

Voltammetric Determination of the Antioxidant Capacity in Wine Samples Using a Carbon Nanotube Modified Electrode

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ABSTRACT: A direct determination of gallic acid was achieved at a carbon paste electrode modified with carbon nanotubes under differential pulse voltammetry conditions. The values obtained for gallic acid were used to estimate the antioxidant properties of the wine sample based on gallic acid oxidation. The proposed method is based on the gallic acid oxidation process at a modified carbon paste electrode (MCPE) containing 30% (m/m) of carbon nanotubes monitored at 0.35 V versus Ag/AgCl (KCl 3 mol L⁻¹). Using the optimized experimental conditions, the calibration curve for gallic acid was linear in the concentration range from 5.0 × 10⁻⁷ to 1.5 × 10⁻⁵ mol L⁻¹ with a detection limit of 3.0 × 10⁻⁷ mol L⁻¹. The MCPE was successfully applied for the determination of the antioxidant capacity for red and white wine samples without interference of glucose and ascorbic acid, and the obtained results were compared with the standard spectrophotometric method.

KEYWORDS: gallic acid, antioxidant capacity, carbon nanotubes, voltammetric determination

INTRODUCTION

Gallic acid (GA, Figure 1) is one of the main phenolic components found in black tea, grapes, humic substances, and several other plants.¹ Recently, polyphenols, especially those naturally present in foods, have been a subject of increasing interest due to their biological properties, including anti-inflammatory, antihistaminic, and antitumor activities, scavenging of free radicals, and protecting against cardiovascular diseases.²

Wine is a complex drink and is a mixture of hundreds of compounds simultaneously present in different concentrations with water, ethanol, glycerol, sugars, and organic acids as the major components.³ With the exception of ethanol and glycerol, other aliphatic alcohols, aromatic amino acids, and phenolic compounds are present at much lower levels of concentration. Moreover, the concentration of phenolic compounds in wine depends on the practice of winemaking, climate, and parasites in the field, among other factors.⁴ Due to the effect of phenolic antioxidants in human health, the development of robust and sensitive methods for their determination gains more importance. The “determination of total phenols” is very difficult because of the chemical complexity and difficulties in the extraction process, as well as the complexity of the matrix for the food samples.³ Thus, the use of a reliable index that expresses the level of total polyphenols is sufficient to know the total amount of polyphenols in a given sample.^{5,6} In this way, the measurement of “total polyphenols” is a good indication of the level of antioxidants present in the sample.⁷ The term “total phenolics” refers to the total phenolic content obtained by the spectrophotometric protocols (Folin–Ciocalteu method). The above-mentioned methods are based on the reaction of phenolics with a colorimetric reactant, thereby allowing their measurement in the visible region of the spectra.⁸

The importance of the phenolic compounds has led to the development of some methods for its determination in various types of samples, including chemiluminescence,⁹

spectrophotometry,¹⁰ and capillary electrophoresis^{11,12} as well as chromatography.¹³

Several electroanalytical methods have been described for the determination of GA in plant extracts and food and beverage samples.^{14,15} Gunckel et al.¹⁶ investigated the electrochemical behavior of GA in an aqueous solution. The authors suggested that oxidation occurred via two steps of electron transfer with an irreversible process, which was diffusion controlled and pH-dependent. Di Fusco et al.¹⁷ developed and characterized an electrochemical biosensor using laccases as the biocatalytic material for the determination of the polyphenol content in wines based on the GA detection. This amperometric biosensor showed a good response to gallic acid, with a linear concentration range of 0.1–18.0 mg L⁻¹ and a lifetime of 10 days. Piljac-Zegarac et al.¹⁸ proposed the electrochemical determination of the antioxidant capacity of fruit teas, a procedure used to estimate the antioxidation capacity based on the measurements of the charge passed to 600 mV (*Q*₆₀₀ parameter) using a glassy carbon electrode (GCE). Blasco et al.⁵ proposed a method for the determination of total polyphenols in foods based on the use of a flow injection analysis system with a glassy carbon electrode. Using a wide and representative range of food samples and comparing with a spectrophotometric protocol, the total content of polyphenols obtained by electrochemical measurements was defined as the “electrochemical index”. For a qualitative analysis, three potential values (0.3, 0.5, and 0.8 V) were applied.

The purpose of the present study is to investigate the voltammetric behavior of the GA using a carbon paste electrode modified with carbon nanotubes, with the aim of developing its simple and rapid determination at low concentration levels using

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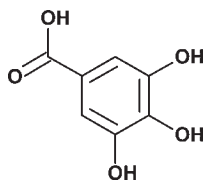


Figure 1. Molecular structure of gallic acid (GA).

differential pulse voltammetry (DPV). In recent years, the use of nanometer-sized materials (so-called nanomaterials) for electrode modification has grown exponentially, owing to their very specific physical, chemical, and electrochemical properties. Metal nanoparticles and carbon nanotubes are the main representatives of the nanomaterials group often used in the construction of electrochemical sensors with good performance.¹⁹ The advantages of using chemically modified electrodes with carbon nanotubes for the development of electrochemical sensors were reported.^{20,21}

To validate the proposed procedure, the determination of total polyphenols in the samples of red and white wines was performed and the obtained results were compared with the recommended protocols.

MATERIALS AND METHODS

Reagents. All of the solutions were prepared using water purified in a Millipore (Milli-Q) system. All of the chemicals were of analytical reagent grade and used without further purification. Phosphate buffer solutions, 0.1 mol L^{-1} of different pH values, were used as the supporting electrolyte in all of the experiments. The carbon nanotubes were prepared by chemical vapor deposition (CVD), starting from pure ferrocene, as previously described by Zarbin et al.²² These CNTs are essentially multiwalled carbon nanotubes filled by long crystals of iron-based species (mainly $\alpha\text{-Fe}$, $\alpha\text{-Fe}_2\text{O}_3$ (hematite), and Fe_3O_4). Other carbonaceous species, such as carbon nanopolyhedra, are also present in small quantities in the sample.

Instruments. Cyclic and differential pulse voltammetric measurements were carried out with a potentiostat/galvanostat microAUTO-LAB (Ecochemie) controlled by a personal computer using GPES 4.9 software. A 15.0 mL capacity glass voltammetric cell was used. The working electrode was the carbon paste, the Ag/AgCl KCl saturated was the reference electrode, and a platinum plate was the counter electrode. The pH measurements were carried out using a Metrohm pH-meter with a Metrohm combined pH reference electrode. All of the experiments were done at room temperature ($25 \pm 1^\circ\text{C}$).

Sensor Construction. Carbon paste electrodes were prepared by carefully mixing the graphite powder ($1\text{--}2 \mu\text{m}$ particle size (Aldrich)) with carbon nanotubes at ratios varying from 0 to 50% (m/m). This mixture was homogenized by magnetic stirring in a 50 mL beaker containing 20 mL of hexane, and the final paste was obtained by solvent evaporation. The modified carbon paste was packed into a plastic cylindrical tube, o.d. 5 mm, i.d. 3 mm, equipped with a copper rod, which served as an external electric contact. Appropriate packing was achieved by pressing the electrode surface against a filter paper.

Cyclic and Differential Pulse Voltammograms. Prior to each measurement, the surface of the carbon paste modified electrode was submitted to 10 cycles of potential between 0 and 1500 mV in a phosphate buffer, 0.1 mol L^{-1} at pH 4.0, to promote the activation of the electrode surface. For gallic acid detection, cyclic voltammograms (CV) were recorded in the 0–1000 mV potential window at different scan rates from 5 to 200 mV s^{-1} and at pH 2–7. After the optimization of

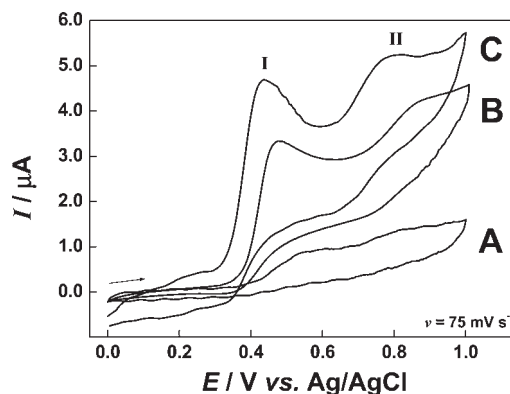


Figure 2. Cyclic voltammograms resulting from the electrochemical oxidation of $1.0 \times 10^{-4} \text{ mol L}^{-1}$ gallic acid in a pH 2.5 phosphate buffer solution at glassy carbon (A), carbon paste (B), and modified carbon paste (C).

these parameters, an analytical curve was obtained in the range of 5.0×10^{-7} – $9.9 \times 10^{-3} \text{ mol L}^{-1}$ GA concentration.

Differential pulse voltammograms were obtained at a potential window of 0–1000 mV with different modulation times (5–100 ms) and pulse amplitudes from 5 to 150 mV. The experiments were carried out in $5.0 \times 10^{-5} \text{ mol L}^{-1}$ GA solutions.

Sample Preparation and Determination of Gallic Acid and Total Polyphenols. Analyses of gallic acid in the red and white wine samples by the proposed voltammetric method were carried out using triplicate samples of wines commercially available in Brazil. For comparison reasons, all of the samples examined were produced recently, stored in the dark at 10°C , and analyzed shortly after being opened. An aliquot of $40 \mu\text{L}$ of white wine or $70 \mu\text{L}$ of diluted red wine ($10\times$) was added into the voltammetric cell containing 10 mL of phosphate buffer, pH 2.5, and homogenized with a magnetic stirrer. The differential pulse voltammograms were recorded in the potential range from 0.2 to 0.6 V, using a scan rate of 20 mV s^{-1} , a modulation time of 10 ms, and a pulse amplitude of 75 mV. The content of gallic acid in these samples was determined by the standard addition method and compared with the spectrophotometric Folin–Ciocalteu method. The above-mentioned method is based on the reduction of phosphotungstic acid in an alkaline solution, which yielded the phosphotungstic blue. The absorbance of the formed phosphotungstic blue is proportional to the number of aromatic phenolic groups and is used for their quantification, using gallic acid as a standard. An aliquot of 1.0 mL (red wine) or 5.0 mL (white wine) was transferred into a calibrated flask and diluted to a final volume of 100 mL with deionized water. A volume of 1.0 mL of this solution, 0.5 mL of Folin–Ciocalteu's reagent, 2.5 mL of deionized water, and 1.0 mL of 20% (m/m) Na_2CO_3 were added. After 2 h, the absorbance was measured at 734 nm, and the content of gallic acid was determined using a calibration curve.

RESULTS AND DISCUSSION

Electrochemical Behavior of Gallic Acid at Carbon Electrodes. Figure 2 shows the typical cyclic voltammograms of $1.0 \times 10^{-4} \text{ mol L}^{-1}$ GA in a phosphate buffer solution, pH 2.5, at a glassy carbon electrode GCE (A), carbon paste electrode CPE (B), and modified carbon paste electrode MCPE with 10% (m/m) of carbon nanotubes (C). In general, the cyclic voltammograms indicated that the electrochemical oxidation of GA was represented by two anodic peaks for all of the electrodes tested. Peak I was attributed to the formation of the semiquinone radical, followed by its oxidation to the quinone form (peak II). Both

Scheme 1. Pathway Proposed for Gallic Acid Oxidation

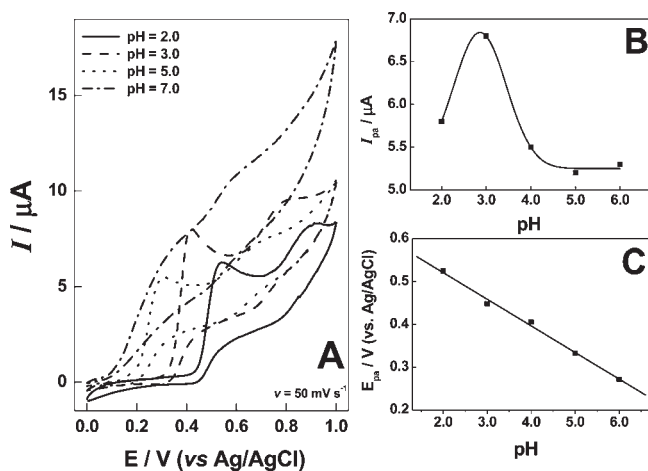
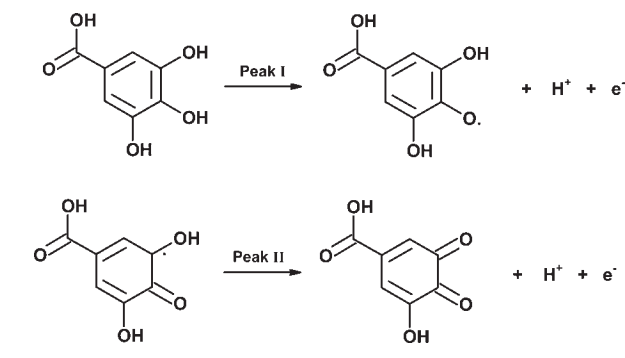


Figure 3. (A) Effect of pH on the cyclic voltammograms recorded for a 5.0×10^{-4} mol L $^{-1}$ concentration solution of GA at a scan rate of 50 mV s $^{-1}$. pH values: 2.0, 3.0, 5.0, and 7.0. Reference electrode = Ag/AgCl. (B) Plot of I_{pa} versus pH. (C) Plot of E_{pc} versus pH; solid line corresponds to a slope of 61 mV per unit of pH.

processes involved one proton and one electron, with no peaks on the reverse scan, indicating an irreversible process, as demonstrated previously.¹⁶ The above-mentioned oxidation process can be attributed to the well-described route for the oxidation of phenols, hydroquinones, and derivatives,²³ as shown in Scheme 1.

In Figure 2A, the use of a GCE yielded a cyclic voltammogram with two anodic peaks at 0.59 V (peak I) and 0.84 V (peak II), with a small current value. Using a CPE (Figure 2B), it was observed that these peaks were shifted to 0.48 and 0.88 V, respectively, and a significant increase on the anodic current peaks was achieved. To improve these results in terms of selectivity and sensitivity, a modified carbon paste electrode with 10% (m/m) of carbon nanotubes (Figure 2C) was evaluated. An important decrease in the overvoltages (0.42 and 0.80 V) and an increase in the anodic peak values for the gallic acid oxidation were observed. Thus, it was suggested that the carbon nanotubes can effectively improve the electron-transfer kinetics, which can be attributed to the nanotubes' dimensions, electronic structure, and/or topological defects.^{24–26}

Optimization Studies. The effect of several parameters, such as the effect of the potential window, composition of the carbon paste, pH variation, and scan rate, on the voltammetric response for GA determination using the MCPE was investigated.

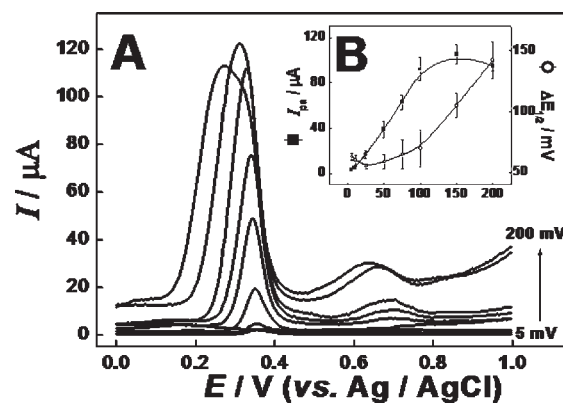


Figure 4. (A) Differential pulse voltammograms obtained using different pulse amplitude values. (B) Effect of pulse amplitude on the anodic current peak and $\Delta E_{1/2}$.

The amount of carbon nanotubes in the carbon paste had a significant influence on the voltammetric response. The peak currents increased with an increasing amount of carbon nanotubes up to 30% (m/m). For amounts higher than 30% (m/m), the anodic peak current became almost constant, probably due to a saturation of the conductive area at the electrode surface. In this way, the best carbon paste composition was achieved with 30% (m/m) carbon nanotubes, 45% (m/m) graphite powder, and 25% (m/m) mineral oil.

The effect of pH on the oxidation of GA (1.0×10^{-4} mol L $^{-1}$) was investigated over a pH range between 2.0 and 7.0 using phosphate buffer solutions. It was observed that GA presented a well-defined peak at pH values of <4.0 (Figure 3A). In addition, pH values of >7.0 were avoided because there are no defined peaks for GA oxidation. The obtained results indicated that the current values of the anodic peak reached a maximum value at pH 2.5, as shown in Figure 3B. The anodic peak potential (peak I) obtained for GA oxidation at pH values ranging from 2.0 to 7.0 presented a shift of 61 mV by pH unit to more negative values (Figure 3C), thereby indicating that the electrode process is influenced by the protonation reactions, as observed by other authors.¹⁶ For further studies, a phosphate buffer solution at pH 2.5 was selected.

The effect of the potential scan rate on the voltammetric response for the oxidation of 1.0×10^{-4} mol L $^{-1}$ of GA using the MCPE was investigated in the range of 5–200 mV s $^{-1}$. The anodic peak current varied linearly with the square root of the scan rate, thereby demonstrating that GA oxidation was controlled by diffusion to the electrode surface. From the obtained results, a scan rate of 75 mV s $^{-1}$ was chosen for further studies.

Under the optimization conditions, an analytical curve was obtained using cyclic voltammetry and a linear relationship (I_{pa} (μ A) = $0.551 + 17.6 \times C_{GA}$ (mmol L $^{-1}$)) between the anodic peak current and the GA concentration ranging from 1.5×10^{-5} to 9.9×10^{-3} mol L $^{-1}$. A detection limit²⁷ of 4.0×10^{-6} mol L $^{-1}$ (3 times the standard deviation of the intercept/slope) was obtained.

Differential Pulse Voltammetric Analysis. To obtain the best performance of the MCPE, its voltammetric response was investigated using differential pulse voltammetry. Under these conditions, the gallic acid oxidation was observed at 0.35 V (vs Ag/AgCl).

The effect of the scan rate on the voltammetric response of the MCPE with carbon nanotubes (solution containing

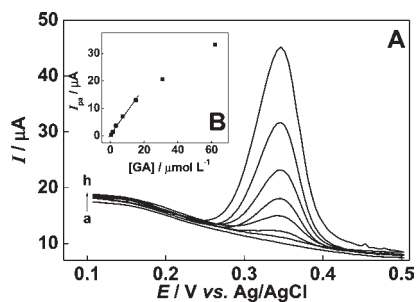


Figure 5. Differential pulse voltammograms (A) obtained under optimized conditions in 0.1 mol L^{-1} phosphate buffer (pH 2.5) solution containing (a) 0.0, (b) 5.0×10^{-7} , (c) 1.5×10^{-6} , (d) 3.5×10^{-6} , (e) 7.4×10^{-6} , (f) 1.5×10^{-5} , (g) 3.1×10^{-5} , and (h) $6.2 \times 10^{-5} \text{ mol L}^{-1}$ of GA. (B) Analytical curve.

$5.0 \times 10^{-5} \text{ mol L}^{-1}$ gallic acid) was investigated in the range of $1\text{--}50 \text{ mV s}^{-1}$. For scan rates higher than 20 mV s^{-1} , the increase in the values of the anodic peak currents was accompanied with a broadening and distortion of the peaks. As a result, the optimum scan rate of 20 mV s^{-1} was chosen, and this value was adopted throughout the subsequent studies. Differential pulse voltammograms (Figure 4A) recorded at several potential pulse amplitude values ($5\text{--}200 \text{ mV}$) showed that the values of the anodic peak currents increased with an increase in the potential pulse amplitude. However, the use of a potential pulse amplitude higher than 75 mV led to an increase in the background currents as well as the $\Delta E_{1/2}$ (Figure 4B). The above-mentioned values were linear with the potential pulse amplitude, thereby suggesting that there was no parallel reaction in the electrode surface. From the obtained results, a potential pulse amplitude of 75 mV was chosen due to the best voltammetric profile and higher sensibility.

After optimization of the operating conditions for the MCPE (scan rate of 20 mVs^{-1} , pulse amplitude of 75 mV , pH of 2.5, electrode composition of 30% (m/m) carbon nanotubes, and modulation time of 5 ms), differential pulse voltammetric measurements were carried out in solutions containing different GA concentrations. Figure 5 shows the voltammograms obtained and the respective analytical curve (inset). A linear relationship ($I_{\text{pa}} (\mu\text{A}) = 0.306 + 8.27 \times 10^2 \times C_{\text{GA}} (\text{mmol L}^{-1})$) between the anodic peak current and the GA concentration ranging from 5.0×10^{-7} to $1.5 \times 10^{-5} \text{ mol L}^{-1}$ with a detection limit of $3.0 \times 10^{-7} \text{ mol L}^{-1}$ was observed. The nonlinear region observed for the peak current as a function of GA concentration suggests that the electrochemical oxidation of the GA at the electrode surface is limited by the saturation of active sites. This could probably be due to a slow process associated with the diffusion of the GA molecules.

To investigate the concomitant effects of compounds usually present in wines, DP voltammograms for the MCPE with carbon nanotubes were carried out in a 0.10 mol L^{-1} phosphate buffer solution, pH 2.5, containing $5.9 \times 10^{-6} \text{ mol L}^{-1}$ (1 mg L^{-1}) GA in the absence and presence of glucose, caffeic acid, and ascorbic acid ($0.1, 1.0, \text{ and } 10 \text{ mg L}^{-1}$). In the present study, no significant influence in the voltammetric response for the GA was observed.

Analytical Application. An aliquot of $40 \mu\text{L}$ of white wine or $70 \mu\text{L}$ of diluted red wine sample was added directly in an electrochemical cell containing 10 mL of the phosphate buffer solution, pH 2.5, and the differential pulse voltammograms were recorded, as described under Materials and Methods. Typical results can be seen in Figure 6, where the dashed curves represent

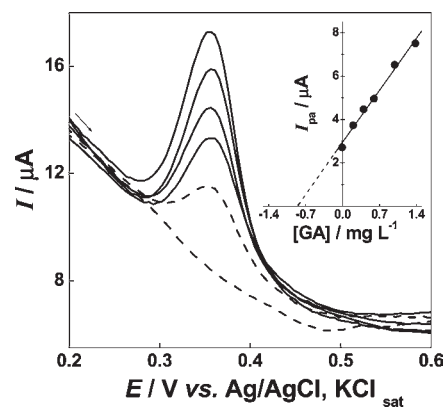


Figure 6. Differential pulse voltammetric response and standard addition curve (inset curve) obtained for determination of GA in the commercial white wine sample.

Table 1. Determination of Total Polyphenol in Wine Samples Using the Proposed CPE Modified with Carbon Nanotube and Spectrophotometric Methods

sample	voltammetric	spectrophotometric	E_r^a (%)
red wine 1	1682 mg L^{-1}	2179 mg L^{-1}	-22.8
red wine 2	2414 mg L^{-1}	2301 mg L^{-1}	+4.91
red wine 3	1865 mg L^{-1}	1991 mg L^{-1}	-6.36
white wine 1	229.1 mg L^{-1}	244.2 mg L^{-1}	-6.59
white wine 2	219.6 mg L^{-1}	224.5 mg L^{-1}	-2.18
white wine 3	265.8 mg L^{-1}	275.3 mg L^{-1}	-3.45

^a E_r , relative error voltammetric vs spectrophotometric.

a blank measurement and a real sample measurement, respectively. The GA content in the wine samples was determined by using the standard addition method and compared with the spectrophotometric method. The anodic current peak observed around 350 mV could be attributed to *o*-dihydroxyphenol and gallate groups. Thus, the above-mentioned species are the major contributors to the antioxidant capacity in wine. In the present study, the estimate of the antioxidant properties of the sample wine was realized on the basis of gallic acid current oxidation. A similar approach was proposed by Seruga et al.,²⁸ whereby the authors used a catechin as a standard for estimating the polyphenol content.

The obtained results with the MCPE for three samples each of white wine and red wine are summarized in Table 1. The given values correspond to the mean value of the three determinations. The same wine samples were also analyzed using the spectrophotometric method involving the use of Folin–Ciocalteu reagent. The total amount of polyphenols was estimated in the above-mentioned method by comparison with a calibration curve with the gallic acid standard solutions. Despite the relative differences observed for the polyphenol index value obtained for one red wine sample, as a consequence of the completely different analytical methodologies employed, a good correlation was found ($r = 0.980$) when the results obtained with the MCPE were plotted versus the results achieved with the Folin–Ciocalteu method. The results showed that the MCPE can be used to give a good estimation of the content of phenolic compounds in wines. Moreover, as expected, important differences were found in the polyphenol content of red and white

Table 2. General Characteristics of the Electrochemical Methods Used for Antioxidant Estimation in Several Samples

electrode	technique	LDR	sample	ref
tyrosinase biosensor using glassy carbon electrode modified with gold nanoparticles (Tyr-nAu-GCE)	amperometry	2.5×10^{-5} – 9.0×10^{-5} mol L ⁻¹	wines	29
platinum based on change of a [Fe(CN) ₆] ³⁻ /[Fe(CN) ₆] ⁴⁻ redox-reagent solution	flow injection potentiometric detection	1.0×10^{-6} – 1.0×10^{-2} mol L ⁻¹	tea and fresh fruit extracts	15
glassy carbon electrode at different potential of detection	flow injection amperometric detection	0.4–60 mg L ⁻¹ (caffeic acid)	apple, pear, wine, and green bean	5
carbon paste electrode modified with carbon nanotubes	differential pulse voltammetry	5.0×10^{-7} – 1.5×10^{-5} mol L ⁻¹ or 0.085–2.55 mg L ⁻¹ (gallic acid)	wines	^a
glassy carbon electrode ^a Procedure proposed.	differential pulse voltammetry	1.0–15 mg L ⁻¹ (catechin)	wine	31

wines by both methods, which confirmed the higher antioxidant capacity of red wines. The statistical calculations realized for the obtained results for white wine suggested a good precision for the adopted voltammetric procedure when used for this type of a sample. According to the *t* test, there were no significant differences between the recovery and added values at the 95% confidence level and within an acceptable range of error. The relative standard deviation (RSD) for 15 replicates of a 5.0×10^{-5} mol L⁻¹ GA solution was 5.3%, thereby indicating a very reproducible response.

The analytical characteristics of the proposed and other electrochemical methods applied to the estimation of the antioxidant properties are shown in Table 2. For the first time, the present study related the application of a carbon paste electrode modified with carbon nanotubes for the antioxidation estimation in the wine samples by using the differential pulse voltammetry technique. The proposed MCPE represents a good and easy method for monitoring polyphenols in real samples exhibiting a good analytical performance due to its stability and reproducibility associated with an easy and rapid preparation, low cost, and longer lifetime when compared with biosensors.^{17,29} Moreover, in comparison with the spectrophotometric procedures, the proposed method exhibited better results in terms of selectivity, sensitivity, and amount of waste yielded. The practical usefulness of the MCPE was demonstrated by the estimation of the content of phenolic compounds in white wine, using an extremely simple procedure involving the direct addition of a sample aliquot in the electrochemical cell dispensing any sample pretreatment.

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