Dissolved Oxygen Sensing Using an Optical Fiber Long Period Grating Coated With Hemoglobin

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Abstract—A long period grating fiber optic sensor coated with hemoglobin is used to detect dissolved oxygen. The sensitivity of this sensor to the ratio of dissolved carbon dioxide to dissolved oxygen is demonstrated via the conversion of carboxyhemoglobin to oxyhemoglobin on the sensor surface. The sensor shows good repeatability with a ±5% error [2]. However, dissolved oxygen measured in this way is only partially useful as a clinical indicator [3]. A measurement of the hemoglobin-available dissolved oxygen would provide a better indication of the actual dissolved oxygen reaching the cells of patients. While rare this is an issue in patients with a range of conditions that cause changes in pH or temperature of the blood system where normal oxygen uptake is not maintained [4].

Optical fiber based approaches to the sensing of dissolved oxygen include the measurement of changes in the reflectivity of Ru(bpy)$_3^2$ deposited onto the ends of optical fiber or via tapered evanescent wave sensors [5]. Optical fiber approaches are attractive as a sensor as they can be implanted in specific locations such as cancer growths or specific organs to monitor the blood oxygenation at those sites, which is highly clinically relevant [4].

Long period grating (LPG) have been applied to a wide range of sensing applications from the detection of toluene contamination [6] to the measurement of ammonia gas concentration [7]. They exhibit a high refractive index sensitivity. Without a secondary recognition system, an LPG is inherently nonspecific and will only function as a local refractive index sensor.

I. INTRODUCTION

DISSOLVED oxygen sensing is used on a daily basis in a large number of applications in health care and medicine. The most commonly used approach to the measurement of dissolved oxygen in the blood stream is the pulse oximeter, which exploits the absorption of light in the infrared by hemoglobin [1] and typical have a range of 10–90% oxygenation with a ±5% error [2]. However, dissolved oxygen measured in this way is only partially useful as a clinical indicator [3]. A measurement of the hemoglobin-available dissolved oxygen would provide a better indication of the actual dissolved oxygen reaching the cells of patients. While rare this is an issue in patients with a range of conditions that cause changes in pH or temperature of the blood stream where normal oxygen uptake is not maintained [4].

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II. MATERIALS AND METHODS

Preparation of the optical fiber sensor was carried out in two stages. The fabrication of the LPG was followed by the immobilization of hemoglobin onto the surface of the section of the optical fiber containing the LPG.

A. LPG Fabrication

An LPG was fabricated using a point-by-point irradiation method. A length (~2 m) of photosensitive fiber (Fiber core PS750) was prepared by stripping the buffer jacket off a short section (40 mm) in the middle of a 2 m length. This was then mounted on a translation stage which could be moved with ~0.05 μm accuracy. The LPG was inscribed in the fiber core by exposing it to the output from a frequency quadrupled Nd:YAG laser operating at 266 nm. The beam was passed through a slit.
of controllable width, manufactured in-house, which was set to a width of 54.85 μm (±0.05) which equates to half the desired LPG period of 109.7 μm (50% duty cycle). This period was chosen to ensure the coupling to the LP019 mode near to the phase matching turning point in this fibre type [9]. The fiber was exposed for 40 s before being translated by a distance equivalent to the desired period.

B. Hemoglobin Coatings

The hemoglobin used in this experiment was a lyophilized prep from Sigma (H7379) made up to 5 mg/ml in phosphate buffered saline (PBS) solution. Prior to coating, the fiber was pre-treated with a 0.18 M KOH solution made up in 60% (v/v) ethanol (details) and distilled water. This KOH treatment is known as a hydroxide etch and ensures that the silica has an abundance of –OH groups on the exterior surface for protein immobilization. The use of hydroxyl groups to promoted protein absorbance has been well typified [10] and used in ELISA assay design [11].

The treated fiber was then rinsed in distilled water and dried before being immersed in the 5 mg/ml solution of Hemoglobin. The sensor was then incubated at room temperature (22.5 °C) for 12 h in sealed container to limit evaporation. After 12 h the sensor was removed and stored in PBS until use.

Once all the sensor testing had been completed, the fiber was cleaved in the middle of the sensing region and the coating was examined by an environmental scanning electron microscope (ESEM). An image of the coating is shown in Fig. 2.

As can be seen at point B, there is a thin coating layer surrounding the fiber, a flake of the coating disturbed by cleaving the fiber is also identified as A. The size of these features (∼1 μm) suggest that more than one layer of hemoglobin (∼100 nm) was deposited.

Unlike other protein based selective coatings, such as antibodies, hemoglobin doesn’t have external active binding groups. This means that hemoglobin can be deposited in any orientation via passive adherence. Oxygen and carbon dioxide will permeate the protein and reach their binding sites.

C. Sensor Testing

For testing, the sensor was mounted in a 3-D printed trough with a volume of ∼3 ml. The trough was constructed in-house so as to allow the fiber to be held securely either side of the sensing region with the entire sensing region suspended in the center of the container. One end of the fiber was connected to an Ocean Optics tungsten-halogen light source and the other to an Ocean Optics H4000 spectrophotometer with a range of 600–1100 nm. Each spectrum recorded was an average of the ten scans.

All experiments were carried out in PBS solution (Sigma PBS tablets) dissolved in deionized water. Deionized water will rapidly absorb CO₂ from the air after it is produced. To avoid any variation in the dissolved gas content of the water, a single bulk PBS solution was made up which was then ‘aerated’ by bubbling it with zero-air (BOC) for 3 h.

Previous work by Zhernovaya et al. [12] to typify the refractive index change of hemoglobin used sodium bicarbonate to ensure that all solution-state hemoglobin was converted to oxy-hemoglobin, by converting the dissolved carbon dioxide into carbonate groups [13]. 7.5 and 12 mg/ml solutions of sodium bicarbonate were prepared in aerated PBS.

While the sodium bicarbonate acts on the CO₂ converting it to carbonate ions, it has no effect on the dissolved O₂. Thus the dissolved oxygen concentration of the solution remains constant throughout the experiment.

III. RESULTS AND DISCUSSION

A. LPG Fabrication

The spectrum of the optical fiber was recorded before and after LPG inscription. The graph shown in Fig. 3 shows these spectra overlaid. The LPG fabrication process took around 3 h, to eliminate any drift in the output from the light source the data was normalized to the maximum value.

The data in Fig. 3 shows the formation of the LP019 phase matching turning point appearing around 865 nm. LP018 and LP017 can be seen appearing at 700 and 610 nm respectively. LP019 is the most sensitive to local refractive index changes and so only that portion of the spectra is used for subsequent experiments.
B. Non-Specific Reaction Testing

As previously discussed, LPGs are highly sensitive to refractive index change. To rule out any interference from the bulk refractive index being mistaken for selective sensing, comparator data was taken before coating the sensor.

Prior to KOH treatment and coating, the LPG was immersed in alternating solutions of PBS and 7.5 mg/ml sodium bicarbonate solution five times. The position of the peak around 820 nm was recorded and is plotted in Fig. 4. The peak position was determined by custom data analysis software based on minima location.

There is no discernable difference between the spectra of PBS solution and 7.5 mg/ml solution, Fig. 4. Without a coating, the LPG cannot differentiate between these solutions.

C. Hemoglobin Coating

Fig. 5 shows the normalized transmission spectrum of the LPG recorded before and after coating with hemoglobin. The protein coating causes a local refractive index change significant enough to cause the spectra to shift away from the phase-matching turning point (PMTP) to where the coupled modes are shown as separate peaks.

D. Bicarbonate Testing

In order to demonstrate that the coated sensor can distinguish between carboxy and oxyhemoglobin, the sensor was immersed in alternating solutions of PBS and 7.5 mg/ml sodium bicarbonate solution ($n = 6$). The addition of the sodium bicarbonate should have the effect of shifting the balance from preferential carboxy hemoglobin to oxyhemoglobin [3]. The spectra are plotted in Fig. 6 and the position of the peak around 820 nm is shown in Fig. 7.

The movement of the coupled LP019 peaks inwards towards the PMTP is indicative of a decrease in the refractive index of the coating [14]. This is in keeping with the decrease in the refractive index of hemoglobin when it converts to oxyhemoglobin [15].

Fig. 7 further demonstrates the discrimination between solutions both of which shows two separate populations of results ($P \leq 0.01$). By cycling the sensor between the two solutions these results also demonstrate that the sensor is reversible. The standard deviation of 0.3 nm for PBS and 0.2 nm for PBS + NaHCO₃. There is no trend in either set of results. There is a modulation in the data but this is present in both sets and is possibly due to other conditions affecting the testing. This highlights the need when using LPG sensors to have a secondary control with a non-reactive coating in order to correct for any
The final goal of this work is to produce an implantable sensor system that can produce real-time feedback on the availability of dissolved oxygen in the blood stream.

REFERENCES


Matthew Partridge received the Ph.D. degree from Cranfield University, Cranfield, U.K., in a joint project between Engineering Photonics and Cranfield Health. He is currently a Research Fellow with the Engineering Photonics Centre, Cranfield University. He has worked extensively in sensor development, including immunoassays, micro arrays, microfluidics, and optical sensors. His current research area is primarily focused on combining novel photonic tech-niques with Langmuir monolayers to develop improved sensor systems. In addition, he also runs an open science blog and webcomic (errantscience.com) aimed at disseminating complex science to a wider audience.

IV. CONCLUSION

A hemoglobin coated LPG sensor for dissolved oxygen has been demonstrated. Initial data shows the sensor’s insensitivity to sodium bicarbonate and the deposition of hemoglobin. Further testing with sodium bicarbonate concentrations showed a reaction indicative of the conversion of carboxyhemoglobin to oxyhemoglobin with a shift in the refractive index. A number of possible non-specific chemistries were also ruled out.

The experiments were designed such that the dissolved oxygen content was constant for all solutions. The only changing factor was the dissolved carbon dioxide in solution. This mimics the same pressures on hemoglobin in the blood stream and shows that the hemoglobin deposited on the LPG responded as expected.

This is the first time that hemoglobin has been used as a direct sensing element in conjunction with an optical fiber. It has been previously applied to electrochemical studies [17] but as a hemoglobin characterization method and not as a sensing element. Future work will examine the response of hemoglobin to other parameters such as pH and temperature. Also using sodium dithionite [18] it is possible to completely remove dissolved oxygen from samples and demonstrate fully CO2 saturated hemoglobin.
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