A Simple and Rapid Estimation of Totals Polyphenols Based On Carbon Paste Electrode Modified with Ruthenium Oxo-Complex

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Abstract: A simple and fast method based on electrochemical measurements was proposed for estimation of total polyphenols using a carbon paste modified electrode. The method was based on catalytic response exhibited by a ruthenium complex $[(bpy)_2(NH_3)Ru^{III}(\mu\text{-}O)Ru^{III}(NH_3)(bpy)_2]^{4+}$ in presence of gallic acid (GA). Calibration plots using chronoamperometry (CA) showed a linear response for GA concentrations ranging from 6.6×10^{-6} to 1.9×10^{-4} mol L^{-1} with a detection limit of 4.9×10^{-7} mol L^{-1}. Comparative studies using the official method revealed a good agreement between methods suggesting that the proposed method can be applied for polyphenol estimation without any sample treatment.

Keywords: Carbon paste modified electrode · Ruthenium oxo-complex · Gallic acid · Polyphenols estimation

1 Introduction

Antioxidants can be defined as compounds easily oxidizable that play an important role in reduction of free radicals. In general, these compounds are naturally found in tea and wine and the most representative structures are polyphenolic compounds and flavonoids [1]. Several important properties for human health are attributed to antioxidant characteristics of polyphenolic compounds [2].

In last decades, several reports have indicated that moderate consumption of wine is associated with beneficial effects on the immune system and others physiological functions. Wines are beverages which contain numerous components that influence their organoleptic characteristics and final quality. These characteristics can be affected by many factors involved in the production and wine storage such as grape maturing and barrels for ageing [3].

There are two general approaches to examine and to quantify the polyphenolic content in wines: total polyphenols and individual antioxidant species [4]. In the first case, Folin-Ciocalteu spectrophotometric method is the most frequently employed. This methodology is based on absorbance at 280 nm by the resulting product formed after reaction between polyphenols and phosphomolybdenium complex [5]. Similar spectrophotometric method based on reaction between copper II complex by polyphenols [6], yielding a Cu(I) complexes with maximum absorption peak at 450 nm was recently proposed by Tutem et al. [7]. In second approach, separation techniques such as chromatography or electrophoresis with UV, fluorimetric, mass spectrometry or electrochemical detection are the most commonly used [8–10].

Besides of spectrophotometric and/or separations techniques, there are several electroanalytical strategies for antioxidant estimation in wine samples. Information about antioxidant properties are related to their ability to donate electrons after electrochemical oxidation at low potentials values and it has been investigated by voltammetric techniques [11]. Recently, Arribas et al. [10] published a manuscript reviewing different electrochemical approaches for quantification of polyphenols. Such review discusses the electrochemical approaches most reported involving polyphenols detection are performed by carbon and metallic electrodes as well as biosensors, and eventually coupled to flow systems. There are few works reporting the use of chemically modified electrodes (CME) with inorganic species for this purpose [12,13]. These devices could provide advantages to sensors such as improving sensitivity, selectivity and stability (long lifetime) [14].

Ruthenium polypyridyl complexes have been investigated in great detail in different fields of application. The redox properties of the dimeric oxo-bridged ruthenium(III) complex are studied with special attention for sta-

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bility of metallic redox center. Dimeric complexes of polypyridine-Ru(III) bridged by a variety of N-heterocyclic ligands have been used in (photo)chemistry and photophysical devices [15], electrocatalytic process [16], solar cells [17], electrochemistry [18], biosensors applications [19], among others. Concerning electroanalytical applications, such class of coordination compounds is presented as an interesting system for electrochemical sensors construction due to the presence of metallic center redox couple Ru(III)-O-Ru(III)/Ru(III)-O-Ru(IV) that presents reversible behavior and presents good performance as redox mediator hence it can be employed to improve the electronic transfer between the analyte and the modified electrode surface [20,21]. Currently, several electrochemical systems with high performance, repeatability, reproducibility and stability, inorganic matrices such as zeolites have been employed as support of above-mentioned complexes [22].

Based on previously reported, the goal of present work is application of a carbon paste electrode (CPE) modified with a dinuclear ruthenium oxo-complex $[(bpy)_2(NH_3)Ru^{III}(\mu\text{-}O)Ru^{III}(NH_3)\ (bpy)_2](ClO_4)_4$ incorporated at zeolite matrix, as sensor for detection of totals polyphenols using Gallic acid (GA) as electrochemical probe for polyphenol estimation.

2 Experimental

2.1 Apparatus

All voltammetric measurements were carried out in a 50 mL thermostated glass cell at $25\,°C$, with a three-electrode configuration: carbon paste modified electrode (CPME) as the working electrode, saturated calomel electrode (SCE) as reference electrode and platinum wire as auxiliary electrode. During measurements, the solution in the cell was neither stirred nor aerated. Cyclic voltammetry (CV), linear sweep voltammetry (LSV) and amperometry were conducted with a µAutolab type III (Eco Chimie, Netherlands) connected to a microcomputer and controlled by GPES software.

2.2 Reagents and Solutions

All solutions were prepared using water purified with a Millipore Milli-Q system. All chemicals were of analytical grade and used without further purification. Silver nitrate, sodium phosphate, zeolite (NaY), graphite, mineral oil, potassium chloride, $cis\text{-}(bpy)_2Ru^{II}Cl_2$, ammonium sulfate and gallic acid were purchased from Sigma-Adrich (St. Louis, MO, USA). Sodium perchlorate was obtained from Merck (Darmstadt, Germany). The supporting electrolyte used for all experiments was a $0.5\ mol\,L^{-1}$ KCl solution. The gallic acid $(1.0 \times 10^{-3}\ mol\,L^{-1})$ was prepared in solution of KCl $0.5\ mol\,L^{-1}$ and used as stock solution.

2.3 Synthesis of Dimeric Oxo-Bridged Ruthenium(III) Complex and Preparation of Zeolite-Encapsulated Complex

The bipyridyl dinuclear ruthenium oxo-complex $[(bpy)_2(NH_3)Ru^{III}\text{-}\mu O\text{-}Ru^{III}\ (NH_3)(bpy)_2](ClO_4)_4$ was synthesized as proposed by Ishitani et al. [23]. The ruthenium complex was prepared from its monomeric $cis\text{-}(bpy)_2Ru^{II}Cl_2$ precursor following well-established synthetic procedures and used without further purification [21]. The encapsulated of ruthenium oxo-complex was prepared as previously described in literature [24]. Ruthenium was ion-exchanged into the support to achieve a weight loading 6.7 % Ru/NaY [25]. Then a typical procedure involving slow adding of a dilute solution of $[(bpy)_2(NH_3)Ru^{III}(\mu\text{-}O)Ru^{III}(NH_3)\ (bpy)_2]^{4+}$ $(1.0\ mmol\,L^{-1})$ to a rapidly stirred treated zeolite (1.0 g) at room temperature over 12-h period was performed. This has been followed by additional stirring for 24 h. The zeolite encapsulated oxo-complex was collected by filtration, washed with purity water and dried in inert atmosphere.

2.4 Sensor Preparation

Carbon paste modified electrode was prepared by carefully mixing 70 % (w/w) of graphite powder (1–2 µm particle size), 10 % (w/w) ruthenium oxo-complex encapsulated in zeolite and 20 % (w/w) of mineral oil. This mixture was mixed by magnetic stirring in a Becker (50 mL) containing 20 mL of hexane [26]. The final paste was obtained with the evaporation of the solvent. The carbon paste modified was packed into an electrode body consisting of a plastic cylindrical tube (o.d. 7 mm, i.d. 4 mm) equipped with a stainless steel staff serving as an external electric contact. Appropriate packing was achieved by pressing the electrode surface (surface area of 12.6 mm^2) against a filter paper.

3 Results and Discussion

3.1 Electrochemical Characterization of CPME with Dimeric Oxo-Bridged Ruthenium(III) Complex

The voltammetric behavior of the modified electrode was investigated in $0.5\ mol\,L^{-1}$ KCl solution (pH 5.8). Cyclic scans were conducted in unstirred solution at scan rate of $25\ mV\,s^{-1}$ from 0.10 V to 0.60 V vs. SCE (Figure 1A). Measurement presents a typical cyclic voltammogram with two peaks at $+0.442\ V$ (E_{PA}) and $+0.325\ V$ (E_{PC}), which remained stable after the second cycle. The peaks can be attributed to the processes reversible single electron reduction/oxidation of the pair Ru^{III}–O–Ru^{III}/Ru^{III}–O–Ru^{IV}, revealing a similar voltammetric behavior observed previously for this complex in solution [21]. Well-defined redox peaks using a modified electrode can testify an effective incorporation of ruthenium complex at electrode surface [24,25].
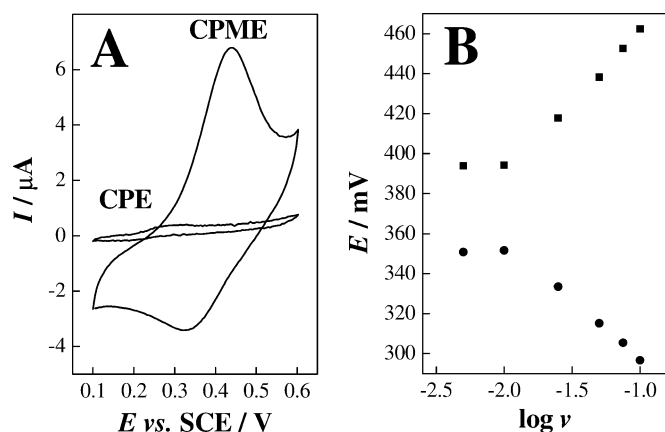
Fig. 1. Cyclic voltammograms obtained for CPE and CPME with ruthenium oxo-complex in 0.5 mol L^{-1} KCl solution (pH 5.8) at scan rate of 25 mV s^{-1} (A). Plot indicating the variation of potential peak (anodic and cathodic) as function of logarithm of scan rate (B).
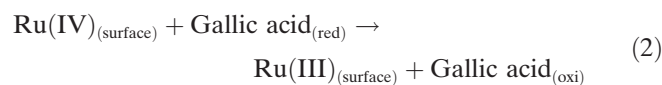
Concerning about the content of Ru-complex on CPE, previous works have showed that content higher than 25% (m/m) of Ru-complex/NaY leads to resistive profile due to reduction of conductive material (45% of graphite). Based on such information, the content of 10% (w/w) of Ru-complex was used aiming good voltammetric presenting well defined peaks and absence of resistive profile [24].

The effect of potential scan rates (5–100 mV s^{-1}) on the voltammetric response for a modified electrode with ruthenium complex was investigated. The recorded cyclic voltammograms revealed that the anodic peak current increases and the peak potential shifts as the scan rate increases. The anodic and cathodic peak currents varied linearly with the square root scan rates indicating that the process redox follows a diffusion-controlled mechanism [27,28]. This behavior suggests the importance of mobility of the counterions of the supporting electrolyte which is necessary for charge transport and/or to keep the electroneutrality at the electrode surface during the redox process [29,30]. The relationship between variation of peak potential with logarithm scan rate may be used to extract parameters such as electron transfer coefficients (α_a) and the apparent electrochemical rate constant (k_e) for immobilized redox center in the electrode surface using the method described by Laviron [31] (Figure 1B). Linear dependences between peaks potential and scan rate were verified for anodic processes and slope of linear segment was used for α_a estimation. The evaluated value for α_a was of 0.201. The apparent electrochemical rate constant, which is a measure of the kinetic facility of a redox couple, was determined applying the equation $k_e = 2.303\alpha_a nFv_a/RT$, in defined scan rate ($v_a = 5.92 \times 10^{-3}$ V s^{-1}) was determined by extrapolation of the anodic linear branch at higher scan rates and its intersection with the constant peak potential. The apparent electrochemical rate constant was calculated to be 0.11 s^{-1}. A system with a large electrochemical rate constant will achieve

equilibrium on a short time scale. Compared with the apparent electrochemical rate constant at the dinuclear of ruthenium(III)-bipyridyl complex in aqueous solution (1.87×10^{-7} s^{-1} [21]), it is clear that the modified electrode with zeolite matrix encapsulated with complex can promote the electrochemical reaction significantly [24].

3.2 Electrochemical Performance of CPME with Dimeric Oxo-Bridged Ruthenium Complex for Gallic Acid Oxidation

The direct oxidation of polyphenols such as gallic acid at an unmodified electrode could offers some problem for analytical application due to slow electrode kinetics and relative high overpotentials required for redox reactions of gallic acid [32]. By other side, redox mediators have been widely used in order to decrease the overpotential and increase the kinetics of electron transfer. In order to verify the electrocatalytic activity of the CPME with oxo-bridged [(bpy)$_2$(NH$_3$)Ru^{III}-µO-Ru^{III}(NH$_3$)(bpy)$_2$]$^{4+}$ complex encapsulated in zeolite matrix, cyclic voltammograms were obtained in the absence and presence of gallic acid, as shown in Figure 2. In the presence of gallic acid the anodic peak current of the CPME increasing significantly as well as a decrease in the overvoltage (0.442 V). Increasing in the anodic peak current clearly shows the process of catalytic oxidation of gallic acid by central metallic cation of the complex immobilized in the zeolite-encapsulated dimeric oxo-bridged ruthenium(III) complex. The voltammetric response of the modified electrode for gallic acid is based in two redox steps that can describe the electrocatalytic mechanism: the first involves the electrochemical oxidation of ruthenium(III) in the zeolite-encapsulated producing ruthenium(IV) on the modified electrode surface, followed by the electron transfer of the gallic acid and consequently regeneration of the ruthenium(III) in the complex as showed in Equations 1 and 2 [24,29,30]. The anodic peak current obtained in 0.442 V (vs. SCE) is proportional to the gallic acid concentration in solution:

$$Ru(III)_{(surface)} \rightarrow Ru(IV)_{(surface)} + e^- \qquad (1)$$

$$Ru(IV)_{(surface)} + Gallic\ acid_{(red)} \rightarrow$$
$$Ru(III)_{(surface)} + Gallic\ acid_{(oxi)} \qquad (2)$$

The chemical structure of gallic acid is composed by benzene 1,3,4,5-substituted with carboxyl group in position 1 and three phenolic groups. The deprotonation of gallic acid is related to four different pKa values of 4.0 to carboxylic acid, 8.7, 11.4, and upper to 13 to phenolic groupments [33]. Therefore in all the cases mentioning gallic acid in solution of support electrolyte (pH 5.8) the majority of species are found in gallate form.

Souza et al. [34] and Gunckel et al. [35] describe detail the electrocatalytic mechanism for polyphenols, specifically gallic acid. The above-mentioned oxidation process
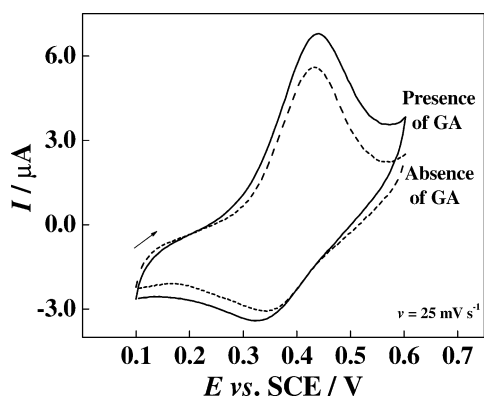
Fig. 2. Cyclic voltammograms obtained in $0.5 \, mol \, L^{-1}$ KCl solution (pH 5.8) in the absence and presence of $5.0 \times 10^{-4} \, mol \, L^{-1}$ gallic acid (GA) for CPME.

Fig. 3. Typical chronoamperometric response obtained for CPE and CPME in $0.5 \, mol \, L^{-1}$ KCl (pH 5.8) containing GA at concentrations varying from $0.0 \, mol \, L^{-1}$ to $4.0 \times 10^{-4} \, mol \, L^{-1}$ (A). Analytical curves constructed for CPE and CPME (B).

can be attributed to the well-described route for the oxidation of phenols, hydroquinones and derivatives. Additionally, the voltammetric profile obtained using unmodified CPE in presence of gallic acid presented only a poor defined oxidation peak with low anodic current value. The peak current of the catalytic oxidation of gallic acid shifted linearly with the square root of the scan rate suggesting that the oxidation process follows a diffusion-controlled mechanism. For scan rate higher than $100 \, mV \, s^{-1}$ there was a small decrease in anodic peak current, suggesting the existence of restrictions in the reaction kinetics between ruthenium and gallic acid, for high scan rates. The catalytic cycle, as observed for many reactions catalyzed by metallic complexes most likely involves the interaction of the molecule with the metal active sites on the surface-confined complexes. This results shown that the use of CPME could provide some improve in limit of detection and it should be investigated for analytical application.

3.3 Influence of Experimental Parameters and Analytical Performance of the Proposed Sensor

In order to evaluate the performance of CPME for direct electrocatalytic oxidation of gallic acid, experiments involving linear sweep voltammetry (LSV) and chronoamperometry were performed. Firstly, effect of applied potential was investigated in order to optimize chronoamperometric response using CPME, which was polarized at potentials values between 0.1 V to 0.6 V vs. SCE. The anodic current values increase in the potential range of 0.2 V to 0.45 V reaching the highest amperometric response for gallic acid at 0.45 V (vs. SCE); and then is not possible observe increasing of current at higher potential values however current reach a steady state. Analytical curves were constructed using an unmodified CPE and CPME with ruthenium complex under applied potential of 0.45 V in order to compare the analytical performance. Typical hydrodynamic chronoamperometric response obtained by successive addition of gallic acid under continu-
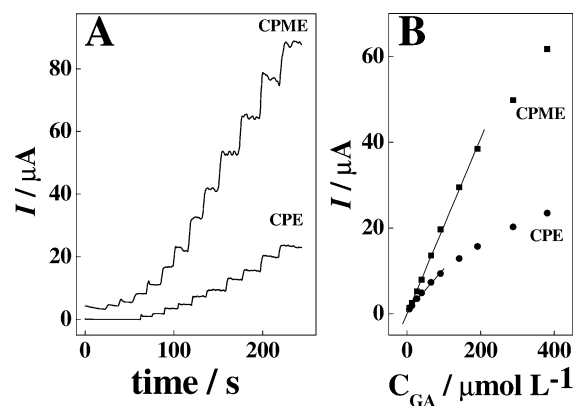
ous stirring at 300 RPM is shown in Figure 3A. Such results have been demonstrated that use of CPME promotes a significant improvement on amperometric response compared to unmodified CPE (Figure 3B). Similar results were obtained using LSV for both electrodes (CPE and CPME with ruthenium complex). Analytical results such as linear dynamic range (*LDR*), calibration sensibility (*S*) and limit of detection (*LOD* – 3 times the standard deviation of the intercept/slope) [36,37] obtained for CPME under voltammetric and chronoamperometric conditions are shown in Table 1.

To investigate the concomitant effects of compounds usually present in the wines, chronoamperometric measurements for CPME were carried out in a $0.5 \, mol \, L^{-1}$ KCl solution (pH 5.8) containing $6.0 \times 10^{-5} \, mol \, L^{-1}$ ($0.1 \, mg \, L^{-1}$) gallic acid in the absence and presence of glucose and ethanol at three different concentration levels (0.1, 1.0 and $10 \, mg \, L^{-1}$). Recovery result varied from 93.8 to 101.0 % indicating a not-significant interference of these species in wine matrices. In presence of other antioxidant species as ascorbic acid, pyrogallol, quercetin or caffeic acid an increase of anodic peak current was observed similar those previously reported for gallic acid. These results suggested that voltammetric response of the proposed sensor is not selective, but it can be applied for antioxidant estimation based on gallic acid concentration as standard for polyphenols. A similar approach was proposed by Seruga et al. [4] and Souza et al.

Table 1. Analytical parameters obtained using CPME with ruthenium oxo-complex for gallic acid determination under linear sweep voltammetry (LSV) and Chronoamperometry (CA) conditions.

Electrode	Technique	Linear range ($\mu mol \, L^{-1}$)	Sensitivity ($\mu A \, L \, \mu mol^{-1}$)	Limit of detection ($\mu mol \, L^{-1}$)
CPME	LSV	2.64–11.7	0.727	1.43
	CA	6.61–191	2.89	0.49

Table 2. Comparative information about electrochemical methods applied for analysis of antioxidant. CPE: carbon paste electrode; SPE: screen printed electrode; MWCNT: multiwalled carbon nanotubes. DPV: differential pulse voltammetry; CA: chronoamperometry, *LRD*: linear region of determination; *LOD*: limit of detection.

Sensor	Technique	*LRD* (mg L^{-1})	*LOD* (mg L^{-1})	Application	Reference
CPE/MWNTC	DPV	0.08–2.55	0.05	Red and white wine	[34]
CPE/TiO$_2$	DPV	0.44–25.5	0.15	Tap water; green and black teas	[32]
CPE/MWNTC	DPV	0.17–1.06	0.04	*Myrtus communis* leaves	[38]
SPE/MWCNT-Laccase	CA	0.1–18.0	0.09	Wine samples	[39]
CPME/Ruthenium oxo-complex	CA	1.12–32.5	0.08	Wine samples	This work
SPE/Au-Laccase/Nafion	CA	0.34–1.19	0.25	*Salvia officinalis* extract	[40]

[34] whereby the authors used a catechin and gallic acid as standard for estimating the polyphenols content, respectively.

3.4 Analysis of Total Polyphenols in Wine Sample

In order to verify its analytical applicability, the proposed method was applied for polyphenols estimation in some wine samples. Aliquots of wine samples were added directly into electrochemical cell without any treatment followed by four successive addition of gallic acid standard solution. Content of polyphenols present in wine samples was provided as mg L^{-1} of gallic acid equivalent.

In general, found values are slightly lower than obtained from Folin–Ciocalteu method because in the spectrophotometric approach a stronger oxidant agent is used when compared to electrochemical detection at ~0.45 V. In spite of the relative differences observed for the polyphenol estimation obtained for wine samples, as a consequence of the completely different analytical methodologies employed, a good correlation was found when the results obtained with the electrochemical methodology were plotted versus the results achieved with the spectrophotometric method. These results suggest that proposed procedure could be applied for polyphenols estimation using GA as standard for calibration. The analytical characteristics of the proposed and other electrochemical methods applied to the estimation of the antioxidant proprieties are summarized in Table 2.

4 Conclusions

The results obtained using a CPME with dinuclear of ruthenium(III)-bipyridyl complex applied for antioxidation estimation allow us to provide a significant improvement in analytical performance of the sensor when compared with an unmodified electrode. The use of chronoamperometry procedure promotes a fast and sensitivity determination demonstrating that electroanalytical techniques may provide quantitative information about antioxidant capacity of wine samples using gallic acid as electroactive standard. When the proposed sensor was compared with other electrochemical sensors reported in literature (Table 2) is possible to verify that the present work represents a good and easy method for monitoring poly-

phenols in real samples exhibiting a good analytical performance due to its stability (more than 100 measurements with the same electrode surface) and reproducibility ($RSD < 5.0\%$, $n = 10$) associated with an easy and rapid preparation, low cost, and longer lifetime when compared with biosensors. Moreover, the proposed method can be applied to estimate total polyphenols in food samples with minor interference effects, minor waste generation and no need of sample pretreatment.

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