

# A recirculating-flow fluorescent oxygen sensor

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**Abstract:** An immersible oxygen sensor was constructed by circulating small quantities of ruthenium tris-(2,2'-bipyridyl) II dichloride oxygen-sensitive fluorescent dye through a loop of oxygen-permeable silicone tubing immersed in test medium. The fluorescence intensity of the dye was subsequently measured as it exited the flow loop and related to oxygen tension. This method of measuring the oxygen tension, through diffusive transport to a flowing stream of dye and recirculating it in the sensor, has been found to give a stable response and relatively long sensor lifetime without major recalibration. The sensor showed good stability over at least a week's duration and showed no degradation due to leaching of the dye through membranes or photobleaching that commonly affects fluorescent sensors with immobilized chemistries. © 1996 Elsevier Science Limited

**Keywords:** oxygen sensor, fluorescent biosensor

## INTRODUCTION

NASA space-bioreactor cell-culture applications require sensors to be compact, withstand lift-off and zero gravity conditions, have negligible calibration drift over time while immersed in complex protein-bearing cell culture solutions, and operate essentially unattended over space shuttle missions of several weeks extending to months. Long-term sensor stability is particularly

needed to employ computer feedback control systems to maintain oxygen tension in culture within preset limits. These kinds of operating criteria are difficult to meet with present commercial oxygen sensors such as Clark-type oxygen sensors, as well as with more recent fluorescent oxygen sensors marketed for biomedical applications.

Although fluorescent oxygen sensors have been under development for many years (Wolfbeis, 1991; Wolfbeis & Leiner, 1988; Wolfbeis *et al.*, 1988; Trettnak, 1988; Gehrich *et al.*, 1986; Peterson & Fitzgerald, 1984), most reported fiber

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optic-coupled sensors for oxygen targeted for biomedical applications must be recalibrated periodically; at least every several days. Reasons for this include leaching of the encapsulated fluorophore from behind membranes upon long-term exposure to solution, and photobleaching of the fluorophore in the fiber tip upon exposure to intense ultraviolet excitation needed to achieve adequate sensor signal-to-noise ratios.

## MICRODIALYSIS GAS TRANSPORT

Chemical sensing by microdialysis is a method of introducing a membrane hollow fiber into a solution under test and then passing a dialysate (a sweep fluid such as water) through the tube. Diffusive transport across the membrane will create an equilibrium of gases between the dialysate and the test solution (Keck *et al.*, 1991; Kissinger & Kissinger, 1990; Mascini *et al.*, 1992). The flow of the dialysate carries it further downstream to a location where its gas concentration is more conveniently analyzed. The use of silicone membrane tubing allows gases like oxygen and carbon dioxide to diffuse readily through its thickness and then dissolve in the dialysate.

The degree of concentration equilibrium between the external solution  $C_{bulk}$  and the dialysate moving through a semipermeable membrane tube  $C_d$  is given by:

$$C_d/C_{bulk} = 1 - \exp[-1/Q_d R]$$

where  $Q_d$  is the dialysate flow rate and  $R$  is the overall resistance to gas transport across the membrane as well as through the solution next to the membrane. Minimizing the resistance term in the equation is strongly dependent on the achievement of a well-stirred environment next to the membrane. This is so that convection replaces oxygen that may otherwise be locally lost to the dialysate. Oxygen-depleted boundary layers next to the silicone tubing will tend to increase the resistance value.

## METHODS

A sweep fluid carrying a 10 mM solution of the fluorescent dye ruthenium tris-(2,2'-bipyridyl) II dichloride (formula weight 640.5) was passed through a length of thin-walled silicone membrane

tubing immersed in various test solutions. The silicone tubing isolates the dye yet permits diffusive equilibrium with the solution. The fluorescence of the dye is monitored as it exits the test solution and this is then related to solution oxygen tension by a conversion formula.

The silicone tubing used is 1.65 mm o.d. by 0.76 mm i.d. (Dow Corning) and typically 7.5 cm in length. Silicone tubing has a high oxygen permeability  $50 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1} \text{ cm}^{-2} \text{ cm-Hg}$  (Zero Corp., 1993). When the tubing is exposed to oxygen either dissolved in a solution under test or in the atmosphere, an equilibrium of oxygen with the dye occurs in residence times on the order of a minute. By pumping the dye through the tubing at a flow rate in the range of 0.25–5  $\mu\text{l}/\text{min}$ , the dye exiting the silicone tubing is in equilibrium with the environment oxygen tension. Immediately measuring the dye fluorescence exiting the tubing gives an optical signal proportional to its oxygen tension.

Figure 1 shows a diagram of the sensor. We use a compact solenoid micro-pump of dimensions  $29 \times 8 \text{ mm}$  (Wilson Greatbatch Inc.) which provides a dye flow rate of 500 nl per pulse through the flow loop. The portion of the dye exiting from an in-line optical cell is returned to a several milliliter holding reservoir for reuse.

An optical fluorescence cell is constructed of square quartz tubing  $1 \times 1 \text{ mm}$  internal dimensions and mates directly to the silicone tubing entering the sensor body. Blue excitation light,

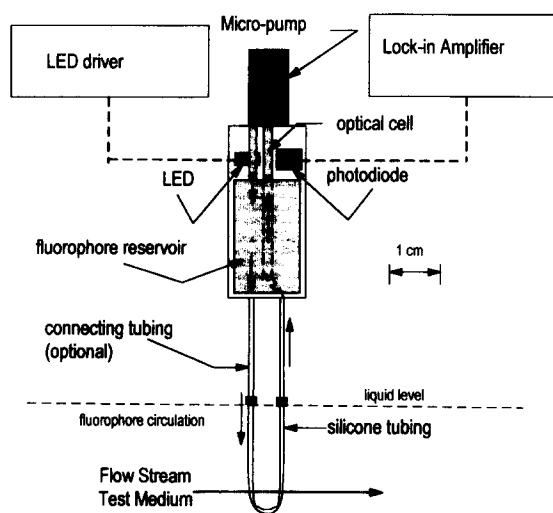


Fig. 1. A diagram of the prototype sensor body and the silicone tubing flow loop containing the fluorescent dye.

470 nm, from a 13 millicandella intensity light emitting diode (LED) passes through the dye in the flow cell. Light from the LED is blocked from direct illumination of the photodetector by a red celluloid filter (broad bandpass) that transmits the dye red fluorescence but blocks the blue excitation source. The photodiode sensitive area is approximately 4 mm square while the total volume of the dye contributing to the detected fluorescence signal is approximately 5  $\mu$ l.

The electronics support system for the optical fluorescence cell consists of an oscillator-driver which applies a 400 Hz square wave current to the blue LED for dye fluorescence excitation. The resulting chopped fluorescence signal is detected by the photodiode and is demodulated by a lock-in amplifier. The chopping of the light and following demodulation improves noise rejection from many sources including 60 Hz power line interference and from small amounts of interfering ambient light which scatters up the silicone tubing. Three second signal integration time constants were generally used on the lock-in amplifier.

The ruthenium dye used was reported by Wolfbeis *et al.* (1988) in his investigations of fiber optic oxygen sensors as having a relatively large Stokes shift of fluorescent emission to 620 nm (into the red) relative to the peak absorbance of the dye (463 nm). This makes separation of the induced fluorescence from the excitation light by simple colored filters fairly easy to achieve with low optical filter losses.

### SENSOR RESULTS

Figure 2 shows a plot of the measured dye fluorescence as a function of oxygen tension in a test solution. As is common with fluorescent oxygen sensors, the response is non-linear with the greatest sensitivity of the dye to oxygen quenching in the region below about 150 mm-Hg.

Figure 3 shows an approximate 5 min rise time of the sensor to 95% of the final value to a change in oxygen tension from 21 to 0%. We observe that when the sensor was exposed to a similar concentration step in oxygen in the gas phase, there was a faster response; about 3.5 min to 95% equilibrium.

Figure 4 tracks the oxygen sensor response to

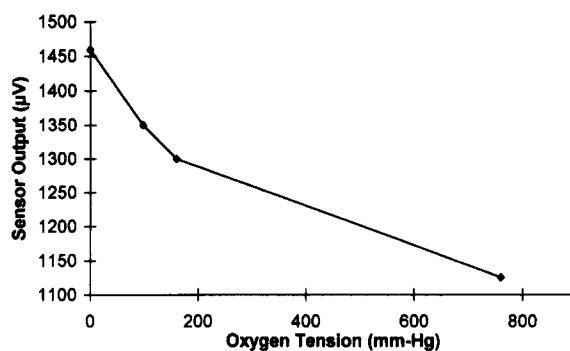


Fig. 2. A plot of the measured Tris 2,2'-bipyridyl ruthenium II dichloride (RTDP) dye fluorescence as a function of oxygen tension. The increasing slope of the response curve towards 0% oxygen tension is consistent with the behavior of fluorescent dyes.

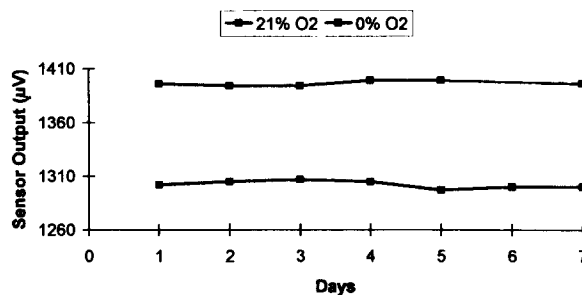


Fig. 3. The stability of sensor responses to 0 and 21% oxygen in solution over a week's interval.

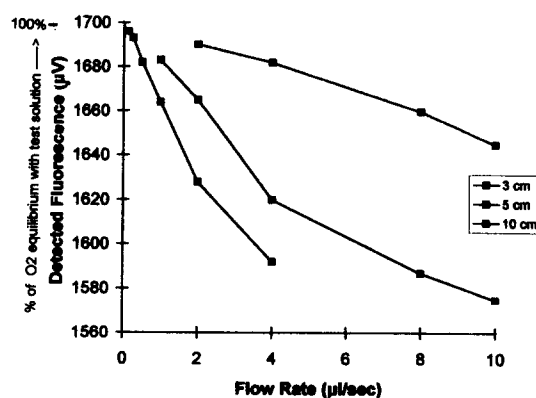


Fig. 4. The figure shows that the dye flow rate and silicone tubing length affect the percentage of oxygen equilibrium that the dye achieves by diffusion with the test solution. An asymptotic approach to 100% equilibrium is seen as dye flow rates decrease and tubing lengths increase.

both 0 and 21% oxygen concentrations in solutions exposed to the ambient laboratory environment over a week's interval. No trend towards a decaying response or other systematic change in the sensor output is seen.

Figure 5 shows the detected fluorescence from the optical cell as a function of several different lengths of flow loop silicone tubing exposed to a 0% oxygen stirred solution. There is a tradeoff between sensor response time, flow rate and length of silicone tubing exposed to solution.

## DISCUSSION

Reproducibility of the sensor response is excellent, generally settling back to within  $\pm 1\%$  of its initial reading after exposure to a transient change in oxygen tension. No measurement hysteresis or unaccountable baseline shift is seen and there is no trend of sensitivity change or apparent change in characteristics of the dye over time.

The electronic noise in the fluorescence measurements with 10 s electronic filter time constants results in  $\pm 2$  mm-Hg accuracy in the oxygen tension range (0–150 mm-Hg) of interest for bioreactors. The curvature of the fluorescence versus oxygen tension plot in this sensor is consistent with the hyperbolic nature of the Stern–Volmer relationship (1919). This non-linear response is not a particular problem since it is predictable and corrected by subsequent signal processing.

The residence time of the moving dye in diffusive contact with the test solution is the important variable defining the achievement of

a needed gas equilibrium and is the major factor in determining the overall response time of the sensor. The data show that dye flow rates of  $1 \mu\text{l/s}$  using 10 cm lengths of the membrane tubing exposed to the test solution yielded an acceptable overall oxygen equilibrium of greater than 95% and a 5 min response time.

The  $\pm 8\%$  sensor overall gain variation over the week's duration can be largely attributed to temperature variations in the environment, since temperature control of the flow stream at the optical cell was not performed in this work. For a typical photodetector voltage response of  $1.03 \mu\text{V}$  per mm-Hg  $\text{O}_2$  tension, the variation in the sensor data over time can be explained by an uncompensated temperature variation of  $2.3^\circ\text{C}$  in the laboratory over the week. There is also possibly some of the variation (perhaps up to  $\pm 1.5\%$ ) results from uncompensated barometric pressure changes over the week's duration. We do not view the temperature sensitivity to be a major problem since space bioreactors, as well as most cell culture reactors on the ground, are temperature regulated at  $37^\circ\text{C}$ . Placement of the optical cell in this environment should eliminate any thermal-induced drifts in the sensor.

The silicone oxygenator tubing showed no ill effect of week-long exposure to the dye and did not seem significantly to build up any residues or deposits in the micro-flow system. We found the ruthenium-based oxygen-quenched dye to be very stable in an aqueous carrier solution and spectrophotometry showed no leaching of the dye through the hydrophobic silicone membrane into the test solution.

The basic approach of recirculating microquantities of fluorescent dye through silicone tubing

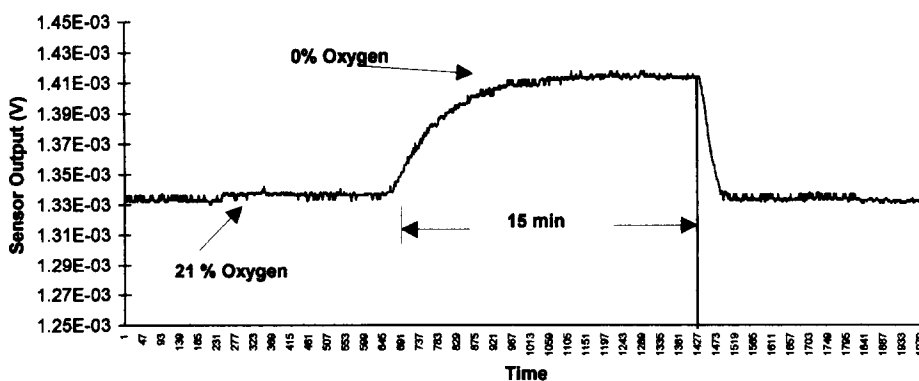


Fig. 5. The response of the sensor in water solution to changes in oxygen tension caused by bubbling nitrogen gas through water.

contacting the test medium appears to eliminate the problem of long-term photobleaching and fluorescent decline through dye loss by leaching out of entrapment membranes. The principal reason for this is the low intensity of excitation light that can be used with a relatively larger volume optical cell. The larger optical cell has a volume in the microliter range compared with perhaps picoliters of dye commonly entrapped at the end of a small fiber optic catheter. The recirculating dye also has a low duty cycle of exposure to the medium under test compared with its otherwise continuous exposure by using simple dye entrapment.

The principal tradeoff of this sensor approach is the additional hardware needed to add a micro-flow loop and the need for a several centimeter-long silicone membrane exposed to the test medium rather than a small sensor tip. The postage-stamp size of more recently available micropumps creates a compact overall sensor package.

We observe a decline in sensor sensitivity with insufficient local stirring. There is a need to maintain a flow environment around the membrane tubing, much like that required by Clark oxygen electrodes having thin membranes. Flow sensitivity results from the dye-carrying sweep fluid creating a boundary layer around the membrane tubing because of differences in oxygen concentration between the sweep fluid and the solution under test. Disturbances of this boundary layer affect the equilibration time of the sensor. In cell culture bioreactors, the tubing would need to be introduced either directly into the stirred bioreactor or into a media flow loop used to recirculate and replenish nutrients.

## CONCLUSIONS

The recirculation of the fluorescent dye through an oxygen-permeable silicone tube exposed to

solution appears to give a relatively robust sensor that does not appear to exhibit any unaccountable drift due to reagent loss or bleaching over week-order intervals. The overall compactness and low energy consumption of this kind of sensor approach is an advantage compared with other types of fluorescent optical sensors using lasers, high intensity halogen lamps, lenses and beam splitters.

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