Bioinspired Laccase-polydopamine Films for Amperometric Biosensors

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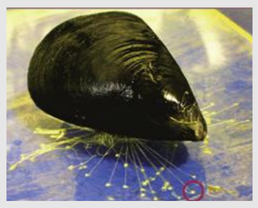
Introduction

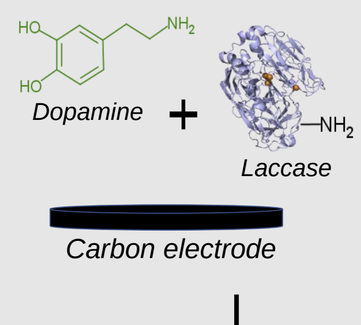
- Electrochemical biosensors have a tremendous potential to become cheap, fast and reliable analytic tools in many fields[1], eliminating the need of expensive equipment, highly trained personnel and time-consuming steps, however biofunctionalization strategies and biocompatible materials are still needed to achieve a proper immobilization of the biorecognition element of the sensor (e.g. enzyme).
- Synthesis of biomimetic adhesive polymers, inspired by mussel foot proteins, in commonly achieved by the standard polymerization of dopamine (DA) in alkaline medium, yielding a melanin-like semiconducting thin film – **polydopamine (PDA).**[2,3]
- In recent work, we have highlighted the advantages of the electrochemical routes over standard chemical methods, aiming the construction of ePDA-based biosensing matrices with optimized redox responses and ability to robustly immobilize biomolecules.[3,4]
- We present a one-step electrode modification where the enzyme Laccase (Lac) is co-immobilized during the potentiostatic deposition of a PDA film. The fast and efficient one-pot procedure proposed in this work is further explored in the detection of phenolic compounds.



Polydopamine films - Mussel-Inspired Surface Chemistry for Multifunctional Coatings[5]

Methods and techniques

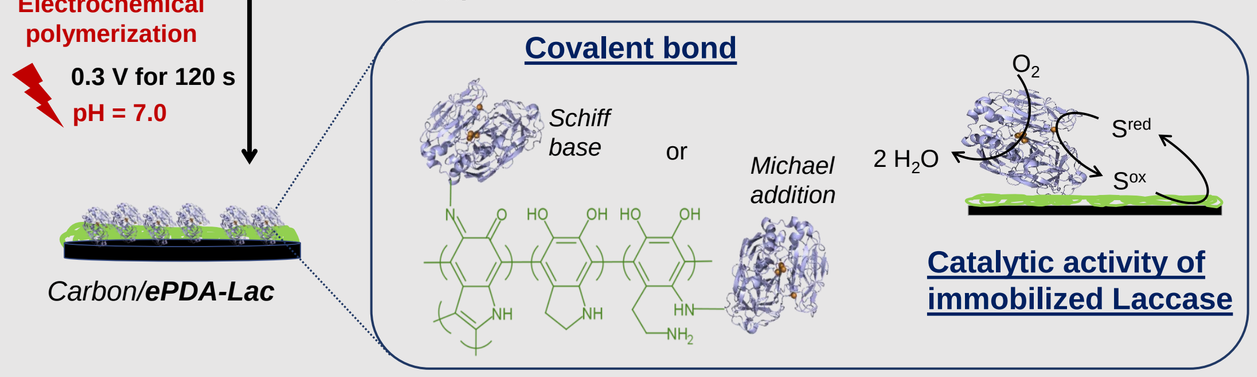
Carbon/ePDA/Lac preparation: Potentiostatic growth was performed at 0.3V for 120s, using a N_2 saturated citrate-phosphate buffer (pH = 7.0) containing 5 mM dopamine and Laccase booster (478 U mL^{-1}, pH = 4.6, 22 °C) diluted by a ratio of 1:500.



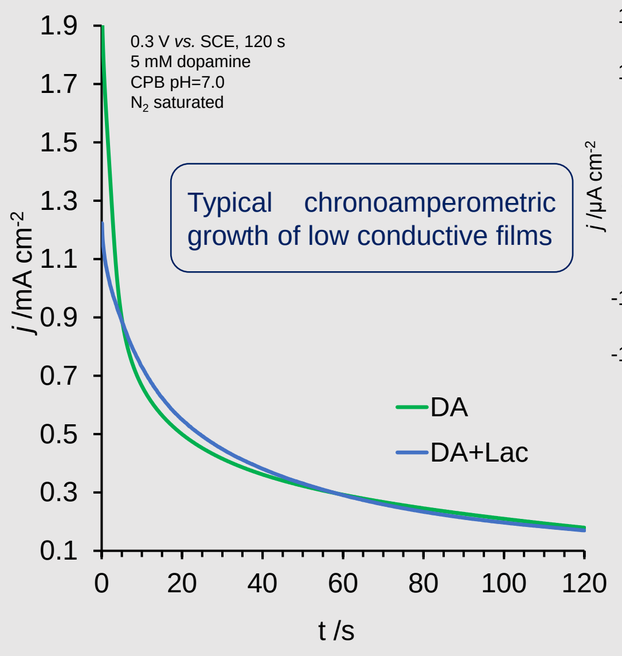
Electrochemistry: CE - Pt foil; RE - SCE; WE - Glassy carbon (GC) (0.44 cm^2) or Graphite (≃ 3.0 cm^2); CHI600A Electrochemical Analyzer.

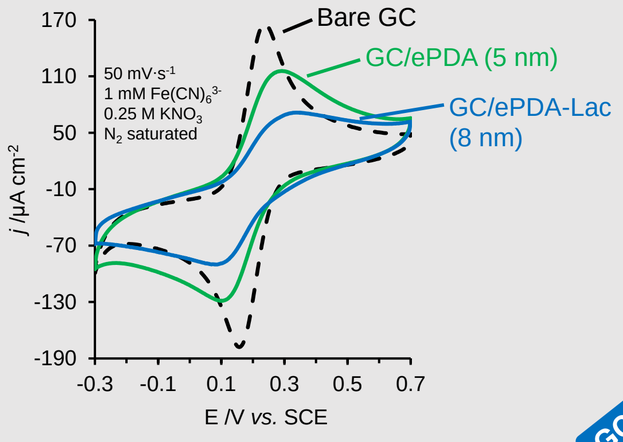
Ellipsometry: multi-incident angles from 40° to 70°, He-Ne laser at 632.8 nm; SENTECH Instruments GmbH SE400.

AFM: Nanoscope IIIa Multimode AFM Microscope (Bruker); Etched silicon tips (~300 KHz) were used for *Tapping* mode AFM.

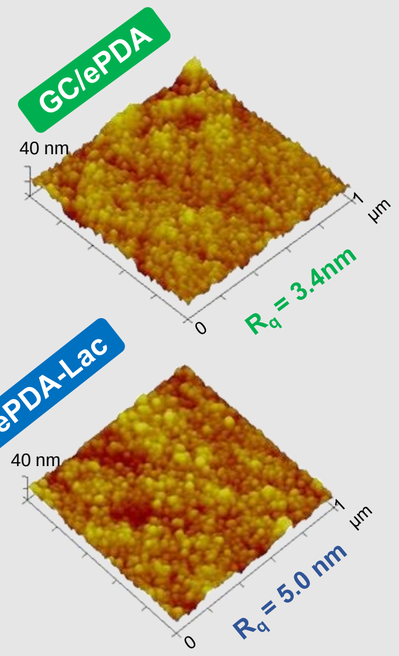


One-step electrosynthesis of Laccase-Polydopamine Films

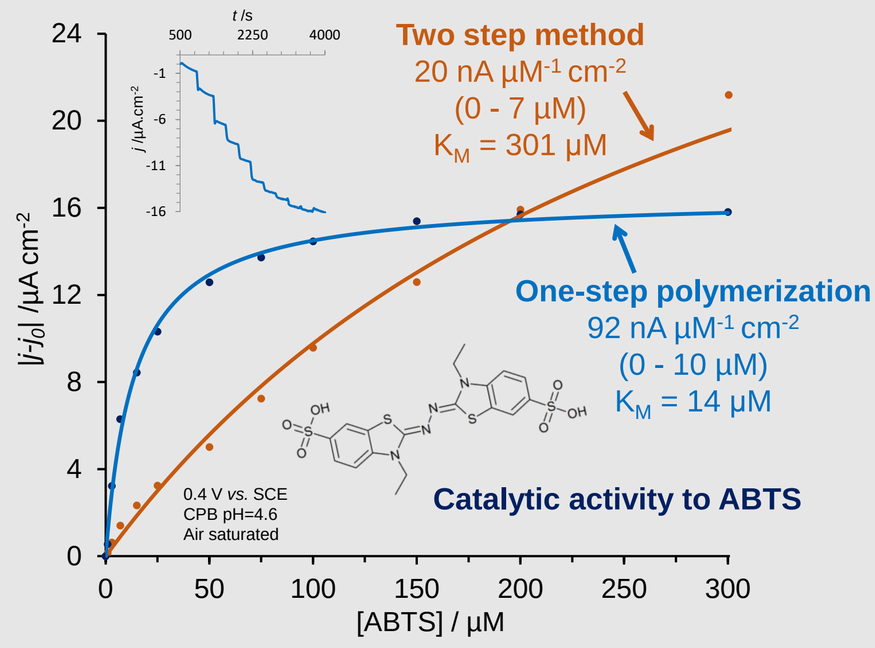




Fe(CN)$_6^{3-}$/Fe(CN)$_6^{2-}$ redox process is more hindered on PDA-Lac modified electrodes, due to the presence of isolating protein.



Enzyme immobilization efficacy



Catalytic performance

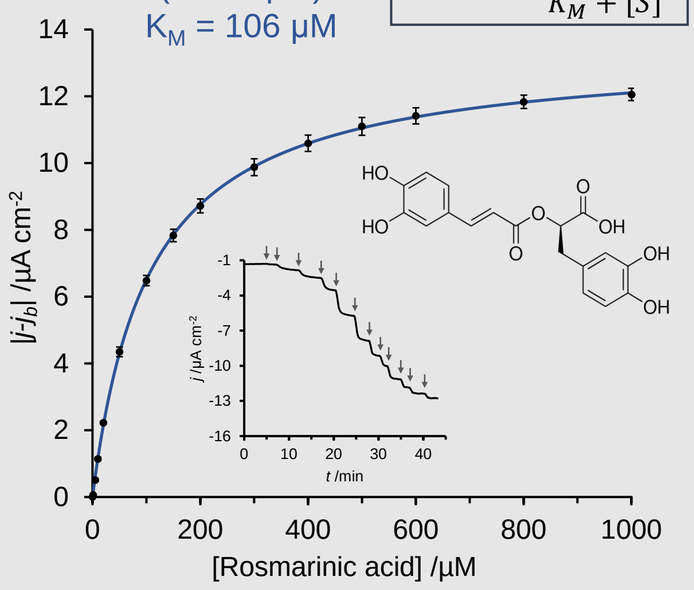
Rosmarinic acid

114 nA μM^{-1} cm^{-2}

(1 - 20 µM)

K_M = 106 µM

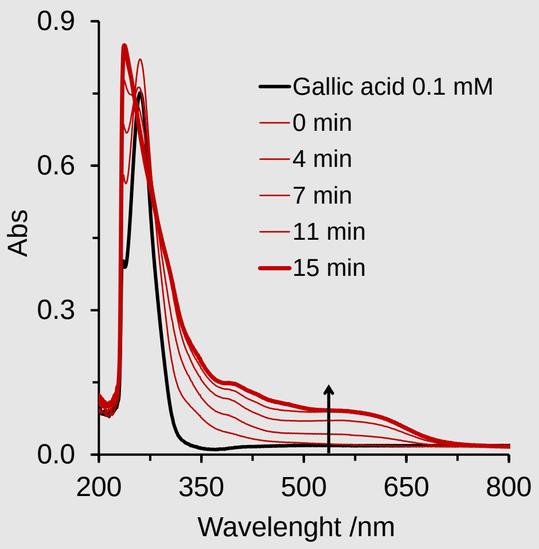
$$|j - j_0| = \frac{j_{max} \cdot [S]}{K_M + [S]}$$



Quantification of phenols on chestnut shells extract

Catalytic oxidation of Gallic acid by Lac – UV-Vis Spectroscopy

Gallic acid (0.1mM) + Lac (1:100000)

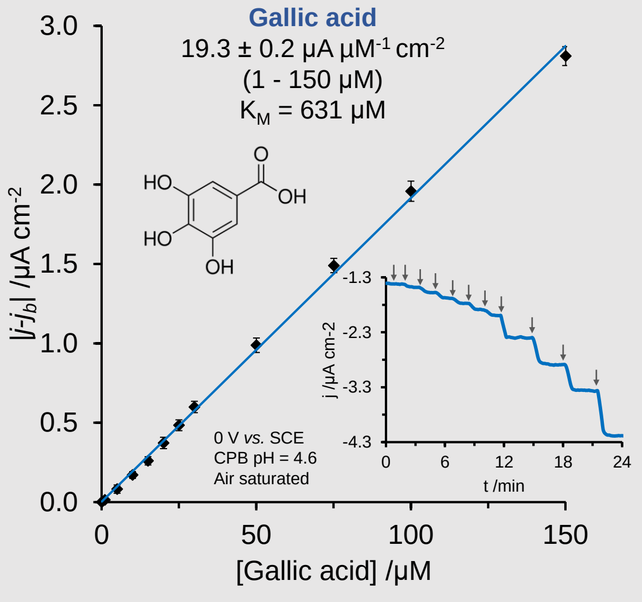


Gallic acid

19.3 ± 0.2 µA μM^{-1} cm^{-2}

(1 - 150 µM)

K_M = 631 µM



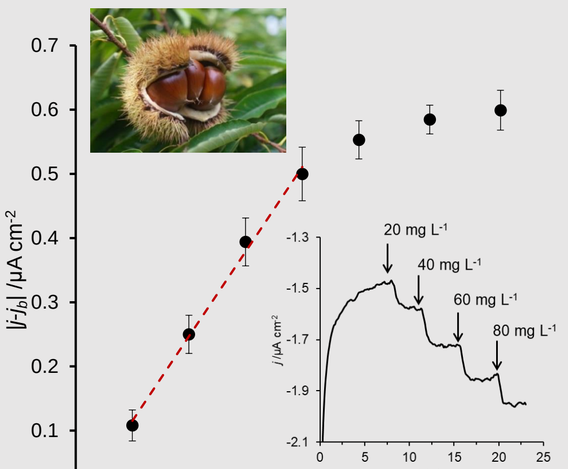
ePDA-Lac biosensor displays a wide linear range for phenolic compounds detection

Chestnut shell extract

56 ± 2 mg GAE g^{-1}

GAE = Gallic Acid Equivalents

(estimated from GA calibration curve)



Conclusions

- Laccase is robustly immobilized through one-step potentiostatic growth of polydopamine (ePDA), with a clear improvement of the analytical parameters (apparent Michaelis-Menten constant and sensitivity) for the catalytic detection of ABTS regarding the two steps method: immobilization of Lac on pre-synthesized PDA films.
- PDA-Lac electrodes display excellent catalytic performances for several phenolic compounds (caffeic acid, rosmarinic acid and gallic acid), allowing the estimation of total phenol content on agro-industrial waste extract.
- This work highlights the promising applications of these biocompatible polymeric interfaces in biosensing field.

References

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Funding

This work was funded by Fundação para a Ciência e a Tecnologia through PhD scholarship SFRH/BD/129566/2017, projects: UID/MULTI/00612/2013, PEst-OE/QUI/UI0612/2013, PTDC/CTM-NAN/0994/2014; and by National Research Council of Italy through the Short Term Mobility program 2017.