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Electrodeposited Prussian Blue on carbon black modified disposable electrodes for direct enzyme-free H_2O_2 sensing in a Parkinson's disease model

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Abstract

The combination of Carbon Black (CB) and electrodeposited Prussian Blue (PB) covered with a Nafion layer on Screen-Printed electrodes (CB/PB-SPE) for non-enzymatic H_2O_2 sensing in SH-SY5Y Neuroblastoma cell line is presented. These cells were challenged with 6-hidroxidopamine (6-OHDA) for modelling Parkinson's disease. Electrochemical sensing of H_2O_2 was carried out at very low potentials (-50mV), with a LOD of 0.01 µM and linear range between 0.2 and 1000 µM, allowing interference-free detection of H_2O_2 in the selected cell culture. The H_2O_2 concentration was successfully monitored in an experimental model of Parkinson's disease and correlated to the cell viability.

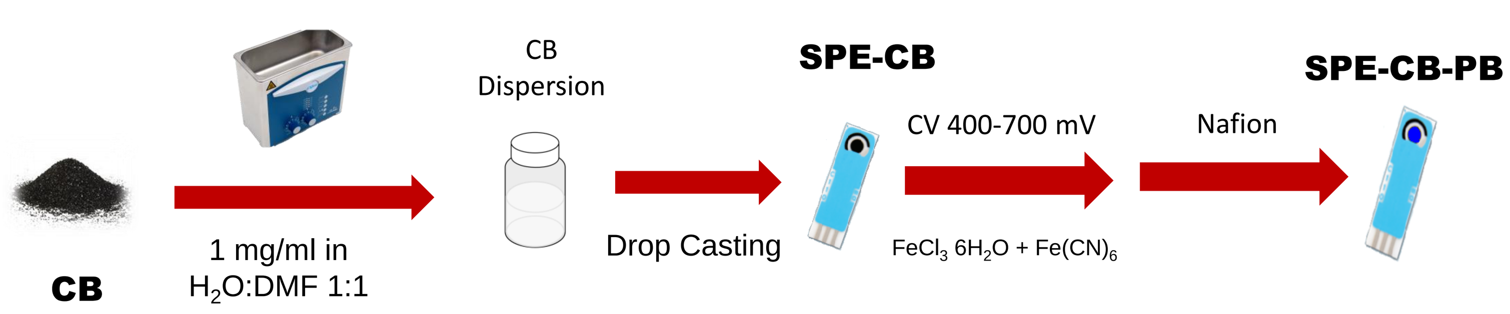
Introduction

Oxidative Stress is defined as an imbalance between oxidant stressors and antioxidant defences, this physiological status leads to several diseases such cancer, ischemia, atherosclerosis, Alzheimer's and Parkinson's disease (PD). Hydroxydopamine (6-OHDA) is a selective catecholaminergic neurotoxin that has been widely used to produce PD models in vitro and in vivo; it induces a toxicity status that mimics the neuropathological and biochemical characteristics of PD. 6-OHDA is rapidly oxidized by molecular oxygen to form the superoxide anion, hydrogen peroxide, and 2-hydroxy-5-(2-aminoethyl)-1,4-benzoquinone. Therefore, a quantification of the hydrogen peroxide produced could give information about the PD mechanism and status. Hydrogen peroxide is commonly used as oxidative stress marker due to its relative stability in contrast to superoxide, nitric oxide or peroxynitrite. Different analytical strategies have been proposed for H_2O_2 detection such as chemiluminescence, fluorescence, and electrochemical techniques. Among these, electrochemical sensors are very appealing for their simplicity, speed, sensitivity, miniaturization and cost-effectiveness. Nanomaterials have emerged as electrode modifiers since are able to shows improve their characteristics compared with their macroscopic counterparts allowing to improve LOD, sensitivity and selectivity. Prussian Blue (PB), also known as "artificial peroxidase" is one of the most known and widely used electrocatalyst for H_2O_2 reduction. PB allows low potential and interference-free detection of H_2O_2 in oxygenated media; nonetheless, has some disadvantages such as poor stability at physiological pH and high crystallization rate which hinder the potential use in nanocomposites and application in biological media

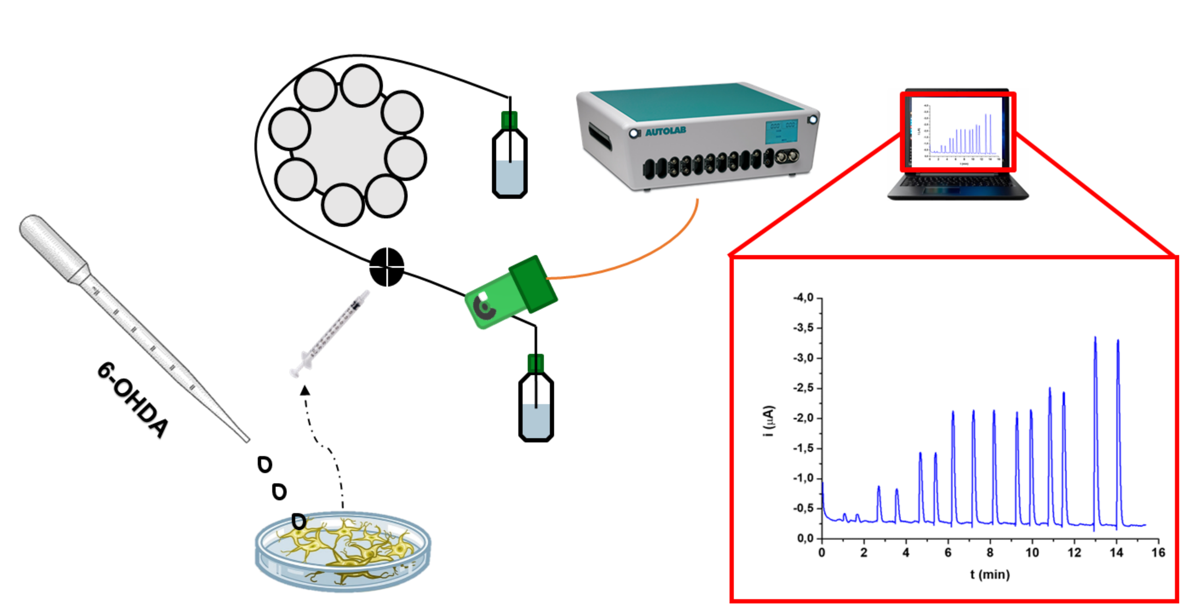
Objectives

- Development and characterization of CB-PB sensor for non-enzymatic H_2O_2 sensing applications in cell cultures
- To test the developed sensor in sensing in SH-SY5Y cell line. These cells were challenged with 6-hidroxidopamine (6-OHDA) for 'modelling' Parkinson's disease

Experimental



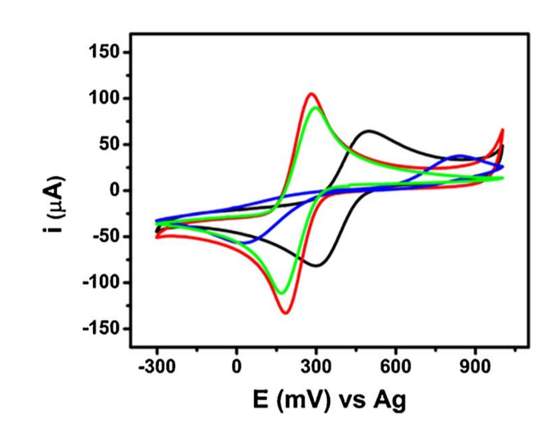
Electrode fabrication



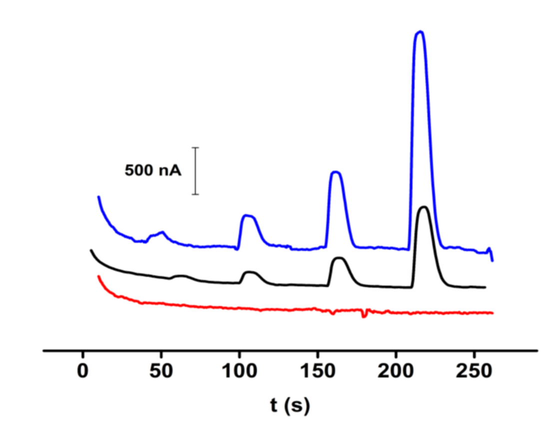
6-OHDA+O_2→p-quinone+ H_2O_2

Scheme of the experimental set-up

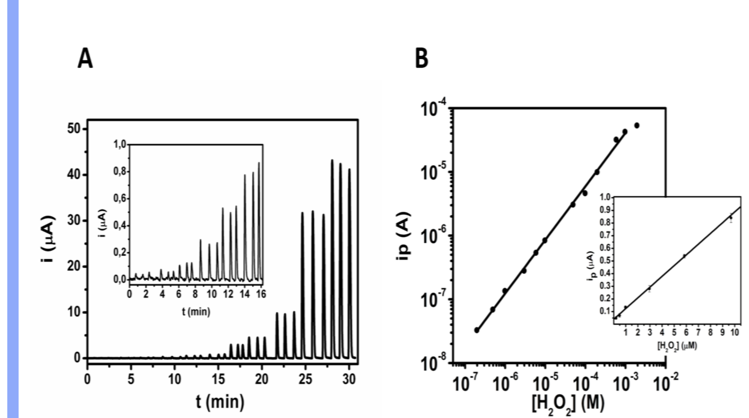
Results



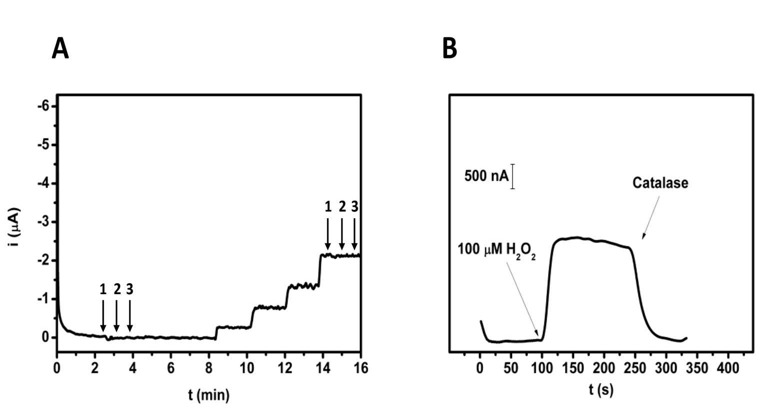
Cyclic Voltammetry of PB precursors solutions, 5 mM Fe^{3+} and 5 mM $[Fe(CN)_6]^{3-}$in a bare SPE (Blue and Green respectively) and SPE-CB (Black and Red respectively) recorded at 40 mV/s in 0.1M HCl and 0.1M KCl.



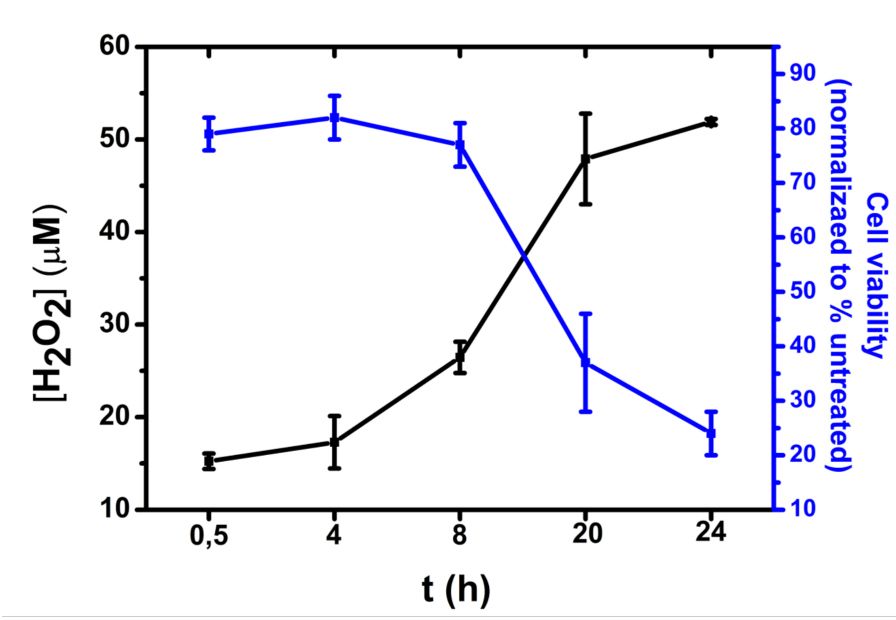
Amperometric signals in FIA for 5, 10, 20 and 50 µM of H_2O_2 in Phosphate Buffer 50 mM, 0.1 KCl (pH=7.4)SPE-CB (red line), SPE-PB (black line) and SPE-CB/PB (blue line).



A) Signals in a FIA system to different concentrations of H_2O_2 B) Calibration plot for wide linear range. Inset: calibration plot for the lowest points. Measurements carried out in phosphate buffer (pH=7.4) flow rate 0.6 ml min-1; E= -50 mV.



A) Amperometry signals due to the addition of FBS (1), L-Glu (2) and P/S (3) in DMEM medium B) Selectivity of the electrode towards 100 µM of H_2O_2 spiked in the cell culture without cells. E=-50 mV vs Ag



Hydrogen peroxide concentration (black) and cell viability (blue) in Parkinson's disease cellular model at different incubation time

Conclusions

- An enzyme-free electrochemical sensing platform was successfully proposed taking advantage of CB properties to enhance PB electrodeposition and improve the signal towards H_2O_2 reduction.
- The described sensor showed detection limit in the nanomolar range and showed excellent selectivity in a complex environment such as the culture medium used, allowing the selective determination of very low amounts of H_2O_2 without interferences.
- These results could pave the way for a better understanding the neurotoxic effect of hydrogen peroxide using an in vitro model of Parkinson's disease.

References

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