

Nanomaterial-based Electrochemical Sensing For Cell Lines Oxidative Stress Evaluation And For Bio-compounds Detection In Food

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Abstract

In this work different electrochemical sensors based on nanomaterials aimed to assess cell oxidative stress and bioactive compounds are proposed. These sensors represents the first step in the development of an integrated lab-on-chip microfluidic platform to study the antioxidant protection action of food components towards oxidative stressors in selected cell cultures. Carbon black (CB)-Prussian Blue (PB) based electrodes for H₂O₂ sensing in a Parkinson's disease model. The excellent analytical performance and selectivity achieved in cell culture media is due to the combination of features of both nanomaterials. Moreover, a new hybrid nanomaterial based on CB and molybdenum disulfide is proposed for class-selective evaluation of ortho-diphenols. This nanohybrid shows improved charge-transfer properties and enhanced electrocatalysis compared to the individual nanomaterials. Moreover, an enhanced fouling resistance is achieved. The sensor features has been exploited for polyphenols analysis in olive oil and cocoa samples. These results are the basis for the realization of an integrated lab-on-chip device able to evaluate the antioxidant protection of food functional components towards oxidative stress.

Introduction

Oxidative Stress is defined as an imbalance between oxidant stressors and antioxidant defenses, this physiological status leads to several diseases such cancer, ischemia, atherosclerosis, Alzheimer's and Parkinson's disease (PD). Hydrogen peroxide is commonly used as oxidative stress marker due to its relative stability in contrast to superoxide, nitric oxide or peroxynitrite. On the other hand, polyphenols are widely know antioxidants that are able to mitigate oxidative stress effects. This is especially evident for polyphenols with ortho-diphenolic structures which have demonstrated the highest antioxidant capacity. Consequently, the determination of o-diphenols in polyphenols rich samples is very interesting. For example, olive oil contains two main o-diphenols hydroxytyrosol (HYT) and oleuropein (OLEU) while cocoa contains catechin (CT), epicatechin (EP) and epigallocatechin (EG). Different analytical strategies have been proposed its detection such as chemiluminescence, fluorescence, and electrochemical techniques. However, electrochemical sensors are very appealing for their simplicity, speed, sensitivity, miniaturization and cost-effectiveness. Their modification with 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.

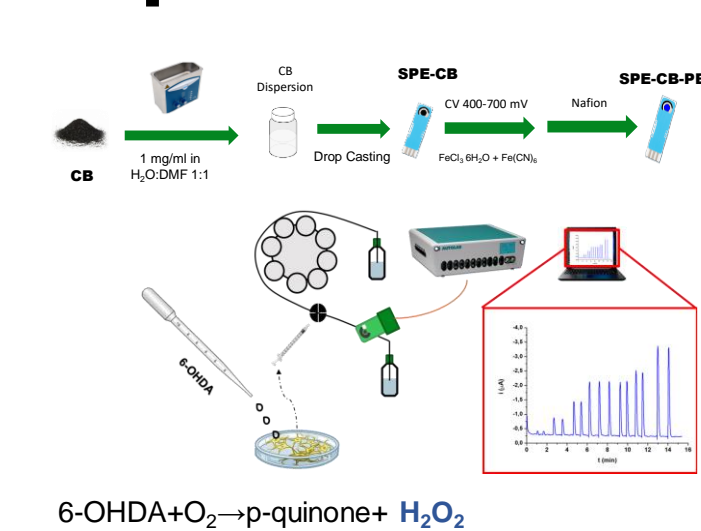
Therefore, a simultaneous quantification of oxidative stress and polyphenols in easy-to-use, low sample consuming and with fast analysis time are very desirable. In this context, lab-on-chip devices together with nanomaterials-based electrodes appears as the platform able meet these requirements

Objectives

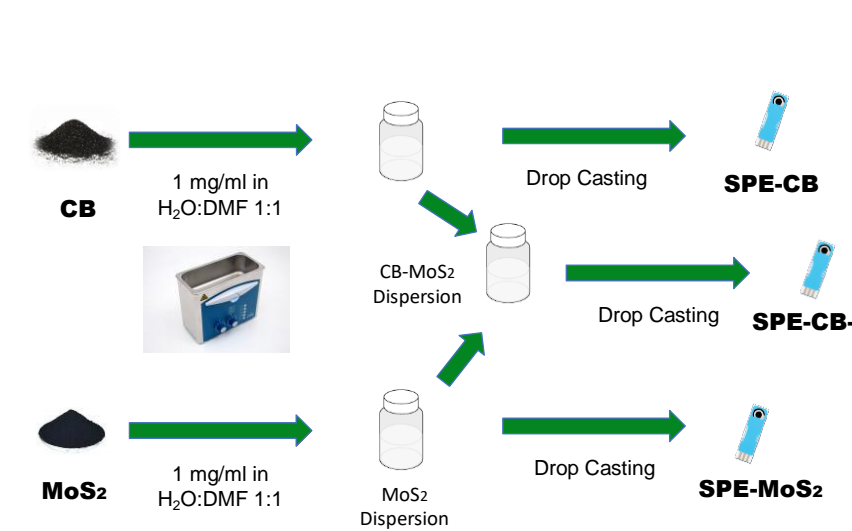
- Develop a sensor able to asses H₂O₂ concentrations in cell culture as oxidative stress marker.
- Apply the sensor to in SH-SY5Y cell line challenged with 6-hidroxidopamine (6-OHDA) for 'modelling' Parkinson's disease.
- Develop a sensor able to perform quantitative analysis of o-diphenols from different foodstuffs with high antioxidant capacity.

Experimental

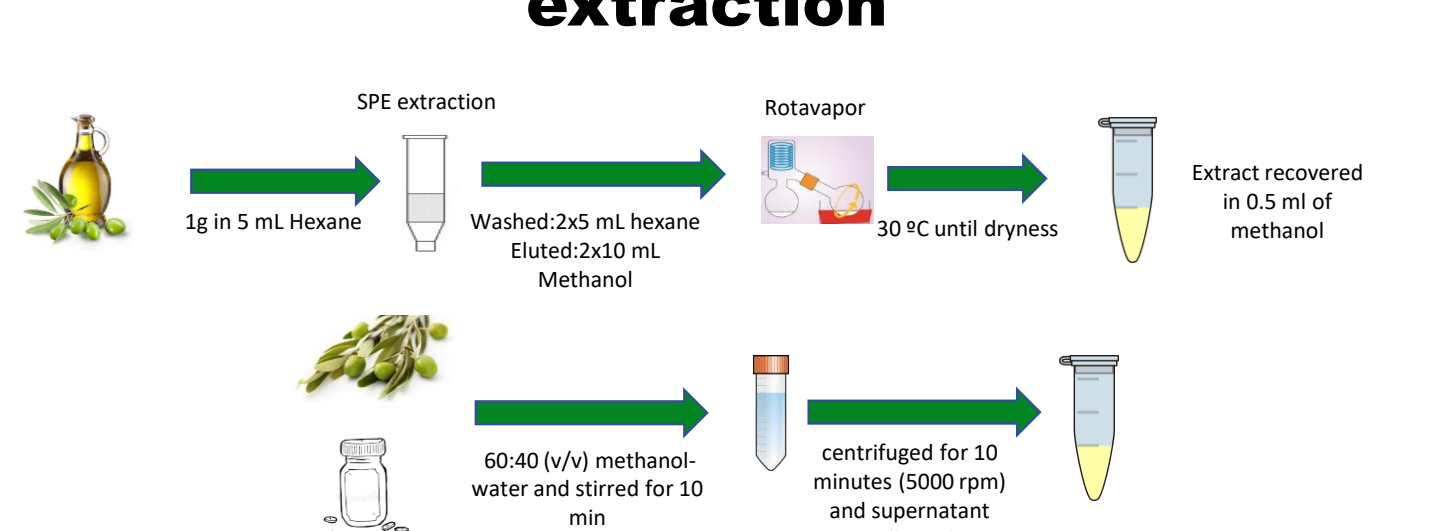
CB-PB electrode fabrication and experimental set-up



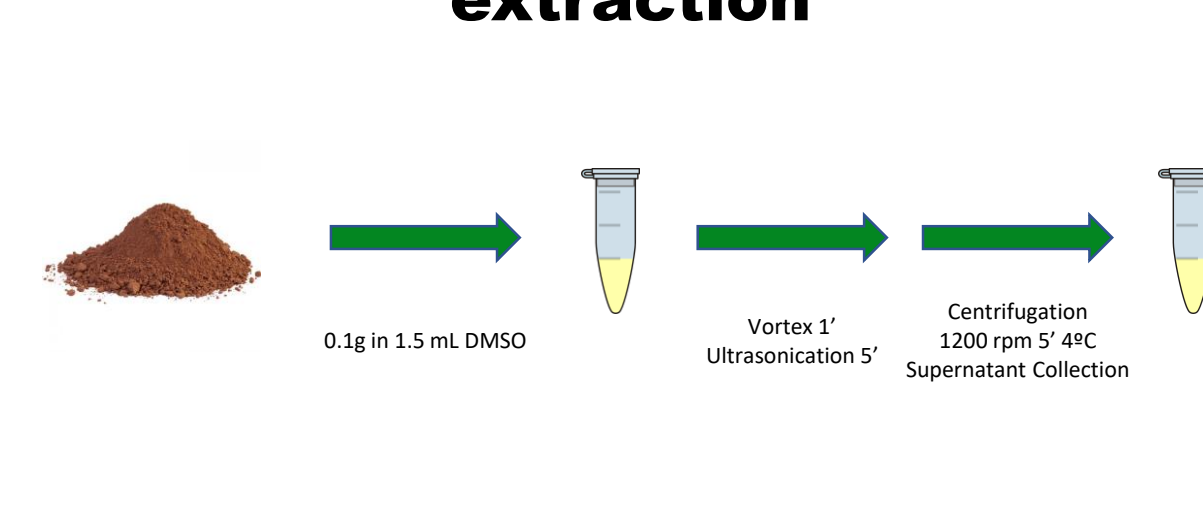
CB-MoS₂ electrode fabrication



Olive Oil and related samples polyphenols extraction



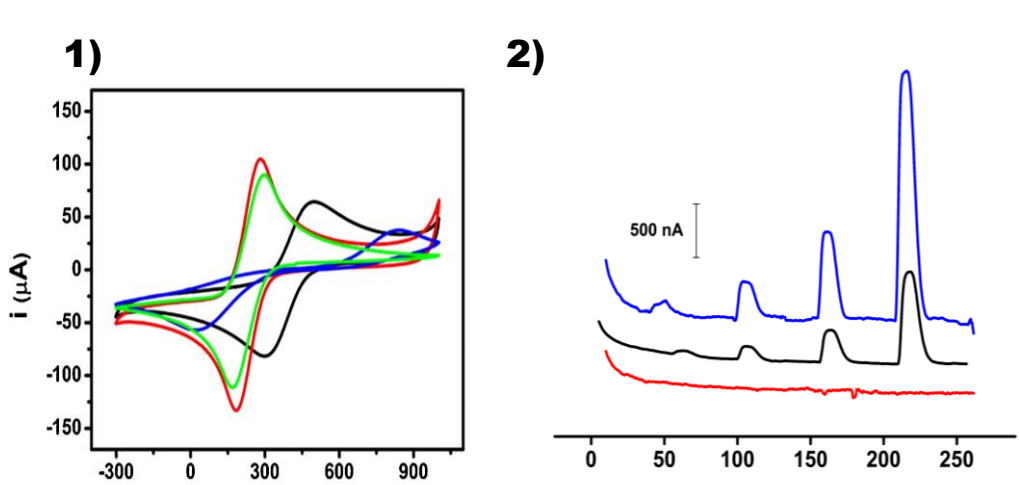
Cocoa samples polyphenols fast extraction



Results

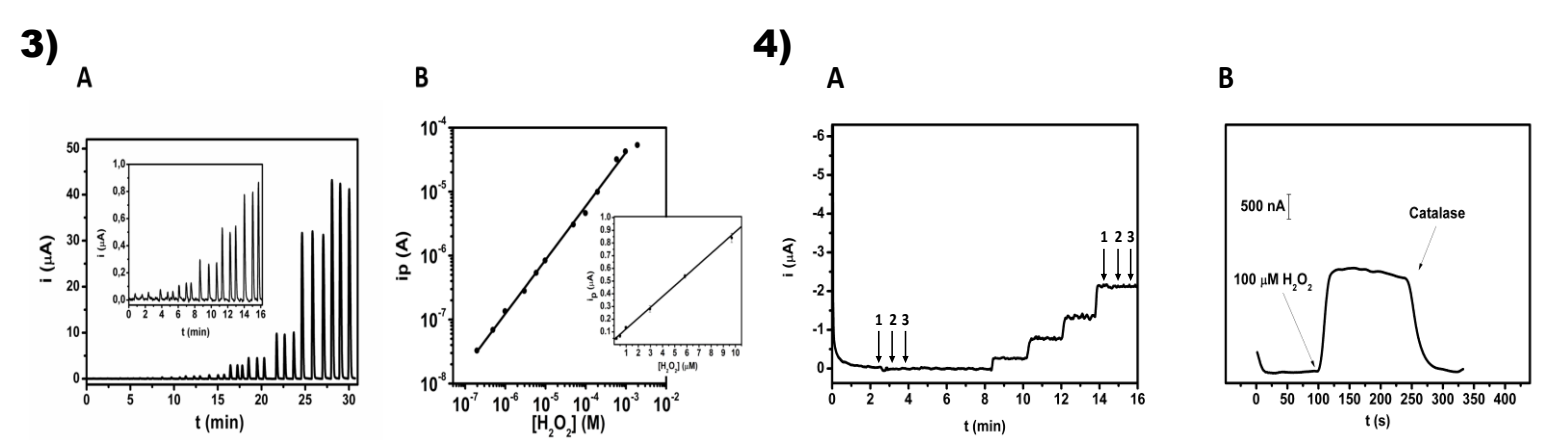
CB-PB electrodes for H₂O₂ sensing

Electrochemical Characterisation



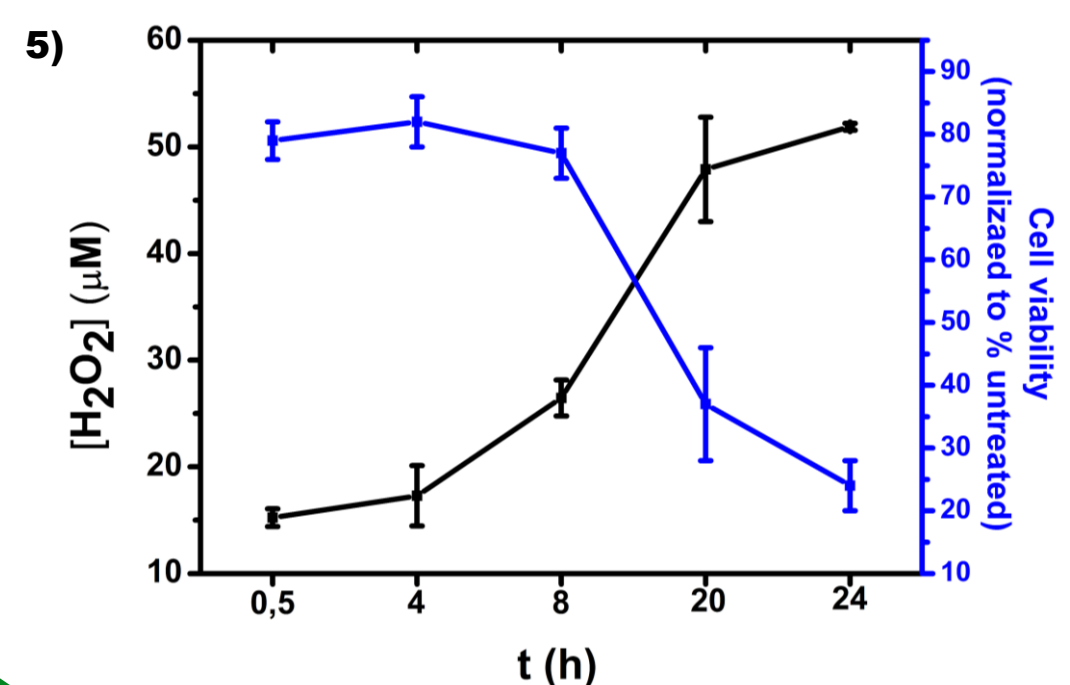
1) Cyclic Voltammery of PB precursors solutions, 5 mM Fe³⁺ and 5 mM [Fe(CN)₆]³⁻ in a bare SPE (Blue and Green respectively) and SPE-CB (Black and Red respectively) recorded at 40 mV/s in 0.1M HCl and 0.1M KCl. 2) Amperometric signals in FIA for 5, 10, 20 and 50 μM of H₂O₂ in Phosphate Buffer 50 mM, 0.1 M KCl (pH=7.4) SPE-CB (red line), SPE-PB (black line) and SPE-CB/PB (blue line)

Analytical Performance



3) A) Signals in a FIA system to different concentrations of H₂O₂ 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. 4) 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 H₂O₂ spiked in the cell culture without cells. E= -50 mV vs Ag

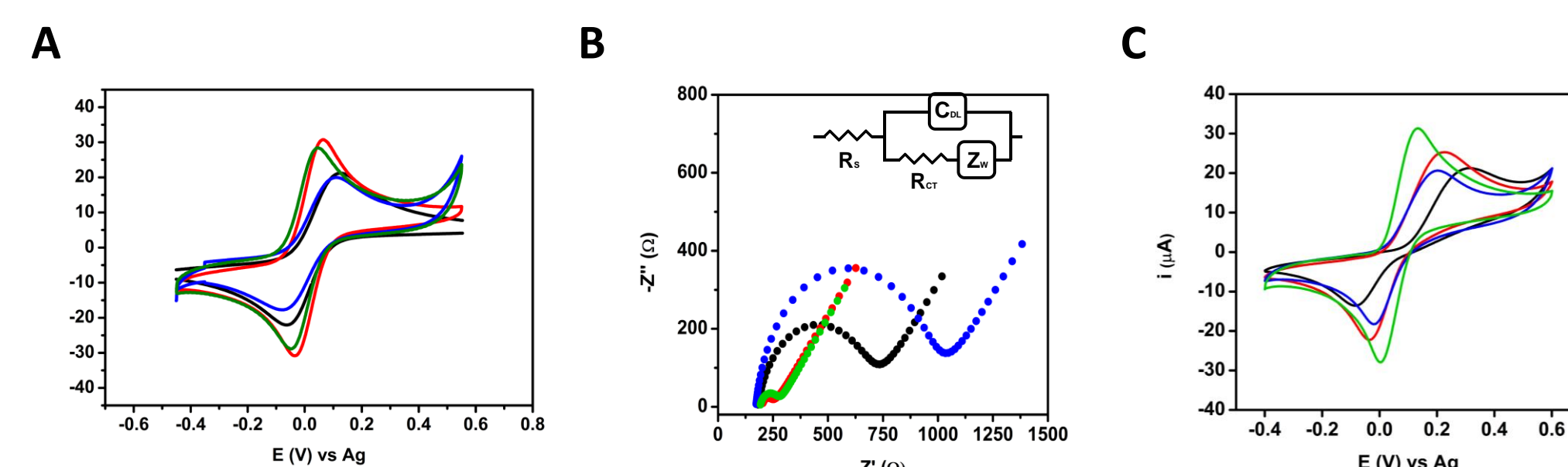
Results in Cellular Model



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

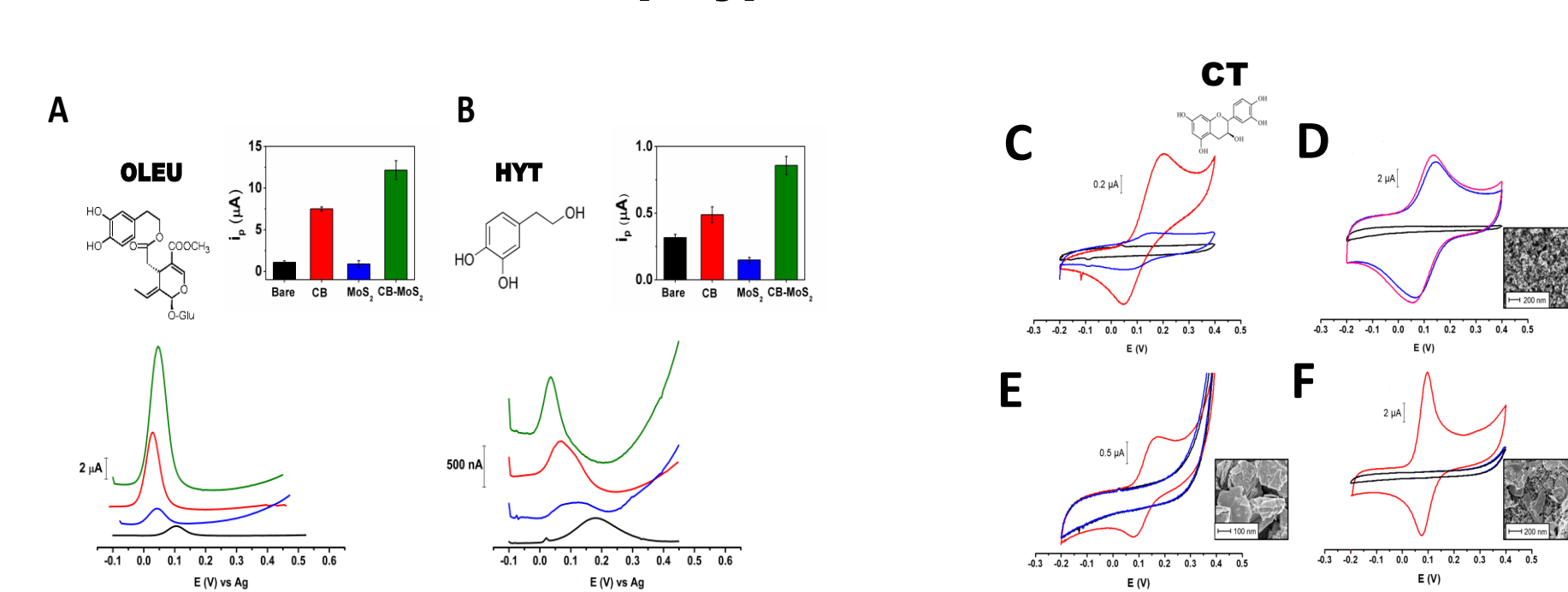
CB-MoS₂ electrodes for olive oil and cocoa polyphenols analysis

Electrochemical Characterisation



Electrochemical characterization employing A) cyclic voltammery and B) electrochemical impedance spectroscopy using [Fe(CN)₆]^{3-/4-} as redox probe C) Cyclic voltammery of a solution containing 1 mM Catechol as representative molecule of o-diphenol structure. For different electrodes: SPE (black), CB (red), MoS₂ (blue) and MoS₂-CB (green)

Olive oil and cocoa polyphenols electrochemical reactivity



Comparison of olive oil and cocoa main o-diphenols using DPV signals for A) OLEU and B) HYT. Inset shows the peak intensity for 5 different electrodes: SPE (black), CB (red), MoS₂ (blue) and CB-MoS₂ (green). Cyclic voltammograms of the bare SPE (C), SPE-CB (D), SPE-MoS₂ (E), and SPE-CB/MoS₂ (F). Demonstration of fouling resistance of each nanomaterial. Black line in blank solution, red line of 50 μM CAT, blue line blank solution after catechins CV.

Comparison of CB-MoS₂ electrochemical sensor with conventional methods

Sample	Sample number	Reference Method	r
Olive oil, leaves extracts and dietary supplements	6	HPLC	0.995
Cocoa	59	Folin-Ciocalteu	0.972
Cocoa	59	ABTS	0.949
Cocoa	59	AuNPs	0.966

Conclusion

- An electrochemical sensing platform was developed taking advantage of CB properties to enhance PB electrodeposition and improve the signal towards H₂O₂ reduction.
- The described CB-PB 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 H₂O₂ without interferences.
- An electrode transducer for o-diphenolic structures has been developed taking advantage of CB and MoS₂ improving the electrochemical and electroanalytical features of individual nanomaterials.
- The CB-MoS₂ were applied to cocoa, olive oil and related olive samples and the results were compared to reference methods, being highly correlated (r>0.9)

References

- [1] D. Rojas, F. Della Pelle, M. Del Carlo, M. d'Angelo, R. Dominguez-Benot, A. Cimini, A. Escarpa, D. Compagnone, Electrodeposited Prussian Blue on carbon black modified disposable electrodes for direct enzyme-free H₂O₂ sensing in a Parkinson's disease in vitro model, Sensors and Actuators B Chem. 275 (2018) 402–408.
- [2] H. Zhang, R. Tsao Dietary polyphenols, oxidative stress and anti-inflammatory effects, Current Opinion in Food Science 8 (2016) 33–42
- [3] F. Della Pelle, D. Compagnone Nanomaterial-based sensing and biosensing of phenolic compounds and related antioxidant capacity in food, Sensors 18 (2018) 462

