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Ratiometric oxygen sensing: detection of dual-emission ratio through a single emission filter

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A new method and device for the ratiometric measurement of oxygen concentration are presented. They are based on the use of a dual-emission oxygen-sensitive dye. The method allows the exclusion of the influence of emission overlap. The detection of the dual-emission ratio is performed using a single long-pass emission filter. The device described is simpler than the widely used lifetime instruments and could easily be a stand-alone low-cost instrument.

Introduction

The measurement of dissolved oxygen concentrations is critical to many industrial, biomedical and environmental monitoring applications. Recent advances in fiber optics and semiconductor sources and detectors have made optical O_2 detection an attractive and reliable method. Oxygen sensors based on luminescence quenching[1–4] allow for reversible and sensitive measurement without consuming the analyte.

Dyes suitable as O_2 indicators exhibit long-lived excited states.[5–34] A broad variety of indicators could be used, such as phosphorescent organic molecules (including polycyclic aromatic hydrocarbons[5,6] and camphorquinone[34]), Pd(II) or Pt(II) porphyrin complexes[7,8] and Ru(II) complexes with polyaza polycyclic chelating ligands.[9–33] The lifetimes of the above compounds span from hundreds of nanoseconds to several milliseconds. All of the above mentioned dyes change in luminescent intensity and lifetime when exposed to different oxygen concentrations. However, the simple intensity measurements are unreliable, as all the dyes suffer from photodecomposition.[5–34] Furthermore, instability of the source or detector, scattering or even minor optical changes can become sources of error.[35–37]

Lifetime measurements have been shown to eliminate most of the problems with intensity measurements. This is because the lifetime of the dye in the absence of oxygen is an intrinsic property, and serves as an internal reference. By modulating the excitation and using a time- or frequency-domain fluorimeter, quenching of the excited state can be measured with great accuracy.[12,14,18–27,31–33] Lifetime methods are very reliable, but the instruments required are sophisticated.

Ratiometric measurements are an alternative to the lifetime detection.[38] They are based on a different kind of internal reference, and the method is well established for measuring pH, Ca^{2+}, Mg^{2+}, Zn^{2+} and heavy metals.[39–47] As the analyte concentration changes, the excitation and/or emission spectrum of the dye changes.[47] The luminescence of the dye is measured at two different (excitation or emission) wavelengths and the ratio of these measurements is related to the analyte concentration.[39–47] The ratio is an intrinsic property of the dye, so it is not affected by photobleaching or dye concentration. Therefore, it is also free from most of the interferences attributed to simple intensity measurements. The method uses significantly simpler instrumentation than the lifetime determination, as it relies on steady-state fluorescence measurements.

Until recently, ratiometric oxygen detection was not used because of the lack of proper indicators. The appearance of a new class of oxygen-sensitive dyes, platinum 1,2-enedithiolates,[48,49] now permits ratiometric measurements of O_2. The dyes possess two distinct emission peaks with differing sensitivity to oxygen quenching. They are luminescent at room temperature, both in solution and when immobilized in plastic films. The dyes have two emissive states, assigned to intraligand charge transfer (ILCT*) singlet and triplet. The lifetimes of the singlet and triplet are 0.5 ns and 15 μs, respectively, when measured in cellulose acetate. The usual lifetime-based method (using the quenching of the triplet only) works poorly with these dyes, since the significant overlap of the singlet and triplet emissions strongly reduces the change of the apparent lifetime upon quenching. However, as the triplet is strongly quenched by oxygen, and the singlet is not, highly sensitive ratiometric O_2 measurements are possible. One member of the class, [1,2-bis(diphenylphosphino)ethane–Pt{$S_2C_2(CH_2CH_2$-*N*-2-pyridinium)}][BPh_4], dppe-Pt2P, was recently used to create a novel ratiometric oxygen analyzer.[50]

A significant drawback of the ratiometric analyzers is that they require two wavelength-selection devices (monochromators or filters) to distinguish between the peaks. This decreases the measured light intensity and complicates the instrument design. Furthermore, it significantly increases the cost of the system, as these optical components can be the most expensive part of the instrument.

In this paper, we present a new ratiometric method for oxygen sensing which utilizes only one optical filter and frequency selection of the emissions. The approach utilizes the large difference between the lifetimes of the two excited states by switching between two frequencies of excitation modulation. Using the method, it is possible to resolve the singlet and triplet emissions and exclude any overlapping effects. A device that operates according to the method is also presented. It uses only one emission filter and is capable of operating in room light.

Theory

Let the fluorescent sample emit two different emissions with steady state intensities A_1 and A_2 and lifetimes τ_1 and τ_2 and $\tau_1 = 1000\tau_2$. The ratio A_1/A_2 is to be determined.

If the sample is excited with modulated light, the ac amplitude A of the emission will be dependent on the excitation

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frequency, the lifetimes and the steady state intensities by the equation[51,52]

$$A = \frac{A_1}{\sqrt{1+\omega^2\tau_1^2}} + \frac{A_2}{\sqrt{1+\omega^2\tau_2^2}} \quad (1)$$

Suppose that $\omega_1 = 0.1/\tau_1$. Then,

$$A_{\omega_2} = \frac{A_1}{\sqrt{1.01}} + \frac{A_2}{\sqrt{1.00000001}} \quad (2)$$

$$A_{\omega_1} = 0.995A_1 + A_2 \approx A_1 + A_2 \quad (3)$$

Thus, at frequency ω_1 the amplitude of the total emission is equal to the sum of both partial emissions.

Now, suppose that $\omega_2 = 100/\tau_1$. Then,

$$A_{\omega_2} = \frac{A_1}{\sqrt{10001}} + \frac{A_2}{\sqrt{1.01}} \quad (4)$$

$$A_{\omega_2} = 0.0099A_1 + 0.995A_2 \approx A_2 \quad (5)$$

Hence, at frequency ω_2 the long–lifetime emission is almost completely demodulated, whereas the short-lifetime emission still retains significant modulation. Consequently,

$$\frac{A_1}{A_2} = \frac{A_{\omega_1}}{A_{\omega_2}} - 1 \quad (6)$$

Experimental

Preparation of the film

The dye, dppe-Pt2P (Fig. 1), was synthesized according to the published procedure[48] and immobilized in plastic film. The immobilization was performed as follows: 1 g of cellulose acetate was dissolved in 15 ml of acetone, then 1 g of triethyl citrate and 2 mg of dppe-Pt2P were added and the solution was thoroughly mixed. The mixture was cast on a glass dish and left overnight. The resulting film had an average thickness of 0.5 mm. All the materials were purchased from Aldrich (Milwaukee, WI, USA) and used without further purification.

Spectral and lifetime measurements

The steady state excitation and emission spectra were taken on an MPF 66 fluorescence spectrofluorimeter (Perkin-Elmer, Norwalk, CT, USA). Phase-modulation lifetime measurements were performed on an ISS Koala instrument (ISS, Urbana, IL, USA), modified to use a blue light-emitting diode (LED) as excitation source.[53] The lowest frequency of the lifetime fluorimeter is 10 kHz, so measurements between 100 Hz and 10 kHz were performed on the ISS Koala optical workbench using an EG&G Princeton Applied Research (Princeton, NJ, USA) Model 5110 lock-in amplifier to measure phase and ac and a D-990 digital multimeter (Protek, Northvale, NJ, USA) to measure dc. Modulation was calculated off-line from sample and reference ac and dc values. A 4000 mcd blue LED MBB51TAH-T (Micro Electronics, Santa Clara, CA, USA) with peak wavelength 470 nm and 40 nm FWHM was used as the excitation source. Its light was filtered with the following set of filters: 500FL07, 550FL07 and 650FL07. These are band stop filters with absorption ~3 in the stop band, width of the band 150 nm, the first number indicating the lower limit of the band stop. The set acts as low-pass filter, eliminating the 'red tail' of the blue LED. A Model 550FH90 long-pass filter was used as an emission filter. It has absorption ~4 in the stop band and cut-off 550 nm. All the filters were obtained from Andover (Salem, NH, USA).

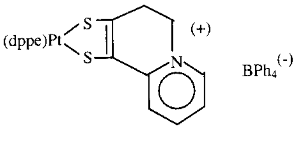

Fig. 1 Structure of the fluorescent dye dppe-Pt2P. dppe = 1,2-bis-(diphenylphosphino)ethane; BPh_4 = tetraphenylborate.

Prototype device

The prototype device was constructed in two parts, hardware and software. The hardware (Fig. 2) consisted of a high current LED driver, fast photodiode detector with a 550 nm long pass filter and PC equipped with PCI 6111E multipurpose A/D card (National Instruments, Austin, TX, USA). The LED driver provided 100 mA of current at frequencies from 10 Hz to 1 MHz. The photodiode detector had overall transimpedance amplification 50 MΩ and cut-off frequency 3 MHz. The A/D card provided a signal at 400 Hz or 100 kHz to the LED driver and acquired analog data from the detector. When 400 Hz was used, the A/D conversion was done at 20 kSPS (SPS = samples per second), and 5 MSPS (the maximum rate of the board) was used for 100 kHz, thus providing the same number of samples per waveform, resulting in equal signal-to-noise ratios at both frequencies.

The software (Fig. 3) collected two data sets, one at each frequency. Next, the average ac component of each data set was determined. Finally, the calculation $(ac_{400\ \mathrm{Hz}} - ac_{100\ \mathrm{kHz}})/ac_{100\ \mathrm{kHz}}$ was performed to extract the triplet-to-singlet ratio.

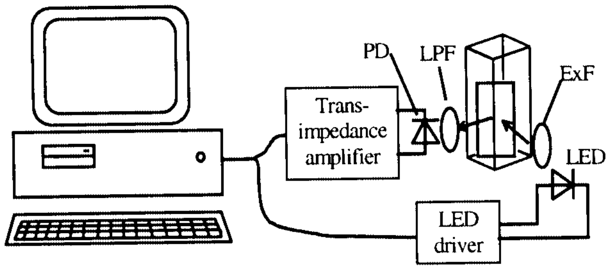

Fig. 2 Hardware set-up of the instrument. The computer controlled the modulation frequency of the LED excitation and measured the output of the photodiode detector detector *via* an A/D card. PD, photodiode; LPF, long–pass filter; ExF, excitation filter.

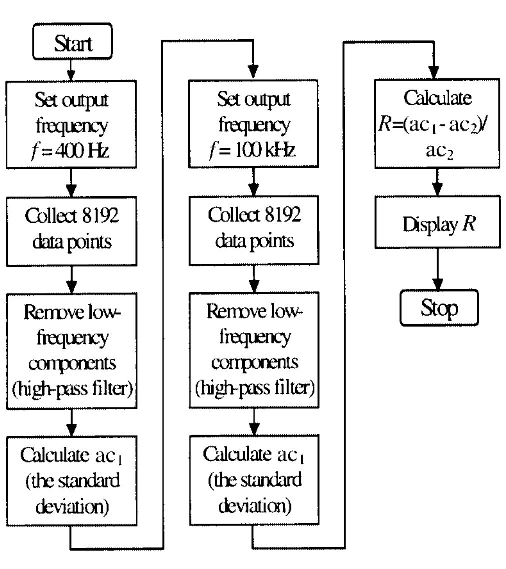

Fig. 3 Algorithm for data processing and determination of ac amplitudes and triplet-to-singlet ratio.

Results and discussion

The dye has two excitation and two emission maxima (Fig. 4). The 470 nm emission of the low-cost blue LED is well suited as an excitation source for this dye. The uncorrected emission spectra are shown at different oxygen concentrations. The triplet intensity (λ_{max} = 680 nm) depends strongly on the oxygen concentration, whereas the singlet intensity (λ_{max} = 570 nm) is essentially unaffected. The triplet intensity decreases approximately 2.5-fold on changing from nitrogen to oxygen; this makes the dye a good sensor for oxygen ratiometric measurements.

However, there is a significant overlap between the peaks. This complicates the straightforward ratiometric detection, as the data for both peaks should be corrected for the extent of intersection prior to ratio calculation. As the degree of the overlap changes with the oxygen concentration, the correction is even more difficult.[50]

Direct lifetime measurements are also cumbersome, as the phase behavior of the dye is complicated by the presence of the short lifetime (Fig. 5, phase data). The frequency–phase plot has a bell-shaped form, complicating the sensor calibration. The maximum phase shift between air and nitrogen occurs at 6.3 kHz. Unfortunately, it is only 16°, only half that observed with most of the Ru based sensors. This phase shift was observed under conditions where most of the singlet intensity was filtered. It would be even smaller if the total emission was observed.

The above mentioned considerations show that for an accurate and linear calibration curve, a different approach is required. One possibility is the so-called 'modulation sensing'.[54] However, this requires measurements of the dc component of the fluorescence. In practical sensing applications it is strongly affected by the ambient light. An optical protective layer over the sensing layer[14] helps, but increases the response time of the sensor. For this reason, the alternative method described in the Theory section was developed. This method allows the measurements to be made even in strong ambient light.

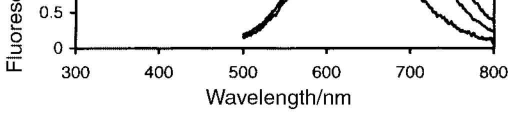

Fig. 4 Uncorrected excitation (left) and emission (right) spectra of (dppe)-Pt2P immobilized in CA–100% TEC film. The excitation spectrum was obtained observing the emission at 680 nm; the emission spectrum was obtained with excitation at 440 nm.

To develop this method, proper selection of the modulation frequencies of the excitation light was required. The low modulation frequency should be sufficiently low to modulate completely both the singlet and the triplet and the high modulation frequency should be sufficiently high to demodulate the triplet completely. For this reason, the frequency responses of the immobilized dye in nitrogen and air were investigated (Fig. 5, modulation data). At very low frequencies it does not depend on the oxygen concentration; however, after 100 kHz the modulation has a plateau. The percentage of the modulation in this frequency region is directly related to the measured oxygen concentration. Based on this data, the high and low modulation frequencies were chosen to be 400 Hz and 100 kHz, respectively.

A prototype device was designed to perform this novel method of measurement. Only bare essentials (excitation source, fast photodetector and A/D card) were realized in hardware, most of the method being performed through software. The device was tested with gas mixtures of known oxygen concentration (Fig. 6). The Stern–Volmer plot is almost linear (correlation coefficient, r = 0.9979), possibly because of the high concentration of plasticizer used (100% of the weight of the cellulose acetate). There are on-going experiments to characterize the dye in different polymers and at different plasticizer concentrations.[55]

The time response of the sensor is shown in Fig. 7. The response time was approximately 3 min, which is adequate for most oxygen measurements and is perfectly reasonable considering the thickness of the film (0.5 mm) and the low diffusion coefficient of oxygen through cellulose acetate.

Conclusion

The presented principle of oxygen detection is robust, as it relies on modulation of the fluorescence at two different frequencies. By choosing frequencies in the modulation plateaus, the output signal is independent of frequency instabilities. Lock-in techniques could easily be added to increase the signal-to-noise ratio and more accurately determine the modulation amplitudes. Also, the algorithm could be easily performed using solid-state multipliers (AD633 or similar) or a microcontroller (PIC) with on-chip A/D converters. Since the instrument uses low-cost all-

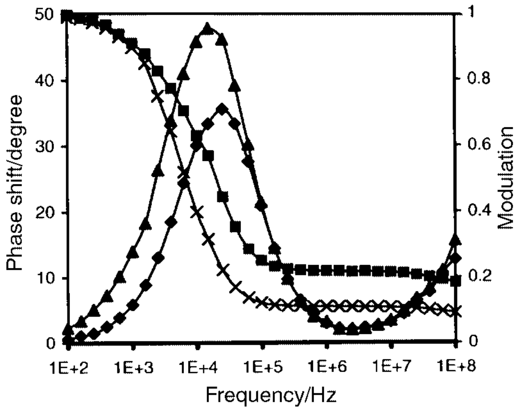

Fig. 5 Frequency-domain behavior of the sensor. ▲, Phase shift in N_2; ◆, phase shift in air; ×, modulation in N_2; ■, modulation in air.

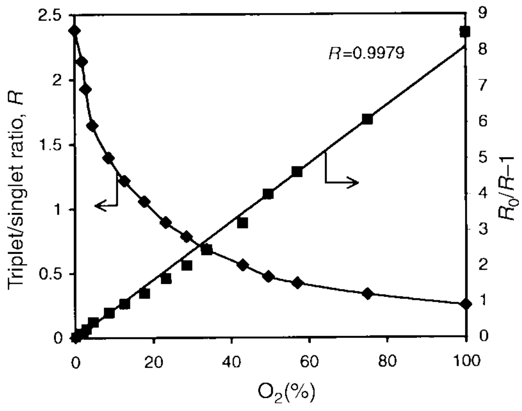

Fig. 6 Calibration curves for the oxygen sensor. ◆, Triplet-to-singlet ratio; ■, Stern–Volmer plot of the ratio.

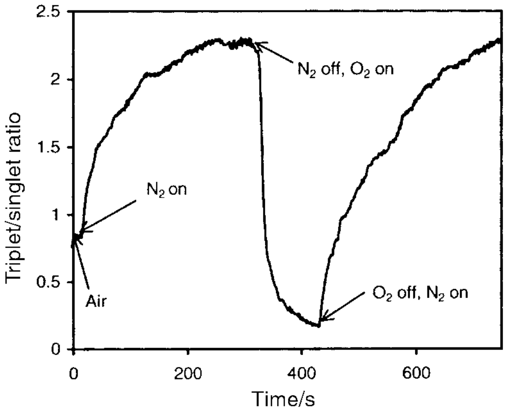

Fig. 7 Time response of the sensor.

semiconductor excitation and emission modules, a stand-alone instrument that costs less than $100 could be easily created.

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