



# Application of polyphenazine films doped with metal nanoparticles for the measurements of antioxidant capacity



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## ABSTRACT

Six glassy carbon electrodes modified with polyphenazine conducting polymers and metallic nanoparticles have been prepared by using electrochemical techniques with the aim of determining antioxidant capacity. The electrodes showed a cathodic peak corresponding to the H<sub>2</sub>O<sub>2</sub> reduction, being adequate for the quantitative studies of this compound. These electrodes can be used to study the interaction between H<sub>2</sub>O<sub>2</sub> and antioxidants. From this interaction it was measured the antioxidant capacity of ascorbic acid. This capacity, in trolox equivalents, was similar for all electrodes studied, and agreed with that previously reported using other contrasted techniques. Platinum nanoparticles modified electrodes are best from the viewpoint of durability but, provided the lower cost of silver salts, silver nanoparticles modified electrodes are also a good selection, though these electrodes must be prepared daily.

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## 1. Introduction

The food industry is interested in antioxidants due their role in disease prevention and nutrition, as well as to their potential toxicity [1]. Radicals generated by peroxides in living organisms, including HO•, HO<sub>2</sub>•, O<sub>2</sub>•<sup>-</sup>, etc. (reactive oxygen species, ROS), are involved in the oxidative stress [2], in the damage to proteins, DNA and lipids [3] and in chronic diseases [4]. The secondary antioxidants, which have radical scavenging activity, prevent the activation of carcinogens by interrupting the free radical propagation, the antioxidant capacity being related to the ability to capture ROS [5]. This activity is measured by using radicals such as the radical cation (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ABTS<sup>+</sup>•, [6], the radical 2,2-diphenyl-1-picrylhydrazyl, DPPH• [7] and also by electrochemical methods, such as measurements of the decrease of the oxidation signal of hydrogen peroxide on mercury electrodes originated by the addition of an antioxidant or food extract [8,9] that react with HO<sub>2</sub>• and O<sub>2</sub>•<sup>-</sup> produced on the electrode [10,11]. There are some restrictions when this methodology is used. First, mercury is restricted by health authorities; second, the measurement pH must be higher than 10 [8,10] and, third, high amounts of ethanol are required [11]. So, the measurements are made far from physiological conditions.

In a previous work, glassy carbon electrodes modified with conducting polymers and electrodeposited platinum nanoparticles

[12] were proposed. The electrode based in the modification of the surface with poly-neutral red (PNR) was stable (for at least 5 months) and reproducible, and assessed the scavenging ability of ascorbic acid. Cyclic Vis-NIR spectroelectrogravimetry and *ac*-electrogravimetry [13] measurements were made and it was concluded that radical cations localized in the inter-monomer bonds of the polymer are stabilized by nanoparticles. In the catalysis of the H<sub>2</sub>O<sub>2</sub> reduction, the polymer acts as a proton reservoir.

Polyphenazines have been widely used in the absence of nanoparticles [14,15], especially as biosensors for hydrogen peroxide [16], to develop redox mediators [17,18] and to study the electrocatalysis in the NADH (reduced nicotinamide adenine dinucleotide) oxidation [19,20].

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is a water-soluble analog of vitamin E. Trolox equivalent antioxidant capacity (TEAC) is a measurement of antioxidant strength based on trolox. Due to the difficulties in measuring individual antioxidant components of a complex mixture, trolox equivalency is used as a benchmark for the antioxidant capacity of mixtures. Trolox equivalency is most often measured using the ABTS assay [21].

Platinum and their salts are expensive, and it seems interesting to explore other metal nanoparticles as electrocatalyst. So, the aim of this work was to compare the performance of electrodes made with glassy carbon covered with conducting polymers obtained from neutral red, safranin and phenosafranin (Fig. 1), modified with platinum and silver nanoparticles. It must be expected that different accommodation of the nanoparticles within the polymer will confer different properties to the electrode due to the chemical structure of the monomers, which will

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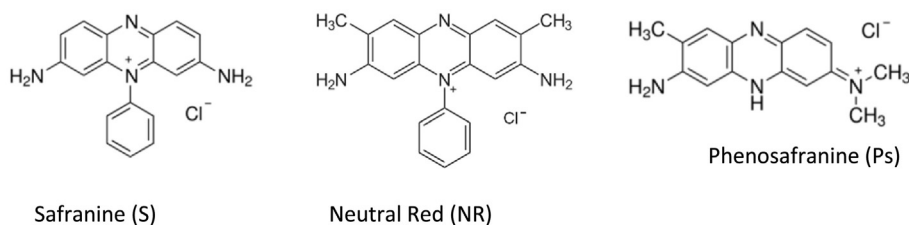


Fig. 1. Chemical structures of the monomers.

play an important role in the properties of electrodes. Such electrodes will be used for the evaluation of antioxidant capacity by detection of hydrogen peroxide voltammetric reduction in neutral media.

## 2. Experimental

### 2.1. Reactants and solutions

All reactants were of analytical grade and were used without further purification. Neutral Red (>90%) was from Amresco, Safranin O (>90%), Phenosafranin 80%, Hexachloroplatinic acid 99.5%, Silver Nitrate ( $\geq 99.8\%$ ), Ascorbic Acid 99% and Trolox 97%, were from Sigma-Aldrich, and the rest of reactants were from Merck.

As supporting electrolyte, it was used 0.1 M phosphate buffer solution, PBS buffer, at pH 7.0. The aqueous solutions were prepared using ultrapure type I (resistivity  $18.2 \text{ M}\Omega \cdot \text{cm}$  at 298 K) obtained from an ultrapure Millipore water system. The pH was adjusted with solid NaOH to keep constant the volume of solutions, and consequently the concentration of the buffer components. Ionic strength was adjusted to 0.5 M with NaCl. Stock solutions were stored in the darkness at 277 K to avoid decomposition and as  $\text{H}_2\text{O}_2$  as ascorbic acid solutions were freshly prepared just before each experiment.

### 2.2. Equipment and electrodes

Electrochemical measurements were made at 298 K with an Autolab PGSTAT302N potentiostat using the software package NOVA 1.7. A three-electrode cell equipped with a Pt wire counter electrode, a BAS MF-2079 Ag/AgCl, 3 M KCl reference electrode was employed.

Glassy carbon electrode was from IJCambria and had a surface of  $7.5 \text{ mm}^2$ . Before preparing a new electrode, the surface was regenerated by polishing with a silicon carbide paper, followed by diamond slurry ( $0.25 \mu\text{m}$ ) and alumina ( $0.3$  and  $0.05 \mu\text{m}$ ) slurries. Residual polishing material was removed from the surface by sonication of the electrode in an ultrapure water bath for 30 min after each polishing. If necessary, to remove the polymer deposited on the surface, the electrode was immersed in a solution of 10% nitric acid, washed with distilled water, immersed in a solution consisting of standard chromic mixture 1:3 in water, during 3–5 min.

Scanning electron microscopy (SEM) images were obtained using FEI Inspect SEM microscope operated at 15 kV, with a  $4 \mu\text{m}$  spot and at a pressure of 0.8 mbar.

### 2.3. Working procedures

For the poly-neutral red (PNR) deposition, after the electrode surface renewal and the electrochemical conditioning, 10 mg of neutral red were added to 40 mL of deoxygenated 0.5 M  $\text{H}_2\text{SO}_4$ . During the polymerization the potential was applied in the range 1.3 V to  $-0.5$  V, at the scan rate  $50 \text{ mV s}^{-1}$  for 10 cycles [12].

For the poly-safranin and poly-phenosafranin, after the electrode surface renewal, 10 mg of monomer were added to 40 mL of 0.1 M PBS solution at pH 7, previously deoxygenated with a nitrogen stream during 10 min. The polymerization was performed by cycling in the potential range 1.2 V to  $-0.8$  V,  $50 \text{ mV s}^{-1}$  for 15 cycles.

A process slightly modified from that given in reference 12 was used for obtaining Platinum nanoparticles. Nanoparticle deposition was carried out by applying a potential of  $-0.2$  V during 90 s, using a  $5 \cdot 10^{-3}$  M hexachloroplatinic acid solution in 0.5 M  $\text{H}_2\text{SO}_4$ . Silver deposition was made at  $-0.2$  V during 90 s using  $1 \cdot 10^{-3}$  M silver nitrate solution in 0.5 M  $\text{H}_2\text{SO}_4$ . After the deposition, the electrode was electrochemically conditioned in a 0.1 M PBS solution, by cycling from  $-0.4$  V to 0.7 V, at  $100 \text{ mV s}^{-1}$  for 2 cycles in most cases.

The electrochemical quartz balance, EQCM, was calibrated by galvanostatic silver electrodeposition in acid media, which gave an experimental Sauerbrey constant equal to  $7.51 \text{ ng Hz}^{-1} \text{ cm}^{-2}$  for a crystal with resonant frequency of 9 MHz. The calibration procedure has been commonly used and described [22–24].

The measurements in the presence of hydrogen peroxide were made after the electrode activation, by applying alternatively the potentials  $-0.3$  V and 0.2 V, during 5 s at each potential, and switching the potentials 5 times.

The measurements of antioxidant activity were made for 0.1 M ascorbic acid or 0.1 M trolox solutions added to 40 mL of  $2.5 \cdot 10^{-3}$  M  $\text{H}_2\text{O}_2$  in PBS buffer at pH = 7.0 and a scan rate of  $0.1 \text{ V} \cdot \text{s}^{-1}$ . These experiments were made with freshly prepared electrodes and solutions, and each measurement was repeated 8 times.

## 3. Results and discussion

First, the electrodeposition of the polymers was optimized. Second, the nanoparticles were deposited on the polymer and, once the sensors were made, the following task was to evaluate their ability for measuring the antioxidant capacity for both ascorbic acid and trolox.

### 3.1. Polymers electrodeposition

The polymerization of safranin on the GCE surface was carried out by cyclic voltammetry, applying a potential scan, starting at 0.0 V, in the direction of negative potentials reaching  $-0.8$  V, next in the positive direction until a potential of 1.2 V, and successively cycling between these two extreme values, as it is shown in Fig. 2. The first step was the optimization of the polymerization medium. To achieve this goal, 250 mg/L solutions of the monomer were used in different media, namely 0.5 M phosphoric acid, 0.5 M nitric acid, 0.5 M potassium chloride and 0.1 M PBS buffer at pH 7.0.

In the experiments made in both phosphoric and nitric acid, a reduction signal corresponding to hydrogen evolution was found. Because this could affect to smoothness of the deposit, it was concluded that the most adequate medium is PBS buffer. On the other hand, potassium chloride has been discarded because as the number of cycles increases, no changes are appreciated on the voltammogram, this implying that a buffered medium is necessary. So, PBS buffer at pH 7 has been chosen to carry out the polymerization.

Two reversible redox systems appeared in the first cycle of the electropolymerization, at  $-0.3$  V and  $-0.5$  V, corresponding the first to the monomer, and the second to the formation of radical and its polymerization, as is shown Fig. 2. During the polymerization it was observed that the signals corresponding to the monomer decrease until

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