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Article

# Determination of Quercetin in a Pharmaceutical Sample by Square-Wave Voltammetry Using a Poly(vinylpyrrolidone)-Modified Carbon-Paste Electrode

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Um eletrodo de pasta de carbono modificado com o polímero poli(vinilpirrolidona) foi avaliado para o estudo eletroquímico e a determinação eletroanalítica da quercetina. Sobre este eletrodo, voltamogramas cíclicos da quercetina apresentaram três picos de oxidação localizados em +0,32, +0,78 e +1,04 V. A eletro-oxidação no primeiro pico conduz à formação da *orto*-quinona correspondente, a qual pode ser reduzida em condições experimentais favoráveis. Nestas condições favoráveis, a reação é quase-reversível e o processo é controlado por difusão. Este comportamento foi explorado para a determinação eletroanalítica da quercetina por voltametria de onda-quadrada. A curva de calibração obtida foi linear na faixa de concentração de 0,5 a 5,5 μmol $L^{-1}$ ($R^2$ = 0,998). Os limites de detecção e quantificação foram de 0,17 μmol $L^{-1}$ e 0,52 μmol $L^{-1}$, respectivamente. O sensor foi usado para determinação de quercetina em produtos farmacêuticos. A exatidão dos resultados fornecidos pelo sensor foi avaliada por comparação com os resultados obtidos pela técnica UV-Vis.

A carbon-paste electrode modified with the polymer poly(vinylpyrrolidone) was evaluated through electrochemical studies and the electroanalytical determination of quercetin. For this electrode, cyclic voltammograms of quercetin showed three oxidation peaks at +0.32, +0.78 and +1.04 V. The electro-oxidation associated with the first peak leads to the formation of the corresponding *ortho*-quinone, which can be reduced under favorable experimental conditions. Under such conditions, the reaction is quasi-reversible and the process is diffusion-controlled. This behavior was exploited for the electroanalytical determination of quercetin by square-wave voltammetry. The calibration curve was linear in the concentration range of 0.5 to 5.5 μmol $L^{-1}$ ($R^2$ = 0.998). The limits of detection and quantification obtained were 0.17 μmol $L^{-1}$ and 0.52 μmol $L^{-1}$, respectively. The sensor was successfully used for the determination of quercetin in a pharmaceutical sample. The accuracy of the results achieved with the sensor was evaluated through comparison with the results provided by the UV-Vis technique.

**Keywords:** quercetin, carbon-paste electrode, poly(vinylpyrrolidone), electroanalysis

## Introduction

Flavonoids are substances of natural occurrence with different chemical structures found mainly in fruits, vegetables, grains, roots, stems, flowers, tea and wine.[1] There are more than 4000 flavonoid compounds and their isolation and identification were pioneered by Szent-Gyorgyi in 1936.[2] The basic chemical structure of flavonoids consists of fifteen carbon atoms distributed in three rings designated A, B and C (Figure 1a). Flavonoids are divided into classes according to their level of oxidation and the number of substitutions of the C ring, whereas the compounds within the same class differ in terms of the number of substitutions of the A and B rings.[3]

Some authors[4] have demonstrated that flavonoids have high antioxidant capacity and this property is attributed to

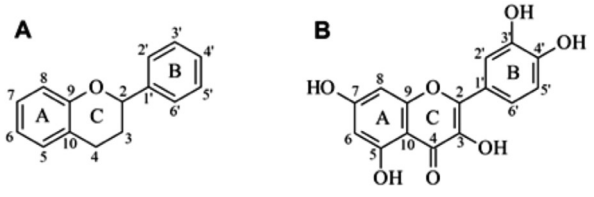


**Figure 1.** Chemical structure of (a) flavonoids and (b) quercetin.

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their ability to scavenge free radicals from both hydrophilic and lipophilic cell components. However, under specific conditions, flavonoids may also present pro-oxidant activiy.[5] The consumption of flavonoids by humans is estimated to be a few hundred milligrams per day.[6] Research indicates[4,6] that a diet rich in flavonoids is related to increased longevity and reduced incidence of cardiovascular disease. In addition to their antioxidant properties, flavonoids have multiple biological properties such as antiviral, antibacterial, anti-inflammatory, vasodilator, anticancer and anti-ischemic.[4,6] Besides their use for biological purposes, there is a growing commercial interest for plants as a source of antioxidants, which can also be used to improve the properties of foods.[7]

Quercetin (5,7,3',4'tetrahydroxi-flavonol) (Figure 1b) is the most abundant flavonoid in the plant kingdom. Its molecule differs from others of the same class, such as rutin and iso-quercetin, for example, through the presence of a hydroxyl instead of a glycoside group in the C ring, which confers the characteristic properties of quercetin.[8] Many researchers[8-12] have studied the electrochemical behavior of flavonoids, particularly of quercetin. In general, published data show that the cyclic voltammogram of quercetin in hydro-alcoholic solution exhibits three oxidation peaks and one reduction peak depending on the experimental conditions. The oxidation of the catechol moiety at the B ring occurs first at low positive potentials, leading to the formation of the corresponding *ortho*-quinone, via a reversible reaction involving two electrons and two protons. The second peak corresponds to the irreversible oxidation of the hydroxyl group at position 3 of the C ring. Finally, the hydroxyl groups at positions 5 and 7 of the A ring appear to have an electron donating effect giving rise to the oxidation at the third peak at higher potentials. However, Timbola *et al.*[9] proposed a more complex multi-step mechanism for the electro-oxidation of quercetin based on electrochemical and spectroscopic experiments. Using the same experimental conditions, Zhou *et al.*[13] reported the isolation, characterization and structural elucidation of 18 intermediates following the electrochemical oxidation of quercetin.

Owing to concerns regarding the electrochemical determination of quercetin, a myriad of sensors and techniques has been proposed. Some selected papers reporting the more recent results obtained are detailed herein. The determination of quercetin and rutin by flow injection analysis and capillary electrophoresis using electrochemical detection has been recently described.[14] Quercetin and rutin have been determined at unheated and heated platinum microelectrodes using cyclic voltammetry. Heated microelectrodes have been used for the determination of the flavonoids in an extract of sea buckthorn and a pharmaceutical preparation. In another study, a molecularly imprinted polymer based on polypyrrole film with incorporated graphene oxide was fabricated and used for the electrochemical determination of quercetin.[15] The calibration curve obtained by differential pulse voltammetry in Britton-Robinson buffer solution of pH 3.5 was linear for concentrations in the range of 0.6 to 15.0 μmol $L^{-1}$ with a detection limit of 48 nmol $L^{-1}$. The electrode showed good stability and reproducibility. Flavonoids with similar chemical structures as rutin or morin did not interfere with the determination of quercetin. The electrochemical determination of quercetin has also been studied using square-wave voltammetry on a glassy carbon electrode modified with gold nanoparticles self-assembled onto the surfaces of p-aminothiophenol functionalized graphene oxide.[16] The linearity range for the calibration curve of quercetin was 1.0 to 10.0 pmol $L^{-1}$ with a detection limit of 0.3 pmol $L^{-1}$. The sensor was applied successfully for the determination of quercetin in pharmaceutical preparations. Also, an electrochemical polymerized 5-amino-2-mercapto-1,3,4-thiadiazole-modified single-use graphite electrode for the electrochemical monitoring of quercetin has been described.[17] A six-fold increase in the quercetin signal was obtained using the modified electrode compared to the unmodified electrode. The electrochemical oxidation of quercetin in citrate buffer has been investigated using a Nafion multi-walled carbon nanotube composite modified graphite-paste electrode.[18] Employing square-wave anodic stripping voltammetry and applying the optimized parameters the content of quercetin was found to be 2.89 mg $g^{-1}$ and 4.21 mg $g^{-1}$ in dry and frozen fruits, respectively, of *Acanthopanax sessiliflorus* (*A. sessiliflorus*). A differential pulse voltammetric procedure has been proposed for the determination of quercetin in aqueous solution using a simple carbon-paste electrode.[19] The detection limit obtained using this carbonaceous material was 38.5 nmol $L^{-1}$. A carbon nanotube-paste electrode modified with copper microparticles has been constructed and used for the determination of quercetin in apple juice.[20] The performance of the modified carbon-paste electrode was compared to the unmodified electrode for the determination of quercetin and a lower limit of detection was obtained with the former. The results obtained for quercetin in apple juice concurred with a spectrophotometric method at the 95% confidence level with recoveries of between 98.9 and 102.8%. Flowerlike $Co_3O_4$ nanoparticles have been used as a modifier on a glassy carbon electrode to fabricate a quercetin sensor.[21] The electrochemical behavior of quercetin at the sensor was studied by cyclic voltammetry and semi-derivative voltammetry. Under the optimum conditions, the catalytic peak currents were

linearly dependent on the concentrations of quercetin in the range of 0.5 to 330 μmol $L^{-1}$, with a detection limit of 0.1 μmol $L^{-1}$. This proposed method was successfully applied to determine the quercetin concentration in Ginkgo leaf tablets and human urine samples.

Poly(vinylpyrrolidone) (PVP) is a polymer with numerous properties, for instance, the ability to extract phenolic compounds from fruit juices and plant extracts is well-documented.[22,23] However, reports on the use of PVP for the construction of modified electrodes are scarce.[24-26] In one study,[24] rutin, a flavonoid with a chemical structure similar to that of quercetin, was determined using a carbon-paste electrode modified with PVP. The authors of the study reported that the polymer PVP enhanced the adsorption of rutin on the electrode surface due to the presence of hydrogen bonding between the imide group in PVP and the hydroxyl group in rutin. This adsorption property was exploited to accumulate rutin on the sensor. After a pre-concentration time of 10 min, the calibration curve obtained by linear sweep voltammetry was linear for rutin concentrations of 0.39 to 13.0 μmol $L^{-1}$. The detection limit was 0.15 μmol $L^{-1}$. However, the sensor has not been tested for other phenolic compounds using different electroanalytical techniques and experimental conditions.

The main goal of this study was to test a PVP-modified carbon-paste electrode (PVP-CPE) as a sensor for the quantitative determination of quercetin. Specifically, our aim was to explore experimental conditions, which differed, from those used in a previous study,[24] in order to reduce the accumulation time necessary to obtain the analytical response. Hence, a previously prepared sensor was employed together with cyclic voltammetry to study the electrochemical behavior of quercetin. Then, square-wave voltammetry was used for the construction of the calibration curve and the electroanalytical determination of this flavonoid in a pharmaceutical sample. The performance of the PVP-CPE sensor in quercetin determination was compared to that of the UV-Vis technique.

## Experimental

### Reagents and solutions

All reagents used in this study were of analytical grade and employed without prior purification. Ultrapure water with a resistivity of 18 MΩ obtained from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all solutions. Phosphate, acetate and Britton-Robinson buffers were tested as the supporting electrolyte. All supporting electrolytes were prepared at a concentration of 0.1 mol $L^{-1}$ and then their pH was adjusted to the appropriate value with 1.0 mol $L^{-1}$ HCl or NaOH. A stock solution of quercetin was prepared in ethanol at a concentration of 0.1 mmol $L^{-1}$. Less concentrated solutions were prepared by dilution with purified water. Supporting electrolytes and stock solutions were kept under refrigeration for a maximum of 30 days.

### Sensor preparation

The PVP-CPE sensor was prepared by macerating 10 mg of PVP (5% w/w) and 160 mg (80% w/w) of graphite powder for 10 min to obtain a uniform dispersion of the polymer in the powdered graphite. Next, 30 mg (15% w/w) of a mineral oil was added and the mixture was macerated for a further 20 min in order to obtain a paste with homogeneous composition. The paste was packed into a 1.0 mL plastic syringe and a copper wire was inserted to obtain the external electrical contact. The PVP-CPE sensor was abraded manually to ensure a renewed surface before each measurement. In all studies, the geometric area of the sensor was kept constant (0.31 $mm^2$). For comparison purposes, an unmodified CPE was also constructed.

### Electrochemical measurements

Cyclic and square-wave voltammetries were carried out with a PalmSens (Palm Instruments BV, The Netherlands) potentiostat/galvanostat interfaced to a personal computer and the software PSTrace (version 2.5.2) was used for data acquisition. A platinum plate, an Ag/AgCl (3.0 mol $L^{-1}$ KCl) electrode and a CPE or PVP-CPE were used, respectively, as the auxiliary, reference and working electrodes in a 15 mL conventional three-electrode cell. Cyclic voltammetry was carried out at 100 mV $s^{-1}$ firstly in the positive direction and then in the negative direction. For the construction of the calibration curve and the determination of quercetin in the pharmaceutical sample, the square-wave voltammograms were recorded at between 0.0 and +0.5 V, with the following optimized parameters: $a$ = 60 mV, $f$ = 100 Hz and ΔEs = 4 mV.

### Preparation of sample and standard addition curve

The pharmaceutical formulation containing quercetin was purchased at a local (Florianopolis-SC, Brazil) drugstore. The content of one capsule was dissolved and diluted in ethanol to give a 200 mL solution. A suitable aliquot of the resulting solution was transferred to the electrochemical cell containing 10 mL of the supporting electrolyte. Quercetin was quantified after successive additions of 0.5 μmol $L^{-1}$ quercetin standard solution.

### Comparative method

UV-Vis spectroscopy was carried out in the wavelength range of 200 to 500 nm using a Cary 60 Agilent (Agilent Technologies, United States) spectrophotometer. A quartz cell with an optical path length of 1.0 cm was used. The maximum absorbance of the solutions containing different concentrations of quercetin was determined at 373 nm.

## Results and discussion

### Voltammetric behavior of quercetin on the PVP-CPE sensor

Cyclic voltammograms recorded at between –0.7 and +1.3 V for 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ phosphate buffer solution (pH 6.0) at a PVP-CPE sensor are shown in Figure 2. The first cycle (Figure 2a) exhibited clearly three well-defined oxidation peaks at +0.32 V, +0.78 V and +1.04 V in the forward scan. No reduction peaks were observed in the reverse scan. This behavior was similar to that previously observed by other authors.[9] For the second cycle (Figure 2b) and the following cycles (data not shown) a significant decrease in the current values was observed, indicating that the electrode surface was blocked by a strong adsorption of the reagent and products of the oxidation reaction. The adsorption of flavonoids has been attributed to the formation of a hydrogen bond between the hydroxyl groups of the phenolic compound and the carbonyl group present in the polymer.[24] In addition, this property has been used to propose a PVP-CPE-based sensor for rutin determination. However, an accumulation time of 10 min was necessary to achieve good results.

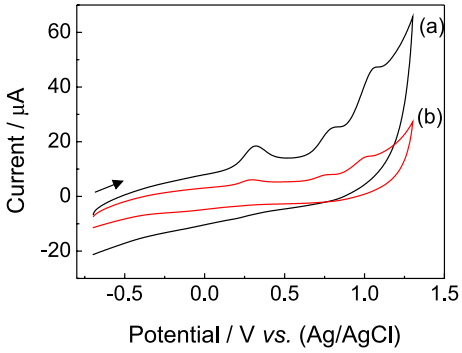


**Figure 2.** Cyclic voltammograms for 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ phosphate buffer (pH 6.0) at a PVP-CPE sensor, (a) first cycle and (b) second cycle, $v$ = 100 mV $s^{-1}$.

Figure 3 shows the cyclic voltammograms recorded between –0.1 V and +0.7 V for 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ phosphate buffer solution (pH 6.0) at the CPE and the PVP-CPE sensors. The potential range chosen corresponds to the potential interval of the first peak shown in Figure 2. As expected, no peaks were observed for the CPE (Figure 3a) and PVP-CPE (Figure 3b) in the absence of quercetin. Conversely, in the presence of the flavonoid, a well-defined oxidation peak at +0.25 V and a small reduction peak at +0.14 V were observed using the CPE (Figure 3c). For the PVP-CPE sensor (Figure 3d) the peaks were located at +0.32 V and at +0.17 V for the oxidation and the reduction reactions, respectively. Furthermore, the currents were at least twice as high using the PVP-CPE sensor.

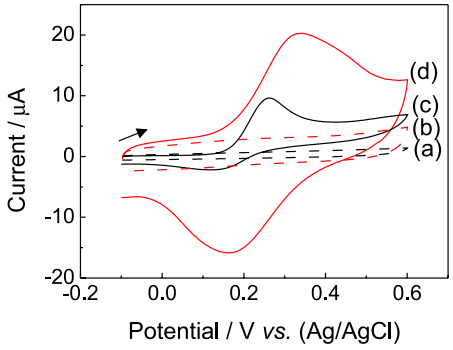


**Figure 3.** Cyclic voltammograms for a CPE (a, c) and a PVP-CPE sensor (b, d) in the absence (a, b) and presence (c, d) of 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ phosphate buffer (pH 6.0), $v$ = 100 mV $s^{-1}$.

As cited earlier, the first peak is associated with the oxidation of the catechol moiety at the B ring, which leads to the formation of the corresponding *ortho*-quinone, via a reaction involving two electrons and two protons. If the potential is reversed before the second peak, the reaction is reversible or, at least, quasi-reversible, and a reduction peak is clearly perceived. In addition, the formation of products and their adsorption onto the electrode surface were not observed. This behavior was investigated with the aim of applying the PVP-CPE sensor for the determination of flavonoids, in particular quercetin. Hence, all experiments described in the next sections were carried out using the potential intervals shown in Figure 3.

### Optimization of the experimental conditions

In order to optimize the response of the PVP-CPE sensor, several experimental conditions were investigated, such as the percentage of PVP (5.0% to 40.0% w/w), pH of the supporting electrolyte (2.0 to 12.0) and the chemical composition of the supporting electrolyte (phosphate, acetate and Britton-Robinson buffers).

The effect of the percentage of PVP, which was varied from 5% to 40 % (w/w), on the response of the PVP-CPE sensor to the oxidation of quercetin was investigated. It was

observed that the current obtained by cyclic voltammetry for the oxidation of 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ phosphate buffer (pH 6.0) at a PVP-CPE did not increase with increasing content of the PVP in the carbon paste (Figure 4). On the contrary, the voltammetric profiles were less defined and the consistency of the paste was less satisfactory for the higher contents of PVP. Thus, the percentage of PVP chosen for the construction of the PVP-CPE sensor was 5%.

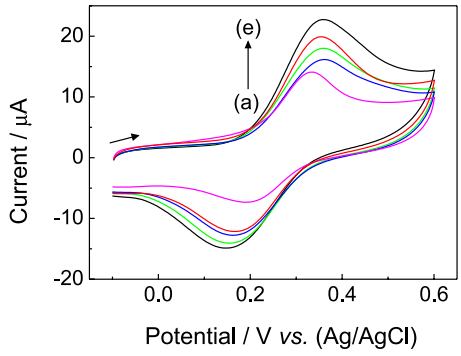


**Figure 4.** Cyclic voltammograms for 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ phosphate buffer (pH 6.0) at the PVP-CPE sensors; (a-e) = 40, 30, 20, 10, 5% of PVP, respectively; $v$ = 100 mV $s^{-1}$.

The influence of the pH of the supporting electrolyte on the electrochemical oxidation of quercetin was also investigated. In this case, the universal Britton-Robinson buffer was used at pH varying from 2.0 and 12.0. As in the previous experiments, the current obtained in the cyclic voltammetry for the oxidation of 1.0 mmol $L^{-1}$ quercetin was monitored using a PVP-CPE sensor. For pH higher than 9.0, no redox signal was observed. The highest currents for the oxidation-reduction reaction of quercetin on the PVP-CPE sensor were observed for pH lower than 5.0, as shown in Figure 5. Thus, we selected pH values in the range of 2.0 to 5.0 to investigate the influence of the chemical composition of the supporting electrolyte on the electrochemical response of quercetin at the PVP-CPE sensor.

The effect of the different supporting electrolytes, such as the Britton-Robinson, phosphate and acetate buffers, with the pH varying between 2.0 and 5.0, was investigated by cyclic voltammetry for the oxidation of 1.0 mmol $L^{-1}$ quercetin. The results are shown in Figure 6. The best voltammetric responses were obtained in 0.1 mol $L^{-1}$ acetate buffer with the pH adjusted to 5.0. Thus, this supporting electrolyte and pH were selected for further experiments.

In summary, the electrochemical oxidation of 1.0 mmol $L^{-1}$ quercetin was investigated by cyclic voltammetry in the potential interval of –0.1 V to +0.7 V applying the PVP-CPE sensor. The best results were

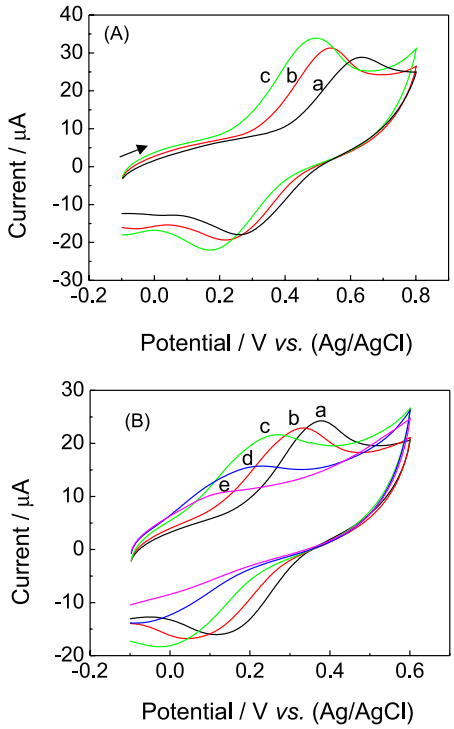


**Figure 5.** Cyclic voltammograms for 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ Britton-Robinson buffer at the PVP-CPE sensor. (A) (a) pH 2.0, (b) pH 3.0 and (c) pH 4.0 and (B) (a) pH 5.0, (b) pH 6.0, (c) pH 7.0, (d) pH 8.0 and (e) pH 9.0; $v$ = 100 mV $s^{-1}$.

obtained for the following experimental conditions, which were used in the subsequent experiments: (i) composition of the PVP-CPE sensor: (80:15:5%, w/w/w) graphite powder:mineral oil:PVP; (ii) composition of the supporting electrolyte: 0.1 mol $L^{-1}$ acetic acid- sodium acetate buffer; and (iii) pH of the supporting electrolyte: 5.0.

## Influence of scan rate

Figure 7A shows the influence of the scan rate (ν) on the voltammetric profile for 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ acetate buffer solution (pH 5.0) obtained with the PVP-CPE sensor. This profile verifies that the quercetin and corresponding *ortho*-quinone co-exist at the sensor surface. The reaction is quasi-reversible because the potentials shifted with increasing scan rate. In addition, the cathodic current-$i_{pc}$/anodic current-$i_{pa}$ ratio deviated slightly from 1.0. The current of both peaks changed linearly with the square root of the scan rate (Figure 7B). The corresponding linear regression equations are $i_{pa}/\mu A = 0.732 + 1.122\ v^{1/2}$ / (mV $s^{-1}$) $^{1/2}$ ($R^2$ = 0.992) and $i_{pc}/\mu A = 4.104 = 1.668\ v^{1/2}/(mV\ s^{-1})^{1/2}$ ($R^2$ = 0.996). These results indicate that the redox reaction of quercetin at the PVP-CPE sensor is a diffusion-controlled process. Moreover, the plot of log i *vs.* log *v* (Figure 7C) exhibited a slope of 0.67 for the oxidation reaction and 0.58 for

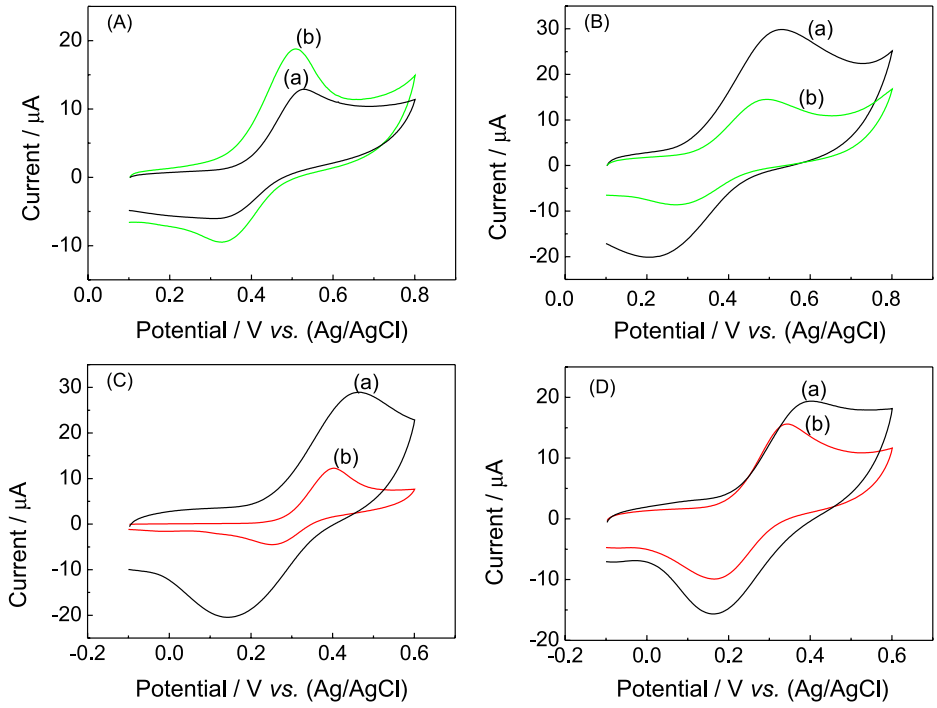


**Figure 6.** Cyclic voltammograms obtained at (A) pH 2.0 and (B) pH 3.0 in 0.1 mol $L^{-1}$ (a) Britton-Robinson and (b) phosphate buffers and (C) pH 4.0 and (D) pH 5.0 in 0.1 mol $L^{-1}$ (a) Britton-Robinson and (b) acetate buffers for 1.0 mmol $L^{-1}$ quercetin at the PVP-CPE sensor, $v$ = 100 mV $s^{-1}$.

the reduction reaction, clearly indicating that the current for both reactions was diffusion-controlled with a small contribution from adsorption.

### Selection of the electroanalytical technique

Different electroanalytical techniques were investigated to evaluate their sensitivity in the oxidation of quercetin on the PVP-CPE sensor. To this aim, the experimental setup of the linear sweep voltammetry (LSV), differential-pulse voltammetry (DPV) and square-wave (SWV) was adjusted to obtain the same scan rate for the three techniques during the measurements. Figure 8 shows the results obtained when a solution of 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ acetate buffer (pH 5.0) was used for the test. It can be clearly observed that the three techniques provided an analytical signal for the electrochemical oxidation of quercetin. However, the maximum current supplied by LSV (Figure 8a) was lower than that supplied by DPV (Figure 8b) which, in turn, was around half of that supplied by SWV (Figure 8c). The highest sensitivity in the latter case is due to the lower capacitive charging current made available in the SWV. Additionally, for reversible or quasi-reversible reactions, as is the case for the quercetin-corresponding *ortho*-quinone couple, the difference between the current produced by the anodic and cathodic pulses ($\Delta i_p = i_{pa} - i_{pc}$) gave a voltammogram with a peak current that was around twice that of the individual currents. Hence, SWV applied in the potential interval of the first oxidation peak, where the reaction studied was quasi-reversible and diffusion-controlled, was chosen for further experiments.

### Calibration curve obtained by SWV/PVP-CPE sensor

Applying the optimized experimental conditions discussed above the square-wave voltammograms shown in Figure 9A demonstrate the excellent response of the PVP-CPE sensor provided as a function of the quercetin concentration. It is evident that the current increased and the peak potential was not shifted with increasing quercetin concentration. The calibration curve obtained from Figure 9A is shown in Figure 9B. The analytical parameters extracted from the calibration curve are given in Table 1.

The calibration curve obtained for quercetin is linear in the concentration range of 0.5 to 5.5 µmol $L^{-1}$, with a correlation coefficient of 0.998. The linear regression equation can be expressed according to the function $\Delta i/\mu A = 0.57 + 3.96$ [quercetin]/µmol $L^{-1}$. The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the equations: LOD = 3.3 $S_b/B$ and LOQ = 10 $S_b/B$, where $S_b$ is the standard deviation of the linear coefficient and B is the slope of the curve. The LOD and LOQ values obtained were 0.17 µmol $L^{-1}$ and 0.52 µmol $L^{-1}$, respectively. The linear range of the calibration curve obtained from this study (0.5 to 5.5 µmol $L^{-1}$) is

somewhat lower than that obtained in previous studies[24] (0.4 to 13.0 μmol $L^{-1}$), but the detection limit is essentially the same, 0.17 and 0.15 μmol $L^{-1}$, respectively. On the other hand, the procedure used allows increasing the speed of analysis, considering that it is not necessary the pre-concentration step. The intra-day repeatability provided by the PVP-CPE sensor was assessed by considering five times the peak current of the square-wave voltammograms

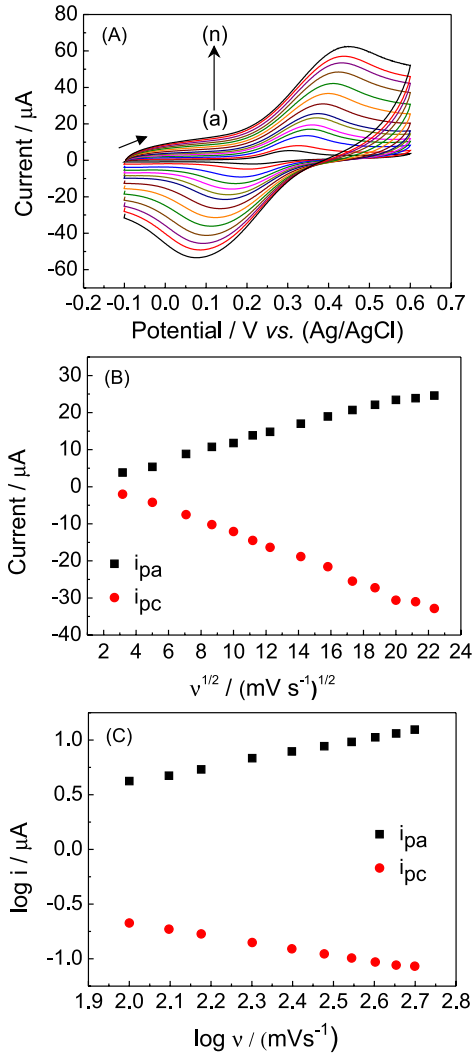


**Figure 7.** (A) Cyclic voltammograms for 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ acetate buffer (pH 5.0) at the PVP-CPE sensor, $\nu$ (a-j) = 10, 25, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450 and 500 mV $s^{-1}$, respectively; (B) plot current *vs.* $\nu^{1/2}$ and (C) plot log i *vs.* log $\nu$.

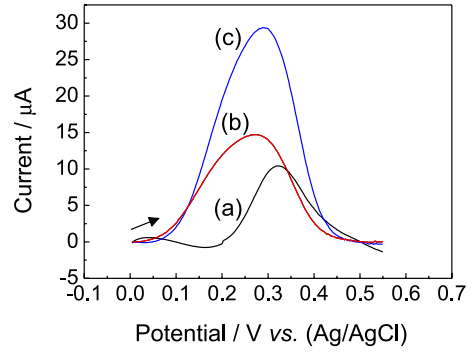


**Figure 8.** Voltammograms for 1.0 mmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ acetate buffer (pH 5.0) at a PVP-CPE sensor obtained by: (a) LSV, (b) DPV and (c) SWV, $\nu$ = 100 mV $s^{-1}$.

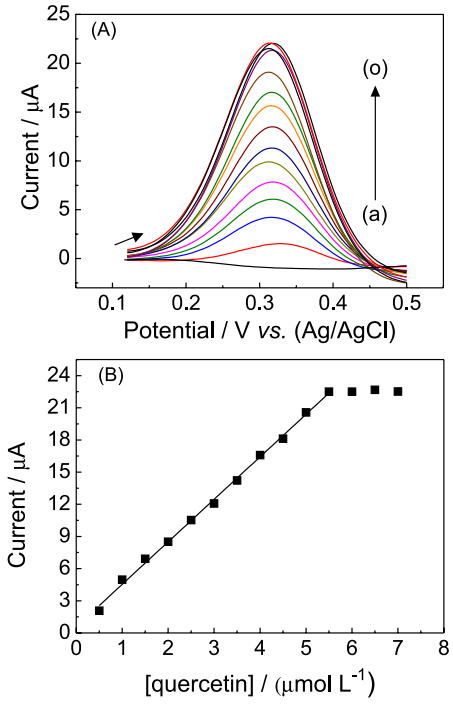


**Figure 9.** (A) Square-wave voltammograms for (a) blank, (b) 0.5 μmol $L^{-1}$, (c) 1.0 μmol $L^{-1}$, (d) 1.5 μmol $L^{-1}$, (e) 2.0 μmol $L^{-1}$, (f) 2.5 μmol $L^{-1}$, (g) 3.0 μmol $L^{-1}$, (h) 3.5 μmol $L^{-1}$, (i ) 4.0 μmol $L^{-1}$, (j) 4.5 μmol $L^{-1}$, (k) 5.0 μmol $L^{-1}$, (l) 5.5 μmol $L^{-1}$, (m) 6.0 μmol $L^{-1}$, (n) 6.5 μmol $L^{-1}$ and (o) 7.0 μmol $L^{-1}$ quercetin in 0.1 mol $L^{-1}$ acetate buffer solution (pH 5.0) obtained at the PVP-CPE sensor, $f$ = 60 Hz; $a$ = 100 mV and ΔEs = 4 mV; (B) calibration curve for quercetin.

**Table 1.** Analytical parameters for the determination of quercetin using SWV/PVP-CPE sensor

| Analytical parameter | Value |
|---|---|
| Peak potential / V | 0.31 |
| Linear range / (μmol $L^{-1}$) | 0.5 to 5.5 |
| Correlation coefficient | 0.998 |
| Slope / (μA L μmol$^{-1}$) | 3.96 |
| Standard deviation of slope / (μA L μmol$^{-1}$) | 6.1 × $10^{-2}$ |
| Intercept / μA | 0.570 |
| Standard deviation of intercept / μA | 0.208 |
| Detection limit / (μmol $L^{-1}$) | 0.17 |
| Quantification limit / (μmol $L^{-1}$) | 0.52 |
| Repeatability of $\Delta i_p$ (intra-day) / % [a,b] | 2.68 |
| Repeatability of $\Delta i_p$ (inter-day) / % [a,b] | 2.05 |

[a]Relative standard deviation; [b]n = 5.

using the same electrode, the surface being renewed after each measurement. The measurements were carried out at three different concentrations of quercetin in 0.1 mol $L^{-1}$ acetate buffer solution (pH 5.0): 1.0 µmol $L^{-1}$, 3.0 µmol $L^{-1}$ and 5.0 µmol $L^{-1}$. The relative standard deviation (n = 5) varied from 0.1% for the highest concentration to 2.7% for the lowest concentration. To assess the inter-day repeatability, a single sensor was used and the same procedure for the measurements was repeated on five consecutive days. The relative standard deviation (n = 5) varied from 0.1% for the highest concentration to 2.1% for the lowest concentration. The results indicate that excellent repeatability was achieved for the determination of quercetin at the three concentrations using the proposed PVP-CPE sensor.

## Determination of quercetin in pharmaceutical preparation

The electroanalytical determination of quercetin in a pharmaceutical formulation and the recovery experiments were performed by adding aliquots of a standard solution of 0.5 µmol $L^{-1}$ quercetin. The sample solution was prepared as described in the experimental section above and diluted to concentrations comparable to those of the calibration curve.

Figure 10A shows the square-wave voltammograms for the sample alone and after successive additions of the standard solution. The voltammograms revealed well-defined peaks at +0.31 V, and the peak currents increased linearly with increasing quercetin concentration. For comparison purposes the calibration curve is shown in Figure10B curve a together with the standard addition curve shown in Figure 10B curve b. It can be observed that the slopes of the two curves are very similar (the slope of the calibration curve was 3.96 µA L µmol$^{-1}$ while that of the standard addition curve was 4.05 µA L µmol$^{-1}$). This verifies the selectivity of the sensor for quercetin compared to the matrix components. In other words, the excipients present in the sample of the pharmaceutical formulation did not interfere in the determination of quercetin. The determination was carried out in triplicate and the mean concentration of quercetin found in the sample was 213 mg, which is consistent with the value provided by the manufacturer of the product (200 mg).

## Accuracy and precision

The accuracy provided by the PVP-CPE sensor for the determination of quercetin was evaluated in two ways: firstly through recovery experiments and then by comparison with the data furnished by the UV-Vis technique. For the

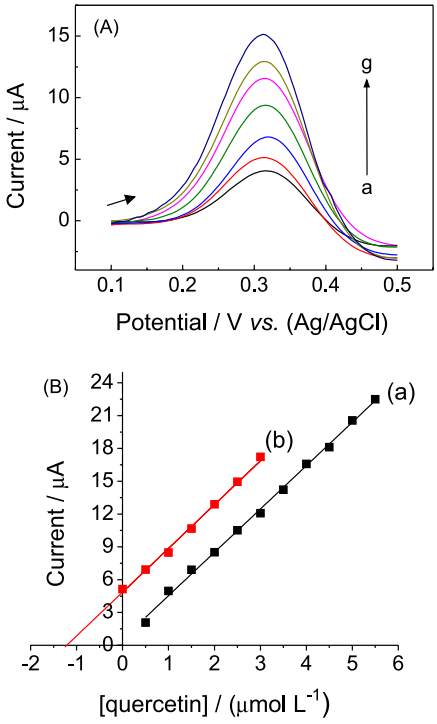


**Figure 10.** (A) Square-wave voltammograms for (a) sample alone, (b-g) sample with successive additions of 0.5 µmol $L^{-1}$ quercetin standard solution in 0.1 mol $L^{-1}$ acetate buffer (pH 5.0) obtained at the PVP-CPE sensor, $f$ = 60 Hz, $a$ = 100 mV and ΔEs = 4 mV; (B) (a) the calibration curve and (b) the standard addition curve.

recovery experiments, six determinations were carried out using different concentrations of the standard solution of quercetin added to the sample. The recovery values were between 96.9% and 102.6% indicating a good accuracy of the proposed sensor. Table 2 shows the results obtained for the determination of quercetin in a pharmaceutical product using the SWV/PVP-CPE sensor and UV-Vis technique.

**Table 2.** Determination of quercetin in pharmaceutical preparation

| Technique | UV-Vis | SWV/PVP-CPE |
|---|---|---|
| Labeled values / mg | 200 | 200 |
| Found values / mg[a] | 211 | 213 |
| RSD / % | 2.08 | 2.51 |
| $Er_1$ / %[b] | 5.50 | 6.50 |
| $Er_2$ / %[c] | – | 0.95 |
| $t_{value}$[d] | 3.22 | 3.12 |
| $F_{value}$[e] | 1.46 | 1.46 |

[a]n = 3; [b]$Er_1$ = relative error between UV-Vis or SWV/PVP-CPE sensor and labeled values; [c]$Er_2$ = relative error between SWV/PVP-CPE sensor and UV-Vis; [d]$t_{theoretical}$ = 4.30; [e]$F_{theoretical}$ = 19.

The average values obtained from three determinations (n = 3) using the two techniques, i.e., 211 mg (UV-vis) and 213 mg (SWV/PVP-CPE sensor), were very close

to the value provided by the manufacturer of 200 mg. The relative standard deviation (RSD) of the three determinations was 2.51% for the measurements taken with the comparative technique and 2.08% using the proposed sensor. The relative error between the UV-Vis technique and the labeled value was 5.50%, while for the SWV/PVP-CPE sensor the relative error was 6.50%. The relative error between the two techniques used for quercetin determination was less than 1.0%. The Student's t-test was used to compare the results obtained by the two techniques with the labeled value (accepted as the true value). At a confidence level of 95%, the calculated value ($t_{value}$) was lower than the theoretical value ($t_{theoretical}$), indicating that there are no significant differences between the results obtained using the two techniques and the true (labeled) value. The precision of the data collected was evaluated using the variance or F test. The $F_{value}$ value obtained for the determination of quercetin was lower that the $F_{theoretical}$ value at a confidence level of 95%, indicating that there is no significant difference between the precision of the data provided by the UV-Vis technique and that obtained with the SWV/PVP-CPE sensor. This set of experiments confirmed that the proposed sensor supplied accurate and precise data for the determination of quercetin in the pharmaceutical sample used for the test.

## Conclusions

The results of this study showed that the PVP-CPE sensor can be used for the electroanalytical determination of quercetin. PVP enhanced the sensitivity of the CPE and under optimized voltammetric experimental conditions also increased the reversibility of the reaction for the quercetin-corresponding *ortho*-quinone couple. Since the reaction rate was diffusion-controlled under these optimized conditions, the determination of quercetin could be easily carried out. The PVP-CPE sensor showed excellent performance for the determination of quercetin in a pharmaceutical sample, with the results comparable to those obtained by UV-Vis spectroscopy. The proposed sensor is a low cost assembly; the surface is easily renewable and in association with square-wave voltammetry it provided low values for the limits of detection and quantification, as well as excellent selectivity, accuracy and precision.

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