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Irreversible sensing of oxygen ingress

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ABSTRACT

Absorption-based opto-chemical sensors for oxygen are presented that consist of leuco dyes (leuco indigo and leuco thioindigo) incorporated into two kinds of polymer matrices. An irreversible and visible color change (to red or blue) is caused by a chromogenic chemistry involving the oxidation of the (virtually colorless) leuco dyes by molecular oxygen. The moderately gas permeable copolymer poly(styrene-*co*-acrylonitrile) and a highly oxygen-permeable polyurethane hydrogel, respectively, are used in order to increase the effective dynamic range for visualizing and detecting oxygen. We describe the preparation and properties of four different types of such oxygen sensors that are obtained by dip-coating a gas impermeable foil made from poly(ethylene terephthalate) with a sensor layer composed of leuco dye and polymer.

1. Introduction

The detection and determination of oxygen in various media is of great importance in medical and environmental sciences, in industrial processing and biochemistry, where oxygen is often involved as reactant or product [1–3]. Amperometric determination of oxygen by Clark-type electrodes suffers from the drawback of oxygen consumption [4,5] except the Ross electrode which can regenerate the consumed oxygen [6]. Potentiometric determination of oxygen based on zirconium dioxide as a solid electrolyte operates only at high temperatures usually in cars (the so-called lambda probe) [7,8]. The paramagnetic detection of oxygen is another attractive method but is interfered by free radicals [9].

Optical sensors for oxygen and, especially, fiber-optic sensors still experience a substantial amount of research and development [10]. The most common and useful types of optical oxygen sensors are those based on the quenching of luminescence of suitable indicators [11]. Kautsky reported that both the phosphorescence and fluorescence of surface-adsorbed dyes such as trypaflavin, benzoflavin, safranin, chlorophyll, porphyrins and others are sensitively quenched by molecular oxygen [12]. Various methods for fluorescence sensing have been reported [13,14]. The fluorescence intensity can vary due to changes of many parameters [15–17].

Baldini et al. [18,19] have reported an absorption-based fiber-optic oxygen sensors based on organometallic compounds which undergo changes in their absorption in the presence of oxygen. The probe bis(histidinato)cobalt(II) is capable of binding molecular oxygen and in doing so undergoes a reversible change in the absorption at 408 nm that is related to the concentration of oxygen. A reversible sensor based on contact charge-transfer absorption of *N*,*N*-dimethyl-*p*-toluidine and oxygen was reported by Choi and Hawkins [2]. This sensor suffers from drawbacks such as long response and recovery times, and also serious interference by chlorine and sulfur dioxide. Chen and coworkers [20] have developed schemes for colorimetric sensing of oxygen, again based on fluorescence and fully reversible. However, an optical oxygen sensor with a visually detectable color change would have several advantages over sensors based on fluorescence quenching. Such a sensor is less influenced by interferences than a fluorescent sensor and read-out can be by reflectometry using LEDs as light sources, and photodiodes as detectors, both of which are inexpensive.

We are presenting here two different absorption-based irreversible but regenerable optical probes for oxygen. They consist of oxygen-sensitive leuco dyes (leuco indigo and leuco thioindigo) incorporated into various polymer matrices. In order to increase the effective dynamic range, the leuco dyes were incorporated either into a moderately gas permeable copolymer or into highly oxygen-permeable hydrogel polymer. We also describe applications of these probes for visually monitoring of oxygen ingress.

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2. Experimental

2.1. Chemicals

Indigo and poly(styrene-*co*-acrylonitrile) (PSAN, with 30 wt% acrylonitrile) were purchased from Sigma (www.sigmaaldrich.com). Sodium hydroxide solution (1 M) and sodium dithionite were supplied by Merck (www.merck.com). Thioindigo was from TCI Europe (www.tcieurope.eu). The 125 μm thick and optically fully poly(ethylene terephthalate) foil (Mylar$^{\text{TM}}$) was purchased from Goodfellow (www.goodfellow.com), polymer hydrogel D4 (a linear polyurethane) from Cardiotech (www.cardiotech-inc.com). All solvents (methanol, tetrahydrofurane) were from Fluka (www.sigmaaldrich.com) and used in analytical purity. All chemicals were used as received. All manipulations were performed under an atmosphere of dry nitrogen gas (from Linde; www.linde-gase.de).

2.2. Instruments

An Edwards Modulyo Freeze Dryer (www.imaedwards.com) was used for lyophilizing the aqueous solution of leuco dye. A Hitachi U 3000 spectrophotometer (www.hitachi-hitech.com) was used to acquire the absorption spectra of planar sensors. The pictures of the sensor layers were acquired with a Canon Ixus 110 IS digital camera (www.canon.com) equipped with a 1/2.3 inch 12-megapixel CCD.

2.3. Preparation of polymer solutions

The hydrogel (D4; a linear polyurethane) is a highly oxygen-permeable matrix polymer, soluble in a mixture of methanol and water [21]. Its permeability coefficient (P) for oxygen is not reported in the literature. However, the oxygen permeability coefficients (P) of closely related polyurethane-based hydrogels typically range from 4.4×10^{-12} to $6.5 \times 10^{-12}\,\text{cm}^2\,\text{Pa}\,\text{s}^{-1}$ which is similar to the oxygen permeability coefficient (P) of pure water [22]. A 10 wt% solution of the hydrogel in a 9:1 (v:v) methanol:water mixture was prepared. The moderately gas permeable copolymer poly(styrene-*co*-acrylonitrile; PSAN; containing 30 wt% acrylonitrile) was also used in the form of a 10 wt% solution in tetrahydrofurane. PSAN possesses an oxygen permeability coefficient (P) of $3.5 \times 10^{-14}\,\text{cm}^2\,\text{Pa}\,\text{s}^{-1}$ [23] which is less than that of polystyrene which is widely used in optical sensors for oxygen.

2.4. Preparation of sensor layers

Leuco dyes. Solutions of leuco indigo and leuco thioindigo, respectively, of approximately 3 M were made by vat dye reduction with alkaline, aqueous solution of sodium dithionite (20 mM) under nitrogen atmosphere at 95 °C [24]. The clear, lightly yellow colored solution of leuco dye was lyophilized over night to give a slightly yellow solid. Scheme 1 gives a schematic representation of the redox reactions.

Sensor layer. Solid leuco dye (10 mg) was mixed with 15 mL of hydrogel solution (or 15 mL of PSAN solution) under nitrogen atmosphere and stirred for about 30 min at room temperature. This "cocktail" was cast on the PET support by dip coating [25]. The sensor was dried under nitrogen atmosphere. Data on the sensor systems, dyes and polymers used are given in Table 1.

3. Results and discussion

3.1. Choice of materials

Indigo (blue) and thioindigo (red) are vat dyes that become water soluble and almost colorless after reaction with a suitable reducing agent. The reverse color change occurs as a result of the reaction of oxygen with the leuco dyes as shown in Scheme 1.

The hydrogel and PSAN were chosen because they differ in their oxygen permeability (which is much higher in case of hydrogel). Thus, it is possible to create various sensor systems depending on which dye and which polymer is being used (Table 1). The polymers also are good solvents for both the dyes and their leuco forms.

3.2. Sensors based on hydrogel

Time-dependent 3-D diagrams of the absorption spectra of the sensors LI-D4 (left) and LTI-D4 (right) are presented in Fig. 1. LI-D4 shows an absorption-peak at 368 nm that decreases with time due to the consumption of leuco indigo. In parallel, the peak at 616 nm increases due to the formation of indigo [26]. LTI-D4 shows a peak at 420 nm that decreases with time due to the oxidation of leuco thioindigo, while the peak at 547 nm increases with time due to the formation of thioindigo [24].

3.3. Sensors based on PSAN

The time-dependent absorption spectra of sensors LI-PSAN (left) and the LTI-PSAN (right) are presented in Fig. 2. The LI-PSAN sensor type shows an absorption-peak at 441 nm that decreases with time due to the oxidation of leuco indigo, and the peak at 609 nm increases with time due to the formation of indigo. Similarly, LTI-PSAN shows an absorption peak at 423 nm that decreases with time due to the oxidation of leuco thioindigo, while the peak at 549 nm increases with time due to the formation of thioindigo.

3.4. Color images of the sensors

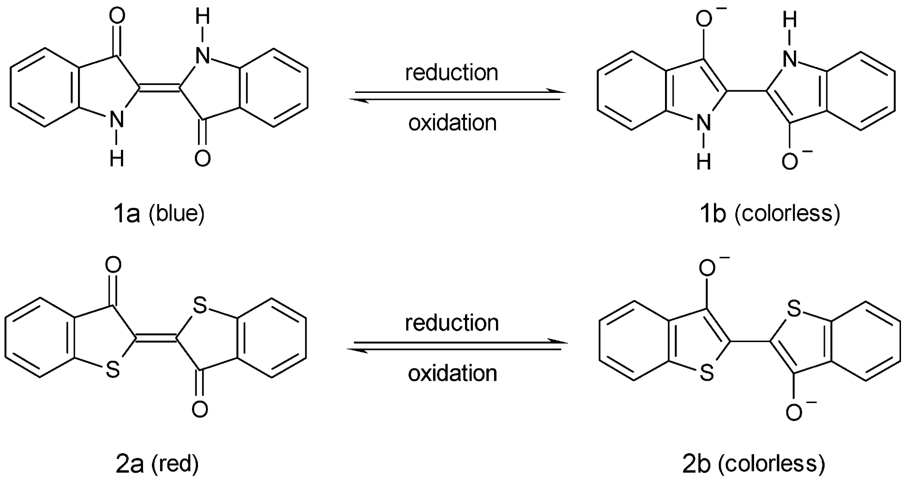
The color change of sensors of the type LI-D4 and LTI-PSAN, respectively, are shown in Fig. 3. The images of the LI-D4 sensor were acquired after 0, 1, 8 and 20 min after the first exposition to air. An irreversible color change (from pale yellow to deep blue) can be seen as a result of the formation of indigo. This sensor can detect the presence of air within a few minutes which is quite important with respect to the detection of leaks in food packed under modified atmosphere. The LTI-PSAN sensor, in contrast, needs several hours to gradually change its color from yellow to red after its exposure to air. Fig. 3 shows the images of the LTI-PASN sensor at 0 min, 4 min, 2 h and 36 h where leuco thioindigo is used as the oxygen-sensitive dye.

3.5. Evaluation of the sensors

Fig. 4 displays the increase of absorbance over time for LI-D4 and LTI-D4 at 616 nm and at 547 nm, respectively. The fit of the data points was made by using the parallel exponential kinetics (PEK) [27] model (Eq.(1)).

$$y = y_0 + y_1\left[1 - \exp\left(\frac{-x}{t_1}\right)\right] + y_2\left[1 - \exp\left(\frac{-x}{t_2}\right)\right] \tag{1}$$

where y is the absorbance at time x of exposure of the sensor to air, and y_0 is the absorbance at time zero. The plot is composed of two exponential terms which represent a fast $y_1\left[1 - \exp\left(\frac{-x}{t_1}\right)\right]$ and a slow $y_2\left[1 - \exp\left(\frac{-x}{t_2}\right)\right]$ process having the time constants t_1 and t_2, respectively. The terms y_1 and y_2 can be related to two processes,



Scheme 1. Chemical formulae of indigo (**1a**) and thioindigo (**2a**), and of the respective (colorless) leuco forms (**1b**, **2b**).

Table 1
Polymers, dyes and respective codes of the sensor systems prepared.

Polyme[a]	Volume[b] [mL]	Leuco-dye[c]	Mass[d] [mg]	Sensor code
D4	15	LI	10	LI-D4
D4	15	LTI	10	LTI-D4
PSAN	15	LI	10	LI-PSAN
PSAN	15	LTI	10	LTI-PSAN

[a] Hydrogel (D4) or PSAN.
[b] Volume of solution of hydrogel (10 wt% in MeOH/H_2O 9:1 v/v) or PSAN (10 wt% in THF).
[c] LI, leuco indigo; LTI, leuco thioindigo.
[d] Mass of leuco dye used for sensor preparation.

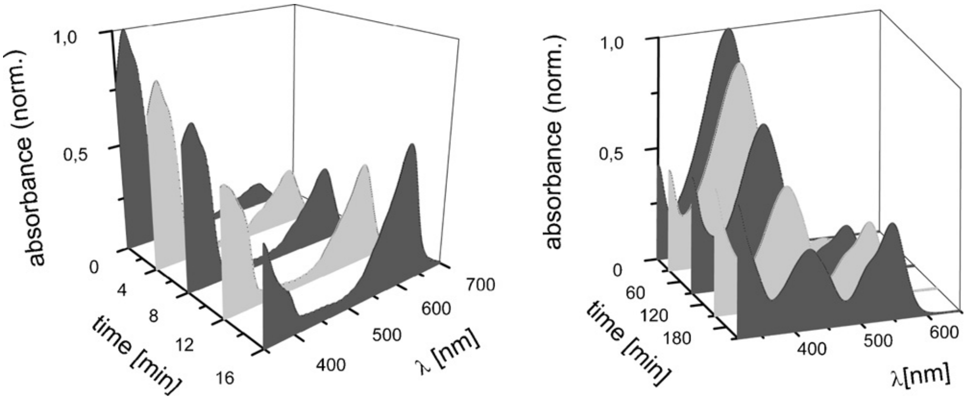


Fig. 1. Time-dependent absorption spectra of sensors at room temperature. Left: LI-D4; right: LTI-D4.

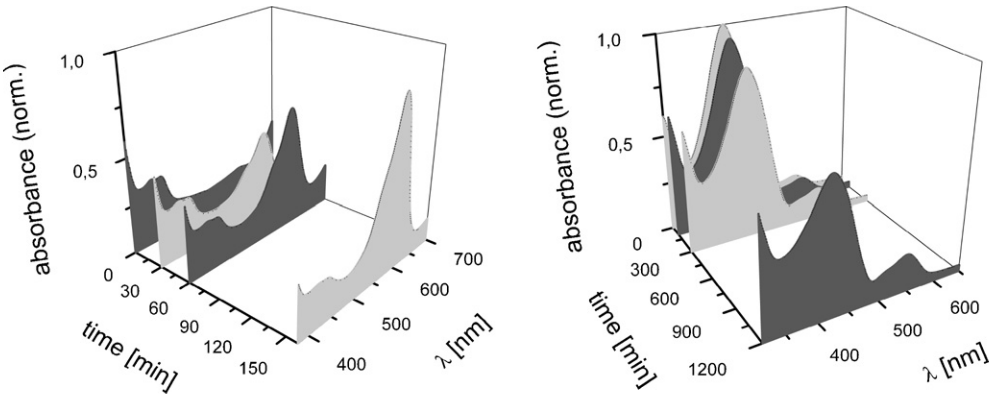


Fig. 2. Time-dependent absorption spectra of sensors at room temperature. Left: LI-PSAN; right: LTI-PSAN.

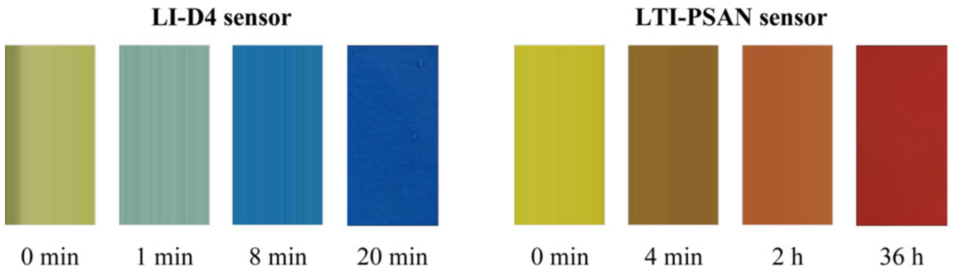


Fig. 3. Real color images of LI-D4 sensor (left) at a time range from 0 to 20 min and LTI-PSAN sensor (right) at a time range from 0 to 36 h after exposition to air at room temperature.

viz. the diffusion of oxygen into the polymer and the oxidation of leuco dye in the polymer.

We also used the parallel exponential kinetic model for the fit of the data obtained with PSAN-based sensors. Fig. 5 displays the kinetics of the increase of absorbance with time for the LI-PSAN at 609 nm (left) and the LTI-PSAN (right) opto-chemical oxygen sensor at 549 nm, respectively.

Table 2 gives the parameters of the parallel exponential kinetic (PEK) model for each sensor type and the coefficient of determination (R^2). The values for R^2 fairly indicate a good quality of the fits.

3.6. Regeneration

The sensors based on hydrogel can be regenerated. This was accomplished by reduction under an inert atmosphere using an alkaline, aqueous solution of sodium dithionite (20 mM) at 95 °C. The reduction of the oxidized dye was completed after a few minutes and the sensor film was colorless again due to the formation of the leuco dye. This sensor, if exposed to air, again shows the expected response to oxygen (air). The regeneration of PSAN-based sensors was not investigated in detail, because the reduction of the dye embedded in PSAN-matrix is too slow.

4. Discussion and conclusions

The main aim of the study was to create a system capable of detecting the ingress of oxygen via an irreversible and visible color change. The results show that qualitative detection of oxygen ingress is enabled by this method in giving and a visually detectable color change. Quantitative analysis is more complicated as it requires precise timing and instrumental readout, for example with a digital camera and exploiting the red–green–blue readout of such cameras [28]. This was not investigated here. Nevertheless, we are suggesting a few options in the following on how quantitative sensing of oxygen may be accomplished. The first option is to place the sensor layer into a closed system. Obviously, oxygen must not be present there in a quantity higher than the equivalent concentration of the leuco dye in the sensor layer. Once all oxygen is consumed, the formation of color will stop and the test strip may be evaluated quantitatively by reflectometry.

If the sensor is placed in an opened system, a second option for quantitative sensing of oxygen exists that is based on the kinetics of the color change. Coloration can be easily measured by reflectometry (or via a digital camera) and its kinetics (i.e., the differential increase in color as a function of time) will increase with oxygen concentrations. This approach may, of course, also be used for closed systems. Another method to quantify oxygen in either open or closed systems consists in the application of more than one sensor, each having different response kinetics. The analytical information may be obtained by comparing the coloration of the different sensors after a certain time of exposure. It has to be kept in mind, however, that this kind of sensor is of the cumulative type, much like portable badges as used to measure the total exposure to toxic gases or radiation. Its signal results from the product of oxygen concentration and time of exposure. Hence, it will provide reliable data on the concentration of oxygen only if the time of exposure is defined and the same as used in the calibration process.

The detection range of the sensors was not studied in detail here because of the primary goal of this study which is detecting leaks. As a result, all sensors were exposed to ambient air only. Possible interferences and the effect of other oxidative reagents were not studied in view of closely related reports on the determination of dissolved oxygen in water using leuco dye chemistry. Interferences were reported for Fe(III), Ca(II) and Cu(II) ions [29–31]. However, our experiments were mainly performed in gaseous phase. Even in this case, interferences may be encountered by oxidizing species such as ozone, hydrogen peroxide (in vapor phase), NO_x, or chlorine. These usually are not found in packed food, but their potential interference needs to be kept in mind when applying the sensor to ambient air.

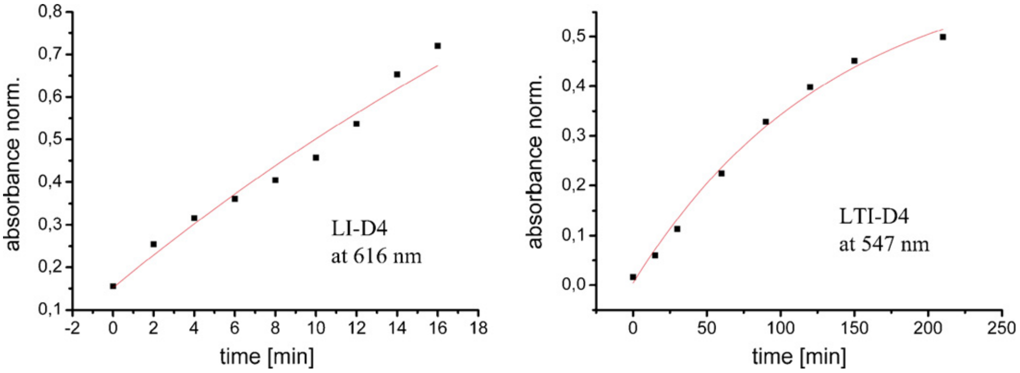


Fig. 4. Sensor kinetics of sensors at room temperature. Left: LI-D4; right: LTI-D4.

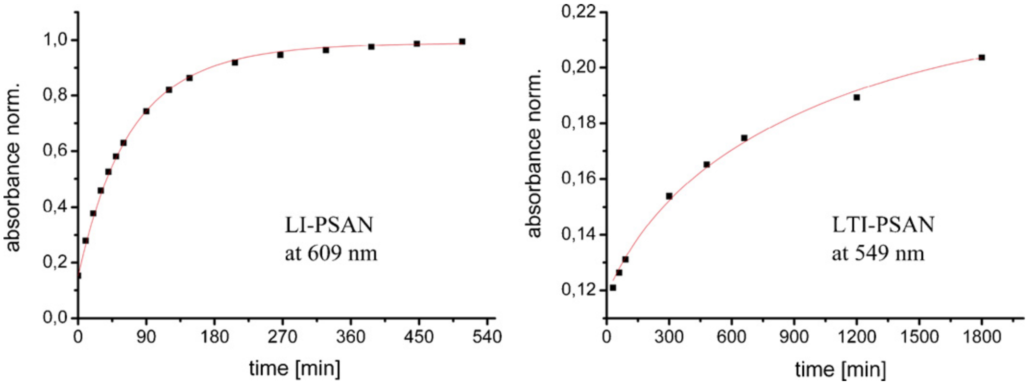


Fig. 5. Response kinetics of sensors at room temperature. Left: LI-PSAN; right: LTI-PSAN.

Table 2
Figure of merits for the four typical sensors.

Sensor code	y_0	y_1	y_2	t_1	t_2	R^2
LI-D4	0.15238	0.66888	0.96982	48.47438	38.0004	0.97019
LTI-D4	0.00435	0.30024	0.34878	144.2277	128.81839	0.99363
LI-PSAN	0.16323	0.43875	0.38807	108.39482	46.16315	0.99973
LTI-PSAN	0.11869	0.01509	0.08516	171.39591	1049.88096	0.99674

In conclusion, we have introduced a simple means for the detection of oxygen on the basis of a color-forming scheme that recently also has been employed to deisgn micro-capillary clocks where the length of a colored segment is indicating the time that has elapsed after one end of the capillary has been exposed to air [32]. Readout can be either visually or instrumental, and responses are fast (~5 min in case of the LI-D4 type of sensor). Such sensors represent a cost-effective means to visually detect and monitor oxygen ingress. Main applications are in the food and pharmaceutical industry in order to determine the tightness of packaged food [33], for example of fish and meat, but also of pharmaceuticals and therapeutics (such as immune reagents), and wherever modified atmosphere packaging is applied.

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Biographies

Stefan Wilhelm, born 1981, studied chemistry at the University of Regensburg and now is pursuing his PhD studies in the group of Prof. Wolfbeis. His research interests are absorption-based detection of oxygen using chromogenic chemistries, and in optical sensors based on magnetic nanoparticles with luminescence upconversion capability.

Otto S. Wolfbeis, born 1947, is a professor of analytical chemistry. He has authored more than 500 articles on topics such as optical (fiber) chemical sensors, analytical fluorescence spectroscopy, and fluorescent probes. He has edited a (widely used) book on Fiber Optic Chemical Sensors and Biosensors, acts as the editor of the Springer Series on Fluorescence, is the Editor-in-Chief of Microchimica Acta, and one of the ten curators of Angewandte Chemie. His h-index is 51, and his articles have been cited >9000 times. Several sensors developed in his group have been commercialized. His present research interests include fluorescent biosensing, the design of novel spectroscopic schemes, new fluorescent probes, beads, and labels, new methods of interface chemistry (such as click reactions), and analytical uses of advanced materials (such as upconverting luminescent nanoparticles, and graphenes). Also see: www.wolfbeis.de.