Cat-O₂ sensor for H₂O₂ determination based on Foxy-R Oxygen Sensor
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1. Introduction

In spite of the substantial amount of work done in optical sensor research, it is difficult to find a biochemical receptor/optical transducer scheme, which can be used for a device permitting the continuous monitoring of a chemical compound in clinical or environmental samples. Perhaps one of the most interesting alternatives is based on using biochemical receptors that, in the appropriate concentration range, give easily reversible reactions.

H₂O₂ is a very interesting analyte in several kinds of biochemical and environmental samples. In biochemistry, in addition to its well-known importance in aging, this compound plays a role as an intracellular messenger [1], in the response of the immune system [2] and there is evidence that it contributes to neurodegeneration in diseased states [3]. In the environmental field, the compound is used (sometimes in combination with organic peroxides) as a water disinfectant as an alternative to chlorine or to chlorine dioxide.

Catalase (Cat) is an oxidoreductase enzyme formed by a heme group containing a ferric (Fe³⁺) central ion; in addition, Cat contains a NADPH group. This enzyme catalyzes substrate oxidation by organic peroxides. Catalase is also able to disproportionate hydrogen peroxide into water and oxygen:

\[ \text{H}_2\text{O}_2 + \text{Cat} \rightarrow \text{Cat} + \text{H}_2\text{O} + \text{O}_2 \]

In this application note, we propose a novel indicating scheme for the detection of H₂O₂ based on detecting the O₂ formed during the enzymatic reaction by coupling a biosensor membrane of catalase into a Fiber Optic Oxygen Sensor System (FOXY-R) [4]. This system is phase fluorometer-coupled chemical sensor for full spectral analysis of dissolved oxygen. A fluorescence method is used to measure the partial pressure of dissolved or gaseous oxygen.

2. Experimental conditions

The enzyme immobilization is made by entrapment in a polymeric matrix of polyacrylamide. 75 mg acrylamide and 5 mg of bis-acrylamide are mixture in 500 µL of Cat 4.12 kU·mL⁻¹ solution in milliQ water. Then 4 µL of (NH₄)₂S₂O₈
(10%) is added. After bubbling with N\textsubscript{2} during a few minutes to remove dissolved oxygen and 0.4 mL TEMED are added. After that the mixture is poured into a PTFE tube (diameter=0.36 mm) covering with a plug. The mixture was left to react during 30 minutes. After the polymer formation the Cat membrane is removed from the tube and is introduce in the Cat-O\textsubscript{2} sensor.

The Cat-O\textsubscript{2} sensor consists on a centre holed (0.3 x 0.4 mm) 0.36 x 20 mm PTFE tube closed by two septum caps (figure 1). The PAA-catalase membrane is placed at the bottom and the Foxy-R Oxygen Sensor is inserted through the top cover. The PAA-probe relative position highly affects the method sensitivity; both should be as near as possible avoiding a direct contact between them. An optical fiber carries excitation light produced by a blue LED (450 nm) to the ruthenium thin-film coating at the FOXY-R probe. Fluorescence generated at the tip is collected by the probe and carried by the optical fiber to the phase fluorometer. When oxygen diffuses into the ruthenium thin-film, it quenches the fluorescence. The degree of quenching correlates to the level of oxygen pressure and consequently with the quantity of hydrogen peroxide.

![Figure 1. Cat-O\textsubscript{2} sensor for H\textsubscript{2}O\textsubscript{2} determination](image)

This device has been designed to use it as an immersion probe. The solution to be tested is placed into a 1.5 mL Eppendorf tube in which the Cat-O\textsubscript{2} probe will be immersed and the minimum value of the fluorescence intensity \(F_{\text{min}}\) is obtained. The \(F_0\) is measurement from a blank solution (milliQ water).
3. Results

Figure 2 shows the shape of the $F=f(t)$ representations obtained. As can be seen a sudden intensity decrease is observed (due to the oxygen generation) that is followed by a short stabilization and the consequently increase of signal. Since the final stabilization time and the final signal depend on the sample container size, we decided to use the $F_{\text{min}}$ as the measurement parameter.

![Graph showing fluorescence intensity changes](image1)

**Figure 2.** Variation of the fluorescence intensity of the Cat-O$_2$ sensor during the enzymatic reaction using different [H$_2$O$_2$]: A) Blank; B) 4.69·10$^{-3}$ M; C) 1.18·10$^{-2}$ M ([Cat-Ru]=4.12 kU·mL$^{-1}$)

Figure 3 shows the calibration line obtained by the successive immersion of the Cat-O$_2$ probe in solutions containing different H$_2$O$_2$ concentration.

![Graph showing calibration line](image2)

**Figure 3.** Calibration line $\Delta F_{\text{min}}$ ([Cat]= 1.22·10$^{-6}$ M)
The lineal adjust obtained by the least square method is:

\[ \Delta F_{\text{min}} = 54.3_{\pm 6.8} \cdot 10^{-4} + 4.510_{\pm 0.078}[\text{H}_2\text{O}_2]_{\text{M}} \quad r=0.999 \]

4. Conclusion

The developed system allows the direct, immediate and selective determination of hydrogen peroxide in a lineal range that goes from \( 5.03 \cdot 10^{-4} \) M at less \( 1.40 \cdot 10^{-2} \) M with DSR = 5% (n=5; [\text{H}_2\text{O}_2]=3.85 \cdot 10^{-3} \) M).

5. References