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Article

Electrochemical Oxidation, Adsorption and Quantification of 1,2-Benzopyrone Employing a Glassy Carbon Electrode

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O processo de eletrooxidação da 1,2-benzopirona (BP) foi avaliado através de medidas de voltametria linear e espectroscopia de impedância eletroquímica sobre o eletrodo de carbono vítreo em tampão fosfato dibásico de potássio. O número de elétrons envolvidos na eletrooxidação é um e o produto formado é uma cetona. O produto da oxidação fica adsorvido na superfície do eletrodo formando um filme, o qual bloqueia os sítios ativos e a espessura do filme aumenta após medidas consecutivas. As condições de analise foram otimizadas usando planejamento fatorial e matriz Doehlert. A metodologia eletroquímica foi comparada com a cromatografia líquida de alta eficiência (HPLC) com um limite de detecção para a BP de 26,4 μmol L^{-1}. Os resultados voltamétricos foram estatisticamente semelhantes aos obtidos por HPLC, mas o método proposto é mais rápido, simples, de fácil aquisição e de alta sensibilidade, e não exige grandes quantidades de solventes orgânicos.

The electrooxidation of 1,2-benzopyrone (BP) was assessed via linear voltammetry and electrochemical impedance spectroscopy on a glassy carbon electrode in dibasic potassium phosphate buffer. The oxidation process for BP requires one electron and forms a ketone. This oxidation product was adsorbed by the electrode surface to form a film that blocks active sites and increases in thickness over consecutive measurements. The oxidation conditions were optimized using factorial design and Doehlert matrices. This electrochemical method was compared to high performance liquid chromatography (HPLC), which has a detection limit of 26.4 μmol L^{-1} for BP. The voltammetric results were statistically similar to those from HPLC; however, the method was faster, simpler, more easily acquired, more sensitive, and required less organic solvent.

Keywords: 1,2-benzopyrone, guaco, adsorption process, multivariate optimization

Introduction

Coumarin, or 1,2-benzopyrone ($C_9H_6O_2$, molar mass of 146.15 g mol^{-1}), is found in a wide variety of plants, microorganisms and animal species. This molecule consists of an aromatic ring fused to a condensed lactone that is soluble in ethanol, chloroform, diethyl ether and oils but is not very soluble in water.[1] Coumarins are secondary metabolites that occur naturally in different plant parts, such as the roots, flowers and fruit.[2] The most important biological effect of coumarins are their anti-microbial,[3] anti-thrombotic, vasodilatory, anti-tumoral, anti-neoplasic, anti-inflammatory,[4] anti-metastatic,[5] and anti-depressant activities.[6,7] These effects have been specifically studied in various organs and the central nervous system.[8,9] Therefore 1,2-benzopyrone is of significant clinical importance due to its potential for treating many diseases and extreme importance for developing analytical methodologies for monitoring this substance in guaco syrup and teas.

Several analytical methods have been proposed for identifying and quantifying BP, including high performance liquid chromatography (HPLC),[10] first order derivative spectrophotometry[11] and gas chromatography.[12] In addition, electrochemical methods have proven to be effective for detecting and quantifying organic compounds. For example, Seruga *et al.*[13] characterized and determined

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the total polyphenol content (gallic acid, caffeic acid, (+)-catechin, (–)-epicatechin and quercetin) in red wine via differential pulse voltammetry.

This paper describes an alternative method for determining 1,2-benzopyrone based on its oxidative voltammetric behavior using a glassy carbon electrode. Thus, this work aims to optimize the analysis conditions for 1,2-benzopyrone via linear sweep voltammetry (LV) and to evaluate its electrooxidation processes to utilize the advantages offered by electrochemical methods. To this end, the influences of the scan rate (SR) and chemical variables (pH and buffer concentration - BC) were studied using a multivariate methodology based on factorial design and the Doehlert matrix. These techniques were used to avoid mistakes while optimizing the experimental conditions. The simultaneous study of several factors facilitates evaluating their interactions and thus affords the best sensitivity.[14] Finally, the electrochemical quantification of BP in guaco extracts was compared to another methodology based on liquid chromatography. To the best of authors' knowledge, this was the first time linear sweep voltammetry associated with a multivariate methodology has been used for BP analysis. Certain aspects related to the electrooxidation process were also investigated.

Experimental

Reagents and chemicals

All chemicals used in this work were of analytical grade from Sigma-Aldrich (St. Louis, MO, USA) and Vetec (Rio de Janeiro, RJ, Brazil). All solutions were prepared using ultra-pure water (resistivity ≥ 18 mΩ cm^{-1}) obtained through a Milli-Q water purification system (Millipore, Bedford, MA, USA). Dibasic sodium phosphate buffer solutions with varying pH values were used as the supporting electrolyte.

A 10 mmol L^{-1} stock solution of 1,2-benzopyrone was prepared in ethanol:water (1:1; v/v) and stored at 4 °C until it was needed for the analysis. This solution was appropriately diluted by mixing with a buffer solution.

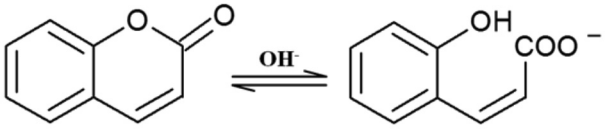
Electrochemical study

Linear and cyclic sweep voltammetry measurements were performed using a potentiostat (model PalmSens PS Trace, PalmSens, Bellefonte, PA, USA) controlled by electrochemical software (PalmSens PS Lite, PalmSens, Bellefonte, PA, USA). The voltammetric measurements were performed using a three-electrode electrochemical cell containing a glassy-carbon (GC) electrode 3 mm in diameter (model MF-2012, BioanalyticalSystems, West Lafayette, IN, USA) as the working electrode, an Ag/AgCl in KCl 0.1 mol L^{-1} as the reference electrode and a platinum wire as the counter electrode. Before each measurement, the surface of the GC electrode was carefully polished with 0.03-0.05 mm alumina powder and rinsed with deionized water. The electrochemical impedance spectroscopy (EIS) experiments were performed using an Autolab/PGSTAT 30 potentiostat-galvanostat (Echo Chemie, Utrecht, Netherlands) apparatus. The results were obtained in the frequency range between 100 kHz and 20 mHz while applying 5 mV of sinusoidal voltage. The solution standard for the experiments was 10 mmol L^{-1} of BP, and dibasic sodium phosphate buffer was used as the support electrolyte. Such analyses showed the film formation on the electrode surface after the BP oxidation process.

Optimization of the linear sweep voltammetry methodology

First, the multivariate studies were performed to screen the significance of three factors (pH, BC and SR) affecting the BP detection using LV. For this purpose, a full factorial 2^3 design was performed in duplicate. The minimum and maximum of the variables were as follows: pH 11 and 13, BC of 0.1 and 0.5 mol L^{-1} and SR of 5 and 50 mV s^{-1}, respectively.

The variable values initially tested were based on preliminary results obtained after a univariate analysis. The pH values ranging from 11 to 13 were evaluated. At pH > 11, the 1,2-benzopyrone was fully hydrolyzed (Scheme 1). These experiments were performed in a random order using 3.34 × 10^{-4} mol L^{-1} of BP. Blank solutions were also prepared for each experiment.



Scheme 1. Coumarin: both forms depend on the pH. The lactone is shown on the left, and deprotonated coumaric acid (carboxylate) is shown on the right.

The significance determined for the variables via the full factorial design were accounted for when performing the final optimization using the Doehlert design. All data were processed using the STATISTICA software package (StatSoft, Tulsa, USA).

Chromatography

The BP separation was completed on an analytical column, LC-18 (250 mm × 4 mm, i.d. 5 mm), from

Supelco (Bellefonte, USA). The mobile phase consisted of acetonitrile (A) and water (B), and the chromatographic separations were performed in the isocratic mode using an acetonitrile:water solution (40:60; v/v). A modular Shimadzu LC-10 system equipped with an LC-10AD pump, CTO-10A column oven, SPD-10A UV-DAD detector, CBM-10A interface and LC-10 Workstation was used to analyze the samples. The column was conditioned for 5 minutes with the mobile phase. The flow rate was held at 1 mL min^{-1}, and the column temperature was maintained at 30 °C. The injection volume was 20 µL, and the detection wavelength was set to 274 nm. This chromatographic method is well established in the literature.[10]

The detection of BP in guaco extract was performed using an external standard. Standard BP solutions at 1, 10, 20, 40, 60, 80 and 100 µg mL^{-1} were used. Each determination was performed in triplicate. A linear calibration curve ($r = 0.99$) was constructed using the peak area from the chromatogram and the standard solution concentration. The limit of detection (LOD) was calculated from the calibration curve parameters using the formula $LOD = 3\ sa / b$, where sa is the standard deviation multiplied by the y-intercept of the regression line, and b is slope of the calibration curve.

Plant material and extraction

Guaco samples (*Mikania glomerata Spreng*) were obtained from the UNIFAL-MG herbarium, Alfenas, MG, Brazil. The leaves were collected in October 2012 and dried at 40 ºC for 3 days (until a constant mass was obtained). Next, the samples were physically homogenized using a mortar and pestle in the presence of liquid nitrogen. Only particles with a diameter of 0.5-1.0 mm were employed in the following extraction procedure. Various extracts were prepared.

Maceration

Powdered dry leaves (DL) and leaves *in natura* form (IN) (1 g) were macerated in an ethanol:water solution (1:1; v/v, 10 mL) for 7 days at room temperature. The material was filtered, and the obtained crude extract was directly analyzed by LV and HPLC. This procedure was repeated in triplicate.

Maceration under sonication

Powdered dry leaves and leaves *in natura* form (1 g) were added to an ethanol:water solution (1:1; v/v, 10 mL) and sonicated at 540 Hz (water bath at 25 °C for 30 min). The material was filtered, and the obtained crude extract was directly analyzed by LV and HPLC. This procedure was repeated in triplicate.

Infusion

Powdered dry leaves (1 g) were added to boiling distilled water (10 mL). The mixture was covered until it reached room temperature (25 °C). This material was filtered, and the obtained crude extract was directly analyzed by LV and HPLC. This procedure was repeated in triplicate.

After each extraction, the samples were filtered through a membrane (Whatman, 0.45 µm), and the extracts were analyzed using the optimized LV conditions and by HPLC.

Statistical analysis

All measurements were performed in triplicate, and the results are presented as the mean value ± the standard deviation (SD). The correlation and regression analyses were performed using Statistica 7.0 (StatSoft Inc., Tulsa, USA) and OriginPro 8.0 (OriginLab Corporation, Northampton, USA) software packages. Any correlations with $p < 0.05$ were considered statistically relevant.

Results and Discussion

Electrooxidation of 1,2-benzopyrone

First, a study was performed using cyclic sweep voltammetry; however, BP exhibited an irreversible oxidation peak at 0.53 V *vs.* Ag/AgCl in alkaline mediums similar to 7-OH–coumarin,[15] which prevents the subsequent use of the active sites. Therefore, the GC electrode was pretreated with alumina to improve the stability and reproducibility of the analytical signal. The cathodic peak at –0.6 V was attributed to a reduction in the oxygen present in the medium. An example of the cyclic voltammetry of BP using the GC electrode is shown in Figure 1. As mentioned above, linear sweep voltammetry was chosen due to its ease and analytical speed. Moreover, no peak reduction was observed for BP in the cathodic direction during cyclic sweep voltammetry. The literature describes a reduction peak at approximately –1.35 V when using a glassy carbon electrode modified with Hg and Pb or unmodified in lithium perchlorate.[16]

Effects of the parameters on 1,2-benzopyrone detection

After verifying that the analyte yielded only one anodic oxidation peak in an alkaline medium, three variables were considered for the first optimization step: the pH, phosphate buffer concentration and scan rate. Table 1 shows the factors and analytical responses for the full 2^3 factorial design. In this study, the variable scan rate was optimized because the oxidation peak disappears at a SR greater than 115 mV s^{-1} due to diffusion limitations.

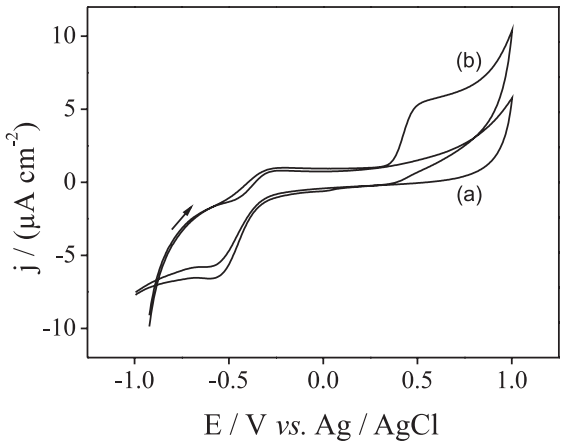


Figure 1. Cyclic voltammograms of an electrode in the absence and presence of BP using a 0.1 mol L^{-1} phosphate buffer solution at pH 12.3 (v = 50 mV s^{-1}). (a) The bare electrode and (b) 10 mmol L^{-1} of the BP solution.

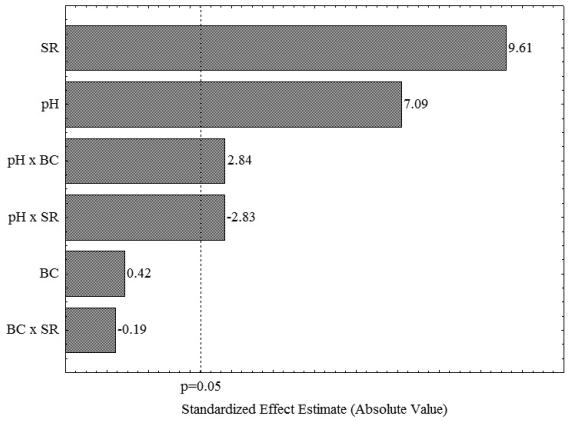


Figure 2. A Pareto chart used to evaluate the effects of the various factors of the electrochemical analysis, including the pH, buffer concentration (BC) in mol L^{-1} and scan rates (SR) in mV s^{-1}.

Table 1. Results of the LV analysis according to the 2^3 factorial design

Assay	pH[a]	BC[b]	SR[c]	j / (μA cm^{-2})
1	(–)	(–)	(–)	22.86 ± 0.01
2	(+)	(–)	(–)	44.29 ± 0.07
3	(–)	(+)	(–)	24.29 ± 0.03
4	(+)	(+)	(–)	44.29 ± 0.09
5	(–)	(–)	(+)	68.57 ± 0.08
6	(+)	(–)	(+)	88.57 ± 0.09
7	(–)	(+)	(+)	44.28 ± 0.06
8	(+)	(+)	(+)	118.57 ± 0.08

[a]Codified values: (–), pH = 11 and (+), pH = 13; [b]codified values: (–), BC = 0.1 mol L^{-1} and (+), BC = 0.5 mol L^{-1}; [c]codified values: (–), SR = 5 mV s^{-1} and (+), SR = 50 mV s^{-1}. All tests were performed using 300 μL of 1,2-benzopyrone at 2.26×10^{-2} mol L^{-1} (final concentration of 3.34×10^{-4} mol L^{-1}).

The significance of these factors and their interactions were evaluated via an analysis of variance (ANOVA) and are represented using a Pareto chart with confidence intervals of 95%, as defined by the vertical line (Figure 2). All effects exceeding this vertical line were considered significant analytical responses. After a brief evaluation, the most relevant factor for the system was SR with a positive contrast (9.61), which indicates that scan rates above 50 mV s^{-1} increase the analytical signal.

The second most important factor in the LV system was the pH with an observed positive contrast (7.09), which also indicates that increasing this variable promotes an analytical response. This result suggests that a higher hydroxide concentration in the reaction medium significantly influences the oxidation reaction between the analyte and electrode surface. However, BC was not a significant variable within the investigated experimental domain. Therefore, the buffer concentration was fixed to 0.1 mol L^{-1} to obtain a satisfactory analytical response.

Considering the results of the full factorial design, further experiments were performed to optimize the pH and SR. The levels of the Doehlert matrix for pH and SR and their responses are summarized in Table 2. The two-factor Doehlert design consists of 7 assays. However, the experiments for the central point were performed in triplicate (assays 1, 2 and 3) to estimate the experimental error (approximately 0.9%). This experimental design indicates that the best results were observed in the central region.

It was then necessary to evaluate the significance of the linear and quadratic regressions. The significance of these models was evaluated by an ANOVA. According to the *F*-test, the ratio of the mean square regression (MSR) to the residual mean square (MSr) was compared to the *F* distribution. For the linear model, the MSR/MSr (0.303) was smaller than the critical $F_{3,5}$ value (5.41) at the 95% confidence level. This means that the linear regression was insignificant and thus unsatisfactory.

In contrast, the results for the quadratic model were highly significant (MSR/MSr = 246.03). In addition, this regression explained 98.5% of the data, which confirms the importance of the quadratic model.

From the ANOVA, it was possible to determine the regression significance and obtain an ratio of the mean square for lack of fit (MS_{lof}) and mean square for pure error (MS_{pe}). The MS_{lof}/MS_{pe} ratio of 2,100 for the quadratic model, which was lower than the 41.440 obtained for the linear model (data not shown). In this sense, the quadratic model is appropriate for describing the experimental data.

The quadratic model (equation 1) was used to build a response surface (Figure 3) and showed the relationship between the factors and anodic peak current.

$$j = -331.97 + 813.07(SR) - 3457.03(SR)^2 + 49.58(pH) - 1.95(pH)^2 - 18.49(SR)(pH) \quad (1)$$

Table 2. Structure of the Doehlert design and the LV results

Assay[a]	Factors		j / (μA cm^{-2})
	SR / (mV s^{-1})	pH	
1	0 (82)[b]	0 (12)	114.67
2	0 (82)	0 (12)	114.46
3	0 (82)	0 (12)	114.44
4	1 (104)	0 (12)	99.69
5	0.5 (93)	0.866 (13)	85.81
6	–1 (60)	0 (12)	81.54
7	–0.5 (71)	–0.866 (11)	69.74
8	0.5 (93)	–0.866 (11)	77.45
9	–0.5 (71)	0.866 (13)	89.72

[a]All tests were performed using 300 μL of 1,2-benzopyrone at 2.26 × 10^{-2} mol L^{-1} (final concentration of 3.34 × 10^{-4} mol L^{-1}); [b]the real factor values are given in parentheses.

Figure 3 shows the response surface from the Doehlert design using two variables. The maximum point was at a pH of 12.3 and a scan rate of 85 mV s^{-1}, according to Lagrange's criterion.[17]

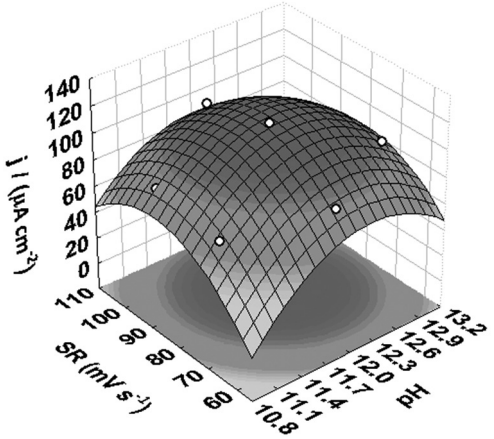


Figure 3. The response surface from the Doehlert design. The buffer phosphate concentration was fixed at 0.1 mol L^{-1}.

The coordinates of the maximum indicate the optimum conditions for detecting BP via the LV methodology. These conditions are consistent with previous results, and the pH optimum suggests that the acid form of the molecule favors oxidation. 1,2-benzopyrone has two forms depending on the environmental conditions. Additionally, the polarographic behavior and absorption spectra of coumarin indicate it is entirely in the lactone form at pH > 11, which is hydrolyzed in alkaline medium.[18] BP is in a lactone at pH values below 6.8, and the acid form, which dominates at pH values above 10.5, is deprotonated once the carboxylic acid reaches a pKa near 4, while alkaline pH values yield a carboxylate (Scheme 1).[16]

Another important parameter that was considered is the scan rate. At speeds above 90 mV s^{-1}, no well-defined anodic peak was formed. Thus, a multivariate optimization was used to obtain the maximum speed to quantify the 1,2-benzopyrone in the shortest possible time. After optimizing the conditions, the effect of the scan speed on the analytical signal was evaluated over the range from 5 to 90 mV s^{-1}. The purpose of this experiment was to determine whether the oxidation of the species in solution is limited by electron transfer or analyte diffusion.

Figure 4 demonstrates that increasing the scanning speed proportionally increases the peak current generated. From this observation, a linear relationship between j and the square root of the speed was obtained according to the Cottrell equation.[19] The plots of j *vs.* v$^{1/2}$ for the peak (Figure 4) followed a linear equation (j = 0.81 v$^{1/2}$ – 0.15 and r = 0.99). Notably, no significant alteration of the oxidation potential was observed at higher scan rates.

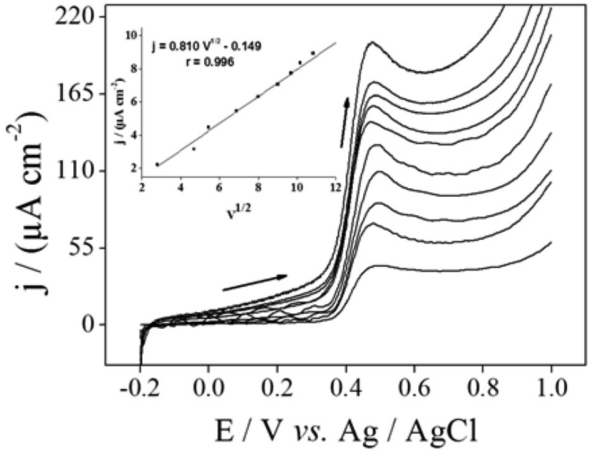


Figure 4. Linear sweep voltammogram obtained at different scan rates (5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 mV s^{-1}) in a 0.1 mol L^{-1} phosphate buffer solution with a pH of 12.3 and 3.34 × 10^{-4} mol L^{-1} of BP. The inset shows the plot of j *vs.* v$^{1/2}$.

The matter transfer properties must be established in diffusion-controlled processes and are described by Randles-Sevick,[20] equation 2:

$$Ip = 0.466nFAC\sqrt{\frac{nFvD}{RT}} \quad (2)$$

where Ip is the peak current, n is the number of electrons transferred, C is the bulk concentration, A is the working electrode area, v is the scan rate, T is the temperature in Kelvin, D corresponds to the diffusion coefficient, and F and R are the Faraday and perfect gas constants, respectively.

Thus, the reaction kinetics at the electrode are sufficiently fast for the oxidation process to be controlled by

mass transfer (diffusion of species) and not electron transfer because the diffusion coefficient of the 1,2-benzopyrone is $D = 2.57 \times 10^{-6}$ cm^2 s^{-1}.[18,21]

To estimate the number of electrons involved during the oxidation of BP in a 0.1 mol L^{-1} phosphate buffer solution (pH 12.3), a low scan rate with a potential of 0.5 mV s^{-1} was used. The resultant potential values were plotted as a function of ln [(j_L – j) / j] (Figure 5), where j_L (μA cm^{-2}) is the steady-state limiting current,[22] which yields a correlation coefficient of 0.99 for n = 20 with a slope (α) of 0.02.

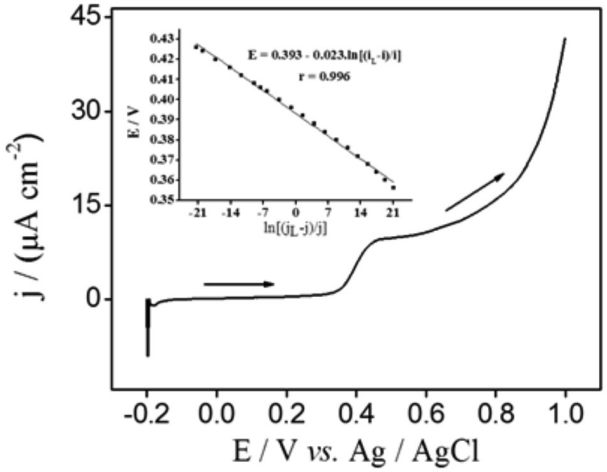
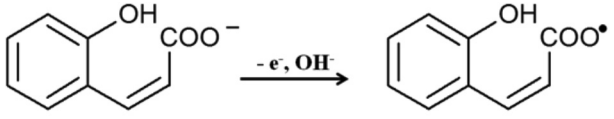


Figure 5. Linear sweep voltammogram for the electrode in a 0.1 mol L^{-1} phosphate buffer solution with a pH of 12.3, 3.34 × 10^{-4} mol L^{-1} of BP and v = 0.5 mV s^{-1}. Inset: linear relation between E (potential) and ln [(j_L – j) / j].

The number of electrons involved was found to be 1.1 (n = RT / αF).[23] This result suggests that the oxidation process of BP involves one electron and forms a radical ketone that can be stabilized as a dimer according to the reaction mechanism presented in Scheme 2.[24,25]



Scheme 2. Coumarin oxidation (coumaric acid form - carboxylate) forming a radical ketone under alkaline conditions (phosphate buffer solution at 0.1 mol L^{-1}, pH 12.3).

Adsorption process

Consecutive LV measurements verified that the well-defined peak in the first potential sweep decreased in intensity during the second cycle. This current density decrease (approximately 84%) can be attributed to the formation of a film that renders the active sites unavailable for subsequent analyses. This conclusion is supported by Figure 6, which shows the LV for the blank solution after a number of consecutive scans in the presence of the analyte.

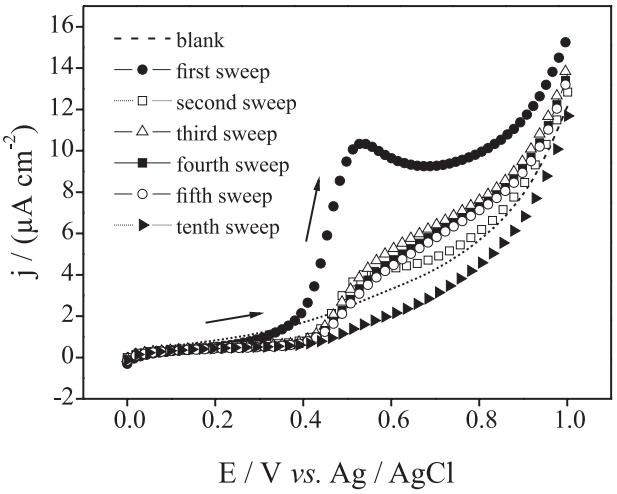


Figure 6. Linear sweep voltammograms in a 0.1 mol L^{-1} phosphate buffer solution with a pH of 12.3, a SR = 85 mV s^{-1} and 3.34 × 10^{-4} mol L^{-1} of BP. --- blank; ● first; □ second; △ third; ■ fourth; ○ fifth; and ▶ tenth consecutive sweeps.

The number of electroactive species on the surface electrode, Γ, expressed as the charge *per* unit area can be calculated by applying equation 3:

$$\Gamma = \frac{Q}{nFA} \quad (3)$$

where Q is the charge integrated at the peak oxidation, F is the Faraday constant, n is the number of electrons and A is the electrode area.[26] The value obtained was 6.88 × 10^{-9} mol cm^{-2} for the first cycle. The subsequent potential scans did not increase in charge, which indicates the adsorbed species block the electrode surface.

Electrochemical EIS measurements were performed to evaluate the interactions of BP on a glassy carbon electrode. The complex impedance can be presented as the sum of its real, Zre, and imaginary, Zim, components that stem from the resistance and capacitance of the cell (Nyquist plot). Figure 7 shows a diagram of the Nyquist for an electrode after cleaning and consecutive potential scans. The clean electrode has a capacitive behavior with strong diffusion effect. After five cycles, a semicircle forms between high and low frequencies, which can be attributed to a film being formed on the electrode surface due to strong molecular interactions after oxidation. After ten and twenty cycles, the semicircle diameter increased, which indicates that the film thickened.

The film resistance (Rf) was determined based on the intersection of the simulated semicircle with the axis in the Nyquist diagram. The resistance was calculated from the equation Rf = Rp – Rs, where Rp is the polarization

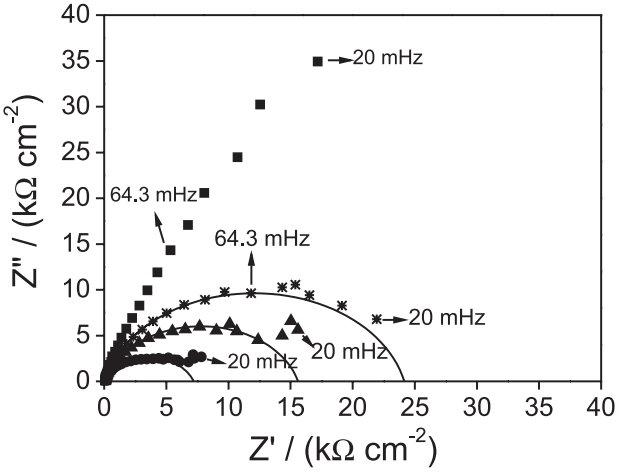


Figure 7. Nyquist diagrams in a 0.1 mol L^{-1} phosphate buffer solution with a pH of 12.3 and 3.34 × 10^{-4} mol L^{-1} of BP. ■) Bare electrode; ●) after five; ▲) after ten; ∗) and after twenty consecutive cycles. The solid lines represent the simulated semicircle. Applied potential: 0.53 V.

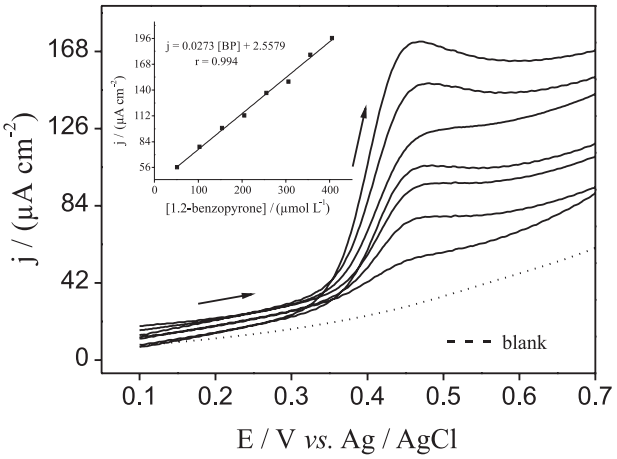


Figure 8. Linear sweep voltammograms for the blank (---) and various concentrations of BP (50; 100; 150; 200; 250; 300; 350; 400 × 10^{-6} mol L^{-1} in 0.1 mol L^{-1} phosphate buffer solution with a pH of 12.3). The SR in this experiment was 85 mV s^{-1}. Inset: calibration curve.

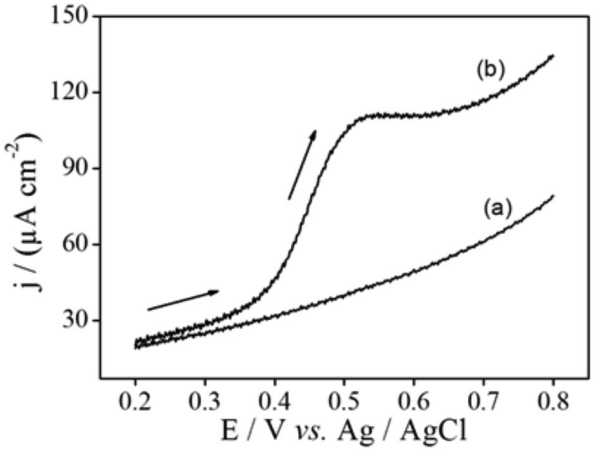


Figure 9. Linear sweep voltammograms of the 0.1 mol L^{-1} phosphate buffer solution with a pH 12.3 and SR = 85 mV s^{-1}. (a) Analytical blank and (b) maceration containing sonicated dry leaves under the same experimental conditions.

resistance and Rs is the solution resistance. The films formed after five, ten and twenty consecutive cycles yielded values of 7, 15 and 24 kΩ cm^{-2}, respectively.

The Nyquist diagrams were characterized by depressed capacitive loops with a theoretical center below the real axis. This feature reflects the surface inhomogeneity of the structural or interfacial origins such as for the adsorption process.

Quantification of 1,2-benzopyrone via LV

The optimization of the voltammetric conditions was evaluated. This was accomplished by analyzing BP across the concentration range from 50-400 μmol L^{-1} (Figure 8). The linear equation j = 2.56 + 0.03c (μmol L^{-1}) was obtained with a correlation coefficient of 0.99. The detection limit was 26.4 μmol L^{-1}, and the relative standard deviation was 4.8% for 10 successive analyses with the analytical recovery of the standard added to guaco samples of 97.5%. After establishing the analytical figures of merit, the methodology was applied to different guaco extracts to quantify BP without interference from the extremely alkaline pH (see example in Figure 9).

Figure 9 shows the determination of 1,2-benzopyrone in guaco samples, the oxidation peak was displaced approximately 45 mV towards the positive potentials due to the presence of other organic compounds in the matrix, which hinder electron transfers. However, this peak was only caused by the hydrolyzation of 1,2-benzopyrone because these other compounds were not electroactive in extremely alkaline medium.

Table 3 shows the BP concentration for different samples obtained via electrochemical and liquid chromatographic analyses. The analyte concentrations were approximately 8 mg g^{-1}, which is consistent with the data reported by Celeghini *et al.*.[10] It should be noted that each sample yielded identical results at the 95% confidence level (Student *t*-test) when comparing the two methodologies (LV and HPLC).

Conclusions

Linear sweep voltammetry was effectively employed for detecting 1,2-benzopyrone using a glassy-carbon electrode. The use of experimental design (factorial design and Doehlert chart) to optimize the LV conditions significantly reduced the length and number of experiments required. The results demonstrated that BP quantification employing LV under the optimized conditions (BC = 0.1 mol L^{-1}, pH = 12.3 and SC = 85 mV s^{-1}) was simple, fast and

Table 3. Results obtained for detection of 1,2-benzopyrone in guaco samples using the proposed method and compared to an HPLC analysis

Extraction method	Solvent (1:1 v/v)	Concentration of 1,2-benzopyrone / (mg g^{-1})	
		LV	HPLC
Maceration/sonication - DL	Ethanol/water	7.5 ± 0.4	7.7 ± 0.1
Infused - DL	Water	7.3 ± 0.2	7.5 ± 0.1
Maceration 7 days - DL	Ethanol/water	8.0 ± 0.2	8.1 ± 0.1
Maceration/sonication - IN	Ethanol/water	2.1 ± 0.3	2.2 ± 0.1
Infused - IN	Water	2.0 ± 0.2	2.1 ± 0.1
Maceration 7 days - IN	Ethanol/water	2.2 ± 0.2	2.3 ± 0.1

DL: dry leaf sample; IN: *in natura* leaf sample.

sensitive (limits of detection and quantification were 26.4 and 87.9 μmol L^{-1}, respectively).

In addition, these experiments were useful for determining certain aspects of the 1,2-benzopyrone oxidation process. This event is controlled by a mass transfer and involves one electron. Furthermore, LV showed a blocking of the electrode active sites after consecutive readings, as confirmed by EIS measurements that showed the formation of a resistive film. This development prevents the multiple measurements from occurring consecutively.

Finally, a very high correlation was found between the data obtained via different analytical methods (LV and HPLC). Therefore, the authors concluded the LV analysis could efficiently replace the analytical methodologies traditionally employed for this task.

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