Several of the methods involve changes in the polymer (e.g., index of refraction) that are sensitive to solute concentration and enable the diffusion coefficient to be calculated. The method involved in the present work measures diffusivity by using the quenching of fluorescence to detect oxygen concentration at a given time and location in the polymer. Thus, oxygen is not measured per sé. Rather, what is measured is the effect of oxygen on the fluorescence intensity of a fluorophor embedded in the polymer matrix. The method is based on the fact that oxygen is a voracious quencher of fluorescence, with every encounter between a fluorophor in an excited state and an oxygen molecule resulting in a non-radiative transfer of energy. The method was originally conceived to measure oxygen concentration in a fluid surrounding the composite fluorophor/polymer matrix (e.g., as an oxygen sensor). However, the method can be readily applied to measure the diffusion coefficient of oxygen in the polymer.

In this work we examine the properties of a fluorophor/polymer composite matrix that utilizes fluorescence quenching to measure oxygen concentration. The results not only contribute to a better understanding of the fluorescence mechanisms operating in the composite matrix, but also provide an important basis for development of a unique biomedical oxygen sensor that is rapid, reversible, and does not consume oxygen.

MATERIALS USED

Poly(dimethyl siloxane) [PDMS] was chosen for these studies. PDMS (filled samples) has both a high solubility and a large diffusion coefficient for molecular oxygen[1]. PDMS has no optical absorption bands in the wavelength regions of interest in this study, and numerous fluorophors can be incorporated in it. Fluorophors can be easily added by dissolving in any one of a number of solvents that are soluble in silicone gels. PDMS is hydrophobic and known to be biocompatible, an important consideration for device applications based on this work.

9,10-diphenyl anthracene [9,10-D] was chosen as the fluorophor additive. 9,10-D has excitation bands from 350 to 400 nm, and emission bands from 410 to 430 nm[2,3]. The spectra changes only slightly upon incorporation into the PDMS, because of the small changes in the refractive index of the host. Furthermore, large concentrations of 9,10-D can be incorporated into the PDMS before concentration quenching occurs. The use of high concentrations is desirable because of the increase in optical signal.

The physical entrapment of the 9,10-D in PDMS prior to curing is straightforward. The fluorophor is dissolved in an intermediate solvent, and the solution is stirred into the silicone gel. Once the solvent is evaporated, which may be done under vacuum if desired, the curing agent is added. To avoid entrapped air bubbles, stirring should be avoided, or curing should be performed under vacuum. The sample may be cured in any suitable mold and fibres may be inserted during the curing.

EXPERIMENTAL RESULTS

Fluorescence Effects

For optimum operation of the devices a large fluorescence signal in the absence of oxygen is desired. Figure 1 shows the variation in fluorescence intensity with fluorophor concentration for 9,10-D in benzene and in 1000 cs velocity silicone fluid. As reported previously[3], the fluorescence properties of 9,10-D in 1000 cs viscosity silicone fluid are identical to those of 9,10-D in fully polymerized silicone. The absolute fluorescence intensity of 9,10-D in silicone is slightly larger than in benzene. This figure shows that the fluorescence intensity increases linearly with fluorophor concentration up to log $C_F = -4$. Concentration quenching then occurs and, finally, an inner cell effect is observed (as these experiments were done in quartz cuvettes). Thus, fluorophor concentrations as large as 0.1 g/l [3×10^{-4} M] can be used.

The fluorescence intensity of 9,10-D is not temperature dependent[4]. Thus, all changes in fluorescence intensity at a given oxygen concentration are due to changes in oxygen solubility in the matrix. Figure 2 shows the change in relative fluorescence intensity as a function of the partial pressure of oxygen above the composite matrix at several temperatures. At a given temperature the variation in relative fluorescence intensity with oxygen partial pressure is linear.

By using these data in combination with an assumed solubility[1,5], we are able to construct Figure 3, which shows the relationship between relative fluorescence intensity and oxygen concentration in the composite material. The relation is linear with the slope being a function of fluorophor concentration. At a fixed fluorophor concentration the slope of the line is the product of the rate constant for bimolecular quenching and the fluorescence lifetime of the fluorophor in the matrix. Figure 3 thus provides the calibration curve for the solubility and diffusion experiments. It is worth noting, however, that increasing the fluorophor concentration does increase the slope of this curve. In miniaturized devices it may be possible to increase the fluorophor concentration above the limits used in these experiments, because inner-cell effects may not occur, and multiple reflections could be used to enhance the fluorescence intensity.

In summary, the fluorescence experiments showed that the 9,10-D/silicone composite matrix was a viable material for measuring free molecular oxygen concentration in a fluid above the matrix. The material is sensitive to small changes in oxygen concentration and does not consume oxygen. Over the time of these experiments, no material degradation of PDMS or fluorophor was observed.

Solution/Diffusion Kinetics

The response time to changes in oxygen concentration of any device based on fluorescence quenching in PDMS will be a function of the solution/diffusion kinetics of molecular oxygen in the composite matrix. Calculations from the values plotted in Figure 2 allow the evaluation of the enthalpy of solution of oxygen in PDMS[6]. Over the temperature range evaluated,

$$\Delta H = -3.0 \text{ kcal/mole.} \tag{1}$$

As the temperature of the material increases, the quantity of oxygen contained in the polymer decreases.

The measurement of the diffusion coefficient of oxygen in PDMS was performed using planar geometry[6]. The composite was cured in a quartz cuvette, which was masked so that a thin planar layer was exposed to light. The cuvette was then placed in an environmental chamber where oxygen concentration could be maintained at a constant level for a prolonged time. Initial oxygen concentrations were varied from zero to 760 mm Hg. Temperatures were kept constant during a given experiment, and experiments were performed over the range 5 to 45°C. Figure 4 shows the absolute fluorescence intensity versus time for two temperatures. Using the calibration curve, the concentration was calculated as a function of time. Data were then fitted to a model for planar diffusion in order to calculate the diffusion coefficient.

The results indicate that the diffusion coefficient for oxygen is independent of both oxygen concentration and fluorophor concentration over the pressure and temperature ranges used in these experiments. Figure 5 indicates that the diffusion coefficient is described by an Arrhenius equation

$$D = D_o \exp[-E_D/RT] \tag{2}$$

The pre-exponential term, D_o, and the activation energy, E_D, were found to be 0.115 cm^2/sec and 4.77 kcal/mole, respectively. The relatively low value of E_D occurs because PDMS in this temperature range is a weakly bonded elastomer. The relatively large value of D_o indicates that the fluorescent compound is not occupying a significant number of sites, allowing the oxygen to move freely in the open polymer.

Extrapolating these results to thin planar samples (thickness of the order of 100 microns) indicates that response times of less than 2 seconds are possible. Moreover, the use of different geometrical configurations, e.g., cylindrical or spherical, would result in faster response times.

SUMMARY

A material suitable for measuring oxygen concentration was developed and characterized. The material consists of a silicone [PDMS] host to which a small amount of a fluorophor [9,10-D] was added. Fluorescence quenching effects were used to sense oxygen in this composite material. The relative fluorescence intensity was found to vary linearly with oxygen concentration, with increases in fluorophor concentration increasing the slope of the curve. The slope of the curve is such that devices using this composite material should be sensitive to small changes in oxygen concentration.

Oxygen is highly soluble in the composite material and has a high diffusivity. Thus, devices should be capable of rapid response times, although this also depends on device geometry. The diffusion coefficient is found to be independent of fluorophor concentration, suggesting that interstices in the composite are available for oxygen motion.

The adaptation of this technique to other composite materials which are suitable for the measurement of different analytes should be readily apparent. The physical trapping in a polymeric matrix of a reagent that exhibits a reversible, equilibrium reaction with a given analyte should be straightforward. Hydrophobic and hydrophilic polymers could be used, and heparinized coatings applied as needed. The optical properties of the reagent in the polymer host should not change dramatically, whether one considers absorbance or fluorescence properties. Finally, it is significant to note that polymer-based composite materials are easily fabricated and applied to optical fibres.

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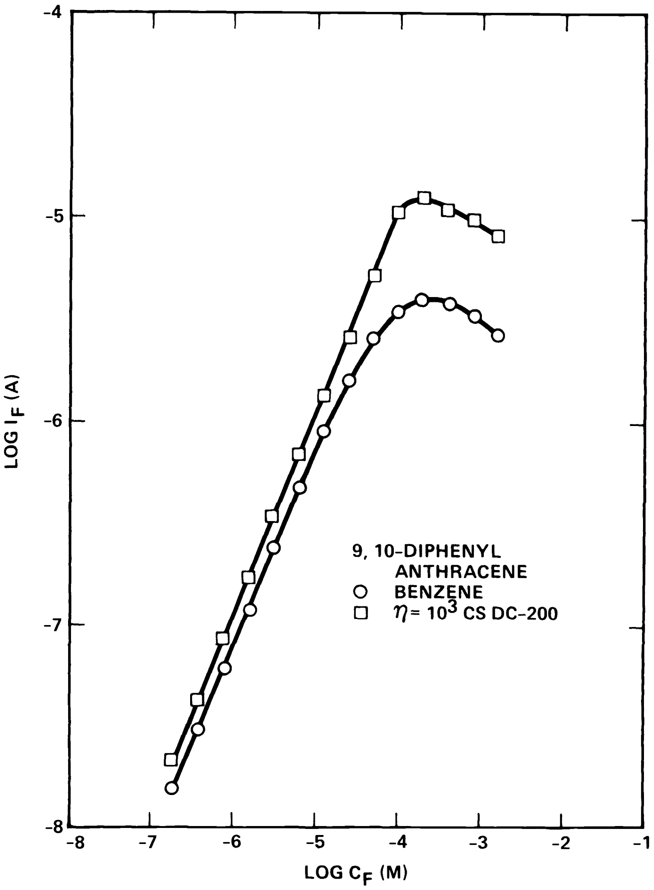

Figure 1. Fluorescence intensity vs. concentration of 9,10-diphenyl anthracene in benzene and $\eta = 10^3$ cs DC-200 silicone fluid.

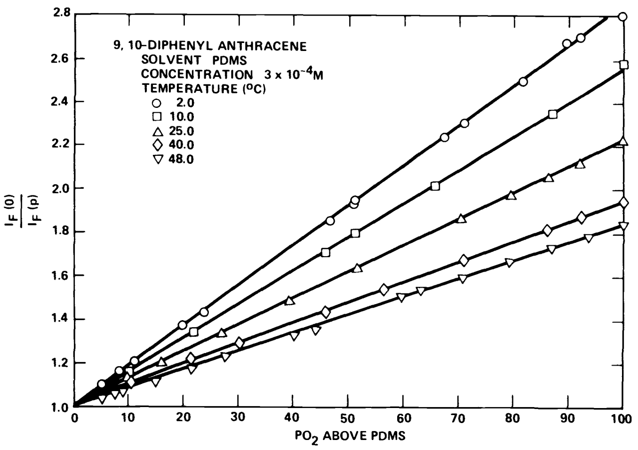

Figure 2. Relative fluorescence intensity vs. partial pressure of oxygen for 9,10-diphenyl anthracene ($C_F = 3 \times 10^{-4}M$) in poly (dimethyl siloxane) at T = 2.0, 10.0, 25.0, 40.0, and 48.0°C.

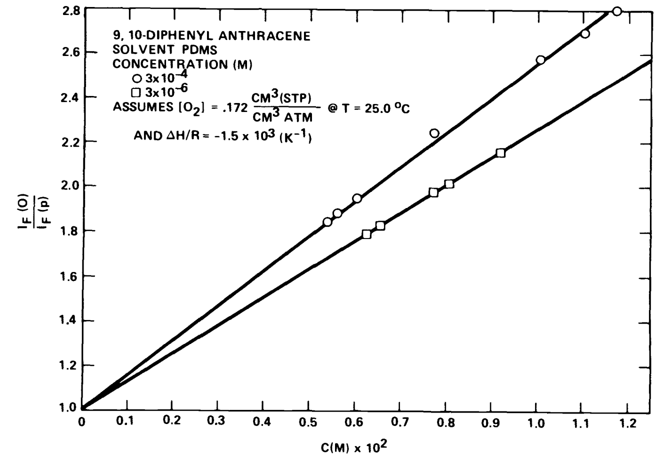

Figure 3. Relative fluorescence intensity vs. calculated absolute oxygen concentration for 9,10-diphenyl anthracene ($C_F = 3 \times 10^{-4}M$) and $3 \times 10^{-6}M$ in poly (dimethyl siloxane).

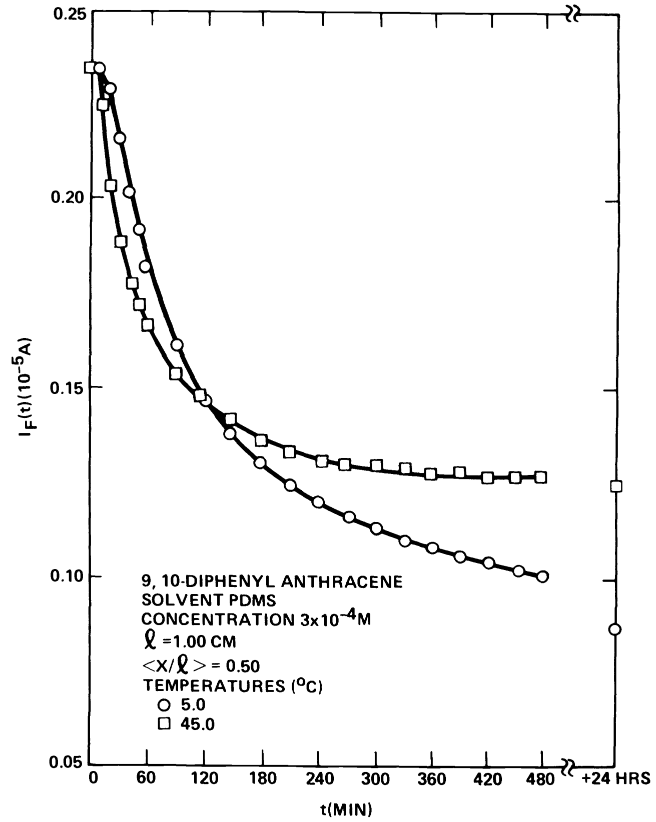

Figure 4. Fluorescence intensity vs. time for $C_F = 3 \times 10^{-4}M$ in poly (dimethyl siloxane) at T = 5 and 45°C.

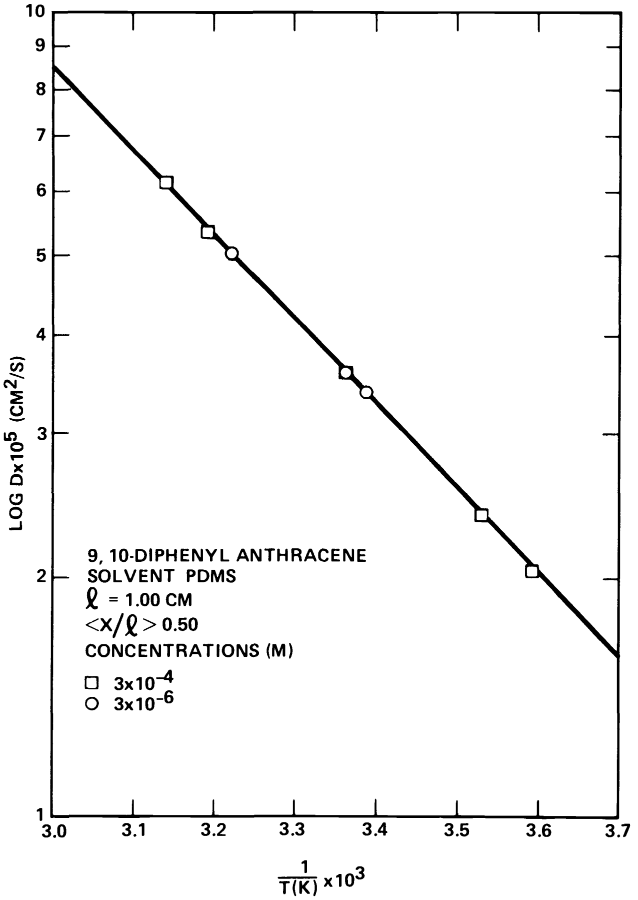

Figure 5. Arrhenius plot of log D vs. 1/T, $C_F = 3 \times 10^{-4}$ and 3×10^{-6}M, for $5 \leq T \leq 45^{o}C$.