Electrochimica Acta 171 (2015) 150–155

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Seeking a reliable electrode for the monitoring of the hydrogen peroxide reduction in the presence of antioxidants

P. Rivas, J.M. Rodríguez Mellado [*]

Departamento de Química Física y Termodinámica Aplicada CEIA3, IUIQFN, Campus Universitario de Rabanales, edificio Marie Curie Universidad de Córdoba
E-14014 Córdoba Spain

ARTICLE INFO

Article history:
Received 22 November 2014
Received in revised form 12 April 2015
Accepted 6 May 2015
Available online 7 May 2015

Keywords:
Hydrogen peroxide
antioxidants
electrocatalysis
polyaniline
poly-neutral red

ABSTRACT

In this work are proposed electrodes modified with conductive polymers and metallic nanoparticles for the determination of antioxidant capacity, with the aim to avoid the use of mercury electrodes. Carbon materials as pure graphite and glassy carbon, covered with polyaniline and poly-neutral red, were used after deposition of platinum on the polymer surface. The best electrode found, glassy carbon + poly-neutral red + Pt, showed a clear signal of H_2O_2 reduction, being stable (more than 5 months) and reproducible. This electrode can assess the scavenging ability of ascorbic acid in a similar way as reported for mercury electrodes, with the advantage of the avoiding the use of mercury.

1. Introduction

Food industry is greatly interested in obtain antioxidants of natural origin rather than synthetic [1,2] frequently used as food additives and that are regulated by law because their potential toxicity associated to excessive consumption. Food antioxidants are of special interest in the prevention of many diseases [3], their toxicity being directly related to the production of radicals.

Reactive oxygen species (ROS) are radicals generated by organic and inorganic peroxides, including those originated by H_2O_2 or water itself, i.e., $HO^{\bullet}$, $HO^{\bullet}_2$, $O^{\bullet}{}_2{}^{-}$ . . . The excessive production of ROS imply the occurrence of oxidative stress [4], such species playing a significant role in the damage to DNA, proteins and lipids [5] and in chronic diseases [6,7]. The reactivity of radicals is associated to the chain reactions they initiate. Secondary antioxidants (that is, antioxidants with radical scavenging activity) interrupt the propagation of free radicals and prevent the metabolic activation of carcinogens [8,9].

Oxidation potentials measured by cyclic voltammetry, have been used to compare the antioxidant strength of compounds such as flavonoids, phenolic acids, cinnamic acids, etc. [10–12], being the electrode most used the GCE (Glassy-Carbon Electrode). Recent findings [13] showed that low oxidation potentials are associated with a greater ability of a given molecule for the electrodonation and, therefore, to act as antioxidant. Such potentials presented good correlation with other techniques such as $DPPH^{\bullet}$. The test involving $DPPH^{\bullet}$ evaluates the scavenging of radicals by substances thermodynamically capable to react with agents having a redox potential below a given value, related to the $DPPH^{\bullet}$ reduction potential. The formal potentials of other less stable radicals of biological interest as $ROO^{\bullet}$ and ROS are much higher, and can react with species with high oxidation potential, this preventing the determination of the antioxidant activity by the $DPPH^{\bullet}$ test. A suitable strategy for the determination of antioxidant capacity is to analyze the interaction (radical scavenging) with ROS.

The polarographic oxidation of hydrogen peroxide on mercury electrodes has been used as a source of ROS to determine the antioxidant nature of phenolic compounds, wines, alcoholic drinks or beers [14–18]. The antioxidant capacity was determined from the decrease in the oxidation signal caused by the addition of antioxidant.

The polarographic (direct current, dc, and differential pulse, DP) and voltammetric oxidation of hydrogen peroxide on mercury electrodes was examined on the basis that radicals ($HO^{\bullet}_2$ and $O^{\bullet}{}_2{}^{-}$) are involved in the process. These species interact with radical scavengers (antioxidants) as carvacrol, vanillin, 3-hydroxycoumarin or Gallic acid [19]. The production of the radicals in the oxidation of the H_2O_2 on mercury electrodes was re-examined under the light of the mechanisms proposed in the literature [14,19,20], and a reaction scheme was proposed and verified [21,22].

---

[*] Corresponding author.
E-mail address: jmrodriguez@uco.es (J.M. Rodríguez Mellado).

http://dx.doi.org/10.1016/j.electacta.2015.05.026
0013-4686/© 2015 Elsevier Ltd. All rights reserved.

The main problem arising with the above described methodology lies in the use of mercury electrodes, since this metal is toxic and its use is restricted to research or highly specialized laboratories, being not desirable in routine tasks in the food industry. Therefore, the replacement of this electrode by other less problematic is desirable. But H_2O_2 oxidation do not takes place (or occurs at very high potentials) on electrodes as carbon (in any of its variations), copper, silver, gold, etc., and the same can be said for the reduction, which appears at very negative potentials. It is important to be in mind that the electrochemical signal must appears at potentials close to 0 V (vs. the Ag/AgCl 3 M KCl electrode) to be useful in this type of determinations [13,21,23]. This could be done with Platinum electrodes where the signal is in the desired range of potentials, but it is a mixed signal of reduction and oxidation [24]. Such signal cannot be used for the determination of antioxidant activity because it is impossible to quantify the effect of the addition of increasing concentrations of antioxidant. However, studies have been published using Platinum electrodes coated with polyaniline (PANI), in which the reduction signal appears at a potential slightly smaller and very separate from the oxidation signal. This signal is overlapped with the formation of Pt oxide and thus cannot be used for the electrochemical study of hydrogen peroxide [25].

The aim of this work was to find stable and reliable electrodes for the detection of hydrogen peroxide voltammetric reduction in neutral media; the signal so recorded will be used for the evaluation of antioxidant activity. To achieve this goal, mainly carbon electrodes will be used, coated with conductive polymers and depositing Platinum in the surface as a catalyst.

2. Experimental

All chemicals, of analytical grade, were used without further purification. Anilinium hydrochloride 99% was from Alfa-Aesar, Neutral red >90% from Amresco, Hexachloroplatinic acid 99.5% from Sigma-Aldrich, and the rest of reactants were from Merck.

0.1 mol·dm^{-3} PBS buffer at pH 7.0 was used as supporting electrolyte for most measurements and solutions containing either sulfuric, acetic and phosphoric acid in different concentrations were also used as indicated in each case. The aqueous solutions were prepared using ultrapure water type I (resistivity 18.2 MΩ.cm at 298 K) obtained from an ultrapure water system Millipore. The *pH* was adjusted with solid NaOH. Stock solutions were stored in the dark at 277 K to avoid decomposition and H_2O_2 solutions were freshly prepared just before each experiment.

The concentration of hexachloroplatinic acid was checked by UV measurements made on a Perkin-Elmer Lambda 750S spectrophotometer with quartz cuvettes Hellma of path-length 1.0 cm.

All electrochemical measurements were made 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. All tests were performed at 298 K.

As working electrodes for electrochemical measurements were used:

- Glassy carbon electrodes (GCE) from IJCambria with 7.5 mm^2 area. To regenerate the surface, the electrode was polished with a silicon carbide paper, followed by diamond slurry (0.25 μm) and alumina (0.3 and 0.05 μm) slurries. Residual polishing material was removed from the surface by sonication of the electrode in a water bath for 30 minutes after each polishing. If necessary, to remove the polymer deposited on the surface, the electrode was immersed in a solution of 10% nitric acid and then in another solution consisting of standard chromic mixture 1:3 in water, during 3-5 min in each case.
- High density graphite rods (GRE) of 99.995% purity form Aldrich (CAS number 7782-42-5C) of 6mm diameter protected with a silicone tube. The electrode was polished with a silicon carbide paper, followed by alumina (0.3 μm) slurry. Residual polishing material was removed from the surface by sonication of the electrode in a water bath for 20minutes after each polishing.

2.1. Working procedures

For the polyaniline (PANI) deposition, after the electrode surface renewal, an electrochemical conditioning was done by cyclic voltammetry in a saturated solution of NaCl (–1 V to 1 V, at 100 mV s^{-1}, 10 cycles). Then, 600 mg of anilinium hydrochloride were added to 40 mL of 0.5 mol·dm^{-3} H_2SO_4 previously deoxygenated with a nitrogen stream during 10 min. The polymerization was performed from –0.2 V to 0.9 V a 50 mV s^{-1} for 8 cycles. A new electrochemical conditioning was made by cyclic voltammetry in deoxygenated 0.5 mol·dm^{-3} H_2SO_4 (0.4 V to 0.8 V, at 100 mV s^{-1}, 6 cycles).

For the poly-neutral red (PNR) deposition, after the electrode surface renewal and the electrochemical conditioning, to 40 mL of deoxygenated 0.5 mol·dm^{-3} H_2SO_4 were added 10 mg of neutral red. The polymerization was performed from 0.4 V to –0.7 V a 50 mV s^{-1} for 6 cycles. A new electrochemical conditioning was made in the same conditions described for PANI.

Platinum depositions were made at –0.2 V during 1 min using a 5·10^{-3} mol·dm^{-3} hexachloroplatinic acid solution in 0.5 mol·dm^{-3} H_2SO_4. After the deposition, an electrochemical conditioning was made by cyclic voltammetry in a 0.1 mol·dm^{-3} PBS solution (–0.4 V to 0.7 V, at 100 mV s^{-1}, 2 cycles in most cases).

3. Results and discussion

3.1. Platinum covered electrodes

The first electrode considered consisted of electrodeposited Pt nanoparticles on GCE. In this case, though a reduction peak was obtained at c.a. –0.15 V in PBS buffer at pH 7.0, it was very difficult to establish with enough precision the dependence of the signal with the H_2O_2 concentration, due to the crossing between the direct and reverse scans shown in Fig. 1. This response is similar to that found for the bulk Pt electrode [24]. The anodic part of the curve is due to the oxidation of H_2O_2. Due to the presence of this combined oxidation-reduction wave, it is very difficult to eliminate

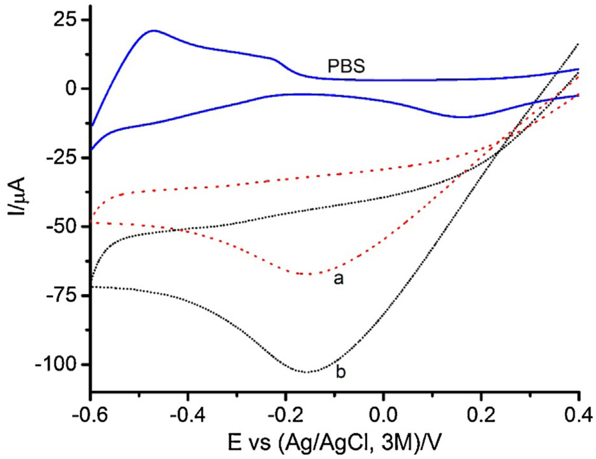

Fig. 1. Cyclic voltammetry on the GCE/Pt electrode in PBS at pH = 7.0 and a scan rate of 0.1 V·s^{-1}. H_2O_2 concentrations in mol·dm^{-3} a) 1.19·10^{-3}; b) 1.89·10^{-3}.