

Phosphorescence characteristics of ruthenium complex as an optical transducer for biosensors

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The saturation intensity, photobleaching, and decay lifetime of tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) chloride [Ru(dpp)₃] are investigated. The saturation point was estimated to be 11.8 W/cm² and subsequently measured to be 11.5 W/cm². Photobleaching of this dye at a peak absorbance equivalence of 6.6 W/cm² is observed over 2 days resulting in a 14% decrease in intensity within 20 min and 47% decrease over two days yielding an unacceptably short operational lifetime under saturated conditions. Lower optical excitation levels in the application of oxygen sensitive fluorescence type fiber optic enzymatic biosensor systems are required and yield acceptable photobleaching rates. © 2008 American Institute of Physics. [DOI: 10.1063/1.2885082]

Ruthenium based phosphorescent molecules are attractive oxygen concentration transducers¹ for use in biosensors which employ genetically engineered enzymes to metabolize target chlorinated ethenes and other analytes.² Tris(4,7-diphenyl-1,10-phenanthroline) ruthenium (II) chloride [Ru(dpp)₃] has been used as an optical transducer in a biosensor for monitoring biochemical oxygen demand in seawater for determination of biodegradable organic compounds in waste water.³ These molecules are photoexcited with blue light and photoluminesce via intersystem cooling of the excited electron to a triplet site prior to recombination which gives rise to long decay lifetimes and photon emission in the red. This process is generally termed phosphorescence rather than fluorescence and this convention is adhered to by the authors here. The phosphorescent intensity and decay lifetime of ruthenium based molecules are quenched in the presence of oxygen and can be described by the dynamic Stern–Volmer relationship.⁴ Specifically, a biosensor with a genetically engineered enzyme as the biocomponent relies on oxygen consumption in the enzymatic conversion of analyte. The resulting decreased oxygen concentration increases the phosphorescence intensity or decay lifetime of the ruthenium complex making them dependent on the concentration of the analyte.

Two design goals for optodes, optical fiber tips coated in the ruthenium complex, are that they provide maximum phosphorescence intensity and that they have a sufficiently long operating lifetime. Operation in the saturation region would maximize the phosphorescent intensity and reduce the effect of excitation source amplitude noise on system sensitivity. This noise could be from the source itself or due to optical misalignments leading to coupling inefficiencies depending on the exact apparatus configuration. To quantify appropriate excitation levels to achieve maximum signal and operating lifetime goals, respectively, both the saturation intensity and photodegradation causing loss of phosphores-

cence, i.e., photobleaching rate, must be known. However, detailed literature searches indicate that such information has not been previously published for any ruthenium containing molecules.⁵ Here we report saturation intensity measurements in good agreement with calculated values and intensity dependent photobleaching rate at high excitation level for Ru(dpp)₃ based optodes. Absorption and emission spectra for Ru(dpp)₃ are shown in Figs. 1(a) and 1(b) respectively.

Typical fiber optic optodes utilizing Ru(dpp)₃ were fabricated by the following procedure. The distal end of a 980 μm core plastic optical fiber (POF) was prepared by polishing with 3 μm grit polishing paper (Industrial Fiber Optics). In a microcentrifuge tube, 1 mg of Ru(dpp)₃ was dissolved into to 1 ml of chloroform. Mixed into this solution was 0.2 g of silicone (Permatex clear RTV 66B) resulting in a concentration of 5 mg/g of Ru(dpp)₃ to silicone. A nominal 2 μl of dye mixture was then transferred by pipette onto the tip of the fiber and allowed to dry. Figure 2(a) shows

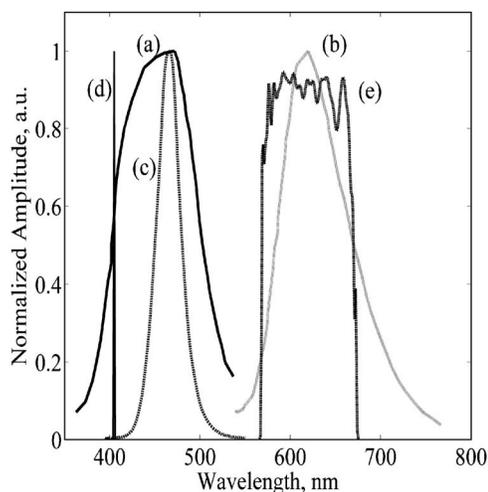


FIG. 1. Ru(dpp)₃ (a) absorption and (b) emission spectra (see Ref. 5), (c) 470 nm LED emission, (d) 405 nm laser emission, and (e) transmission of 620/100 filter.

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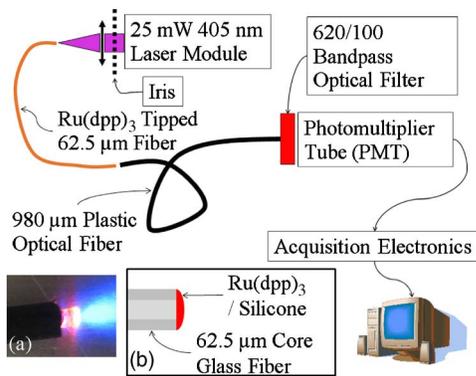


FIG. 2. (Color online) Test apparatus for saturation and photobleaching measurements. Inset (a) is an example of Ru(dpp)₃ optode on 980 μm core POF excited with a blue LED. Inset (b) is a schematic illustration of a 62.5 μm core glass fiber tipped with Ru(dpp)₃/silicone matrix.

the dye's red phosphorescence on an optode when excited with a blue-light emitting diode (LED) with emission spectra shown in Fig. 1(c).

In order to increase the maximum available excitation intensity for both the saturation⁶ intensity and photobleaching⁷ measurements, a special optode was created on 62.5 μm core diameter silica fiber rather than the typical large core POF. Dye was applied by dipping the silica fiber tip into the Ru(dpp)₃ mixture and was allowed to air dry, resulting in a dye layer thickness of approximately 40 μm observed in profile with a microscope. Both the saturation intensity and photobleaching effects were evaluated using the experimental setup shown in Fig. 2. A 405 nm laser was used in lieu of a blue LED to provide higher excitation intensity, and it was assumed that uniform illumination across the fiber surface was achieved. The red phosphorescent light emitted from the 62.5 μm fiber tip was collected with a large core POF arranged coaxially, where the light was then optically filtered with a 620/100 bandpass filter whose transmission spectra is shown in Fig. 1(e), only passing 570–670 nm light which was detected by a H5783-01 photomultiplier tube (PMT).

Prior to saturation measurements, the required excitation intensity to achieve saturation of Ru(dpp)₃ was estimated⁸ by $I_{\text{sat}} = (hc) / (\lambda_0 \sigma \tau)$. This relates an absorbing material's saturation intensity to its resonant wavelength (λ_0), transitional cross section (σ), and decay lifetime (τ) by Planck's constant (h) and the speed of light (c). As the authors could not find published values for the transitional cross section for Ru(dpp)₃, it was assumed that this parameter of Ru(dpp)₃ is equal to the transitional cross section of fluorescein isothiocyanate (FITC). Using a saturation intensity of 1.3×10^4 W/cm² based on prior unpublished work, resonant wavelength of 488 nm, and decay lifetime of 3.7 ns (Ref. 9) for FITC provides a transitional cross section of 8.6×10^{-15} cm².

Reported Ru(dpp)₃ decay lifetimes in the literature are presented only for the unquenched value,¹⁰ only as the ratio of unquenched to quenched lifetimes versus oxygen partial pressure¹¹ or without reference to measurement conditions⁴ including temperature. Direct measurement of the quenched phosphorescent decay of Ru(dpp)₃ in air at 20 °C was obtained by pulsing a blue (470 nm) LED to excite a standard POF optode. Detection was accomplished by using the same 620/100 optical bandpass filter and PMT described above

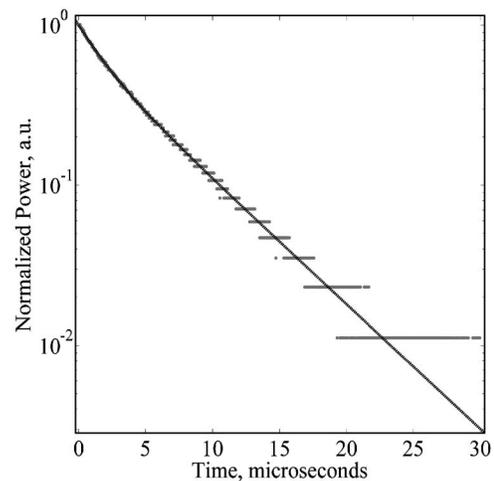


FIG. 3. Normalized phosphorescence decay of Ru(dpp)₃ in air at 20 °C fitted to a sum of two exponentials (dashed).

but with a custom transimpedance circuit with a 3 MHz bandwidth, 10 000 V/A transimpedance and a fall time of <180 ns. The normalized Ru(dpp)₃ decay is shown in Fig. 3 along with a fit to a sum of two exponentials $P(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$, where the preexponentials and lifetimes were then used to calculate a weighted average⁷ found by $\langle \tau \rangle = A_1 \tau_1 + A_2 \tau_2$ with A_1 , A_2 , τ_1 , and τ_2 found to be 0.23, 0.77, 1.59, and 5.0 μs, respectively, resulting in a total, i.e., radiative and nonradiative, decay lifetime of 4.19 μs. Using this lifetime and assuming the same transitional cross section for Ru(dpp)₃, as calculated above, a resonant wavelength of 465 nm⁵, results in an expected saturation intensity of 11.8 W/cm², adjusting for the 405 nm laser excitation according to $I(\omega) = I_0 + 4(I_0/\Delta\omega_a^2)(\omega - \omega_0)^2$. Here, $I(\omega)$ is the saturation intensity at ω , I_0 is the saturation intensity at peak resonance, ω_0 is the resonance frequency, and $\Delta\omega_a$ is the full width at half maximum (FWHM) of the absorption band. Since the absorption band for Ru(dpp)₃ is skewed, a FWHM was found by going from resonance to the half absorbance point for shorter wavelengths and doubled. A saturation power at 405 nm was predicted to be 663 μW.

The results of two repetitions of the saturation intensity measurements are shown in Fig. 4. Measurements were made with an effort to minimize Ru(dpp)₃ exposure at any single excitation level to reduce the potential for photobleaching effects. Both sets of data were collected within 5 min. A plateau in the phosphorescent signal is observed beginning at an excitation power of about 300 μW. The onset of the plateau is more abrupt than the expected parabolic roll off for simple saturation models. It is possible that some photobleaching effect caused this, although the good agreement of the two data sets is inconsistent with this explanation. Based on a linear extrapolation of the least squared error fit to the data for excitation powers <340 μW, as shown in Fig. 4, to a phosphorescent emission power of 680 a.u., i.e., twice the plateau level of 340 a.u., the measured saturation power is at 646 μW corresponding to $I_{\text{sat}} = 21$ W/cm² at 405 nm. Accounting for the off resonance excitation source provides a saturation intensity of 11.5 W/cm² at resonance in very good agreement with the value of 11.8 W/cm² estimated from the calculation discussed above.

Photobleaching in Ru(dpp)₃ was evaluated under excitation conditions in the saturation region to obtain an indica-

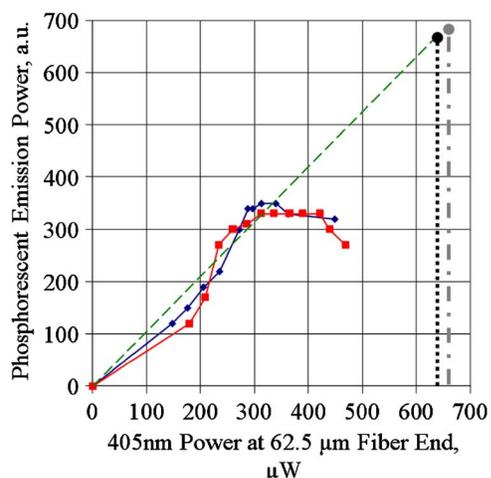


FIG. 4. (Color online) Two data sets showing saturation of Ru(dpp)₃ with 405 nm excitation where the (diamonds) first set and (squares) second set are for the same optode. Linear extrapolation of the linear response of Ru(dpp)₃ emission vs 405 nm excitation power (dashed), expected (dotted), and measured (dash dot) saturation powers are shown.

tion of the operational lifetime of a sensor operated at this elevated excitation intensity. The excitation laser power for the 62.5 μm optode was set to 372 μW which is ~57% of saturation intensity but well into the plateau region for 48 h. The resulting rapid phosphorescence decay can be seen in Fig. 5.

The phosphorescence emission power dropped 14% in the first 20 min and 47% after 48 h. Figure 5 shows a fit to the sum of two exponentials which is in good agreement with the data after the initial 2 h. Fitting the initial rapid decline requires at least one more exponential component.

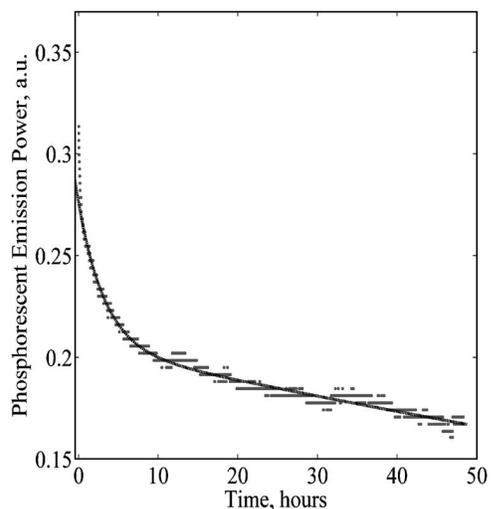


FIG. 5. Photobleaching of Ru(dpp)₃ with 372 μW of 405 nm excitation power over 48 h with sum of two exponential fit (dashed). Fitted photobleaching lifetimes are 3.24 and 236 h with preexponential factors of 0.07, and 0.205, respectively.

Surprisingly, the phosphorescent amplitude started to decrease at a much slower rate after decreasing by ~35% which could be due to the Ru(dpp)₃ adjacent to the fiber that is quickly photobleached and then acting as an attenuator to the laser light, resulting in the dye relatively farther away from the fiber tip photobleaching at a slower rate. The impact of photobleaching was then observed under conditions for use as a biosensor transducer.³ Measurement under excitation from a blue 470 nm LED on a 980 μm core POF optode resulting in an excitation intensity of 159 μW/cm² indicated less than a 3.3% decrease in phosphorescent emission power over 48 h. Operating the excitation source in a pulsed mode would further reduce any remaining photobleaching rate in proportion to the duty cycle.

In conclusion, the long lifetime of Ru(dpp)₃ creates a low saturation intensity which allows for excellent excitation with common LEDs ensuring a usable emission signal. Saturation to maximize emission signal could easily be reached with available laser sources and could reduce the effect of source amplitude noise or optical misalignments, but this advantage is far outweighed by the short operational lifetime due to the rapid photobleaching that would occur. Close agreement of expected and measured saturation intensities suggest that the transitional cross section for Ru(dpp)₃ and FITC are notably similar. Care must be taken when using decay lifetime measurements to take into account the precise conditions in which the data were collected. In particular, the temperature effect on the amplitude and decay lifetime of Ru(dpp)₃ should be considered. Data on temperature dependent quenching of Ru(dpp)₃ will be published elsewhere.

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