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Disposable electrodes for direct enzyme-free H_2O_2 sensing in a Parkinson's disease in-vitro model

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Introduction

Oxidative Stress

- Defined as an imbalance between oxidant stressors and antioxidant defences
- Leads to several diseases such cancer, ischemia, atherosclerosis, aging, Parkinson's and Alzheimer's disease
- 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 oxidized rapidly by molecular oxygen to form the superoxide anion, hydrogen peroxide, and 2-hydroxy-5-(2-aminoethyl)-1,4-benzoquinone.
- Hydrogen peroxide is commonly used as oxidative stress marker due to its relative stability in contrast to superoxide, nitric oxide or peroxynitrite

H_2O_2 sensing

- 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
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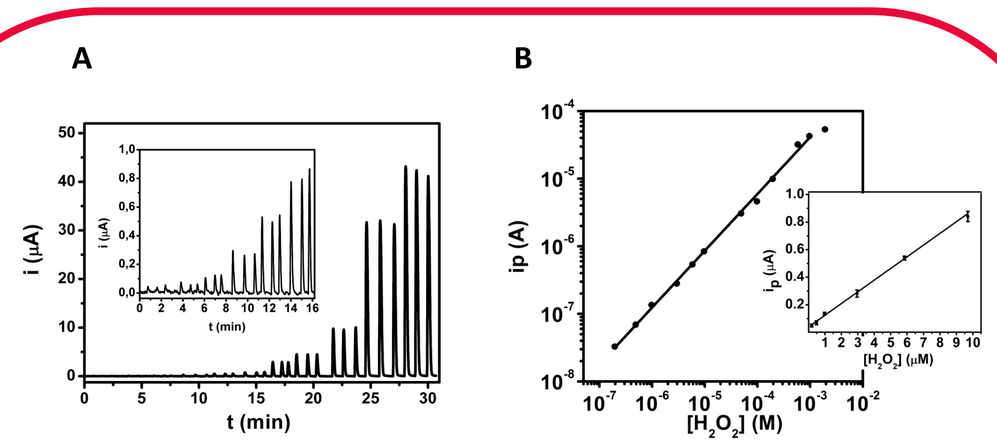
Nanomaterials

- Nanomaterials shows improved 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
- Nanostructured CB exhibit excellent conductivity, unique electrochemical properties and cost-effectiveness (about 1 euro/kg). For these reasons, in the last years CB dispersion hes been used for electrode modification, showing remarkable electrocatalytic properties towards several species of analytical interest.

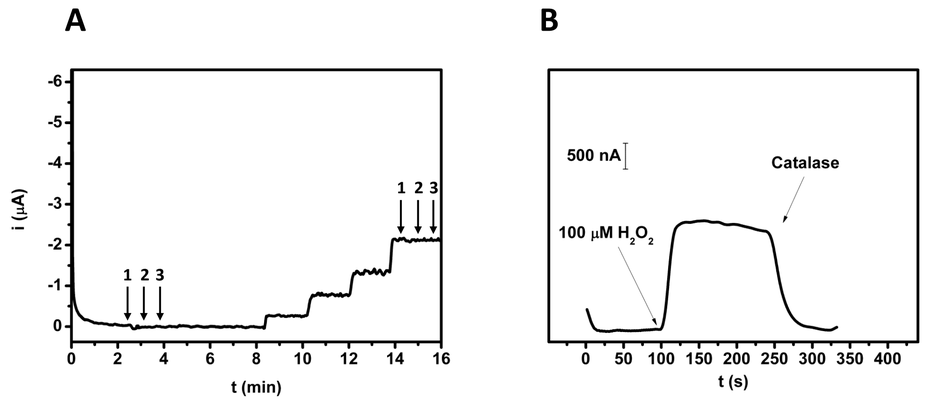
Objectives

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

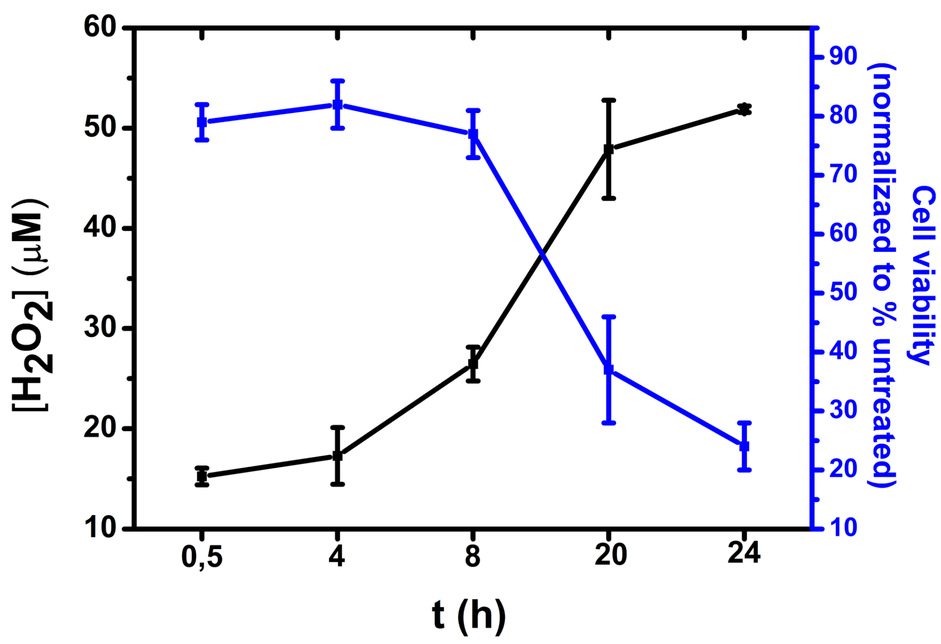
Results



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.

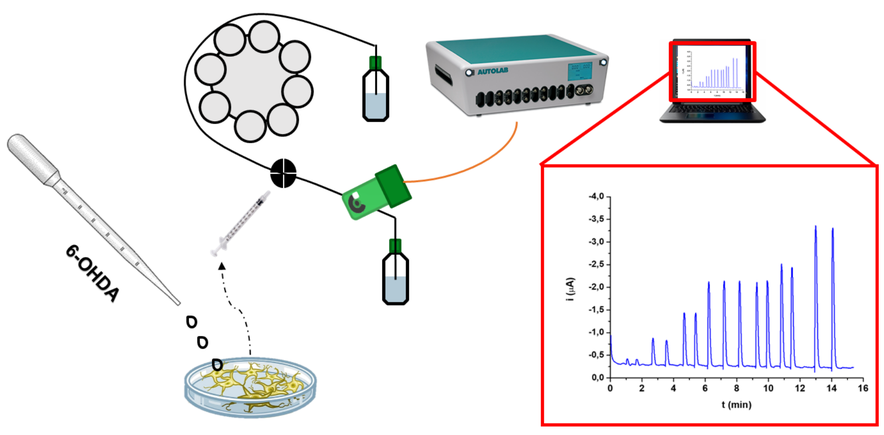


A) Amperometry signals due to the addition of FBS (1), L-Glu (2) and P/S (3) in DMEM medium B) Selectivity of the electrode towards 100 μM of H_2O_2 spiked in the cell culture without cells. E=-50 mV vs Ag



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

Experimental set-up



Scheme of the experimental set up

Conclusions

- An enzyme-free electrochemical sensing platform was successfully proposed for the quantification of H_2O_2 released by SHSY5Y cells.
- 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 ROS using an in vitro model of Parkinson's disease.