Cat-O_2 sensor for H_2O_2 determination based on Foxy-R Oxygen Sensor

Written by Estefanía Ortega, Javier Galbán and Susana de Marcos

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.

H_2O_2 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 oxydoreductase enzyme formed by a heme group containing a ferric (Fe^{III}) 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:

$$H_2O_2 + Cat \rightarrow Cat + H_2O + O_2$$

In this application note, we propose a novel indicating scheme for the detection of H_2O_2 based on detecting the O_2 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 $kU \cdot mL^{-1}$ solution in milliQ water. Then 4 µL of $(NH_4)_2S_2O_8$

(10%) is added. After bubbling with N_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_2 sensor.

The Cat-O_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**.**

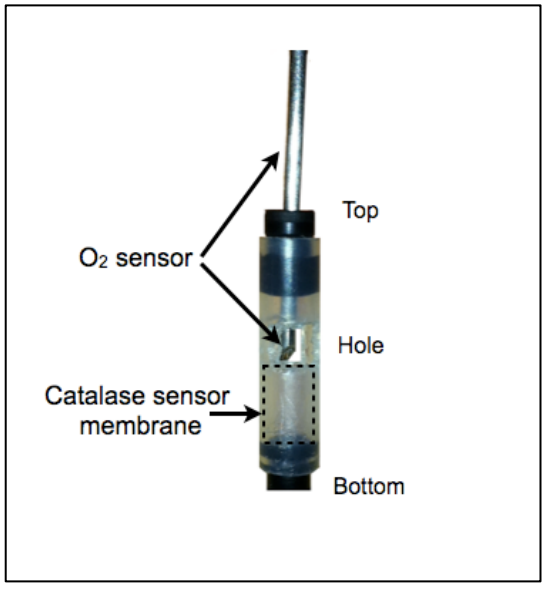

Figure 1. Cat-O_2 sensor for H_2O_2 determination

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_2 probe will be immersed and the minimum value of the fluorescence intensity F_{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_{min} as the measurement parameter.

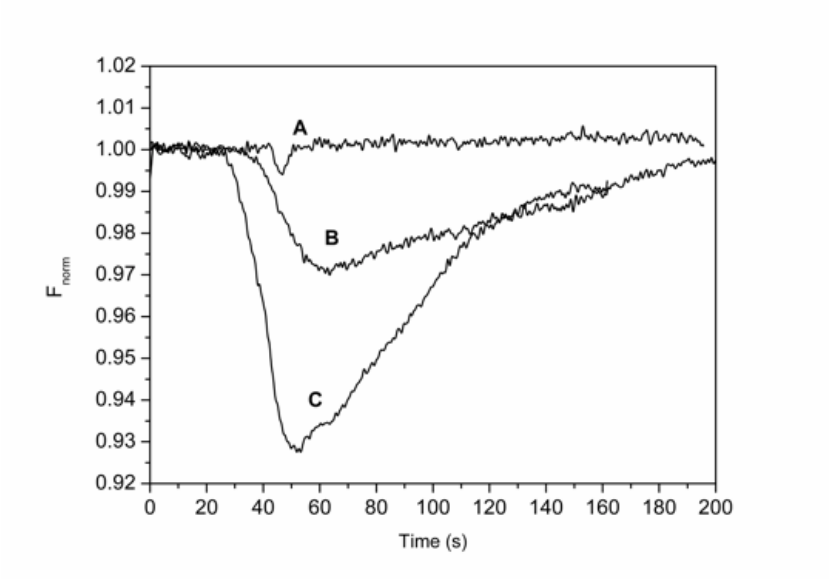

Figure 2. Variation of the fluorescence intensity of the Cat-O_2 sensor during the enzymatic reaction using different [H_2O_2]: A) Blank; B) $4.69 \cdot 10^{-3}$ M; C) $1.18 \cdot 10^{-2}$ M ([Cat-Ru]=4.12 kU·mL^{-1})

Figure 3 shows the calibration line obtained by the successive immersion of the Cat-O_2 probe in solutions containing different H_2O_2 concentration.

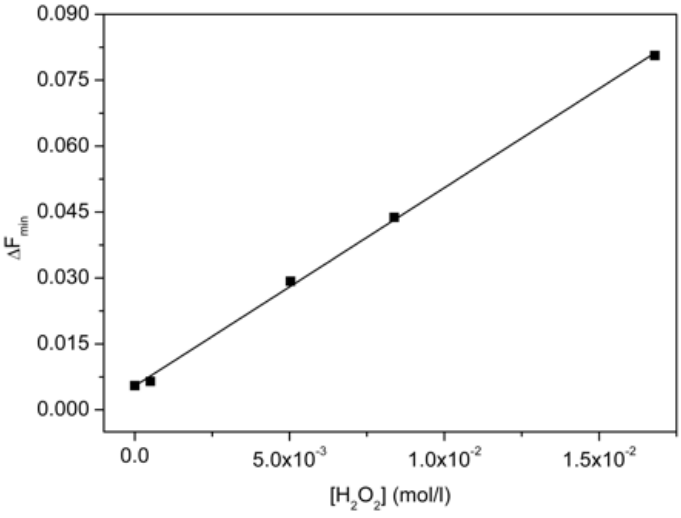

Figure 3. Calibration line ΔF_{min} ([Cat]= $1.22 \cdot 10^{-6}$ M)

The lineal adjust obtained by the least square method is:

$$\Delta F_{min} = 54.3_{\pm 6.8} \cdot 10^{-4} + 4.510_{\pm 0.078} [H_2O_2, M] \quad r=0.999$$

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; $[H_2O_2]=3.85 \cdot 10^{-3}$ M).

5. References

[1] Veal EA, Day AM, Morgan BA. *Hydrogen peroxide sensing and signaling*. Molecular cell **2007**; 26: 1-14.

[2] Niethammer P, Grabher C, Look AT, Mitchison TJ. *A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish*. Nature **2009**; 459: 996-999.

[3] O'Brien KB, Killoran SJ, O'Neill RD, Lowry JP. *Development and characterization in vitro of a catalase-based biosensor for hydrogen peroxide monitoring*. Biosensors & bioelectronics **2007**; 22: 2994-3000.

[4] Ortega E, de Marcos S, Galban J. *Fluorometric enzymatic autoindicating biosensor for H2O2 determination based on modified catalase*. Biosensors & bioelectronics **2013**; 41: 150-156.