Optical Properties of $Ru(dpp)_3$ for Phosphorescence Biosensors

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***Abstract* – The saturation intensity and photobleaching lifetime of tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) choride ($Ru(dpp)_3$) are measured and applied to the design of oxygen sensitive optodes for enzymatic biosensors. Photobleaching limits the excitation intensity in practical sensor systems.**

I. Introduction

Ruthenium based phosphorescent molecules are attractive oxygen concentration transducers [1] for use in biosensors using genetically engineered enzymes to metabolize target chlorinated ethenes and other analytes [2]. Oxygen quenches the 615 nm phosphorescence of ruthenium complexes, and oxygen is consumed in the enzymatic conversion of analytes, making the phosphorescence intensity of the ruthenium complex inversely related to the concentration of the analyte. Two design goals for optodes, i.e. optical fiber tips coated in the ruthenium complex, are that they provide maximum phosphorescence signal, and that they have a sufficiently long operating lifetime. To quantify appropriate excitation levels to achieve maximum signal and operating lifetime goals respectively, both the saturation intensity and photobleaching rate must be known. However, detailed literature searches indicate that such information has not been previously published for any ruthenium containing molecules. Here we report saturation intensity measurements in good agreement with calculated values and intensity dependent photobleaching rates for tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) chloride ($Ru(dpp)_3$) based optodes.

II. Apparatus

A. *Optode preparation*

Fiber optic optodes utilizing $Ru(dpp)_3$ are fabricated by the following procedure. The distal end of a 980 µm core plastic optical fiber (POF) is prepared by polishing with 3 µm polishing paper (Industrial Fiber Optics). In an eppendorf tube 1 ml of $Ru(dpp)_3$ is dissolved into to 1 ml of chloroform. Mixed into this solution is 0.2 g of silicone (Permatex clear RTV 66B). Nominally 2 µl of dye mixture is transferred by pipette onto the tip of the fiber and allowed to dry. Fig. 1 shows the dye's red phosphorescence of an optode when excited with a blue LED.

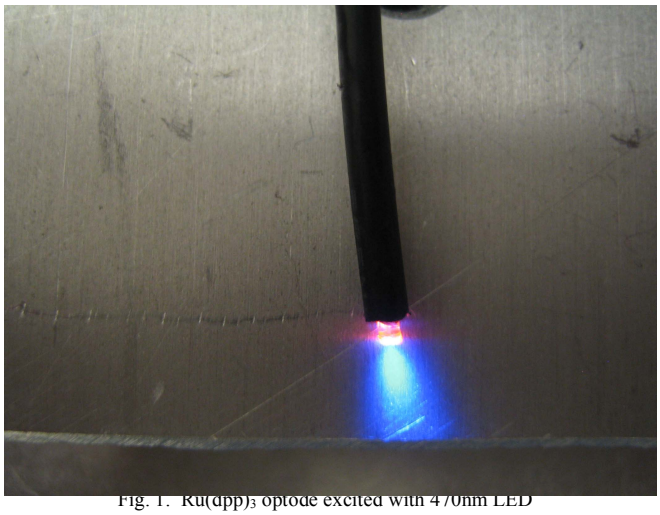

Fig. 1. $Ru(dpp)_3$ optode excited with 470nm LED

B. *Optics configuration*

Both the saturation intensity and photobleaching lifetime were evaluated using a 405 nm laser diode and filtered photomultiplier tube (PMT) as seen in Fig. 2. In order to increase the maximum available excitation intensity, a special optode was created on 62.5 µm core diameter silica fiber rather than the typical large core POF, and the laser was used in lieu of a blue LED. The phosphorescent signal was collected by a large core POF arranged coaxially with the glass fiber optode, filtered to pass the 570 to 670 nm wavelength range, and measured with a PMT.

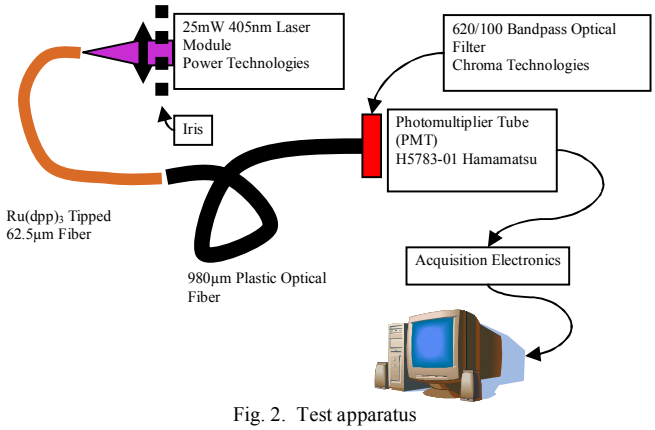

Fig. 2. Test apparatus

III. Saturation Intensity

Prior to the measurements, the required excitation power for saturation intensity of $Ru(dpp)_3$ was estimated by calculating the transitional cross section of fluorescein and applying to $Ru(dpp)_3$. Equation (1) shows the relation of the saturation intensity (I_{sat}) of an absorbing media to its resonant wavelength (λ), transitional cross section (σ), and radiative lifetime (τ) by Plank's constant ($\hbar$) and the speed of light (c).

$$I_{sat} = (hc)/(\lambda \sigma \tau) \quad (1)$$

Using a saturation intensity of 1.27 x 10^4 W/cm^2, resonant wavelength of 488 nm, and radiative lifetime of 3.7 ns [3] for fluorescein provides a transitional cross section of 8.6 x 10^{-15} cm^2. Assuming the same transitional cross section for $Ru(dpp)_3$, a resonant wavelength of 465 nm [4], and a radiative lifetime of 5.34 µs [4] results in an expected saturation intensity of 9.3 W/cm^2.

The results of two repetitions of the saturation intensity measurements are shown in Fig. 3. A plateau in the phosphorescent signal is observed beginning at an excitation power of about 300 µW. Based on the definition of saturation intensity being the excitation level at which the output signal falls to one-half of the linearly extrapolated low excitation response (shown in green in Fig. 3), the measured saturation intensity occurred at 646 µW corresponding to I_{sat} = 21 W/cm^2. Accounting for the off resonance excitation source results in a saturation intensity of 11.5 W/cm^2, this value is in good agreement with the value of 9.3 W/cm^2 estimated from the calculation discussed above.

IV. Photobleaching

The excitation laser power was set to 365 µW which is ~55% of saturation intensity but well into the plateau region, and then the 62.5 µm optode was left for 48 hours (Fig. 4) to evaluate the sensor for operational lifetime.

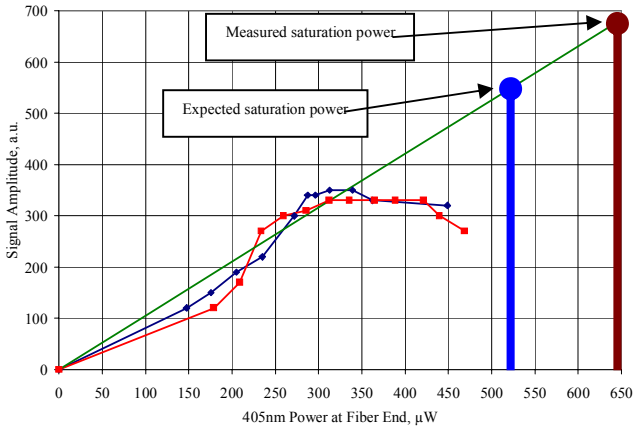

Fig. 3. Saturation of Ru(dpp)3 with 405 nm excitation. Expected and measured saturation powers are shown.

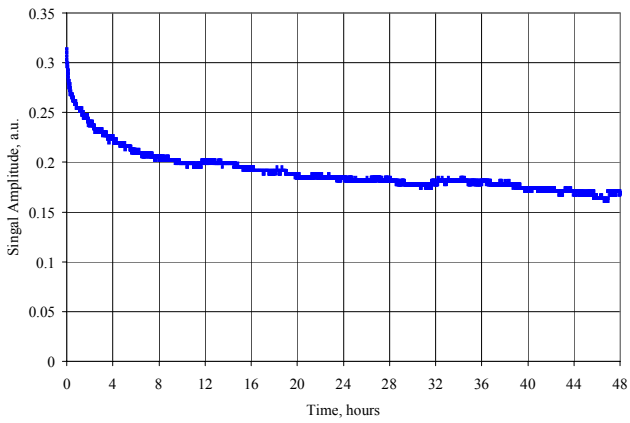

Fig. 4. Photobleaching of Ru(dpp)3 with 365 µW of 405 nm excitation light over 48 hours.

The phosphorescence signal significantly dropped over the first 12 hours and then began a slower rate of decay. Clearly there is more than one exponential decay which could be due to the $Ru(dpp)_3$ adjacent to the fiber is photobleaching quickly and then acting as an attenuator to the laser light, resulting in the dye relatively farther away to photobleach at a slower rate.

V. Conclusions

The long lifetime of $Ru(dpp)_3$ creates a low saturation intensity which allows for excellent excitation with common LEDs. A laser is still needed to reach saturation which can reduce the effect of source amplitude noise but is far outweighed by the short operational lifetime due to photobleaching. Photobleaching characterization of $Ru(dpp)_3$ optodes will be evaluated at low excitation powers utilizing a LED. Having a long phosphorescent lifetime, $Ru(dpp)_3$ could be a good candidate for use in ratiometric phase fluoremetry measurements in order to minimize excitation source noise effects, which will also be explored in the future.

Acknowledgment

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