AP-22: One-Step Chemiluminescent Assay for Hydrogen Peroxide Analysis in Water

Ahlem TENIOU [1], Marwa ZERMANE[1], Ibrahim A. MADI [1], Riane MOUHOUB [1], Jean Louis MARTY [2] and Amina RHOUATI [1]

[1] *Bioengineering Laboratory, Higher National School of Biotechnology, Constantine 25100, Algeria; a.teniou@ensbiotech.edu.dz (A.T.); madiibrahim01@gmail.com (I.A.M.); rianemouhoub@yahoo.com (R.M.)*
[2] *Universite de Perpignan Via Domitia, Unité de formation de recherche Sciences, 66860 Perpignan, France*

Email* : teniouahlem97@gmail.com

Subject description: In the recent decades, biosensors have emerged as valuable tools in various fields: healthcare, environmental monitoring, food safety, agriculture, and biosecurity. In this context, the detection of hydrogen peroxide is of great importance in the environmental field.

Objectives: In this work, we aim to develop a simple, and rapid, nonenzymatic and homogeneous biosensor for sensitive and rapid quantification of hydrogen peroxide.

Methods: In this technique, hemoglobin was used as a bioreceptor, where heme groups act as electroactive centers to catalyze hydrogen peroxide reduction. The chemiluminescence reagent; luminol is also a peroxidase substrate and can be oxidized by hemoglobin thus generating a CL signal. The working principle was based on the competition between hydrogen peroxide and luminol towards hemoglobin. A 96-well microplate was used to perform this assay. For thatat, 25 µL of Hb solution (3 µg mL^{-1}) was added to each well, followed by 25 µL of different concentrations of H_2O_2. After incubation, 25 µL of luminol solution was also added. Then, the chemiluminescence intensity was measured at an emission wavelength of 425 nm.

Results and discussion: The detection principle is mainly based on the chemiluminescence signal diminution in the presence of H_2O_2. Because this latter will react with Hb that is essential for luminol reaction, thus leading to a lower chemiluminescence signal. AlsoUnder optimized conditions, the chemiluminescent signal decreased with increasing hydrogen peroxide concentrations within the linear range of 0.5 to 12 mM, with a correlation coefficient R^2 of 0.99762. The limit of detection was calculated to be as low as 0.308 mM. The selectivity of the biosensor was successfully demonstrated against different interferents.

Conclusion: In this work, an homogenous chemiluminescent assay was developed using Hb as a bioreceptor. The developed strategy provides a one step, simple, and low-cost bioanalytical method which can be applied for the monitoring of hydrogen peroxide.

Keywords: chemiluminescence; hydrogen peroxide; hemoglobin; one-step analysis; environmental monitoring.