Unique Oxygen Analyzer Combining a Dual Emission Probe and a Low-Cost Solid-State Ratiometric Fluorometer

YORDAN KOSTOV, KELLY A. VAN HOUTEN, PETER HARMS, ROBERT S. PILATO, and GOVIND RAO*

Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725W. Lombard St., Baltimore, Maryland 21201 (Y.K., P.H., G.R.); Department of Chemical and Biochemical Engineering, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland (G.R.); University of Maryland, Department of Chemistry and Biochemistry, College Park, Maryland 20742 (K.V.); and LUMET, 1808 Briggs Chaney Rd. Silver Spring, Maryland 20905 (R.S.P.)

A new oxygen analyzer combining a polymer-encapsulated dual-emission probe and a diode-based ratiometric fluorometer is described. The ratiometric fluorometer was configured to measure the relative fluorescence and phosphorescence intensity of the short-lived singlet and long-lived oxygen-quenchable triplet of [(dppe)Pt{$S_2C_2(CH_2CH_2$–N–2-pyridinium)}](BPh_4), where dppe is 1,2-*bis*(diphenylphosphino)ethane. This luminescent dye was immobilized at 0.3% by weight in cellulose acetate/75% triethylcitrate and cast into a 0.5 mm-thick film. The dye molecule was excited with a blue light-emitting diode (LED) and the singlet and triplet emissions monitored by individual photodiodes at 570 and 680 nm, respectively. Oxygen can be accurately measured without interference from nonanalyte-induced intensity changes by monitoring the relative output of the two photodiodes. The output of the device was linear with oxygen concentration. The $PO_2(1/2)$ from the ratio-adapted Stern–Volmer plot was 9.6% oxygen (≈73 torr), offering a dynamic range of 0–90% O_2. The device is stable and the oxygen measurements reproducible. Intentional photobleaching of ≈20% of the sensor dye had little effect upon the reproducibility of the oxygen measurements.

Index Headings: **Oxygen analyzer; Ratiometric sensor; Low-cost fluorimeter.**

INTRODUCTION

The accurate measurement of dissolved oxygen is important to environmental, industrial, and biomedical monitoring. Given the relatively low concentration of oxygen in aqueous media and its role as the terminal electron acceptor in aerobic pathways, oxygen concentrations reflect upon the health of an environment, cell culture, or organism. The most common method for measuring dissolved oxygen is a modified Clark electrode.[1,2] While a long-standing polarographic method, the Clark electrode is adversely affected by signal drift (affecting long-term stability), flow rates (since oxygen is consumed by the electrode), and electrical interference.[3,4]

Given the limitations of the Clark electrode, optical methods are now finding use in measuring oxygen concentration. The long-lived states of many emissive transition metal complexes are quenched at oxygen concentrations of environmental, industrial, and biomedical interest.[5] Furthermore, once polymer encapsulated these emissive dyes can be used to measure oxygen in the gas phase as well as in aqueous or biomedia. Several *tris*(diimine)ruthenium(II) complexes and metalloporphyrins have been polymer encapsulated and used for this application.[6–35] However, simply relying on oxygen-induced changes in emission intensity has proven to be problematic. Such intensity-based measurements are adversely affected by changes in optical clarity, fluctuations in the source and detector, and photobleaching of the emitter.[35–37] These nonanalyte-induced variations in intensity make continual restandardization of intensity-based sensors a requirement.

With the use of a frequency-modulated excitation, a lifetime-dependent phase shift can be use to measure quenching of a long-lived emissive state and hence O_2 concentration.[6,35–40] This method eliminates many of the problems associated with intensity measurements but does add to the complexity of the detector.

As an alternative to frequency-modulated phase-based methods, we describe a new, simple, low-cost, solid-state ratiometric oxygen analyzer which utilizes a dual-emitting sensor dye[41,42] and a diode-based two-wavelength detector.[43] The detector components were chosen to measure the relative fluorescence and phosphorescence of [(dppe)Pt{$S_2C_2(CH_2CH_2$–N–2–pyridinium)}](BPh_4), **1**, where dppe is 1,2-*bis*(diphenylphosphino)ethane.[42] Since only phosphorescence from the triplet state is quenched by oxygen, the fluorescence from the singlet serves as an internal reference. When the triplet/singlet ratio is used in place of intensity, it eliminates problems with nonanalyte-induced intensity changes. While similar detection methods (without the accompanying device) have been developed with two-dye molecules, these are inferior to the method described.[44–46] In the two-dye method, photobleaching of either dye leads to gross changes in the intensity ratio. This problem is eliminated by the dual emitter since the intensity ratio is insensitive to photobleaching of the dye molecule.[42,47]

EXPERIMENTAL

Materials. The dual emitter, BPh_4 (**1**), was prepared according to the literature procedure.[41] Cellulose acetate (CA), triethyl citrate (TEC), and spectroscopic-grade acetone were purchased from Acros and used without further purification. Nitrogen and oxygen (>99%) were purchased from Air Products (Allentown, PA) and used without further purification. Nitrogen–oxygen gas mixtures were prepared with a gas blender equipped with two Series 150 flowmeters from Advanced Specialty Gas Equipment (Middlesex, NJ).

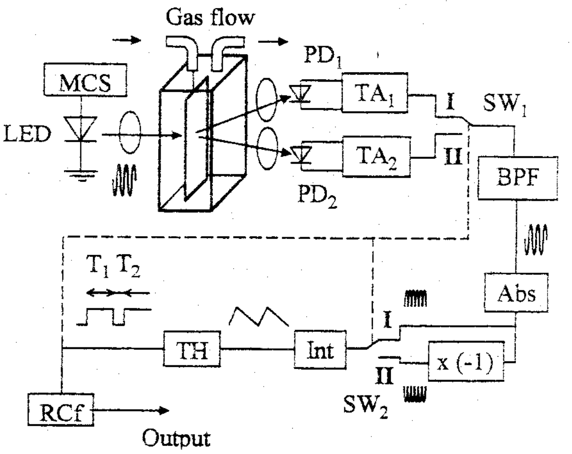
Instrumentation. UV-visible spectra were recorded on a Perkin-Elmer λ2 spectrometer. Room-temperature emis-

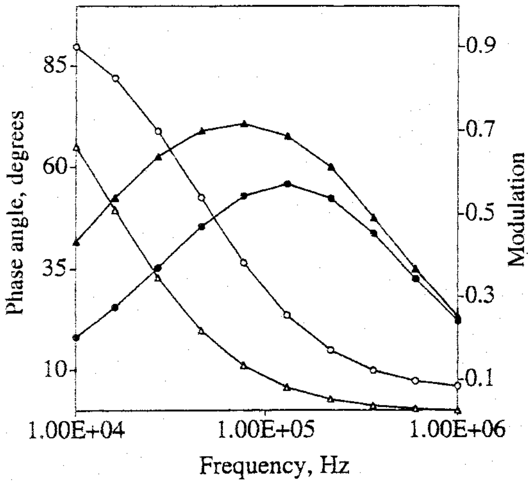
---

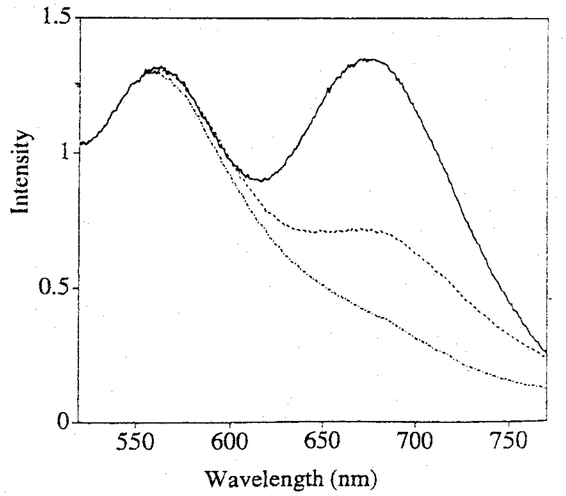
Received 24 November 1999; accepted 2 March 2000.

* Author to whom correspondence should be sent.

0003-7028/00/5406-0864$2.00/0







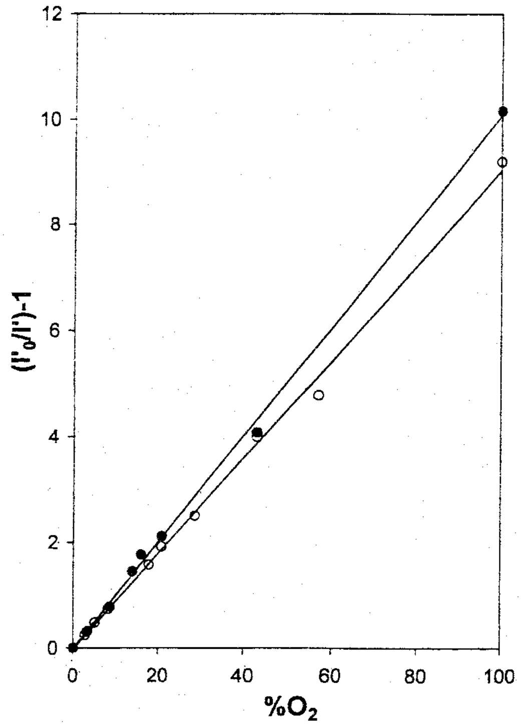


FIG. 1. (Upper left) Schematic diagram of the ratiometric fluorometer. **MCS**, modulated current source; **LED**, light-emitting diode; **PD$_1$** and **PD$_2$**, detection photodiodes; **TA$_1$** and **TA$_2$**, transimpedance amplifiers; **SW1** and **SW2**, switches; **BPF**, bandpass filter; **Abs**, circuit for detection of absolute value of the signal; **x(−1)**, multiplier by 1; **Int**, integrator; **TH**, trigger with hysteresis; **RCf**, RC filter; **T$_1$** and **T$_2$**, integration times for fluorescence and phosphorescence signals.

FIG. 2. (Lower left) Emission spectra of **1** (0.3%/wt) in CA/75% TEC. (———) in N_2; (– – –) in air; (-·-·-·) in O_2. Steady-state measurements were made with an SLM AB2 spectrofluorometer where the sensing film was held at 90° to the incident light. No instrument corrections for either energy or intensity were applied.

FIG. 3. (Upper right) The modulation and phase profiles for **1** (0.3%/wt) in CA/75% TEC. (●) Phase air; (○) modulation air; (▲) phase N_2; (△) modulation N_2. The data were fit to an average ^{3}ILCT* lifetime of 14 μs and 4.6 μs in N_2 and air, respectively.

FIG. 4. (Lower right) Comparative Stern–Volmer plots, $I_0'/I' - 1$ vs. % O_2 {where $I_0'/I' - 1 = ({}^3I_0'/{}^1I')/({}^1I_0'/{}^3I') - 1 = k_q\tau[O_2]$} for **1** (0.3%/wt) in CA/75% TEC. (●) Ratiometric fluorometer; (○) SLM AB2 fluorometer.

sion spectra were acquired with an SLM (Rochester, NY) AB2 fluorescence spectrometer. Emission spectra were corrected for instrumental response using factors supplied by the manufacturer where noted. Luminescent lifetimes were determined with an ISS (Urbana, IL) K2 digital frequency-domain spectrofluorometer modified to use a blue light-emitting diode (LED) excitation source.[48] The standard radio-frequency (rf) amplifier for the photomultiplier tubes was replaced with a ZHL-6A (Mini-Circuits, Brooklyn, NY) to enhance the low-frequency performance. The excitation light was filtered by 500-, 550-, and 650FL07 short wave pass filters (Andover Corp., Sa-

TABLE I. Comparison of ratiometric- and lifetime-based devices for oxygen measurements.

	Ratiometric-based device	Lifetime-based device[57]
Luminophore	$[(\mathrm{dppe})\mathrm{Pt}\{S_2C_2(CH_2CH_2\text{–N–2-pyridinium})\}]^+$	$\mathrm{Ru(DPP)_3}$[a]
Excitation	blue LED (λ_{max} = 470 nm)	Blue LED (λ_{max} = 470 nm)
Support membrane	CA/TEC	Polystyrene
Thickness	500 µm	5 µm
Response time	3.5 min	8 s
Detector	Si photodiode	Si photodiode
Limit of detection	0.2% O_2 (S/N ratio = 3:1)	0.1% O_2 (phase noise = ±0.035°)
Range	0–90% O_2	0–100% O_2
Accuracy	±0.2% O_2	±0.1% O_2
Work in strong ambient light	Yes	Yes
Bleaching problems	No	No
Photostability of the dye	Medium	High
Work with steady-state excitation	Yes	No

[a] $\mathrm{Ru(DPP)_3}$ corresponds to *tris*(4,7diphenil-1,10-phenantroline)$\mathrm{RuCl_2}$.

lem, NH). The emission light was filtered by 650- and 700FH90 long wave pass filters (Andover Corp.) to select the triplet emission.

Preparation of CA/75% TEC-1 Oxygen-Sensing Film. Cellulose acetate, 0.666 g; triethyl citrate, 0.333 g; and **1**, 0.003 g were dissolved in acetone 10 mL to generate the casting solution. The solution was cast into a circular glass well with a 5 cm diameter. The film was cured for 48 h at 25 °C. The 0.5 mm film was stored at −20 °C in the dark prior to use.

Solid-State Ratiometric Fluorometer. The diode-based ratiometric fluorometer was configured for films and the dual emission mode (Fig. 1).[43] The sample compartment was a cuvette (Spectrocell, Oreland, PA) equipped with a screw top and a septum, as well as with an inlet and outlet lines to allow gas flow. To excite **1**, a blue LED, MBB51TAH-T (Microelectronics, Santa Clara, CA), with peak wavelength at 470 nm was used. The modulated light from the LED (f_{mod} = 1.5 kHz) was directed to the sample compartment through a 460 ± 30 nm bandpass filter, mounted on the cell wall. Prior to experiments, the optical power of the LED was measured with a Model 840 optical power meter (Newport Corp., Irwine, CA). The singlet and triplet emissions were monitored through 570 ± 40 nm and 680 ± 22 nm bandpass filters, respectively. The corresponding light intensities were detected by two large-active-area (13 mm^2) PIN-photodiode detectors 1223-01 (Hamamatsu, Bridgewater, NJ). All filters were from Intor, Inc. (Sorocco, NM).

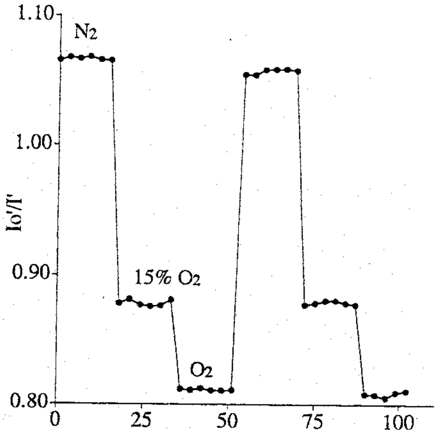


FIG. 5. Monitoring the triplet/singlet ratio of and **1** (0.3%/wt) in CA/75% TEC as a function of time and % O_2 with the use of the ratiometric fluorometer.

The electrical signals, corresponding to fluorescence and phosphorescence, were amplified and passed through a bandpass filter. This procedure rejected components that originated from ambient light and diminished the levels of low- and high-frequency noise. After amplitude detection and sign assignment, the signals were serially fed through the switch SW2 to an integrator (Fig. 1). The consecutive integration of the fluorescent and phosphorescence signals produces a square wave on the output of the trigger. Its duty ratio (T_1/T_2) is proportional to the fluorescence/phosphorescence intensity ratio. The duty ratio was determined by measurement of the dc component of the square wave.

Oxygen Measurements. The CA/TEC film was trimmed and mounted to a quartz cuvette insert with Super 77 spray adhesive (3M, Saint Paul, MN). The insert was mounted in a fluorescence cuvette with a screw-cap septum (Spectracell, Oreland, PA) and held at 90° to the incident light beam. The oxygen concentrations within the cell were varied by purging the cells with the desired nitrogen–oxygen mixture. The singlet and triplet emissions were monitored by using both the diode-based ratiometric fluorometer and an SLM AB2 fluorometer. All measurements were made at 25 °C. A linear progression of the data allowed for a baseline correction of the data. Ratio-adapted Stern–Volmer plots $(I_0'/I') - 1$ vs. percent O_2, where $(I_0'/I') - 1 = ({}^3I_0{}^1I)/({}^1I_0{}^3I) - 1 = k_q\tau[O_2])$ were used to evaluate the data and estimate $P_{O_2(1/2)}$. The limit of detection was calculated as three times the measured effective noise of the device. The accuracy was calculated as twice the limit of detection.

RESULTS AND DISCUSSION

Dual Emitting Dyes. The sensing dye used in this study is a heterocyclic-substituted platinum-1,2-enedithiolate, $L_2Pt\{S_2C_2(\text{Heterocycle})(R)\}$.[41,49–53] Members of this new class of luminescent molecules are both fluorescent and phosphorescent in room-temperature solutions and when polymer encapsulated. The two emissions are assigned to an intraligand charge-transfer singlet, ^{1}ILCT*, and triplet, ^{3}ILCT*, with considerable 1,2-enedithiolate π to heterocycle π* character. Studies of these complexes in room-temperature solutions demonstrate that the quantum yields and lifetimes vary with the heterocycle, the ancillary L_2 group, the R group, and solvent polarity.[41,46,50,51] As a class of molecules, the solution lifetimes of the singlet are generally <1 ns, while the triplet lifetimes vary from 1 to 16 μs.[41,47,51,53] All the emissive dye molecules in this family have an excitation maximum near 470 nm, making them compatible with a device that utilizes a blue LED excitation source.

The particular dye molecule used in this study, BPh_4 (**1**), was immobilized in cellulose acetate which contained 75% by weight triethyl citrate. The TEC percentage is based upon the weight of CA and is used to plasticize the polymer. The lumiphore loading was 0.3% of the combined CA/TEC weight.[41] It is well established that the sensitivity of polymer-immobilized lumiphores to oxygen quenching is controlled, to a large extent, by the polymer and plasticizer content.[14,16,20–22,27,28,32,54,55] It was the intent of this work to make available a new analyzer using **1**, or related dyes, and a solid-state ratiometric fluorometer, while studies of O_2 quenching in a variety of plasticizer combinations are under way.[56] The CA/75% TEC polymer was chosen for this study since it was easily cast into films and allowed instrument development at readily available oxygen concentrations and pressures.[16,18]

Excitation of **1** in CA/75% TEC under N_2 resulted in the dual emission characteristic of this family of complexes (Fig. 2).[41] The singlet and triplet maxima are at 560 and 675 nm, respectively. The triplet/singlet ratio under N_2 is ≈1.03. The selective loss of the ^{3}ILCT* emission in air results in a drop of the triplet/singlet ratio to 0.62. Excitation under O_2 results in nearly complete loss of the ^{3}ILCT* emission. During the lifetime measurements, the singlet emission was strongly filtered—the fractional intensities of ^{1}ILCT* under nitrogen and air were 0.027 and 0.085, respectively. The ^{1}ILCT* lifetime was 0.5 ns. While the average ^{3}ILCT* lifetime under N_2 was 14 μs, it decreased to 4.6 μs in air. Accompanying the decrease in lifetime was a phase shift decrease of >20° at 5×10^4 Hz (Fig. 3). The observations are consistent with oxygen quenching of the ^{3}ILCT*.

Ratiometric Detection of Oxygen. For the evaluation of the performance of the ratiometric fluorometer, oxygen quenching of polymer encapsulated **1** was monitored by using both the ratiometric fluorometer and an SLM AB2 spectrofluorometer. In both experiments the oxygen concentration was gradually increased from 0 to 100% O_2 and the triplet/singlet intensity ratio determined at each point. The data from both experiments were corrected for residual fluorescence and background at 675 nm by using a linear progression.[†] The baseline-corrected data were used to generate the ratiometric Stern–Volmer plots $(I_0'/I') - 1$ vs. percent O_2, where $(I_0'/I') - 1 = ({}^3I_0{}^1I)/({}^1I_0{}^3I) - 1 = k_q\tau[O_2]$) (Fig. 4). The plots for the two methods are in extremely good agreement. From the slopes of the Stern–Volmer plots the $P_{O_2(1/2)}$ values are estimated to be 9.1 and 9.6% O_2 (with $P_{O_2(1/2)} = 69$ and 73 torr) with the use of the SLM AB2 and the ratiometric device, respectively. Several studies of O_2 quenching of long-lived emitters have Stern–Volmer plots that deviate from linearity. The linearity of the Stern–Volmer plots of **1** could be attributed to one of several factors: (1) single-site allocation of the dye, (2) multiple sites with common lifetimes, or (3) high solubility of the dye in the plasticizer. $P_{O_2(1/2)}$, the point of 50% emission loss, is a function of the sensor dye and the encapsulating polymer. As expected, $P_{O_2(1/2)}$ is independent of the method of measurement, validating the performance of the ratiometric fluorometer as a detector for the dual emitting dye, **1.** With the upper limit of detection set at $I_0'/I' = 10$, the dynamic range is approximately 0–90% O_2 for **1** in the CA/75% TEC polymer. This range can be easily adjusted by varying the polymer, the plasticizer, or the emitter.[14,16,20–22,27,28,32,54,55] A summary of the sensor's characteristics, as well as a comparison with the lifetime-based system, is given in Table I.

The high response time of the sensor is reasonable considering the diffusion coefficient of the material used/for support (CA) and its thickness (0.5 mm). The accuracy and the limit of detection are comparable to those of the lifetime-based system. It should be kept in mind that they both could be enhanced significantly by using readily available lock-in amplifier boards.[‡] However, this approach would increase the cost of the device (which is now below $100) approximately tenfold.

The reproducibility and stability of the ratiometric analyzer were probed by repeatedly varying the oxygen concentration in three steps from 0–15%, 15–100%, and 100–0% O_2 at one atmosphere total O_2/N_2 pressure. The oxygen concentration was maintained at a given level for 15 min and monitored every 3 min. The cuvette containing the sensor was purged with a new oxygen concentration over a 3 min detection cycle every six cycles. As can be seen from Fig. 5, the ratiometric measurement varied by only 0.01 over the course of the study.

The measured ratio (at constant oxygen concentration) is independent of the intensity of the illumination source. The LED optical power (after filtering) was varied by changing the LED current (5–20 mA), in the range 50–170 μW. The corresponding changes in the output of the analyzer were 4.2%.

Intentional photobleaching, resulting in ≈20% loss of the emitter molecule, had essentially no effect upon $P_{O_2(1/2)}$ or the response of the dye to variations in oxygen concentration. In a long-term stability test, the measured output ratio changed 3.2% over 5 h of steady-state illumination. The analyzer has an additional advantage in that it permits monitoring of the extent of photobleaching of the dual emitting dye. While the ratio of the integration interval is independent of fluorophore concentration, the length of the integration is directly proportional to fluorophore concentration. Hence, measurement of the square

---

[†] The baseline correction was performed by using a modified Stern–Volmer equation with $(R_0 - B)/(R - B) = 1 + k_{SV}*[O_2]$, where R_0 and R are the triplet/singlet ratios in absence and presence of oxygen, B is the background (originating from the scattered light and peak overlaping), k_{SV} is the Stern–Volmer coefficient, and $[O_2]$ is the oxygen concentration. The equations $(R_{nitrogen} - B)/(R_{oxygen} - B) = 1 + k_{SV}*1$ and $(R_{nitrogen} - B)/(R_{air} - B) = 1 + k_{SV}*0.209$ where 1 and 0.209 are the fractional $[O_2]$ in 100% O_2 and air, were solved for B, which was assumed to be constant.

[‡] There are many manufacturers of dual-phase lock-in amplifier boards: Perkin Elmer (Oak Ridge, TN), Electrosolutions (Flemington, NJ), Scitec (Cornwall, England), Femto Messtechnik (Berlin, Germany), etc.

wave frequency allows degradation of the fluorophore to be estimated.[43]

CONCLUSION

A new class of room-temperature dual emitters, the heterocyclic-substituted platinum 1,2-enedithiolates, has been used with a solid-state diode-based ratiometric fluorometer for the measurement of molecular oxygen. The polymer immobilized dual emitter and the device collectively allow oxygen levels to be measured by monitoring the oxygen quenching of a long-lived emissive state relative to a short-lived nonquenchable emissive state. While such ratiometric measurements are possible with the use of a standard fluorometer, the device described is a lower cost alternative. Furthermore, the device is compact, robust, and immune to the effects of ambient light.[42] This method, like frequency-modulated phase detection, eliminates problems encountered with simple intensity-based methods. Given that the dual emission arises from a single dye, it is superior to methods where two dyes are used to generate the ratio.

While this paper describes an oxygen analyzer, the triplet state of these dual emitting dyes can be selectively quenched by a number of electron, proton, hydrogen atom, and energy transfer processes.[41,51,52] As such, monitoring triplet quenching of dual emitters in this family with a ratiometric fluorometer could allow the development of new methods for a range of analytes. The ability of the dual emitters to work under steady-state illumination could be of great advantage in such fields as microscopy or in multisensor arrays.[58]

ACKNOWLEDGMENTS

We thank Professor Neil Blough, University of Maryland, College Park, for helpful discussions. We are indebted to the NIH (RR10955), the American Chemical Society for the Petroleum Research Fund (Grant No. 28499-G3), Exxon Education Foundation, Genentech, Merck, and Pfizer for supporting this research. This material is based in part upon work supported under a National Science Foundation Graduate Fellowship to Peter Harms.

---

1. L. C. Clark, Trans. Am. Soc. Art. Int. Organs **2,** 41 (1956).
2. Y. H. Lee, and G. T. Tsao, ‘‘Dissolved Oxygen Electrodes’’, in *Advances in Biochemical Engineerring,* T. K. Ghose, A. Fiechter, and N. Blackebrough, Eds. (Springer-Verlag, Berlin, 1979), p. 35.
3. G. Rao, S. B. Bambot, S. C. W. Kwong, H. Szmacinski, J. Sipior, R. Holavanahali, and G. Carter, ‘‘Applications of Fluorescence Sensing to Bioreactors’’, in *Topics in Fluorescence Spectroscopy,* J. R. Lakowicz, Ed. (Plenum Press, New York, 1994), p. 417.
4. G. K. McMillian, Chem. Eng. Prog. **87,** 30 (1991).
5. O. S. Wolfbeis, I. Klimant, T. Werner, C. Huber, U. Kosch, C. Krause, G. Neurauter, A. Durkop, Biosens. Bioelect. **51,** 17 (1998).
6. J. R. Bacon and J. N. Demas, Anal. Chem. **59,** 2780 (1987).
7. E. R. Carraway, J. N. Demas, B. A. DeGraff, and J. R. Bacon, Anal. Chem. **63,** 337 (1991).
8. J. N. Demas, B. A. DeGraff, and W. Xu, Anal. Chem. **67,** 1377 (1995).
9. P. Hartmann, M. J. P. Leiner, and M. E. Lippitsch, Anal. Chem. **67,** 88 (1995).
10. P. Hartmann and W. Trettnak, Anal. Chem. **68,** 2615 (1996).
11. H. D. Hendricks, Mol. Phys. **20,** 189 (1971).
12. I. Klimant and O. S. Wolfbeis, Anal. Chem. **67,** 3160 (1995).
13. E. D. Lee, T. C. Werner, and W. R. Seitz, Anal. Chem. **59,** 279 (1987).
14. X.-M. Li and K.-Y. Wong, Anal. Chim. Acta **262,** 27 (1992).
15. X.-M. Li, F.-C. Ruan, and K.-Y. Wong, Analyst **118,** 289 (1993).
16. H. N. McMurray, P. Dougals, C. Busa, and M. S. Garley, J. Photochem. Photobiol. **80,** 283 (1994).
17. A. Mills and Q. Chang, Analyst **118,** 839 (1993).
18. A. Mills and F. C. Williams, Thin Solid Films **306,** 163 (1997).
19. A. Mills and M. Thomas, Analyst **122,** 63 (1997).
20. A. Mills, A. Lepre, and L. Wild, Anal. Chim. Acta **362,** 193 (1998).
21. A. Mills and M. D. Thomas, Analyst **123,** 1135 (1998).
22. A. Mills, Biosens. Bioelect. **51,** 60 (1998).
23. Y. Kostov, A. Comte, and T. Scheper, Chem. Biochem. Eng. Q. **12,** 201 (1998).
24. D. B. Papkovsky, G. V. Ponomarev, W. Trettnak, and P. O’Leary, Anal. Chem. **67,** 4112 (1995).
25. J. I. Peterson, R. V. Fitzgerald, and D. K. Buckhold, Anal. Chem. **56,** 62 (1984).
26. C. Preininger, I. Klinmant, and O. S. Wolfbeis, Anal. Chem. **66,** 1841 (1994).
27. N. Velasco-Garcia, M. J. Valencia-Gonzalez, and M. E. Diaz-Garcia, Analyst **122,** 1405 (1997).
28. H. Chuang and M. A. Arnold, Anal. Chem. **69,** 1899 (1997).
29. H. Chuang and M. A. Arnold, Anal. Chim. Acta **368,** 83 (1998).
30. O. S. Wolfbeis, M. J. P. Leiner, and H. E. Posch, Mikrochim. Acta **111,** 359 (1986).
31. O. S. Wolfbeis, L. J. Weis, M. J. P. Leiner, and W. E. Zielgler, Anal. Chem. **60,** 2028 (1988).
32. W. Xu, R. C. McDonough, B. Langsdorf, J. N. Demas, and B. A. DeGraff, Anal. Chem. **66,** 4133 (1994).
33. D. B. Papkovsky, A. N. Ovchinnikov, V. I. Ogurtsov, G. V. Ponomarev, and T. Korpela, Biosens. and Bioelect. **51,** 137 (1998).
34. S. B. Bambot, G. Rao, M. Ramauld, G. Carter, J. Sipior, E. Terpetschnig, and J. R. Lakowicz, Biosens. Bioelect. **10,** 643 (1995).
35. S. B. Bambot, R. Holaranahali, J. R. Lakowicz, G. M. Cartes, and G. Rao, Biotech. Bioeng. **43,** 1139 (1994).
36. H. Szmacinski and J. R. Lakowicz, ‘‘Lifetime-Based Sensing’’, in *Topics in Fluorescence Spectroscopy,* J. R. Lakowicz, Ed. (Plenum Press, New York, 1994), Chap. 10, p. 295.
37. P. Hartmann, M. P. J. Leiner, and P. Kohlbacher, Biosens. Bioelect. **51,** 196 (1998).
38. R. B. Thompson and J. R. Lakowicz, Anal. Chem. **65,** 853 (1993).
39. V. I. Ogurtsov and D. B. Papkovsky, Biosens. Bioelect. **51,** 377 (1998).
40. S. B. Bambot, J. R. Lakowicz, and G. Rao, Trends Biotech. **13,** 83 (1995).
41. K. A. Van Houten, D. C. Heath, C. A. Barringer, A. L. Rheingold, and R. S. Pilato, Inorg. Chem. **37,** 4647 (1998).
42. K. A. Van Houten, D. C. Heath, N. V. Blough, and R. S. Pilato, U.S. patent pending (1999).
43. Y. Kostov and G. Rao, Rev. Sci. Instrum. **70,** 4466 (1999).
44. S. L. R. Barker, H. A. Clark, S. F. Swallen, R. Kopelman, A. W. Tsang, and J. A. Swanson, Anal. Chem. **71,** 1767 (1999).
45. I. Koronczi, J. Reichert, G. Heinzmann, and H. J. Ache, Sens. Act. B. **51,** 188 (1998).
46. S. Jayaraman, J. Bilwersi, and A. S. Verkman, Cell Phys. **45,** C747 (1999).
47. K. A. Van Houten and R. S. Pilato, ‘‘Photophysical and Photochemical Properties of Metallo-1,2-enedithiolates’’, in *Molecular and Supramolecular Photochemistry: Multimetallic and Macromolecular Inorganic Photochemistry,* V. Ramamurthy and K. S. Schanze, Eds. (Marcel Dekker, New York, 1999), Vol. 4, p. 185.
48. J. Sipior, G. M. Carter, and J. R. Lakowicz, G. Rao. Rev. Sci. Instrum. **67,** 3795 (1996).
49. K. A. Van Houten and R. S. Pilato, J. Am. Chem. Soc. **120,** 12359 (1996).
50. S. P. Kaiwar, J. K. Hsu, L. M. Liable-Sands, A. L. Rheingold, and R. S. Pilato, Inorg. Chem. **36,** 4234 (1997).
51. S. P. Kaiwar, A. Vodacek, N. V. Blough, and R. S. Pilato, J. Am. Chem. Soc. **119,** 3311 (1997).
52. S. P. Kaiwar, A. Vodacek, N. V. Blough, and R. S. Pilato, J. Am. Chem. Soc. **119,** 9211 (1997).
53. S. P. Kaiwar, J. K. Hsu, A. Vodacek, G. Yap, L. M. Liable-Sands, A. L. Rheingold, and R. S. Pilato, Inorg. Chem. **36,** 2406 (1997).
54. A. Mills and A. Lepre, Anal. Chem. **69,** 4653 (1997).
55. O. S. Wolfbeis, ‘‘Oxygen Sensors,’’ in *Fiber Optic Chemical Sensors and Biosensors* (CRC Press, Boston, 1991), p. 19.
56. Y. V. Kostov, R. S. Rilato, and G. Rao, unpublished results.
57. G. Holst, R. N. Glud, M. Kuhl, and I. Klimant, Sens. Actuators B **38–39,** 122 (1997).
58. D. R. Walt, Science **287,** 451 (2000).