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Polarization-based oxygen sensor

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Abstract

A new approach to oxygen sensing based on the luminescence polarization observed from a novel type of sensor is described. The oxygen sensor consists of an oxygen-sensitive silicone film containing tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride [Ru(dpp)₃Cl₂] and an oxygen-insensitive film of Styryl 7 in poly(vinyl alcohol). Polarizers are used to select orthogonally polarized emission components from Ru(dpp)₃Cl₂ and Styryl 7. The polarization of the combined emission was found to be highly sensitive to the partial pressure of oxygen. This method of polarization sensing is generic and can be used with any fluorophore which displays an analyte-dependent change in intensity.

Introduction

Fluorescence methods have been developed for detection of a wide variety of analytes.^{1–7} Typical analytes detectable using suitable fluorescent probes include metal ions, chloride and other halogens, phosphate, citrate and glucose. One of the earliest applications of fluorescence sensing was the detection of oxygen by quenching of fluorophores with long luminescence lifetimes. The first practical oxygen sensors appear to have been developed by Bacon and Demas⁸ using transition metal–ligand complexes which display luminescence decay times on the microsecond time-scale. The sensors consist of the metal–ligand complex dissolved in an oxygen permeable silicone polymer. Oxygen from a gaseous or aqueous phase dissolves in the silicone in proportion to its partial pressure, reducing the luminescence intensity of the metal–ligand complex according to the Stern–Volmer equation.

Since the initial reports of oxygen sensing using metal–ligand complexes there have been numerous additional reports on this topic,^{9–18} to mention only the more recent references. Because of the long luminescence lifetime, the metal–ligand complexes have also been used for lifetime-based sensing of oxygen.^{19–22} Most of these oxygen sensors are based on various salts of tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II), [Ru(dpp)₃]²⁺, which displays one of the longest observed lifetimes for a ruthenium metal–ligand complex near 5–6 μ s.

In this paper we describe an alternative approach to oxygen sensing. We developed a combination sensor (Scheme 1), which contains both an oxygen-sensitive film of Ru(dpp)₃Cl₂ in silicone, and an oxygen-insensitive film of 2-[4-(4-dimethylaminophenyl)buta-1,3-dienyl]-3-ethylbenzothiazolium *p*-toluenesulfonate (Styryl 7) in poly(vinyl alcohol) (PVA). Polarizers are used to select the vertically and horizontally polarized components of the emission from Styryl 7 and Ru(dpp)₃Cl₂, respectively. Oxygen-dependent changes in the intensity of Ru(dpp)₃Cl₂ result in changes in the polarization, which was found to be a sensitive indicator of the oxygen concentration.

Theory

The theory of polarization sensing is relatively simple, and has been described in a recent paper.²³ However, this concept is new and not widely understood. Hence, we describe the theory in moderate detail.

Consider the sensor shown in Scheme 1. The oxygen sensitive film of Ru(dpp)₃Cl₂ is contained within the cuvet and can thus be exposed to various partial pressures of oxygen. A polarizer ($P_{\perp}$) selects for the horizontally polarized component of the emission from the metal–ligand complex.

The second part of the sensor is a film of PVA containing Styryl 7. Because of the short lifetime of Styryl 7, the intensity from this film is not sensitive to oxygen. This film is stretched to orient the Styryl 7 molecules. As will be shown below, the emission from the Styryl 7-PVA film is highly polarized in the vertical direction, almost independent of the polarization of the excitation light.

Oxygen sensing is accomplished by measuring the polarization of the emission using an analyzed polarizer. The excitation polarized at 45° from the vertical provided measurable emission from both Ru(dpp)₃Cl₂ and Styryl 7. The polarization of the combined emission from the sample and reference is given by

$$P = \frac{I_{\parallel}^T - I_{\perp}^T}{I_{\parallel}^T + I_{\perp}^T}$$

(1)

where the superscript T indicates the sum of the intensities from the Ru(dpp)₃Cl₂ sample and Styryl 7 reference. The subscripts indicate the component parallel (∥) and perpendicular (⊥) to the laboratory vertical axis. The total parallel intensity is given by

$$I_{\parallel}^T = I_{\parallel}^R + I_{\parallel}^S$$

(2)

where the superscripts R and S refer to the reference film and sample, respectively. Similarly,

$$I_{\perp}^T = I_{\perp}^R + I_{\perp}^S$$

(3)

Each intensity depends on a number of chemical and instrumental factors, which we did not explicitly indicate to simplify the presentation.

The use of a polarizer ($P_{\perp}$) in front of the $\text{Ru(dpp)}_3\text{Cl}_2$ sample results in elimination of the vertical component, so that $I_{\parallel}^S = 0$ and $I_{\parallel}^T = I_{\parallel}^R$. Substitution of eqns. (2) and (3) into the definition of polarization [eqn. (1)] yields

$$P = \frac{I_{\parallel}^R - I_{\perp}^R - I_{\perp}^S}{I_{\parallel}^R + I_{\perp}^R + I_{\perp}^S}$$

(4)

The measured polarization depends on the relative intensity of the sample with respect to that of the reference film. If the sample fluorescence is zero then the polarization is given by

$$P = \frac{I_{\parallel}^R - I_{\perp}^R}{I_{\parallel}^R + I_{\perp}^R} = P_R$$

(5)

which is that found for the reference film (P_R). If the observed intensity is dominated by the sample emission, then the polarization approaches a value of -1 :

$$P = \frac{-I_{\perp}^S}{I_{\perp}^S} = -1$$

(6)

The reference films typically display polarization values (P_R) near 0.8. Hence one may expect a wide range of polarization values for this sensor configuration.

Principle of sensor operation

The operational principle of the sensor can be understood by recognizing that we are not measuring the polarization of the emission of $\text{Ru(dpp)}_3\text{Cl}_2$. The $\text{Ru(dpp)}_3\text{Cl}_2$ is in an unoriented silicone film, and its emission is mostly unpolarized. The perpendicular polarizer ($P_{\perp}$) transmits only the horizontally polarized light from $\text{Ru(dpp)}_3\text{Cl}_2$, so the polarization of this component is -1 irrespective of the polarization of the incident light or of the emission from $\text{Ru(dpp)}_3\text{Cl}_2$.

In a similar manner, the emission from Styryl 7 is always vertically polarized. In this case the polarization is due to orientation of the Styryl 7 molecules along the z -axis. For such oriented samples this emission is polarized approximately the same, irrespective of the polarization of the incident light.

When used as a sensor, one measures the combined emission from the stretched film (SF) and from $\text{Ru(dpp)}_3\text{Cl}_2$. These emissions have polarizations of approximately $+1$ and -1 , respectively. The intensity of Styryl 7 is not sensitive to oxygen, whereas the intensity of $\text{Ru(dpp)}_3\text{Cl}_2$ is highly sensitive to oxygen. The intensities of Styryl 7 and $\text{Ru(dpp)}_3\text{Cl}_2$ are adjusted so that $\text{Ru(dpp)}_3\text{Cl}_2$ is the dominant emission in the absence of oxygen, so the polarization is at a minimum. As the partial pressure of oxygen increases the intensity of $\text{Ru(dpp)}_3\text{Cl}_2$ decreases, so the relative intensity of Styryl 7 increases. Consequently, the polarization of the combined emission increases at higher partial pressures of oxygen.

Experimental

All experiments were performed using sensors configured as shown in Scheme 1. Excitation was effected with the 514 nm output of an air-cooled argon ion laser. The laser excitation was polarized 45° from the vertical. The emission was observed through an analyzer polarizer oriented parallel ($\parallel$) or perpendicular ($\perp$) to the laboratory vertical axis. When using an oriented film there is no need to use an excitation polarizer because the emission is highly polarized with polarized or unpolarized excitation. The polarization is given by

$$P = \frac{I_{\parallel} - GI_{\perp}}{I_{\parallel} + GI_{\perp}}$$

(7)

where $I_{\parallel}$ and $I_{\perp}$ are the intensities observed with emission polarizer parallel or perpendicular to the polarized excitation, respectively, and the G factor is the ratio of intensities ($I_{\parallel}/I_{\perp}$) observed with a long lived fluorophore which displays an anisotropy of zero.²⁴ In our apparatus, the G factor was near 1.0.

The oxygen sensor was prepared as described previously.²¹ The ruthenium complex was synthesized in the laboratory. The silicone membrane was prepared by spreading a thin layer of GE Silicone II (Stock CE 5000, GE Silicones, General Electric Company, Waterford, NY, USA) on a microscope slide using a blade. The thin layer (about 0.5 mm thick) was allowed to cure overnight and was then removed from the glass surface, resulting in a thin, transparent, silicone membrane. The membrane was then soaked in a solution of the ruthenium complex in chloroform (typically 1.25 mg mL⁻¹) for 5 min, allowing the silicone matrix to expand and absorb the fluorophore. The membrane was then removed and air dried for 5 min, after which it shrank back to its original size. The membrane was washed with ethanol to remove surface fluorophore molecules. In the final optrode configuration the membrane was positioned on the inner surface of the sensor cuvette.

Styryl 7 was obtained from Aldrich (Milwaukee, WI, USA). Films of PVA were prepared as described previously.^{25,26} The method consists of dissolving the PVA in water and polymerizing at ~360 K. Styryl 7 in methanol was added to a 10–15% aqueous solution of PVA. The PVA solution containing Styryl 7 was then cast on a plate, and dried over the period of several days in dust-free atmosphere. The PVA films were physically stretched at about 350 K up to six-fold to orient the Styryl 7 molecules. To avoid any ambiguity, the PVA films were stretched until they were six times longer than the initial unstretched length. The stretching ratio (R_S) is defined as the axial ratio a/b of an ellipse which is formed when stretching an imaginary circle in the unoriented film.²⁶ The area of the circle or ellipse is assumed to be conserved. Under these conditions,

$$R_S = N^{3/2}$$

(8)

where N is the physical fold of the stretch.

Results

Oxygen-sensitive film

Prior to describing polarization sensing of oxygen, it is valuable to understand the properties of the samples and reference components. Emission spectra of Ru(dpp)₃Cl₂ in the silicone film are shown in Fig. 1. The emission intensity is strongly dependent on the partial pressure of oxygen. An atmosphere of 100% oxygen results in an approximate five-fold decrease in intensity.

We examined the time response and reproducibility of the oxygen-sensitive film (Fig. 2). The intensity changes rapidly and reproducibly as the atmosphere is changed from air to nitrogen. Hence the oxygen-sensitive film responds reversibly and consistently under our experimental conditions.

Polarized reference film

It is also valuable to understand the spectral properties of the reference film. Emission spectra of Styryl 7 in PVA are shown in Fig. 3. Styryl 7 displays a good Stokes shift, which results in minimal loss of polarization due to homoresonance energy transfer. The polarization is high in the PVA film because the Styryl 7 molecules are immobile during the excited state lifetime in this viscous medium. The polarization is mostly constant across the long wavelength absorption band and the emission spectrum, providing a high polarization at all useful wavelengths. The emission maximum of the reference film at 680 nm is considerably longer than that of the oxygen sensitive film, which is near 610 nm.

Although Styryl 7 in the unoriented film could be used as the reference, we chose to obtain a higher polarization using a stretched film. The effect of stretching on the steady state polarization is shown in Fig. 4. A stretching ratio of $R_S = 12$ results in an anisotropy near 0.9 when using vertically polarized excitation. An important property of the stretched films is that they provide highly polarized emission even when excited with unpolarized light. This is shown in Fig. 4 for the excitation polarization oriented at 45° from the vertical, which is equivalent to excitation with unpolarized light. Prior to stretching, the anisotropy values with 45° excitation are half of that found using vertically polarized light, which is the result expected from the theory of photoselection.²⁴ As the film is stretched, the polarization with unpolarized excitation increases rapidly, and approaches the values found with polarized excitation (Fig. 4). This occurs because the electronic transitions are oriented along the stretch axis. The orientation provides the same effect as the polarizer, to orient the emission dipoles vertically in the laboratory axis.

Oxygen sensor

The oxygen sensor contains both Ru(dpp)₃Cl₂ and Styryl 7. Emission spectra from the combined oxygen sensor (Scheme 1) are shown in Fig. 5 (top). When the analyzer polarizer is in the vertical position, the emission spectrum seen through the analyzer is equivalent to that of the Styryl 7-PVA film (—). This is because the horizontally polarized emission from Ru(dpp)₃Cl₂ is eliminated by the analyzer polarizer.

Emission spectra from the sensor were also recorded with the analyzer in the horizontal position (Fig. 5, ---). In this case there is observable emission from both Ru(dpp)₃Cl₂ and Styryl 7. There is a contribution from Styryl 7 because the molecules are not perfectly aligned, so there is a significant horizontal component.

Because of the spectral shift between Ru(dpp)₃Cl₂ and Styryl 7, the relative emission from each will depend on the observation wavelength. We measured the polarization across the emission spectra (Fig. 5, bottom). The polarization increases with increasing wavelength because Styryl 7 emits at longer wavelengths than Ru(dpp)₃Cl₂. Hence the vertically polarized emission of Styryl 7 becomes dominant at longer wavelengths. The polarization values at most observation wavelengths were found to increase with increasing oxygen concentration (Fig. 5, bottom). This occurs because oxygen quenches the emission of Ru(dpp)₃Cl₂ and does not affect the emission from Styryl 7.

The oxygen-dependent polarization values were used to develop a calibration curve for oxygen (Fig. 6). The polarization increases owing to quenching of the horizontally polarized emission from Ru(dpp)₃Cl₂. Importantly, the polarization values display large changes, from -0.33 to +0.45 for the range from 0 to 100% oxygen. Since polarization values are routinely determined to better than ±0.01, one can expect accuracy in the oxygen concentration of 1% or better. The polarization is not linearly dependent on the partial pressure of oxygen, but this is not a problem with modern computerized instruments.

Discussion

It appears that the oxygen sensor described can have numerous applications in clinical and analytical chemistry. This device can be simple and compact, without the need for any liquid components. The absorption spectra of Ru(dpp)₃Cl₂ and Styryl 7 are ideally suited for excitation with light emitting diodes or electroluminescent devices. The long emission wavelengths can be efficiently detected with solid state detectors.

A sensor designed according to Scheme 1 may be less useful with optical fibers. It may be difficult to place the polarizer and two films at a filter tip. We expect this type of sensor to be most useful in hand-held devices, where the sensing cartridge contains the needed films. The fact that the components are in contact will eliminate the need for optical alignment.

It is possible that the sensor described in Scheme 1 could be used without an electronic detector. In a recent paper we described²⁷ a method of visual comparison for polarization sensing. In this method one observes the emission visually through a polarizer. Hence polarization sensing of oxygen may be practical in a variety of home and health care applications, with the only electronic device being the light source for the sensor.

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Fig. 1. Luminescence spectra of the oxygen probe $\text{Ru}(\text{dpp})_3\text{Cl}_2$ in silicone, in the presence of nitrogen, air and oxygen.

Fig. 2. Luminescence intensity of the oxygen-sensitive film of Ru(dpp)₃Cl₂ in silicone upon repeated exposure to nitrogen and air.

Fig. 3. Absorption (A), emission (F) and polarization spectra (---,) of reference Styryl 7 in the unoriented PVA film.

Fig. 4.

Fluorescence polarization at Styryl 7 in the PVA film as a function of the stretching ratio R_s $= N^{3/2}$, where N is the physical fold of the stretch.²⁶

Fig. 5.

Top: emission spectra from the combined oxygen sensor (Scheme 1) observed with the analyzer polarizer in the vertical (V) or horizontal (H) orientation. The dotted lines show the emission spectra of the $\text{Ru(dpp)}_3\text{Cl}_2$ film alone as seen through the horizontal analyzer. Bottom: wavelength-dependent polarization of the combined emission from the oxygen sensor.

Fig. 6.
Oxygen sensing based on polarization.

Scheme 1.

Experimental apparatus for polarization-based oxygen sensing. SF is a stretched film of Styryl 7 in PVA.