Rapid Electrochemical Determination of Antioxidant Capacity Using Glassy Carbon Electrodes Modified with Copper and Polyaniline. Application to Ascorbic and Gallic Acids

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Abstract: This paper proposes an electrochemical technique for determining antioxidant capacity, AOC, based on the interaction of antioxidants with electrogenerated Cu(II) ions. Cyclic voltammetry is used to detect the electrode surfaces characterized with SEM and EDX. When the electrode is covered by polyaniline, the copper particles are more stable, and the performance of the electrode increases. The interaction in the reaction layer between electrogenerated Cu(II) ions and the antioxidants is fast, with the very low concentration of Cu(II) ions preventing the precipitation of ions with the basic components of the buffer. Consequently, physiological conditions can be used to determine pH close to 7 and the absence of non-aqueous solvents, contrary to what happens in the classic CUPRAC method. The decrease in the peak current corresponding to the reduction of Cu(II) to Cu(I) is used for the AOC determination. The method is tested with well-known antioxidants such as ascorbic and gallic acids.

Keywords: antioxidant; CUPRAC; electrochemical detection; conducting polymer, gallic acid, ascorbic acid.

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1. Introduction

Among the food additives, antioxidants constitute a very important class, being substances that protect foods against deterioration caused by oxidation [1]. Antioxidants can be chain-breaking, primary antioxidants, and preventive or secondary antioxidants. Primary antioxidants act by scavenging the so-called reactive oxygen species, ROS, and secondary antioxidants work by suppressing the oxidation promoters such as singlet oxygen, metal ions, enzymes, and other antioxidants [2,3]. The antioxidant action, related to the reaction mechanism, is called antioxidant activity, whereas its antioxidant capacity, AOC, quantifies the efficacy of a given antioxidant.

A wide variety of methods can be used to measure total antioxidant capacity (TAC) [3–10]. The CUPRAC assay (cupric reducing antioxidant capacity) [9–12]. This assay is based on the reduction reaction of Cu(II) ion to Cu(I) ion in the presence of 2,9-dimethyl-1,10-
phenanthroline (neocuproine), used to stabilize the ions. This method must be performed at low pH to avoid the precipitation of copper salts and in the presence of great amounts of a non-aqueous solvent (ethanol) to solubilize the neocuproine.

On the other hand, Electrochemistry is a powerful and versatile technique for antioxidant capacity determination [13–30] because antioxidants generally act as reducing agents, being consequently oxidized on the electrode. This was used to propose electrochemical methods based on the monitorization of hydrogen peroxide on mercury [13,14] or carbon electrodes whose surface was modified with metal nanoparticles supported on a layer of phenazine conducting polymers [15–17].

A previous work [31] reported an electrochemical method based on the monitorization of the Cu(II) ion reduction signal, able to assess the antioxidant activity of gallic acid. The method consisted essentially of the CUPRAC assay but with electrochemical detection instead of neocuproine. The reverse mode of square wave voltammetry was selected due to reproducibility, sensitivity, and absence of interferences. Nevertheless, when it was intended to extend the technique to other antioxidants, which must be dissolved in non-aqueous media (e.g., ethanol), or to more complex matrices such as food extracts, achieving reproducibility was very difficult, which causes the time spent to increase excessively. Furthermore, the experimental conditions of the method make it impossible to work under physiological conditions of pH (around 7) and relatively high ionic strength.

This paper aimed to propose an electrochemical method based on the interaction of antioxidants with electrogenerated Cu(II) ions on the surface of an electrode (modified or not with a conducting polymer), whose measurements can be carried out under physiological conditions. The method is tested with well-known antioxidants such as ascorbic and gallic acids.

2. Materials and Methods

Cu(NO₃)₂, ascorbic acid, gallic acid, neocuproin, and trolox, were purchased from Sigma-Aldrich, the rest of the reactants being obtained from Merck. All chemicals were of at least analytical quality and were not subjected to further purification.

An Autolab PGSTAT101 with the software NOVA 1.8 was used with 15 mL thermostated glass cell to perform the electrochemical measurements. The working electrode was a glassy carbon electrode, GCE, from IJCambria, with an area of 7.5 mm², a reference electrode, Metrohm 6.0733.100 Ag | AgCl | KCl (3m), and an auxiliary electrode consisting of a platinum rod. Glassy carbon disk electrodes of 23.7 ± 0.5 mm² area and 10 mm thick were also used. To remove the molecular oxygen, which could originate undesired redox reactions on the electrode surface, the solutions used in the electrochemical measurements were purged with purified nitrogen for at least 10 min.

The electrode surfaces were characterized with a Field Emission Scanning Electron Microscope JEOL JSM-6300 scanning electron microscope (SEM) equipped with an energy-dispersive X-ray (EDX) detector at the Central Service for Research Support (SCAI) of the University of Córdoba, having an acceleration voltage 5.0 kV and being its emission current 61.5 μA.

The working solutions were prepared using Ultrapure water type I (resistivity 18.2 MV.cm at 298K) obtained from a MilliporeMilli Q system. Ionic strength was fixed to 0.5 M with solid KNO₃.
In the CUPRAC assay, 1.0 mL of 1M ammonium acetate buffer with the pH adjusted to a value of 5.5 were added 1.0 mL of 10 mM CuCl$_2$ water solution and 1.0 mL of 7.5 mM neocuproine solubilized in ethanol, the adequate volumes of extracts. The volume was completed with water to a final value of 4.1 mL. To construct the calibration curve, different volumes of a 0.25 mM ethanolic stock solution of trolox were used. Samples were incubated at 298 K for 1 hour in darkness, and the absorbance was measured after incubation at 450 nm. To correct the matrix effect, the absorbance measured in the absence of Trolox or antioxidant was subtracted. From the absorbances of the samples and trolox, antioxidant capacities were expressed as trolox equivalents.

UV measurements were performed at room temperature using a Perkin-Elmer Lambda 750-S double beam spectrophotometer equipped with Hanna quartz cuvettes of 10 mm path length.

The next section will give the experimental conditions concerning the electrodeposition of copper and polyaniline.

3. Results and Discussion

Electrode reproducibility is essential for comparing measurements; this reproducibility was monitored by cyclic and differential pulse voltammetry. To achieve the best possible reproducibility, the cleaning and preparation conditions of the electrodes and the parameters of the copper electrodeposition were investigated. Surface preparation was found to be the most critical problem in electrode reproducibility. The deposition time was also important because by increasing this parameter, the response increases, but above a given time, the signal increase slows down. After optimizing the parameters, the following protocol was used: the electrode was immersed in a 1:3 diluted chromic mixture for 30 seconds and then in a 1:3 diluted aqua regia for another 30 seconds. The electrode was cleaned with diamond paste, followed by 0.3 and 0.05 mm alumina slurries. The remaining polishing residues were removed from the electrode surface by sonication in a water bath for 3 minutes. The use of the aqua regia ensures the removal of Cu traces from the electrode surface. The electrodeposition of Cu was made at –0.3V using a solution 1·10$^{-3}$ mol·dm$^{-3}$ in Cu(NO$_3$)$_2$ and 0.5 mol·dm$^{-3}$ in HNO$_3$.

![EDX images of electrodeposited Cu on GCE rods at an acceleration voltage of 5.0 kV and a work distance of 18 mm. Deposition conditions given in the text. Deposition times: (a) 30 s; (b) 60 s.](https://biointerfaceresearch.com/)

The deposited copper layer was characterized by using EDX and SEM measurements. Figure 1 shows the results corresponding to EDX at electrodeposition times of 30 s and 60 s.
At 30 s, isolated and disseminated copper particles were appreciated, whereas, at 60 s, the coverage of the electrode by copper particles is high.

Figure 2 shows the SEM images corresponding to the same experiments. It can be observed that for the lowest deposition time, copper aggregates of 0.2-0.3 µm are distributed on the electrode surface. This picture was also observed at lower and higher deposition times: the size of the aggregates is nearly the same, but the electrode coverage increases as the deposition time increases. At 60 s, the electrode is covered by an almost compact layer of copper formed by metal aggregates.

Figure 2. SEM images of electrodeposited Cu on GCE rods at an acceleration voltage of 5.0 kV and a work distance of 18 mm. Deposition conditions given in the text. Deposition times: (a) 30 s; (b) 60 s.

Figure 3 shows the successive cyclic voltammograms after the deposition of Cu. The anodic peak that was observed in the direct scan is due to the oxidation of the Cu atoms of the electrode surface to Cu(II) ions, and the cathodic peak observed in the reverse scan corresponds to the reduction of the Cu(II) ions generated in the direct scan to Cu(I) ions [31]. As it can be seen, the reproducibility is very good; the values of the peak currents of the reduction peak of the first 12 measurements differ by less than 1% from the mean value and decrease slightly as the number of scans increases. This means that the surface of the copper coating is not significantly altered, and the electrode can be reused at least 10 times after copper deposition. This is important because electrode preparation is the most time-consuming step of measurements.

Figure 3. Cyclic voltammograms at 0.25 V·s⁻¹ of electrodeposited Cu for 60 s on the GCE in 0.1 M PBS buffer at pH 7.0. Each scan was initiated manually after the surface was cleaned with a water jet. Arrow indicates the initial scan direction.
The addition of an antioxidant modifies the intensity of the reduction peak, and in less extension, that of the oxidation peak, as can be seen in figure 4, in which are presented the results obtained after the addition of increasing volumes of gallic and ascorbic acid solutions to a PBS buffer at pH 7.0. This decrease must be related to the interaction of the antioxidant with Cu(II) ions generated in the oxidation scan from the copper deposited on the electrode surface. The reaction of such ions with the antioxidant decreases the concentration of Cu(II) in the medium, and the reduction signal decreases.

![Figure 4. Cyclic voltammograms at 0.25 V·s⁻¹ of electrodeposited Cu for 60 s on the GCE in 0.1 M PBS buffer at pH 7.0 after the addition of increasing volumes of antioxidant. The antioxidant concentrations are indicated in the figure. Cu means no antioxidant added. Arrow indicates the initial scan direction. (a) Gallic acid; (b) Ascorbic acid.](https://doi.org/10.33263/BRIAC131.023)

The interaction between Cu(II) ions and the antioxidants occurs near the electrode in the reaction layer. Therefore, because Cu(II) ions are continuously generated in the oxidation scan, the reaction with the antioxidant is fast and occurs simultaneously with the generation of ions. Moreover, since the concentration of Cu(II) ions is very low, the precipitation of these ions with the components of the buffer (H₂PO₄⁻ and/or HPO₄²⁻) does not take place and, consequently, a physiological pH can be used, contrary to what happens in the classic CUPRAC method.

In the same pH, electrode, and antioxidant concentration, the decrease of the reduction signal is directly related to the AOC, as was reported in other cases [14,15,32–34]. The concentration of antioxidants needed to decrease the reduction signal by a given percentage (e.g., 10%) is inversely related to the AOC, because a lower concentration of antioxidant that causes the same diminution implies a higher antioxidant capacity. In the present case, the ratio between the concentrations of ascorbic and gallic acids required to decrease a 10% the reduction signal is 1.9, this being the value of the relative AOC of gallic acid to ascorbic acid.

The antioxidant capacities measured by the CUPRAC method described in the experimental section, in Trolox equivalents, were 3.1 and 1.7 for both gallic and ascorbic acids. So, the relative antioxidant capacity of gallic to ascorbic acid obtained by CUPRAC measurements was 1.8. The convergence of the values can be appreciated.

The inclusion of metal particles in a conductive polymer protects the metal against deterioration [15,16,35]. To develop a more stable and reproducible electrode, a layer of polyaniline conductive electrode (PANI) was deposited on the GCE, and copper was electrodeposited on the PANI layer. In this case, the following protocol was used: the GCE was immersed in 1:3 diluted aqua regia for 30 seconds, and it was cleaned with diamond paste, followed by 0.3 and 0.05mm alumina slurries, removing polishing residues by sonication.
Electropolymerization of aniline was performed in the same way as in the reference [15] for 20 cycles. The electrodeposition of Cu was performed at \(-0.2\) V for 60 s using the same solution as in the deposition on the bare electrode.

In figure 5, it can be observed that the morphology of the PANI layer does not change appreciably after copper deposition. This was also found when several cyclic voltammetry scans were applied to the composite electrode. Moreover, EDX does not show the presence of copper, which is easily detected by voltammetry. All these facts are compatible with the hypothesis that copper particles are included inside the polymer, remaining protected and able to interact with both the solution and the electrode through the conductive polymer.

In figure 6 are gathered the successive cyclic voltammograms of the Cu-PANI electrode showing the same peaks as in the preceding case but accompanied by an oxidation peak at c.a. 0.5 V in the first scans.

SEM images are not significantly altered in these scans. After 10-12 scans, the signal evolves, and the shape of the voltammogram is the same as in the absence of polymer. However, the reduction potential of Cu(II) is more positive than in the absence of PANI, probably due to the metal-polymer interaction. This evolution can be attributed to a reordenation of the copper particles in the polymer. The values of the peak currents of the reduction peak of scans 10-12 to 50-55 differ by less than 1% from the mean value and increase.
slightly as the number of scans increases, stabilizing after 110 scans. This increase could be attributed to a decrease in particle size as oxidation and reduction of copper takes place on their surfaces, increasing the specific surface of the metal. In addition, the nesting of the Cu particles in the PANI matrix protects them from their release from the electrode, this being the cause of the stability concerning the bare electrode.

In conclusion, conditioning must be performed using the electrode after copper deposition by applying 20 cyclic scans from –0.4 V to 0.4 V at 0.1 V·s⁻¹ in PBS buffer at pH=7.0. After this, the electrode can be used for approximately 40 measurements.

The addition of antioxidants modifies the intensity of the reduction peak in a very similar way to that shown in figure 4. In this case, the relative AOC of gallic and ascorbic acids obtained from the concentrations required to decrease a 10% the reduction signal is 2.0, in good agreement with those reported above.

4. Conclusions

Glassy carbon electrodes covered by electrodeposited copper, either on the bare electrode or in a polyaniline (PANI) layer, can be used to determine the antioxidant capacity (AOC) of ascorbic and gallic acids. The interaction between the electrogenerated Cu(II) ions and the antioxidants in the reaction layer is fast and allows the determination of AOC. The very low concentration of Cu(II) ions prevents the precipitation of ions with the basic components of the buffer. Consequently, physiological conditions can be used in the determination, that is, pH close to 7 and the absence of non-aqueous solvents, contrary to what happens in the classic CUPRAC method. In addition, the method consumes much less time than CUPRAC. The composite electrodes covered by PANI are more stable than those obtained with the bare electrode.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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