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Letters to *Analytical Chemistry*

O_2/pH Multisensor Based on One Phosphorescent Dye

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A new sensor is described based on a phosphorescent metalloporphyrin dye incorporated in a polymeric membrane, which allows measurement of the two important analytes, oxygen and pH. In such a sensor, the bifunctional dye is quenched by O_2 altering its phosphorescence intensity and lifetime (0−21 kPa O_2), whereas protonation of the dye causes a major change in the absorption spectrum and also reduces the phosphorescence intensity. As a result, quantification and continuous monitoring of the two analytes can be achieved by (i) simultaneous phosphorescence intensity (O_2, pH) and lifetime (O_2) measurements and (ii) parallel phosphorescence lifetime (O_2) and ratiometric absorbance (pH) measurements. Although the first method generally requires sensor calibration in intensity mode, the second provides internal referencing and calibration-free capabilities for both analytes. This approach can be extended to sensing of some other analytes.

Optochemical sensors have advanced remarkably in recent years, many of them are used successfully in different areas and applications.[1,2] Sensor research is now shifting toward the development of multiparametric sensing, particularly of core analytes such as O_2, pH, CO_2, temperature, humidity, and ions, as well as of more simple, robust, versatile, and cost-efficient systems tailored to specific applications.[3] Internal referencing schemes, such as the ratiometric absorbance/reflectance/fluorescence and luminescence lifetime based sensing represented by direct quenching, dual-lifetime referencing, or fluorescence resonance energy transfer (FRET) formats, are preferred detection modalities for such systems.[3] Rapid development of imaging techniques and low-cost optoelectronics provide information-rich data, miniaturization, and integration, while still retaining sensor accuracy, reliability, and affordable costs.[4−6]

On the chemistry side, the use of arrays of discrete sensors and/or composite materials with several indicator dyes has proved efficient for O_2/T, O_2/pH, O_2/T/CO_2, O_2/T/pH, and some other analyte panels.[3,7] However, an increased number of ingredients, wide bands of most of the indicators which tend to overlap in the usable spectral region (350−1000 nm), cross-sensitivity, and multiple practical restrictions limit multiplexing potential, compromise performance, and boost manufacturing costs of such sensors.

One way to overcome these bottlenecks is to apply multifunctional reporter molecules together with multiple detection modalities. Here, supramolecular structures possessing long-decay luminescence, large spectral shifts, and internal referencing capabilities are particularly advantageous, providing greater scope for multiplexing. We demonstrate this concept with a simple dual-analyte O_2/pH sensor based on one phosphorescent porphyrin dye.

Porphyrins have attractive photophysical properties for use in sensor systems.[8−15] Their chromophoric moiety consists of an aromatic tetrapyrolic macrocycle which can accommodate central ligands (metal ions, protons) and peripheral substituents

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in pyrrole and meso-positions. Modifications can involve the macrocycle itself giving rise to chlorins, porphyrin−ketones, benzoporphyrins, aza-benzoporphyrins, etc. which have distinct spectral characteristics.[10,11] These features provide flexibility in tuning the optical (absorption, emission) and physical−chemical (functionality, hydrophilicity, linkers) properties of porphyrin dyes and in designing new reporter molecules for sensor applications.[12−15]

Pt(II) and Pd(II) porphyrins have been actively exploited in phosphorescence lifetime based O_2 sensing, providing simple, robust, and versatile systems.[1−3,7,8] Quenched-phosphorescence detection of some other analytes (SO_2, NOx, relative humidity) was also demonstrated;[8,9] however, cross-sensitivity with O_2 hampered their utility. Protonation and binding properties of porphyrin dyes have been studied in absorbance and fluorescence based sensors;[16,17] these, however, still have limited usability. Herein, we present new solid-state materials based on the bifunctional phosphorescent porphyrin dyes, which provide simultaneous, reversible sensing of the two principal analytes, dissolved O_2 and pH, and potential for further multiplexing.

MATERIALS AND METHODS

Pt-octaethylporphyrin (PtOEP)-Schiff-base group (SB) and Pd-coproporphyrin-I tetraester (PdCP)-SB were synthesized at the Institute of Biomedical Chemistry, Moscow. Tetrahydrofuran (THF), chloroform, high molecular weight poly(vinyl chloride) (PVC), bis(2-ethylhexyl) sebacate (DOS), and sodium sulfite were from Sigma Aldrich. Tetrakis(4-chlorophenyl)borate (TCPB), tetrakis (4-*tert*-butylphenyl)borate (TBPB) and tetra(*p*-tolyl)borate (TTB) were from Fluka. Standard gas mixtures (O_2 balanced with N_2) were from Irish Oxygen. Sensors were fabricated by dissolving 120 mg of PVC and 240 mg of DOS in 3 g of THF and adding PtOEP-SB or PdCP-SB dye and ion transfer reagent (borate salt) in the required quantities (Table S1, Supporting Information). The cocktail was spotted in 2 μL aliquots on polyester film Mylar and dried to produce thin film sensors of ~10 mm in diameter.

UV−vis absorption spectral measurements (range of 350−700 nm) were carried out on a HP8453 diode-array spectrophotometer (Agilent). Fluorescence spectral measurements (range of 350−600 nm for excitation and 600−750 nm for emission) were carried out on a Cary Eclipse fluorescence spectrometer (Varian). Time resolved fluorescence (TR-F) measurements were performed on a Victor^2 multilabel reader (Perkin-Elmer), using 340 nm excitation and 665 nm emission filters. Phosphorescence lifetime measurements on the Victor reader were carried out by taking TR-F intensity readings at two different delay times, 30 and 70 μs with a window time of 30 μs, and calculating the lifetime according to following formula: $\tau = t_1 - t_2/\ln(F_1/F_2)$.[18] Measurements on a Cary Eclipse fluorometer were made using a built-in short phosphorescence decay option and lifetime determination by single or double exponential fits.

Measurement of optical responses of the dual-analyte sensors to pH and O_2 were conducted using 13 × 60 mm pieces of sensor membranes inserted diagonally in a standard 1 cm quartz cell or placed in the wells of a standard 24-well plate (Costar) and submerged in an aqueous buffer.

pH calibrations were conducted by adjusting the pH of the buffer inside the cuvette or microwell to different pH values (using calibrated pH meter Jenway 3310) and measuring after ~10 min equilibration changes in sensor absorption on the UV−vis spectrophotometer HP8453 or changes in emission intensity on the Victor^2 reader. From this data, sensor pK_a values were determined by plotting intensity vs pH and finding the inflection point that would point to the pK_a value. This is done at a certain wavelength after correcting the intensity values. To reduce influence of the sensor matrix, control sensors were prepared and a blank reading without dye was performed. Sensor response time to changes in pH was typically around 3 min.

Oxygen calibrations were performed on a Cary Eclipse spectrometer. The cuvette with sensor and buffer of known pH was bubbled with standard O_2/N_2 gas mixtures (0−21 kPa oxygen) produced and delivered by a precision gas mixing unit (LN Industries SA). Temperature control was set at 30 or 37 °C. Upon gas equilibration, phosphorescence decay was measured and lifetimes were calculated from double exponential fits with a subsequent calculation of average lifetime.

RESULTS AND DISCUSSION

The reporter dyes comprise the derivatives of hydrophobic Pt-octaethylporphyrin (PtOEP) and Pd-coproporphyrin-I tetraethylester (PdCP) which contain an additional pH-responsive moiety, Schiff-base group (SB) at one meso-position proximal to the macrocyle (Figure 1A). In an unprotonated state, these dyes display normal porphyrin type of electronic spectra, with intense Soret and minor visible absorbance bands and bright room temperature phosphorescence in the red region which is readily quenched by O_2. Like for normal porphyrins, the spectra of PdCP-SB are slightly red-shifted compared to PtOEP-SB (Figure 1B), and emission lifetime is several-fold longer (Table 1). Protonation of the peripheral SB group is accompanied by a major change in electronic spectra due to the formation of a delocalized carbocation.[19] This shifts absorption maximum from approximately 398 to 443 nm, with the disappearance of porphyrin-type spectra and phosphorescence. The protonation is reversible (though very high pH can degrade the dye), thus allowing sensing of pH by absorbance or phosphorescence measurements.

For optochemical sensing of physiological O_2 concentrations (range of 0−21 kPa or 0−250 μM) and pH (range of 5−9) in a convenient format, the reporter dye has to be embedded in a polymeric matrix which provides the desired sensitivity and selectivity for the two analytes and robust optical responses.[1,2] Hydrophobic polymers with moderate O_2 permeability commonly used in O_2 sensors (e.g., polystyrene and alike) are not suitable for being impermeable to protons. Likewise, many polymers employed in conventional pH sensors show inadequate O_2 quenching (in ethyl cellulose, Pt-porphyrins are quenched too much by ambient O_2 producing low phosphorescent signals). After testing different polymeric matrixes, we found plasticised PVC to possess the required characteristics and selected it as

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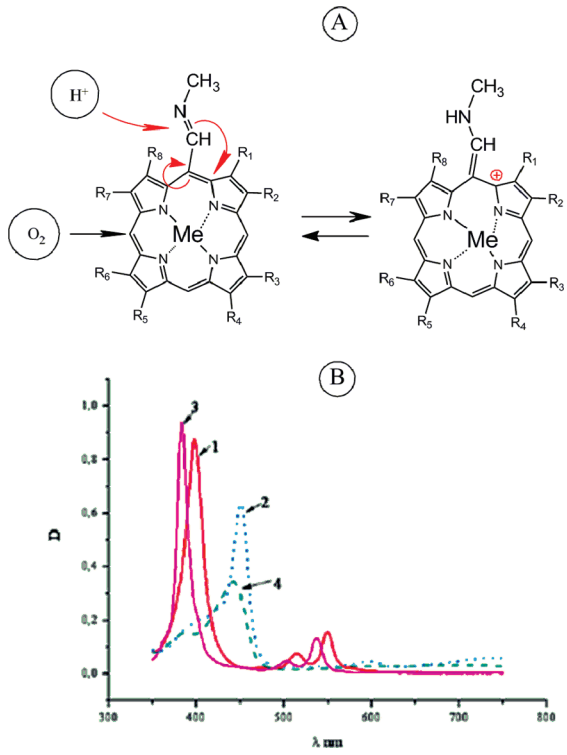

Figure 1. (A) General chemical structures of the PtOEP-SB (Me=Pt^{2+}, R_1-R_8=CH_2CH_3) and PdCP-SB (Me=Pd^{2+}, R_1,R_3,R_5,R_7=CH_3, R_2,R_4,R_6,R_8=$CH_2CH_2COOCH_3$) showing interaction sites for H^+ and O_2. (B) Changes in absorption spectra in methylene chloride upon the addition of trifluoroacetic acid (1-PdCP-SB, 2-PdCP-SB + TFA, 3-PtOEP-SB, 4-PtOEP-SB + TFA).

Table 1. Main Characteristics of the PtOEP-SB and PdCP-SB Sensors

reporter dye/sensor no.	absorbance maximum [nm]	ion transfer additive [%] (w/w)	pK^a	emission lifetime[a,b] [μs]
PtOEP-SB	398(pH8.0)			
N1	443(pH2.0)	2.4(TCPB)	5.9	32.8
N2		4.1(TCPB)	6.5	31.0
N3		5.7(TCPB)	7.0	n.m.
N4		7.6(TCPB)	6.1	n.m.
N5		7.6(TBPB)	<4.0	n.m.
N6		7.6(TTB)	<4.0	n.m.
PdCP-SB	398(pH8.0)			
N7	443(pH2.0)	4.1(TCPB)	6.9	60.3
N8		5.7(TCPB)	7.2	n.m.

[a] Phosphorescent measurements in 0.1 M K_2HPO_4, 21 kPa O_2, 30 °C. Standard deviations were ~0.2 μs or 0.1 pH, respectively, ($N = 3$). [b] n.m.: not measured.

sensor matrix. Plasticiser content is known to affect O_2 quenching in polymers;[20] therefore, it was maintained constant (63 ± 1.5% w/w). The availability of two phosphorescent dyes with different lifetimes and sensitivity to O_2 facilitated the development of O_2/pH sensitive materials and tuning their characteristics. Possible self-quenching in semiliquid PVC membranes was also assessed to optimize dye concentration. Phase transfer additive such as potassium tetrakis(4-chlorophenyl)borate (TCPB) was introduced to allow proton transfer.[1,2] Following the initial selection, sensors of different composition

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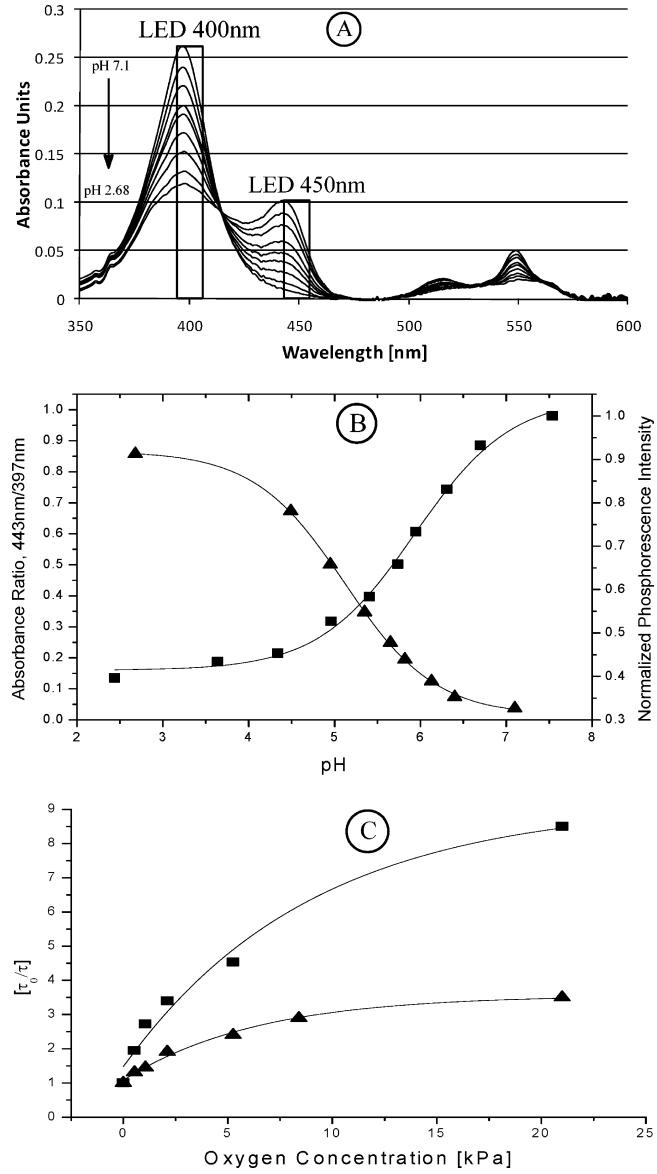

Figure 2. (A) Absorption spectra of PtOEP-SB *N1* sensor at different pH, 24 °C. The bars show the bands of standard 400 and 450 nm LEDs. (B) pH calibrations for the PtOEP-SB sensor in ratiometric absorbance (▲) and phosphorescence intensity (■) scale, 0.1 M acetate buffer, 24 and 30 °C, respectively. (C) Stern−Volmer O_2 calibrations for the PtOEP-SB (▲) and PdCP-SB (■) sensors, in 0.1 M K_2HPO_4, pH 8.5, and 30 °C.

were prepared and studied with respect to their photophysical, O_2, and pH sensing properties and operational performance. Sensors were made by dissolving their components in organic solvent (THF and $CHCl_3$) and casting the cocktail on polyester Mylar film, to produce ~5 μm thick coatings (Supporting Information, Table S1).

When embedded in plasticized PVC membranes, the two dyes showed similar spectral characteristics (absorption and emission), while their sensitivity to O_2 was different. Both sensor types produced the anticipated spectral response in the useful range of pH. Figure 2A shows absorption spectral changes associated with dye protonation, and Figure 2B shows pH calibration produced by ratiometric absorption measurements (443/397 nm). The nature and concentration of ion transfer reagent (and temperature) had a profound influence on sensor response to pH.

Thus, PtOEP-SB sensors increased their pK_a from 5.9 to 7.0 when TCPB content increased from 2.4 to 5.7% but then decreased to 6.1 at 7.6% TCPB. Two other phase transfer reagents, potassium tetrakis (4-*tert*-butylphenyl)borate (TBPB) and sodium tetra(*p*-tolyl)borate (TTB), produced significantly lower pK_a values. Similar values were observed for PdCP-SB sensors showing slightly more basic pK_a than PtOEP-SB. Such dependence of calibration on the nature and concentration of ion transfer reagent can be due to different access of protons to the dye (also seen in other ion-selective membranes.[19,21] Therefore, by selecting the indicator dye and ion transfer additive, pH sensitivity of the sensor can be tuned to cover the range of practical interest (pH 5−8 in this case). On the basis of these results, PtOEP-SB *N2* and *N3* and PdCP-SB *N7* and *N8* sensors (Table 1) were selected for further testing of their O_2 sensitivity and phosphorescent characteristics.

According to the mechanism of protonation (Figure 1), the changes in absorption were accompanied by a marked reduction in phosphorescence intensity signals (Figure 2B). At low pH values in air-saturated buffer at 30 °C, the intensity of the PtOEP-SB sensors decreased by almost 70%. Residual phosphorescence was attributed to incomplete protonation of the dye in polymer membrane.

With respect to the sensitivity to dissolved O_2, the PtOEP-SB sensors showed a moderate response. Phosphorescence lifetime of the sensor with unprotonated dye in O_2-free buffer was 84 μs at 30 °C (92 μs at 24 °C) and was reduced by ~70% in air-saturated solution.

The PdCP-SB sensors showed several-fold longer unquenched lifetimes (360 μs at 24 °C and 340 μs at 30 °C) and, therefore, stronger quenching by O_2. Phosphorescence of the PtOEP-SB and PdCP-SB sensors showed double-exponential decay and a pronounced nonlinearity of Stern−Volmer calibrations (Figures 2C, S1, and S2, Supporting Information). Such behavior is similar to the other O_2 sensors based on Pt and Pd porphyrins.[8,9,20] Both sensor types were deemed suitable for lifetime based sensing of physiological O_2 concentrations (0−250 μM), while PdCP-SB sensors better work in the low O_2 range. Importantly, the sensors showed practically no changes in emission lifetime upon protonation; variations were within measurement error (2 to 3%).

Similar to the traditional pH and O_2 sensors based on similar principles,[16,17] operational performance of the new sensors is influenced by a number of factors. Temperature has a prominent effect affecting both pH and O_2 calibrations, while pH calibrations are influenced by ionic strength. These are inherent features of the sensing schemes, which need to be considered during sensor operation. Sensor photostability is moderate: bleaching under illumination with 150 W Xe-lamp was in the region of 4% per hour (Figure S3, Supporting Information). Although not as good as for traditional O_2 sensors based on highly photostable dyes, this parameter is not critical for the sensing schemes used. Response times to pH and O_2 were within the anticipated range (2 to 3 min).

Therefore, the PtOEP-SB or PdCP-SB sensors perform very satisfactory covering the ranges of high practical significance (pH 6−8 and 0−200 μM O_2). In absorbance (or reflectance) modality, sensor response to pH is independent of O_2, while ratiometric measurement (400/450 nm) provides internal referencing with stable calibration, reduced dependence on sensor alignment, measurement geometry, and dye concentration. Likewise, in phosphorescence lifetime modality sensing of dissolved O_2, with internal referencing and no cross-sensitivity, pH was secured. Thus, the two analytes can be measured independently and continuously with one sensor.

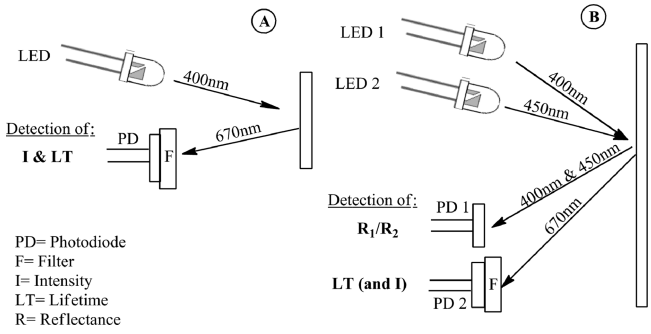

Figure 3. Proposed optical setups for the dual-analyte pH/O_2 sensing. (A) Phosphorescence intensity (O_2, pH) and lifetime (O_2) measurements via one optical channel. (B) Alternating ratiometric absorption/reflection (pH) and phosphorescence lifetime (O_2) measurements.

Practical realization of the dual O_2/pH sensor system might be achieved with relatively simple optical schemes. Figure 3A shows a setup with one light-emitting diode (LED) and one photodiode with an optical filter to monitor phosphorescence intensity and lifetime (in time or frequency domain[1,2]) signals from the sensor. When known relationships are applied (calibrations for each analyte, compensation algorithms which account for dual sensitivity of the intensity signal), the two readings can be related to pH and O_2. Since the intensity signal is not referenced, this scheme requires fixed alignment and consideration of possible signal fluctuations (detector, sample optical properties, dye photobleaching[1,2]).

The alternative scheme (Figure 3B) involves two LEDs shining sequentially coupled with ratiometric absorbance/reflectance based sensing of pH via PD1 and phosphorescence lifetime based sensing of O_2 via PD2. This scheme does provide interference-free, dual-analyte O_2/pH sensing with internal referencing being more advantageous than the first one. It can be implemented with two common LEDs matching the excitation and absorption maxima of the neutral and protonated forms of the dye (Figure 2A, http://www.roithner-laser.com).

CONCLUSIONS

Overall, this study presents simple realization of a dual-analyte optochemical sensor for dissolved O_2 and pH with one bifunctional reporter dye, meso-substituted Pd- or Pt-porphyrin, embedded in plasticized PVC membrane. Such sensor chemistry allows sensing of each analyte with internal referencing and no cross-sensitivity. Moreover, it leaves wide spectral windows (500−650 nm and 800−1000 nm) for multiplexing with other indicator dyes including fluorescent lanthanide chelates[22] and inorganic phosphors.[23] This approach can be applied to other types of sensor materials

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(e.g., nanosensors,[24,25] magnetic particles[26]), analytes (temperature, CO_2, NH_3, ions, enzyme biosensors based on O_2 and pH transducers[1]), and sensing schemes[1,2,27] and integrated with optical imaging systems.

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