Laccase biosensor based on screen-printed electrode modified with thionine-carbono black nanocomposite, for Bisphenol A detection

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Article

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Laccase biosensor based on screen-printed electrode modified with thionine–carbon black nanocomposite, for Bisphenol A detection

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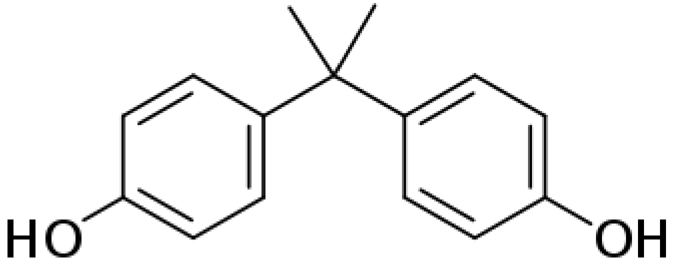
Introduction



Bisphenol A

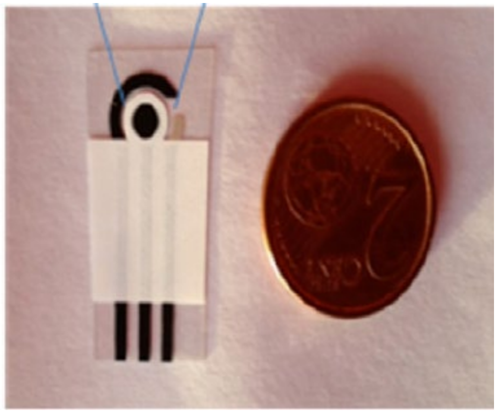
BPA's ability to mimic the effects of natural estrogen, being capable to interrupt the network that regulates the signals which control the reproductive development in humans and animals.

How to detect BPA?

Traditional methods:
- mass spectrometry,
- capillary electrophoresis
- solid phase microextraction

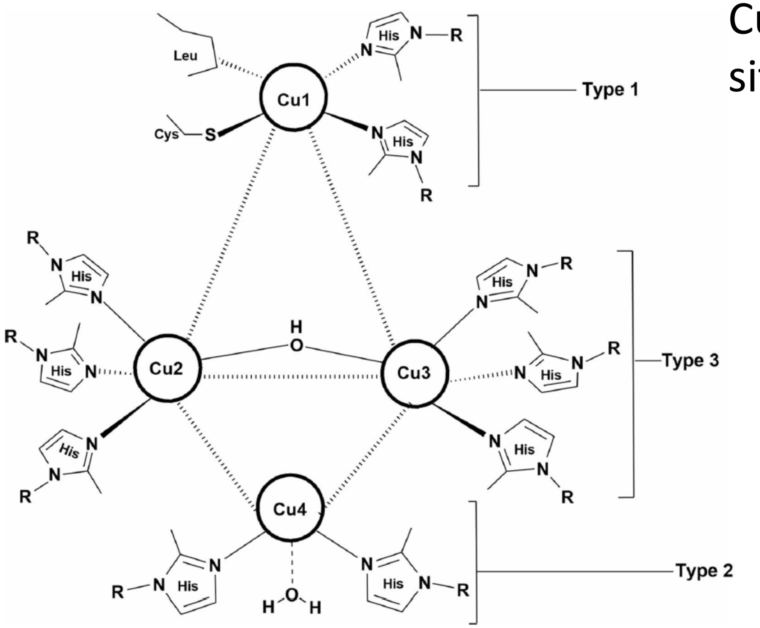
- ➢ takes a lot of time
- ➢ cannot be performed on-site
- ➢ require sample pre-treatament

The goal of this work is produce a biosensor based on a bionanocomposite (laccase–thionine–carbon black)-modified screenprinted electrode (SPE).

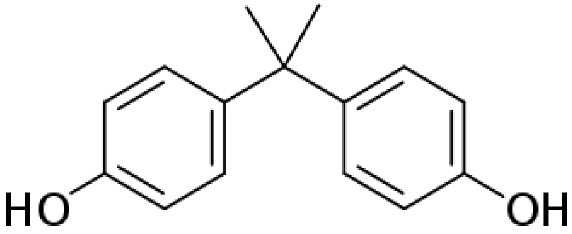


Materials

It is assumed that the catalysis firstly involves T1 Cu reduction by the substrate, followed by internal electron transfer from T1 Cu to T2 and T3 Cu and, finally, dioxygen reduction at T2 and T3 sites

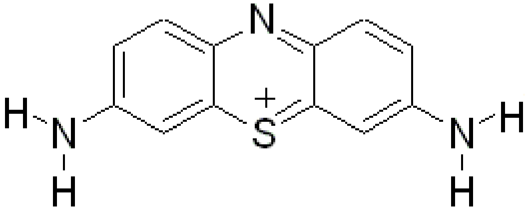


LACCASE



Bisphenol A

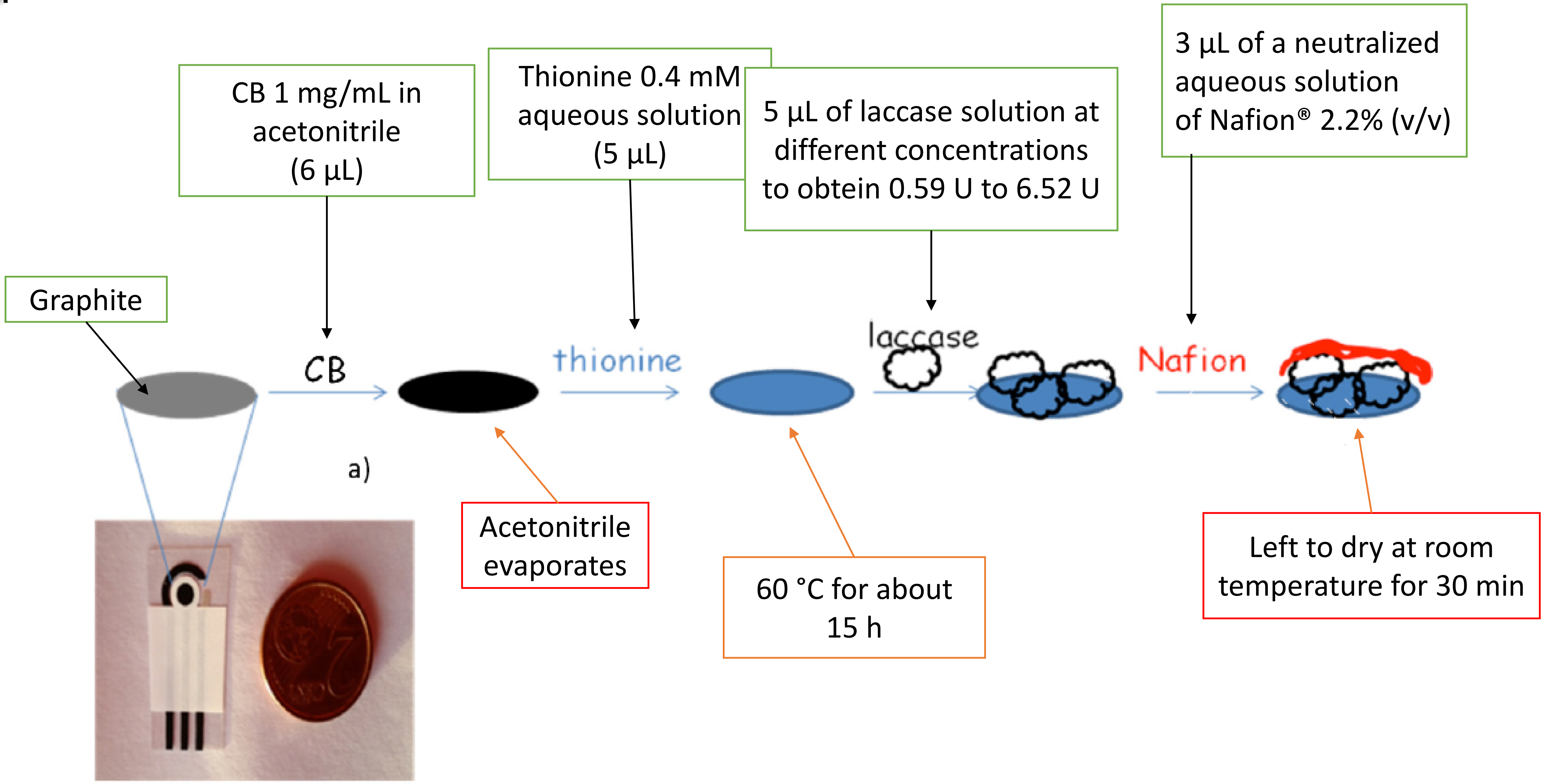
Carbon Black N220



Thionine

The electrode was made of graphite and its diameter was 0.3 cm.

Methods: Modified SPE



1 U = 1 μmol/min

Methods

BPA/laccase reaction products:

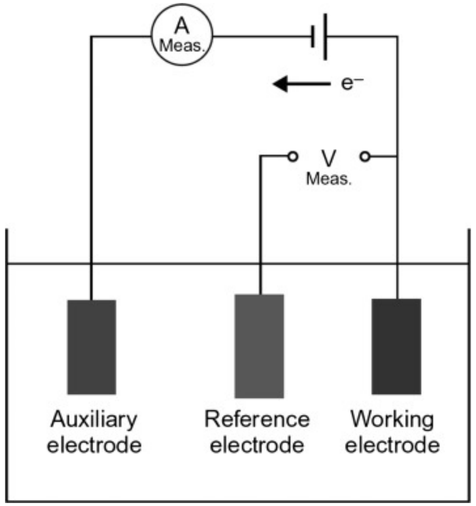
500 µL of 0.05 M citrate buffer at pH 4.5 were added to 100 µL of 1 mM BPA and 100 µL of laccase (40 mg/mL). Left on 37 °C for 1 h

Thionine–CB-modified SPE:

(1º) 6 µL of a dispersion of CB 1 mg/mL in acetonitrile were placed on SPE
(2º) Thionine was made to adsorb on the modified SPE as previously described.

PalmSens instrument

Amperometric experiments

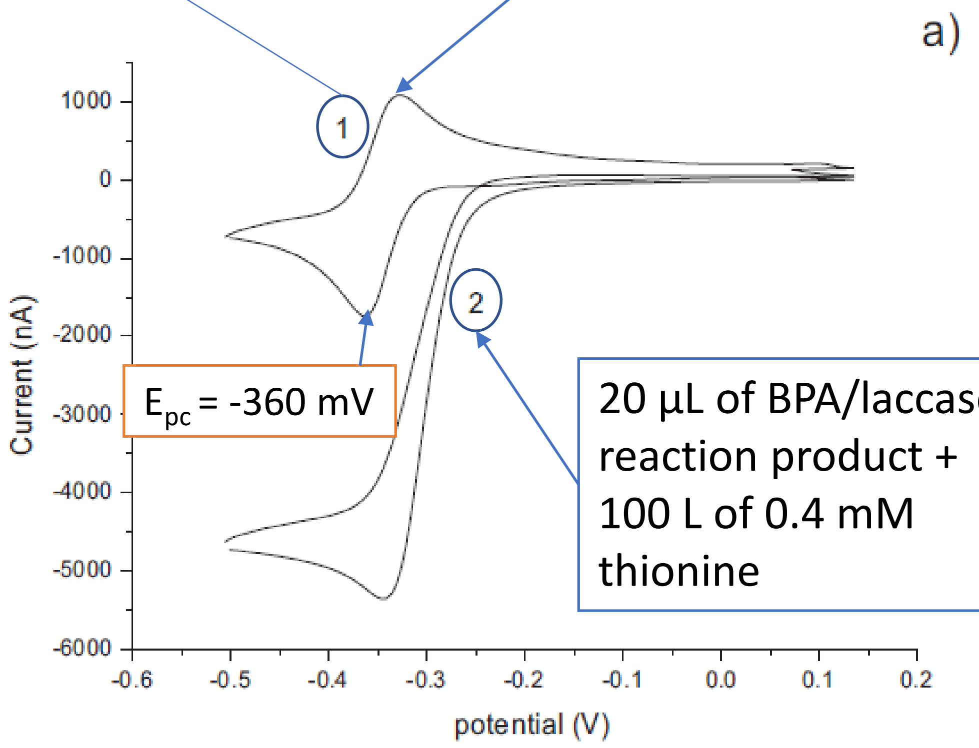


Scanning electron microscopy (SEM)

Voltammogram unmodified SPE

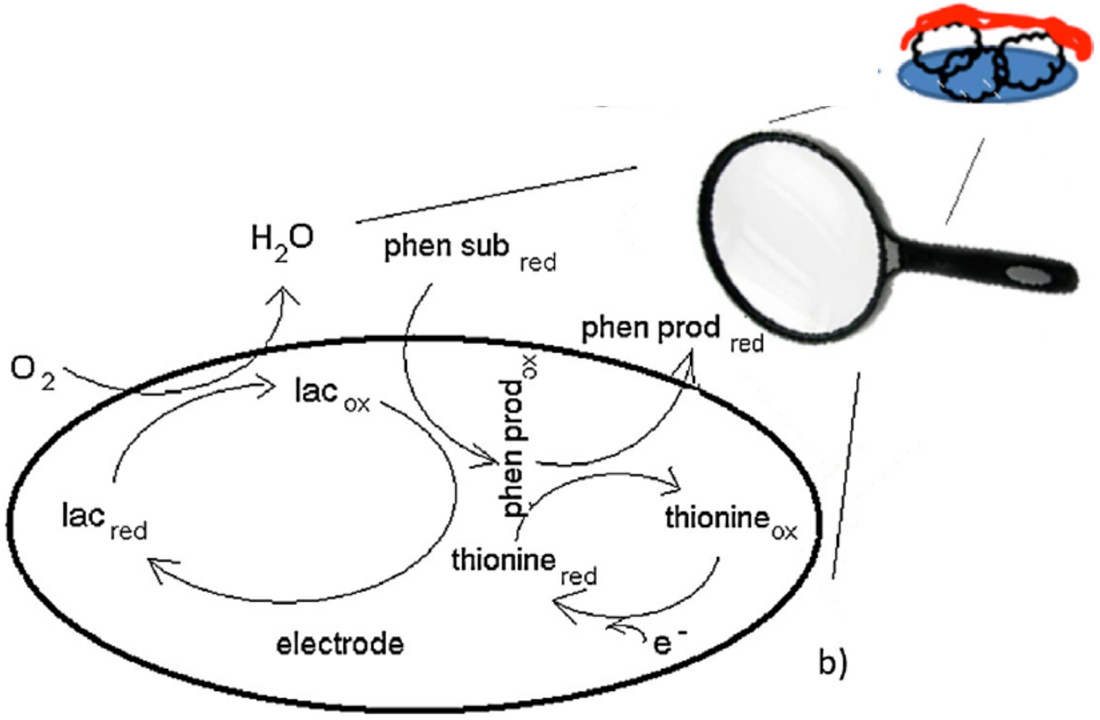
100 µL of 0.4 mM thionine without modification

E_{pa} = -330 mV



E_{pc} = -360 mV

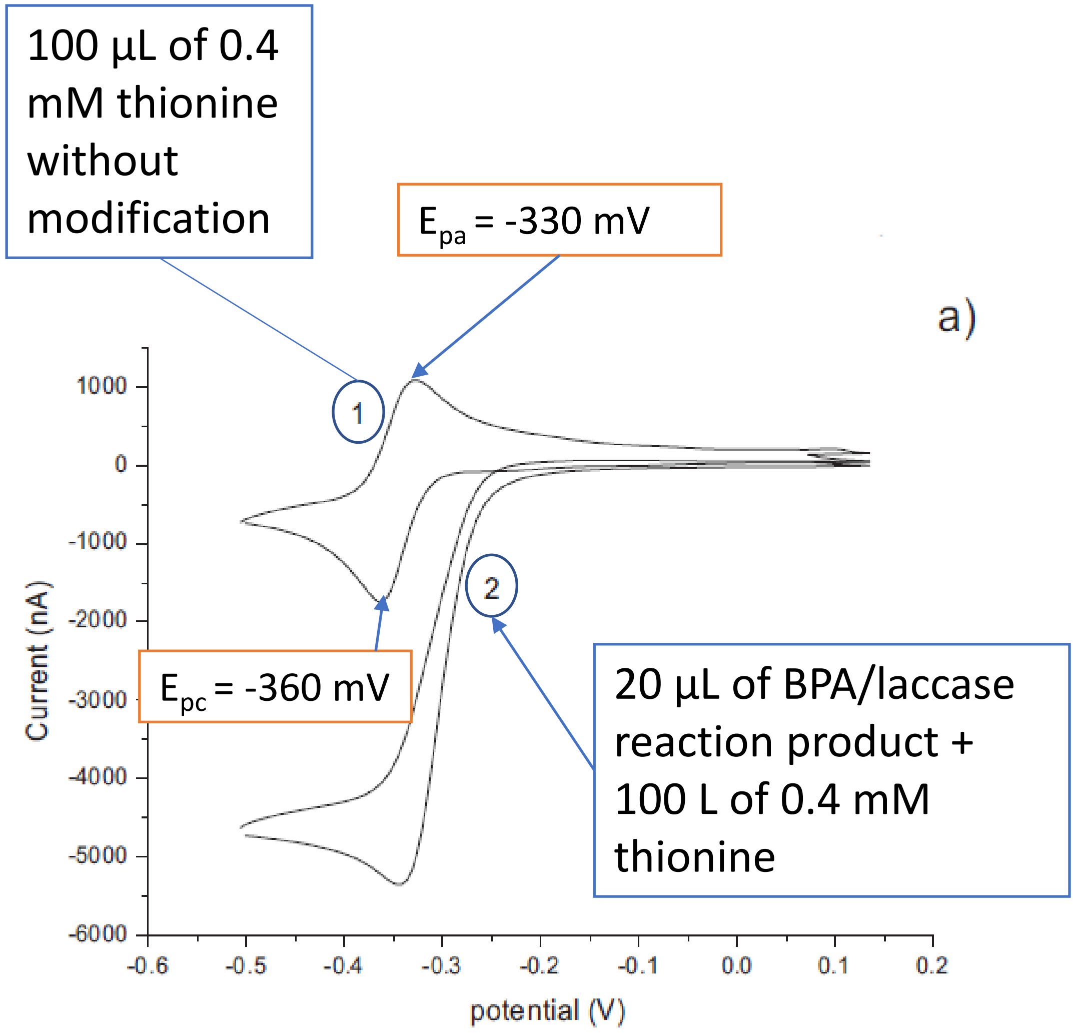
20 µL of BPA/laccase reaction product + 100 L of 0.4 mM thionine



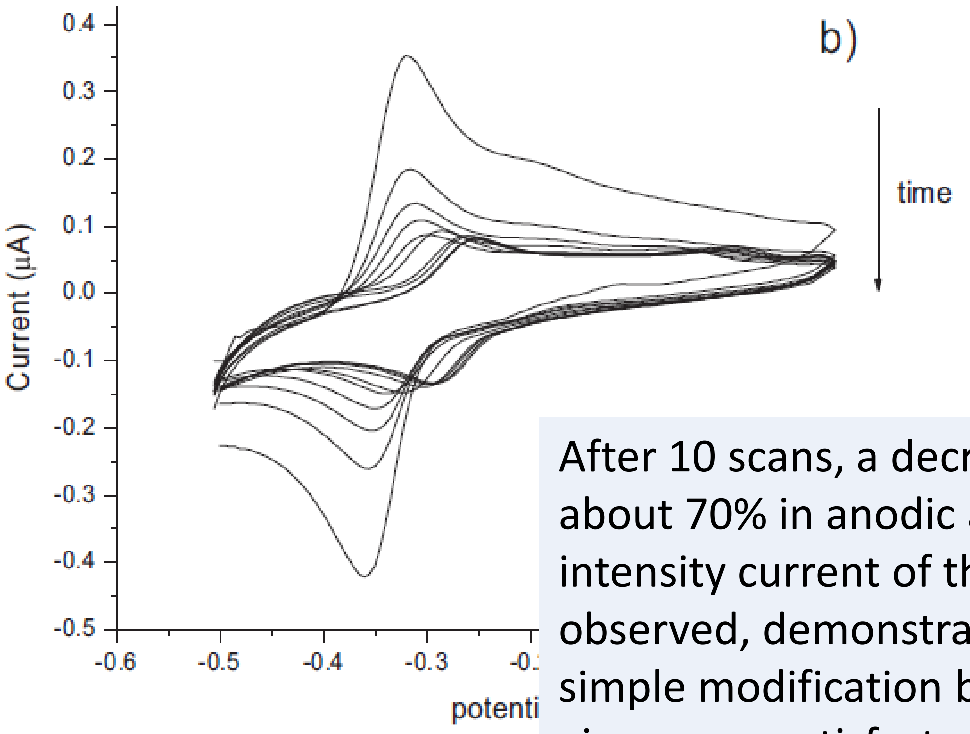
Electric patway in the production of the biosensor response

For all the measurements v_{scan} = 100 mV/s.

Voltammogram unmodified SPE



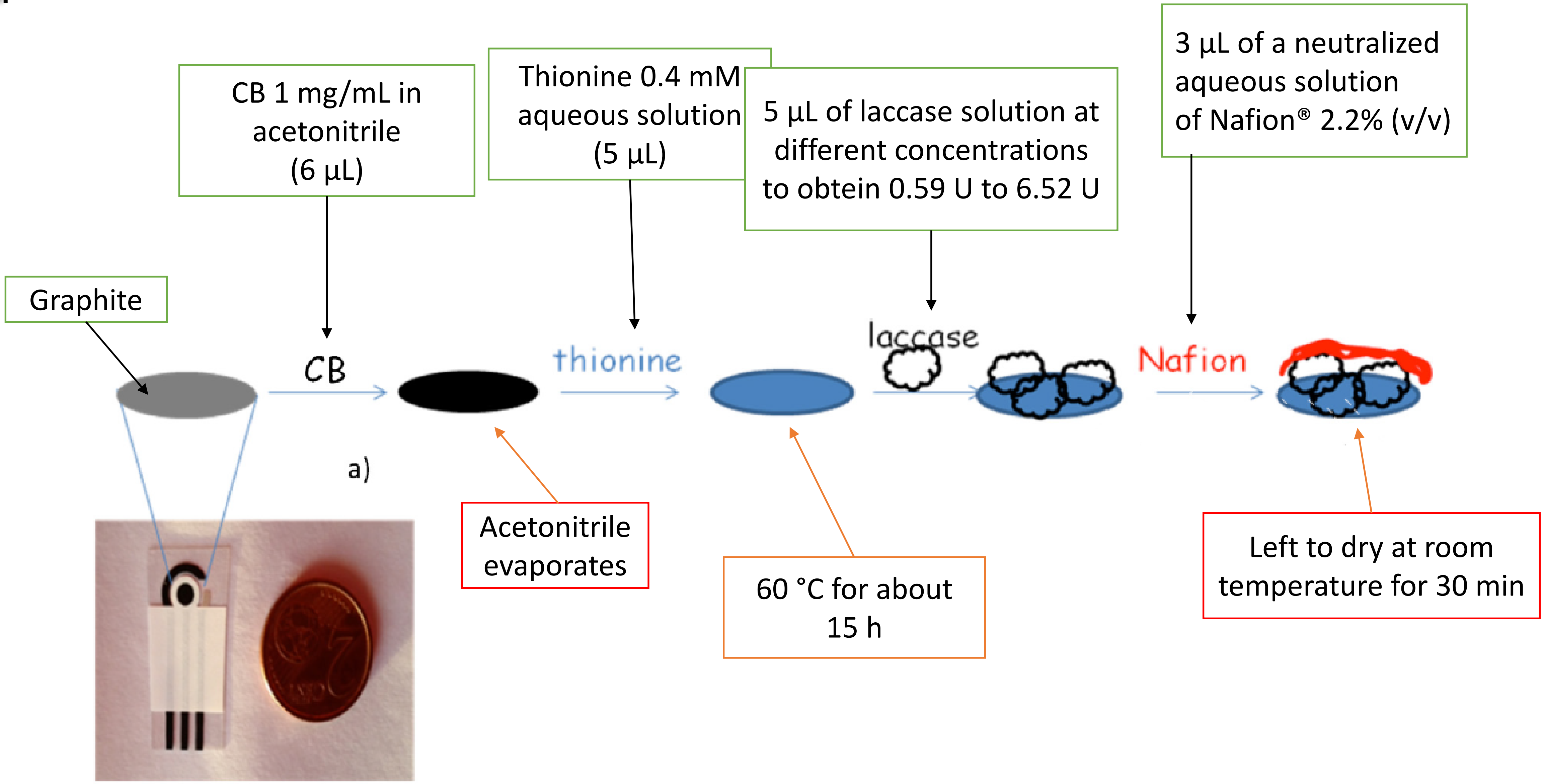
large increase in the peak currents



After 10 scans, a decrease of about 70% in anodic and cathodic intensity current of thionine is observed, demonstrating that the simple modification by adsorption gives an unsatisfactory working stability

For all the measurements v_{scan} = 100 mV/s.

Methods: Modified SPE



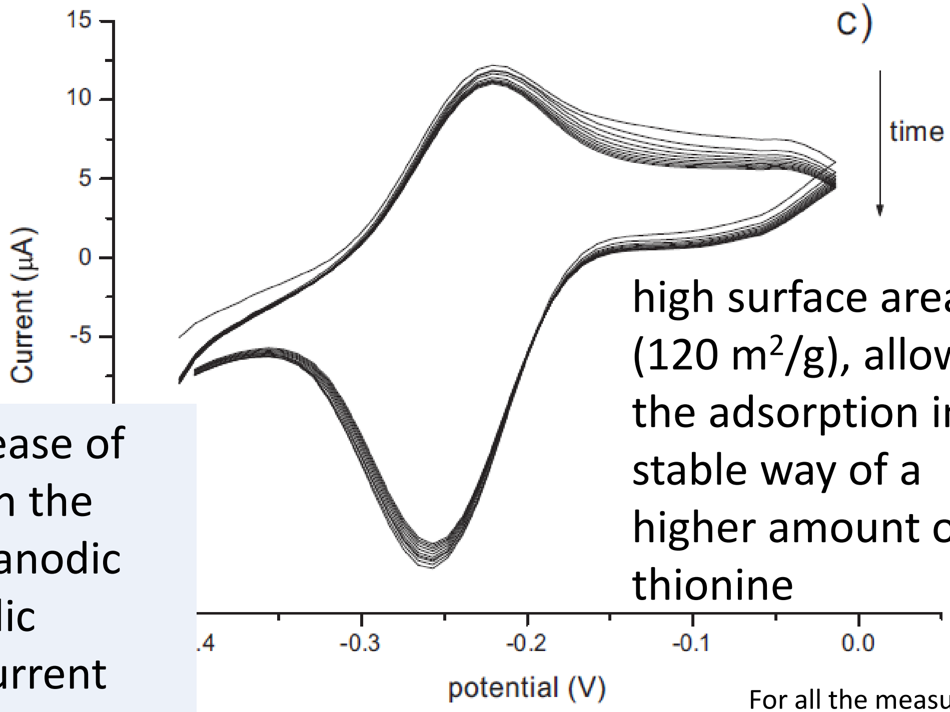
1 U = 1 µmol/min

Biosensor modified with CB-Thionine nanocomposite

Cyclic voltammetry for an adsorbed thionine and CB-SPE

stable cyclic voltammograms were obtained

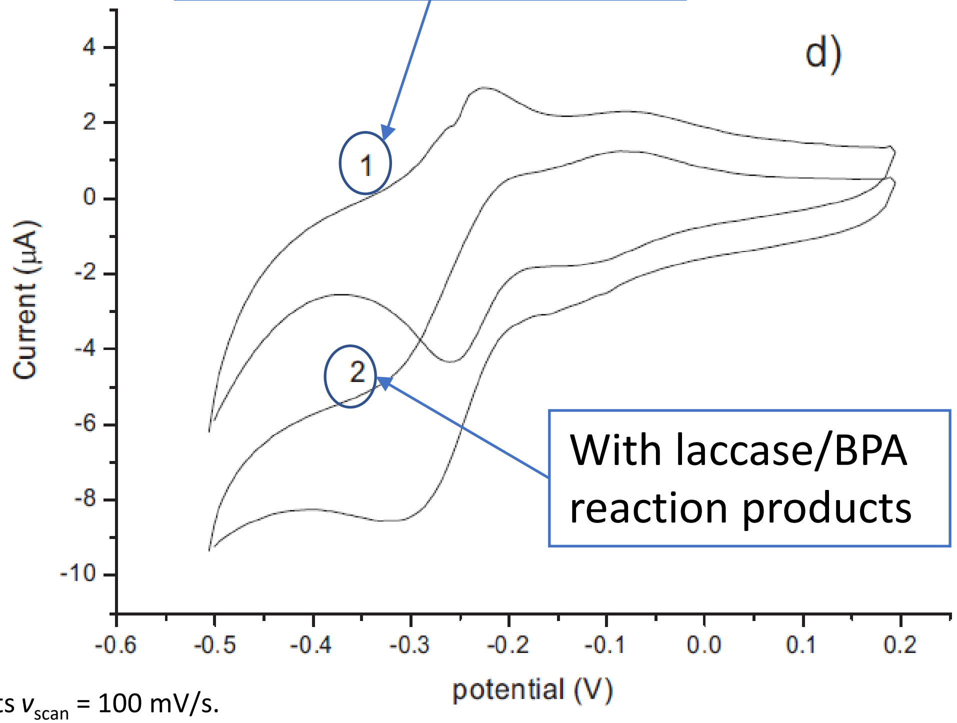
thionine–CB–SPE



high surface area (120 m^2/g), allows the adsorption in a stable way of a higher amount of thionine

small decrease of about 5% in the measured anodic and cathodic intensity current

Without laccase/BPA reaction products

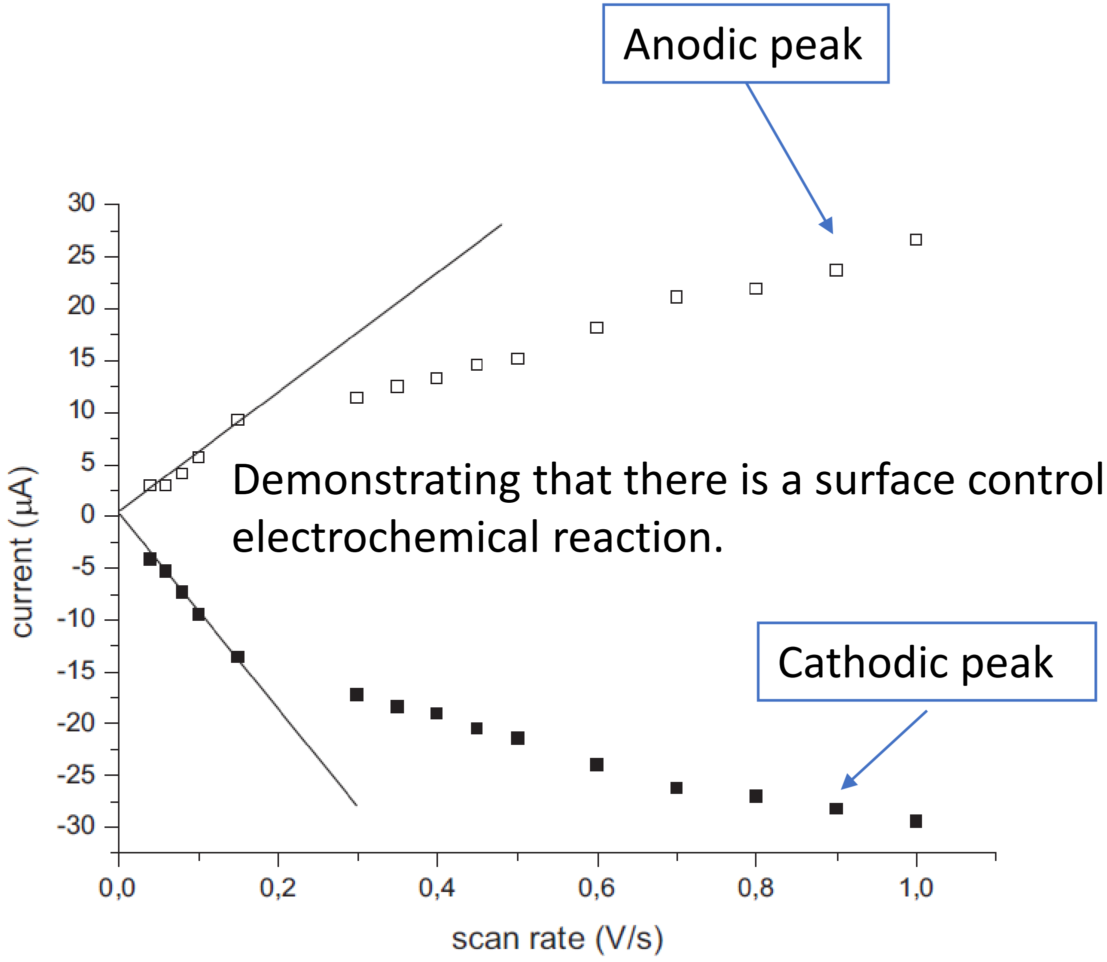


With laccase/BPA reaction products

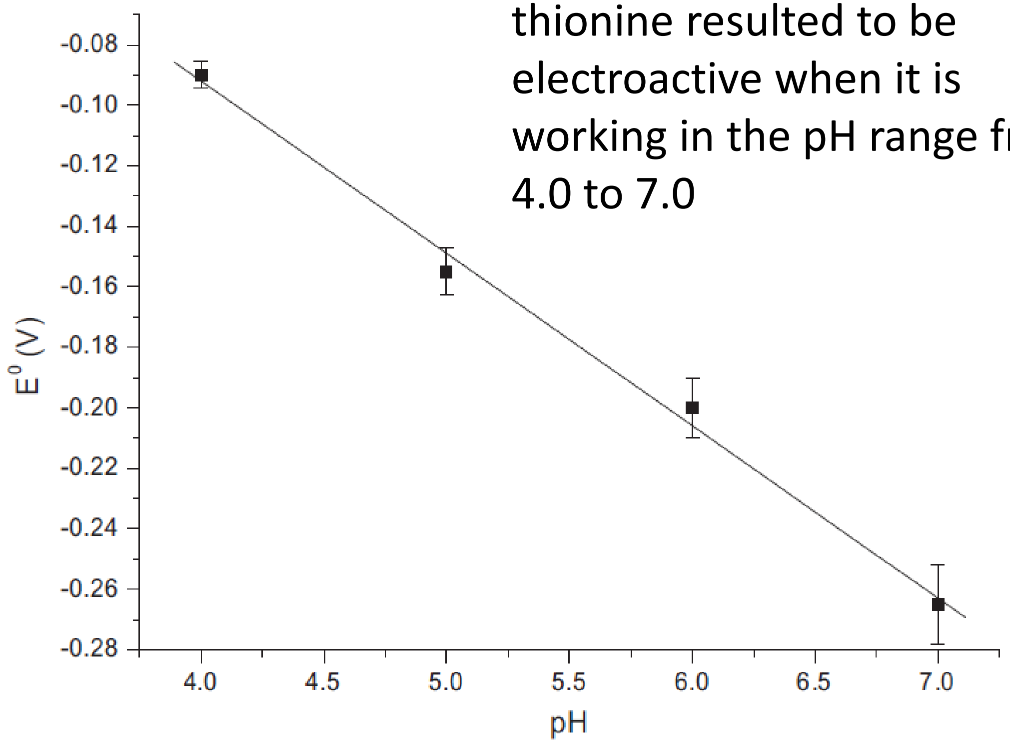
For all the measurements v_{scan} = 100 mV/s.

Electrochemical characterization of thionine-CB-SPE

The slope value is equal to (−57 ± 3) mV/pH and it is in good agreement with the theoretical value of −59 mV/pH (at 20 °C) for a reversible electron transfer process coupled with identical number of protons and electrons.

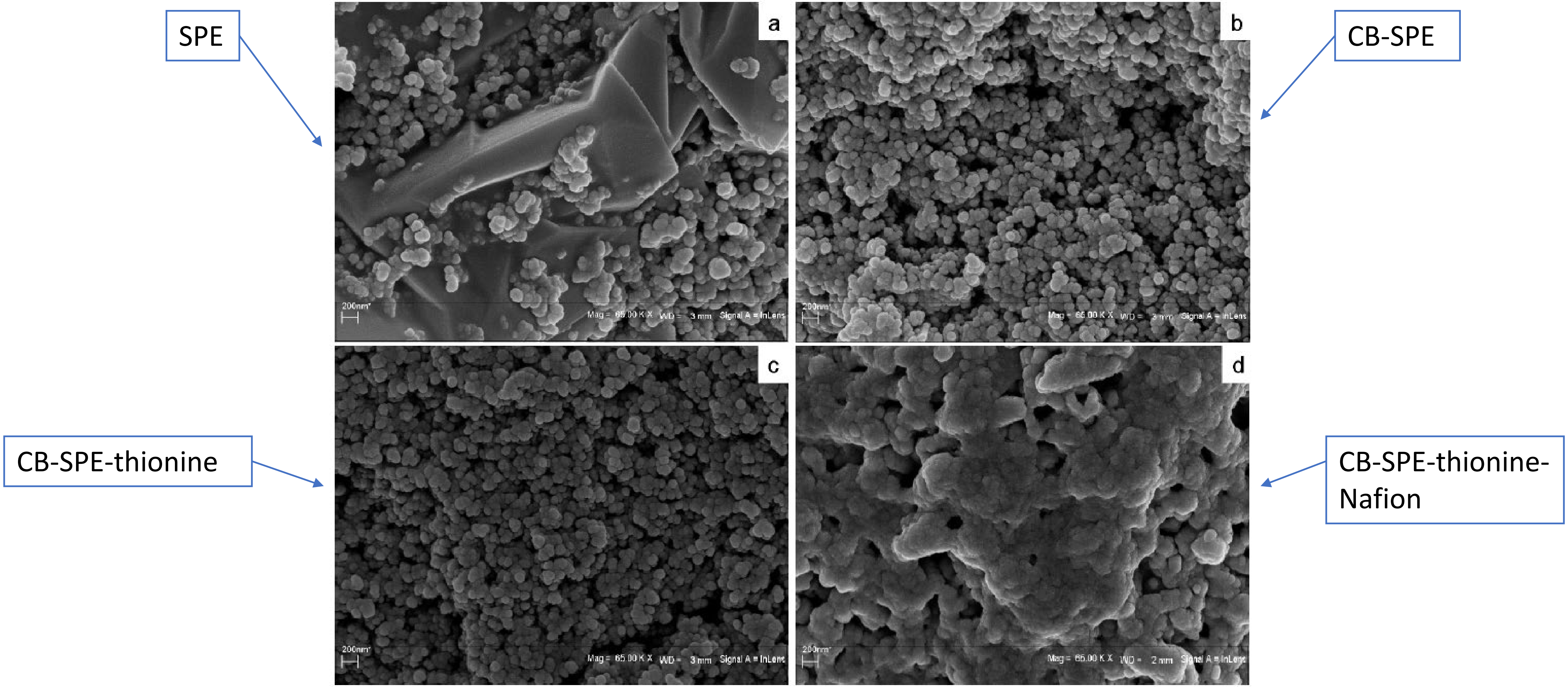


Demonstrating that there is a surface controlled electrochemical reaction.



thionine resulted to be electroactive when it is working in the pH range from 4.0 to 7.0

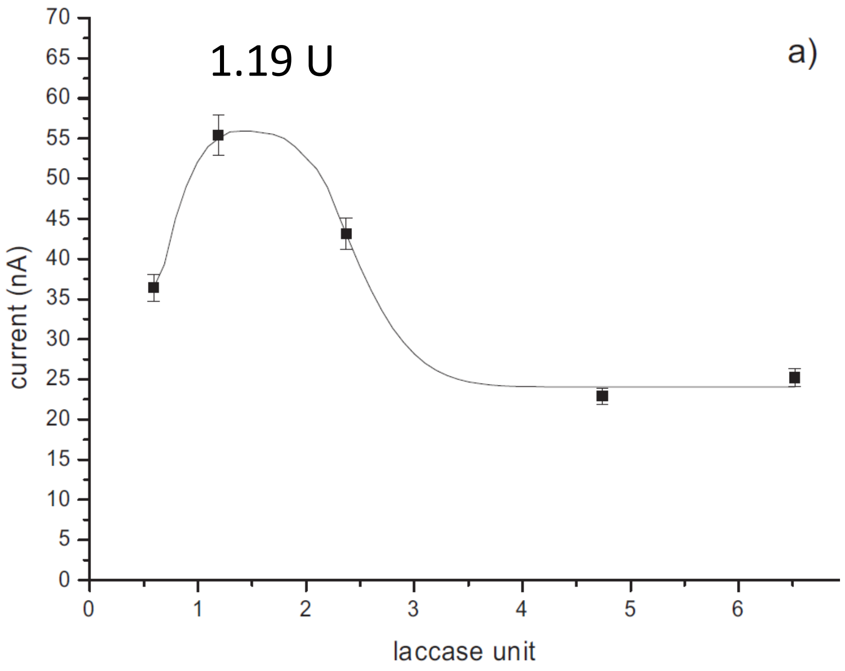
SEM micrographs



SEM images of: bare SPE (a); CB-SPE (b); CB-SPE modified with thionine (c); laccase biosensor based on SPE modified with CB and thionine and Nafic

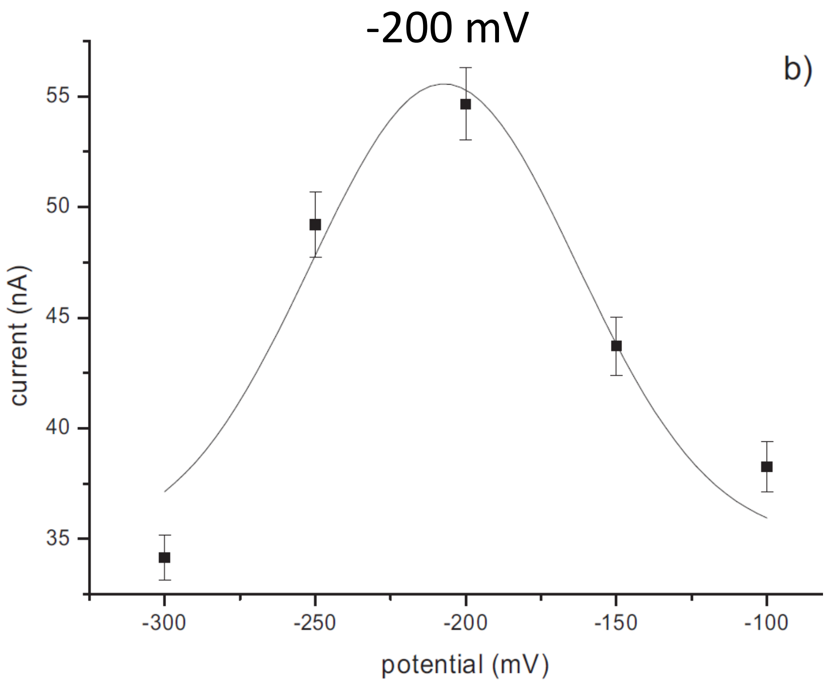
Optimization

Effect of enzyme loading



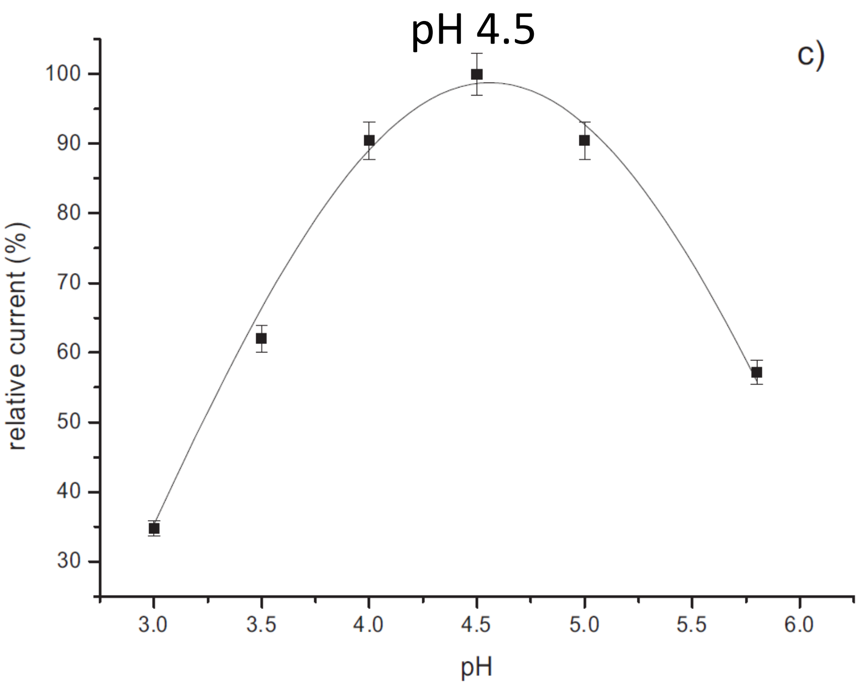
Effect of enzyme units on the biosensor response in presence of 10 µM BPA in 0.05 M citrate buffer, pH 4.5, applied potential equal to −200 mV, T = 25 ◦C.

Effect of applied potential



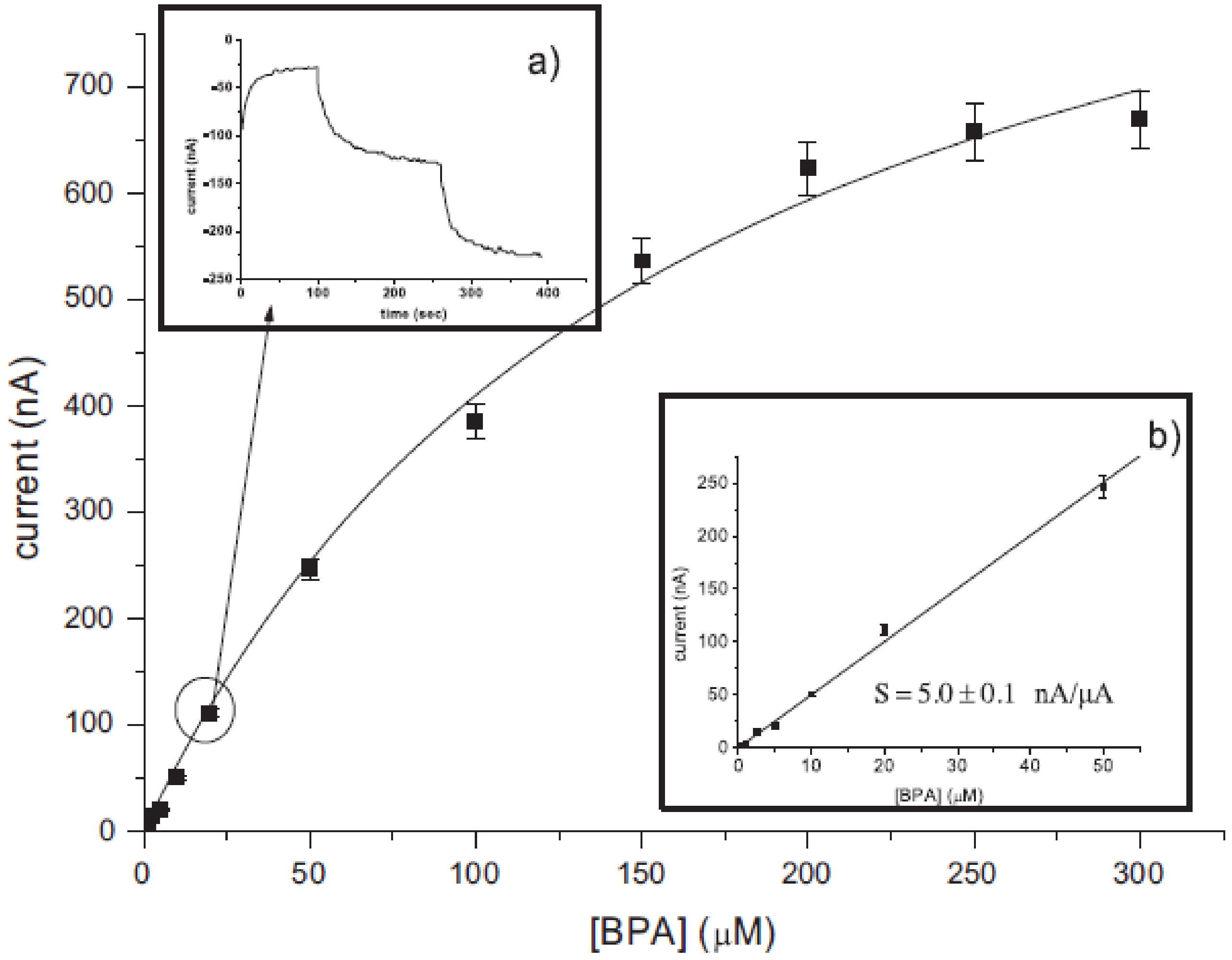
Dependence of biosensor response on the applied potential in the presence of 10 µM BPA in 0.05 M citrate buffer, pH 4.5, T = 25 ◦C and 1.19 U laccase.

Effect of pH



Effect of pH on the biosensor response in presence of 10 M BPA in 0.05 µM citrate buffer, applied potential equal to −200 mV, T = 25 ◦C and 1.19 U laccase.

BPA concentration determination



Dependence of biosensor response on the concentration of BPA in 0.05 M citrate buffer, pH 4.5, applied potential of −200 mV, 1.19 U of laccase. Inset: (a) amperometric signal obtained with two successive addition of BPA standard, of 20 M; (b) linear range for biosensor response.

BPA measurements in real samples

Tomato juice coming from metal cans coated with epoxic resins, which are known to contain and leak BPA

No appreciable current signals were obtained, according to the circumstance that the same samples analyzed with HPLC gave a BPA concentration smaller than the value of our LOD. To evaluate the accuracy of our biosensor, the samples were spiked with known amount of BPA.

Recovery studies of spiked tomato juice samples.

Sample	BPA concentration added (μM)	BPA concentration found (μM)	Recovery (%)
A	10	12	120
A	20	18.5	92
B	10	9	90
B	20	19	95

Experimental conditions: 0.05 M citrate buffer, pH 4.5, applied potential of −200 mV, 1.19 U of laccase. All the values are mean of triplicate measurements.

Conclusions

In this paper it has been reported, for the first time to our knowledge, the design and development of a biosensor based on a bionanocomposite laccase–thionine–carbon black for BPA detection.

The thionine seems to be an effective electrochemical mediator towards the BPA detection in both tyrosinase and laccase based biosensors.

The biosensor proposed here shows better results when compared with laccase carbon paste biosensor [52] and tyrosinase–thionine glassy carbon electrode [10]. The coupling of thionine with nanostructured materials (carbon black in our case) thus allows the development of a stable, miniaturized and low cost device for BPA detection.

Thank you!