Square-wave voltammetric determination of rutin in pharmaceutical formulations using a carbon composite electrode modified with copper (II) phosphate immobilized in polyester resin

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A carbon composite electrode modified with copper (II) phosphate immobilized in a polyester resin (Cu₃(PO₄)₂-Poly) for the determination of rutin in pharmaceutical samples by square-wave voltammetry is described herein. The modified electrode allows the determination of rutin at a potential (0.20 V vs. Ag/AgCl (3.0 mol L⁻¹ KCl)) lower than that observed at an unmodified electrode. The peak current was found to be linear to the rutin concentration in the range from 9.9 × 10⁻⁸ to 2.5 × 10⁻⁶ mol L⁻¹. The response of the electrode was stable, with no variation in baseline levels within several hours of continuous operation. The surface morphology of the modified electrode was characterized by scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) system. The results obtained are precise and accurate. In addition, these results are in agreement with those obtained by the chromatographic method at a 95% confidence level.

Uniterms: Rutin/determination in pharmaceuticals samples. Square-wave voltammetry/determination of rutin. Modified carbon composite electrode/application.

INTRODUCTION

Rutin (C₂₇H₃₀O₁₆) is one of the most abundant flavonoids in the human diet (He, 2007) and concerns the kind of flavonoids, glycoside, commonly found in plants such as Flos Sophorae buds and others species of plants (Chen et al., 2000; Xu, 2007). Rutin is also founded in orange, grapefruit, lemon, berries and so forth (Packer, 2001). It has been widely used as a therapeutic agent and more than 130 preparations containing rutin are registered as drugs worldwide (Dapkevicious et al., 1999; Erlund, 2000). Some related investigations show that rutin has a broad range of biological activities, such as anti-inflammatory, anti-tumor and anti-bacterial (Gene et al., 1996; Nuengchamnong et al., 2004).

Due to its importance, several methods for rutin determination based on spectrophotometry (Li et al., 2005), chromatography (Nuengchamnong et al., 2004; Wang
et al., 2003), and electrochemistry (Franzoi et al., 2009; Santos et al., 2008; Xu, 2007), have been reported. Electrochemical techniques can be used as alternative methods for the determination of rutin because they are simple, fast, and low-cost. Among these, square-wave voltammetry (SWV) was proved to be extremely sensitive for the detection of organic molecules, with low non-Faradaic current, high sensitivity and very low detection limit (Freitas et al., 2010; Freitas, 2010).

The sensitivity, selectivity and operational stability of electrochemical analysis can be enhanced using chemically modified electrodes (CMEs) (Pereira et al., 2002). CMEs have attracted a lot of attention due to the possibility of modifying the electrode surface including composite generation, polymer coating, and so forth. The modification of carbon paste or composite electrodes is an attractive research area in analytical chemistry with potential applications in electrodeanalysis and chemical sensors (Fatibello-Filho et al., 2000; Freitas et al., 2009; Pereira et al., 2002). The introduction of different compounds into the carbon paste or composite electrode has been demonstrated during in the analysis of numerous organic and inorganic compounds. In recent years, molecular recognition at the surface of solid materials has attracted interest of researchers who are trying to develop functional materials for chemical sensors. The use of nanoparticles, including carbon nanotubes, shows a trend toward improving the applicability (Liu et al., 2010; Yang et al., 2010).

Brett and co-workers studied the electro-oxidation of rutin (Ghica et al., 2005), quercetin (Brett et al., 2003), and catechin (Janeiro et al., 2004) using different voltammetric techniques, and revealed that the various hydroxyl groups of these compounds can be oxidized in different cyclic voltammetric peaks by adsorption-involved and pH-dependent transitions. The oxidation mechanisms are very complex due to the formation of multiple products (He et al., 2007; He, 2007). Volikakis and Efstathiou (2000) examined the possibility of determining twelve flavonoids using adsorptive stripping voltammetry in a flow injection system. In their study, carbon paste electrodes based on mixtures of Nujol-graphite and diphenylether-graphite were used. Zu et al. (2006) identified flavanols in leaves by HPLC with diode array detection (DAD).

In the present work, we describe a simple, fast and low-cost square-wave voltammetric method for the determination of rutin in pharmaceutical samples. A modified carbon composite electrode (MCCE) with copper (II) phosphate immobilized in a polyester resin (Cu$_x$(PO$_4$)$_2$-Poly) was used for this task.

## EXPERIMENTAL

### Apparatus

All voltammetric measurements were carried out in a single-compartment glass cell with a three electrodes system and degassing facilities for bubbling N$_2$. A modified carbon composite electrode (MCCE) with copper (II) phosphate immobilized in a polyester resin (Cu$_x$(PO$_4$)$_2$-Poly) was used as working electrode, Ag/AgCl (3 mol L$^{-1}$ KCl) as reference electrode, and platinum wire as auxiliary electrode. Square wave and cyclic voltammetric measurements were performed using AUTOLAB PGSTAT-30 (Ecochemie) potentiostat/galvanostat controlled with the GPES 4.0 software.

Physical characterization and electrode surface composition were investigated using a Philips model XL30 TMP scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) system. The equipment was calibrated with metallic nickel.

HPLC was used as the comparative method for the determination of rutin, which was performed using a LC-10AT Shimadzu system, with an UV/Vis detector (SPD-M10-AVP) set at the wavelength of 257 nm. A Shim-Pack CLC-ODS (60 μm x 150 mm, 5 μm) chromatographic column was used. The mobile phase consisted of a methanol/ acetonitrile/water (40/15/45, v/v/v) solution containing 1.0% v/v formic acid at a flow-rate of 1.0 mL min$^{-1}$, while the injection volume was 30 μL.

### Reagents and solutions

All solutions were prepared using ultra-purified water supplied by a Milli-Q system (Millipore®) with resistivity equal to or higher than 18 MΩcm. All chemicals used such as sodium phosphate, sodium nitrate, potassium nitrate, sodium chloride, potassium chloride, and phosphoric acid (Merck, Germany), were of analytical reagent grade. Methyleneylketone peroxide (MEK peroxide) was purchased from Iberc, Ramires and Cia (Taboão da Serra, SP) and, rutin, graphite powder (1-2 μm), and solid paraffin were purchased from Sigma-Aldrich. Polyester resin (Resapol T-208) was acquired from Reichhold (Mogi das Cruzes, SP). Phosphate buffer solutions (pH range between 4 and 9), sodium nitrate, potassium nitrate, sodium chloride, and potassium chloride were prepared for the study of the supporting electrolyte in several concentrations.

Rutin working standard solutions (9.9 × 10$^{-8}$ to 2.5 × 10$^{-4}$ mol L$^{-1}$) were freshly prepared by dilution of a 1.0 × 10$^{-4}$ mol L$^{-1}$ rutin stock solution in supporting electrolyte.
Immobilization of Cu₄(PO₄)₂ in polyester resin and preparation of the electrode

Cu₄(PO₄)₂(2S) was prepared by reacting CuCO₃, Cu(OH)₂ with concentrated phosphoric acid. The immobilization of Cu₄(PO₄)₂ was performed using a polyester resin and methylethylketone peroxide as a catalyst. Graphite powder (1-2 μm particle size; Aldrich) and solid paraffin (Aldrich), both of high purity, were used in the preparation of carbon composite.

The immobilization of Cu₄(PO₄)₂ in polyester resin (Cu₄(PO₄)₂-Poly) was achieved using a method similar to that proposed by Pereira and Fatibello-Filho (1998). Five grams of polyester resin were mixed with five grams of Cu₄(PO₄)₂(2S) and 0.3 mL of methylethylketone peroxide (catalyst). A rigid solid was obtained after three hours, which was initially broken with a hammer and then ground in a Tecnal multiuse mill, model TE 631/1 (Piracicaba, Brazil). Particles smaller than 100 μm were selected by sieving on a known mesh sieve.

The MCCE with (Cu₄(PO₄)₂-Poly) used as working electrode was prepared according to the building method proposed by Freitas and Fatibello-Filho (2010). In these studies, the graphite: solid paraffin compositions of 75:20, 70:20, 65:20, 60:20, 55:20, 50:20, 45:20, 40:20 and 20:20 (%) m/m, and the Cu₄(PO₄)₂-Poly percentages of 5, 10, 15, 20, 25, 30, 35, 40, and 60% m/m relating to the total mass of the carbon composite, were evaluated. The carbon composite electrode was initially prepared by mixing 0.40g of (Cu₄(PO₄)₂-Poly) (40%, m/m), 0.40g of graphite powder (40%, m/m), and 0.20 g of melted paraffin (20%, m/m) at 60 °C.

The resulting modified carbon composite was placed in a 1 mL plastic syringe and a copper wire was inserted to obtain external electric contact. A unmodified carbon composite electrode (UCCE) containing 80% (m/m) graphite and 20% (m/m) solid paraffin was prepared for the purpose of comparing with a modified electrode containing 40% (m/m) Cu₄(PO₄)₂-Poly.

Cyclic voltammetry and square wave measurements were performed in 0.1 mol L⁻¹ phosphate buffer solution containing 10% (v/v) ethanol (conditional pH of 6.9). Then, 100 μL aliquot of this solution was transferred to the electrochemical cell containing 5 mL of this supporting electrolyte.

An aliquot of 500 μL of liquid sample was transferred to a 10 mL volumetric flask and diluted with 0.1 mol L⁻¹ phosphate buffer solution containing 10% (v/v) ethanol (conditional pH of 6.9). Then, similarly, 100 μL aliquot of this solution was transferred to the electrochemical cell containing 5 mL of supporting electrolyte. The SW voltammograms were obtained after each aliquot addition.

Statistics

All experiments were carried out in triplicate. All data are presented as means ± SD. Student’s t-test at a 95% confidence level was used. P<0.05 was considered a significant result.

RESULTS AND DISCUSSION

Effect of electrode composition

The effect of the carbon composite composition on the voltammetric profiles of a 5.0 × 10⁻⁶ mol L⁻¹ rutin in 0.1 mol L⁻¹ phosphate buffer solution containing 10% (v/v) ethanol (conditional pH of 6.9) was initially evaluated.

The analytical signal (peak currents) increased with increasing amount of chemical modifier up to 40% (m/m). For Cu₄(PO₄)₂ amounts higher than 40% (m/m), the analytical signal decreased significantly (results not shown). This event occurred owing to a decrease in the graphite powder content in the composite and the consequent reduction of the conductive electrode area. The highest quality carbon composite structure was found for an electrode composition of 40% (m/m) Cu₄(PO₄)₂-polyester resin, 40% (m/m) graphite, and 20% (m/m) solid paraffin.

Electrode surface characterization

The modification of electrodes with functional groups, inorganic or biologic catalyst, polymeric films etc., has improved the performance of electrochemical electrode in analytical chemistry. These changes may increase the electrode characteristics such as selectivity, sensitivity and/or operational stability. In this sense, it is fundamental the surface characterization of the electrode surface.

Figure 1 shows the scanning electron micrographs (SEMs) of the modified electrode with Cu₄(PO₄)₂ before and after use of the electrode in one hundred of determinations rutin. The micrographs obtained in backscatter-mode...
(BSE) allowed the chemical contrast of the electrode surface; the clearest points indicate the chemical element of higher density, which can be related to copper. In this Figure, it can be seen that the electrode surface is uniform and presented little porous showing a very good homogenization of the components. Thus, this electrode can be classed as dispersion composite in accordance with Cespedes and co-workers (1996) due to conducting material is randomly distributed in the electrode surface. After using the electrode for rutin determination in 0.1 mol L\(^{-1}\) phosphate buffer solution (conditional pH 6.9) containing 10% (v/v) of ethanol, the electrode shows regularity of the surface, despite with lower amount of material (compare both images). The final profile, with regularity, can represent suitable operational stability.

Results obtained from EDX (results not shown) indicated that the composition percentage of \(\text{Cu}_3(\text{PO}_4)_2\) onto the surface of electrode was 38.2% (m/m), slightly lower than the 40% (m/m) used in the preparation of the carbon composite. Moreover, the oxygen content was kept constant even after high number of determinations, indicating that the oxide formation in the electrode surface was not favored, resulting in an extraordinary stability and extended lifetime of the electrode.

**Effect of supporting electrolyte**

The voltammetric behavior of the modified carbon composite electrode was investigated in five different supporting electrolytes (phosphate buffer, sodium nitrate, potassium nitrate, sodium chloride, and potassium chloride) in several concentrations by cyclic voltammetry (Figure 2). The presence of the voltammetric peak was observed in all these supporting electrolytes. Moreover, in phosphate buffer solution the anodic peak current was higher and the peak shape was better defined. Therefore, this buffer solution was selected for further experiments.

![FIGURE 1 - SEM micrographs of MCCE-Cu\(_3\)(PO\(_4\))\(_2\) surface (A) before and (B) after rutin voltammetric determination in 0.1 mol L\(^{-1}\) phosphate buffer/10% ethanol (v/v) (pH\(_{cond}\) 6.9); BSE detector; scale: 500 µm; zoom 60 times.](image1)

![FIGURE 2 – Effect of supporting electrolyte using MCCE-Cu\(_3\)(PO\(_4\))\(_2\) for 4.0 \(\times\) 10\(^{-5}\) mol L\(^{-1}\) rutin in (a) phosphate buffer; (b) potassium nitrate; (c) sodium nitrate; (d) sodium chloride and (e) potassium chloride solutions. Concentration of electrolyte solutions: 0.1 mol L\(^{-1}\)/10% ethanol (v/v) (pH\(_{cond}\) 6.9).](image2)

The catalytic action of copper has been reported to be the result of Cu(II)/Cu(I) at very high positive potential, or Cu(III)/Cu(II) redox system and a discussion of the potentialities of this redox system has been discussed by Freitas and Fatibello-Filho (2010). Furthermore, some studies have described the influence different factors, such as supporting electrolyte, concentration and pH of supporting electrolyte, number of donor atoms of the analyte, redox potential of the analyte and voltammetric
parameters, which affect the Cu(II)/Cu(I) couple and/or analytical signal (Domenech et al., 2000; Somasundrum et al., 1996; Vazquez et al., 1994).

In our study, the voltammetric response of the modified carbon composite electrode with \( \text{Cu}_3(\text{PO}_4)_2-\text{Poly} \) was not affected by the concentration of the supporting electrolyte. The 0.10 mol L\(^{-1}\) phosphate buffer solution was chosen to further experiments.

**Effect of pH**

The analytical response of MCCE with \( \text{Cu}_3(\text{PO}_4)_2-\text{Poly} \) (40% m/m) was studied over a wide pH range between 4 to 9 using phosphate buffer solution in the presence and absence of 4.0 \( \times 10^{-5} \) mol L\(^{-1}\) rutin (results not shown). The peak current increased with increasing pH from 4.0 to 7.0, but decreased at pH greater than 7.0, fact attributed to the formation of copper(II) hydroxide at the electrode surface (eq. 1).

\[
\text{Cu}_3(\text{PO}_4)_2(\text{es}) + 6 \text{OH}^- \rightarrow 3\text{Cu(OH)}_2(\text{es}) + 2 \text{PO}_4^{3-}
\]  

(1)

Since the best definition of peak and maximum analytical response were obtained at pH 6.9, this pH was chosen for optimization of other variables and for the analytical determination of rutin.

**Electrochemical characteristics**

Figure 3 shows the cyclic voltammograms obtained using an unmodified carbon composite electrode (UCCE) (A) and a modified carbon composite electrode with \( \text{Cu}_3(\text{PO}_4)_2-\text{Poly} \) (40% m/m) (MCCE) (B) in the absence and in the presence of 4.0 \( \times 10^{-5} \) mol L\(^{-1}\) rutin. It can be seen from these voltammograms that the current peak obtained with MCCE was much higher than that obtained using UCCE (20 times higher) with a decrease of over potential of about 150 mV (from 400 mV to 250 mV), indicating the existence of an electrocatalytic process. Moreover, the signal/noise was improved at the modified electrode and the possibility from interference of electroactive species present in the sample matrix of interest may be lower.

The MCCE- \( \text{Cu}_3(\text{PO}_4)_2 \) presented a reversible behavior for rutin, once presented a ratio of peak currents, \( \frac{I_{ap}}{I_{cp}} \) equal to one and a separation of peak potentials, \( E_{pa} - E_{pc} (\Delta E_p) \) equal to 56 mV, according to the accepted theory of cyclic voltammetric (Brett et al., 1993).

A linear plot of the peak current vs. the square root of scan rate was obtained, with a 0.999 correlation coefficient for both anodic and cathodic peak, indicating that the rutin oxidation and reduction process was controlled by diffusion (Figure 4) (Brett et al., 1993; Scholz, 2002).

The \( E_p \) vs pH study shows a linear plot with slope of 29.6 mV pH\(^{-1}\) (Figure 5 A) indicating that two electrons were involved in the oxidation process at electrode surface. It can also be calculated the electron number involved in the rutin oxidation applying the SWV theory (Krause, 1969; Mirceski et al., 2007; Osteryoung et al., 1985; Ramaley, 1969); which \( \Delta E_p \) vs \( \Delta \log f \) (logarithm of frequency) for reversible systems is linear in agreement with equation 2.

\[
\frac{\Delta E_p}{\Delta \log f} = -2.3 \frac{RT}{nF}
\]  

(2)

The slope obtained was 0.0145 V (from Figure 5B). When substituting this value in the equation 2, the num-
number of was obtained as 2. This result confirms the results obtained in the Ep vs pH study (Figure 5A).

Thus, considering the Ep vs pH study and the theory developed for the SWV (Krause, 1969; Mirceski et al., 2007; Osteryoung et al., 1985; Ramaley, 1969) for reversible systems, a plausible mechanism for the voltammetric response of the proposed electrode for rutin is postulated. This mechanism is based on two processes outlined in Figure 6. Initially, a chemical oxidation of the 3′,4′-dihydroxy substituent on the B-ring of rutin by Cu(II) at electrode surface with the formation of 3′,4′-diquinone and 2 H+(H3O+) and Cu(I) was observed, followed by an electrochemical process of oxidation of Cu(I) to Cu(II).

### Optimization of SWV parameters and analytical characteristics

The peak current obtained in square-wave voltammetry depends on various instrumental parameters such as the square-wave frequency (f), scan increment (ΔEs) and square-wave amplitude (a). These parameters are interrelated and have a combined influence on peak current response. The influence of these instrumental parameters on the peak current response of 4.0 × 10^-5 mol L^-1 rutin in 0.10 mol L^-1 phosphate buffer/10% ethanol (v/v) (conditional pH 6.9) was investigated. Table I shows the SWV parameters studied and the optimum values obtained for rutin determination. A baseline increase was observed when the frequency was increased. Thus, a f value of 50 s^-1 was selected for all determinations due to a stable baseline and higher voltammetric signal obtained.

Pulse amplitude (a) is another parameter that strongly influences the peak current in square-wave voltammetry. A better voltammetric definition of peak current was observed in a value of 40 mV, which was chosen for the subsequent analytical applications.

The effective rate of potential variation in square-wave voltammetry is the product between f and ΔEs. The latter parameter will also increase the signal and, therefore, the sensitivity of the technique (Mirceski et al., 2007; Osteryoung et al., 1985). However, for large values of ΔEs, a broadening of the peaks may occur, thus diminishing the resolution of the determinations. In this work, for the analytical applications, ΔEs was set at 1 mV.
The effect of several species, such as sodium saccharine, methylparaben, starch, calcium carbonate and magnesium stearate using the MCCE-\(\text{Cu}_3(\text{PO}_4)_2\) electrode in the presence of \(4.0 \times 10^{-6}\) mol L\(^{-1}\) rutin solution in the supporting electrolyte was evaluated in 1:1 and 10:1 concentration ratio (interference species: rutin).

 Addition-recovery experiments were also performed, and the results (Table II) show that average recoveries varied from 98.2 to 104%, demonstrating the accuracy of the proposed method. It is important to note that the concomitants present in this sample did not cause important matrix interferences for the samples analyzed by the proposed method.

 Under optimized conditions shown in Table I, the SW voltammograms for different rutin concentrations were obtained and are presented in Figure 7. As shown in the insert of this Figure, the analytical curve was linear in rutin concentration range from \(9.9 \times 10^{-8}\) to \(2.5 \times 10^{-6}\) mol L\(^{-1}\), with a detection limit (three times the standard deviation of signal blank/slope) of \(1.2 \times 10^{-7}\) mol L\(^{-1}\).

 The linear regression equation was: \((\Delta I_{pa}/A) = 7.36 \times 10^{-7} + 5.40 \text{[Rutin]}, r = 0.9994\), where \(\Delta I_{pa}\) is the analytical signal minus the background current and [Rutin] is rutin concentration in mol L\(^{-1}\).

 Table III shows the analytical characteristics such as work potential (mV), linear range of the analytical curve (mol L\(^{-1}\)) and the limit of detection (mol L\(^{-1}\)) of the electrode described in this work in relation to those electrodes reported in the literature (Franzoi et al., 2008; Santos et al., 2008; Sun et al., 2008; Chen et al., 2010; Yang et al., 2010; Freitas et al., 2009). As can be observed from this Table, the proposed electrode exhibited a low working potential relation to those reported in the literature, an analytical calibration curve for the low concentration range, detection limits lower than those reported in this Table.

 On a comparison with our previous studies for rutin determination in pharmaceutical formulations using a different electrode (carbon paste electrode modified with
cation-exchange resin) (Freitas et al., 2009), the present study offered some advantages, such as: the higher concentration of linear range, extended lifespan of 15 months (around 2,000 determinations), and simple building method (fewer stages of fabrication of electrode) what also imply less total cost of the method proposed.

The repeatability (n = 10) and reproducibility (five different days) were determined by using a standard solution of rutin, $4.0 \times 10^{-5}$ mol L$^{-1}$. Between each voltammetric measurement, the surface of the electrode was not renewed. The relative standard deviations were less than 1% and 2%, respectively.

**Analysis of pharmaceutical formulations**

Table IV shows the results obtained using a HPLC, comparative method for the determination of rutin in pharmaceutical formulations, the proposed voltammetric method and label value. The statistical calculations for the assay results showed good accuracy of the method according to the t-test ($t_{exp} = 2.234 < t_{label} = 2.571$). Thus, there are no significant differences between the results obtained by either procedure at the 95% confidence level, indicating

**TABLE III**

<table>
<thead>
<tr>
<th>Ep (mV)</th>
<th>Linear range (mol L$^{-1}$)</th>
<th>LD (mol L$^{-1}$)</th>
<th>LD solutions*</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>870</td>
<td>$3.9 \times 10^{-7}$ to $1.3 \times 10^{-5}$</td>
<td>$1.5 \times 10^{-7}$</td>
<td>0.1 mol L$^{-1}$ phosphate buffer (pH 6.0)</td>
<td>(Franzoi et al. 2008)</td>
</tr>
<tr>
<td>370</td>
<td>$5.0 \times 10^{-8}$ to $5.0 \times 10^{-7}$</td>
<td>$1.8 \times 10^{-8}$</td>
<td>Britton-Robinson buffer (pH 4.0)</td>
<td>(Santos et al. 2008)</td>
</tr>
<tr>
<td>448</td>
<td>$5.0 \times 10^{-8}$ to $5.0 \times 10^{-7}$</td>
<td>$3.6 \times 10^{-7}$</td>
<td>0.1 mol L$^{-1}$ PBS (pH 2.5)</td>
<td>(Sun et al. 2008)</td>
</tr>
<tr>
<td>440</td>
<td>$2.5 \times 10^{-7}$ to $1.0 \times 10^{-6}$</td>
<td>$1.0 \times 10^{-7}$</td>
<td>0.1 mol L$^{-1}$ PBS (pH 4.0)</td>
<td>(Chen et al. 2010)</td>
</tr>
<tr>
<td>487</td>
<td>$4.8 \times 10^{-8}$ to $9.6 \times 10^{-7}$</td>
<td>$3.2 \times 10^{-8}$</td>
<td>0.1 mol L$^{-1}$ phosphate buffer (pH 3.5)</td>
<td>(Yang et al. 2010)</td>
</tr>
<tr>
<td>-50</td>
<td>$9.9 \times 10^{-7}$ to $8.0 \times 10^{-6}$</td>
<td>$2.6 \times 10^{-8}$</td>
<td>0.10 KNO$_3$/10$^{-6}$ HNO$_3$ mol L$^{-1}$ (pH 6.0)</td>
<td>(Freitas et al. 2009)</td>
</tr>
<tr>
<td>190</td>
<td>$9.9 \times 10^{-8}$ to $2.5 \times 10^{-6}$</td>
<td>$1.2 \times 10^{-8}$</td>
<td>0.1 mol L$^{-1}$ phosphate buffer / 10% ethanol (v/v) (pH$_{cond}$ 6.9)</td>
<td>This work</td>
</tr>
</tbody>
</table>

Ep = work potential; LD = Limit of detection; Ref = Reference; LD solutions* = solutions where the limit of detection was estimated; PBS = phosphate buffer saline

**TABLE IV**

Determination of rutin in pharmaceutical formulations by voltammetric and HPLC methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rutin</th>
<th>Relative error %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Label value</td>
<td>HPLC</td>
</tr>
<tr>
<td>A</td>
<td>300</td>
<td>299 ± 2</td>
</tr>
<tr>
<td>B</td>
<td>20.0</td>
<td>19.9 ± 0.2</td>
</tr>
</tbody>
</table>

$n = 3$; confidence level of 95%; A = mg of rutin per tablet; B = mg of rutin per milliliter; $E_r$% = voltammetric method versus label value; $E_{r2}$% = voltammetric method versus HPLC method.
that the modified carbon composite electrode is suitable for the determination of rutin in pharmaceuticals.

CONCLUSIONS

The modified carbon composite electrode with Cu$_3$(PO$_4$)$_2$ immobilized in a polyester resin showed to be a very suitable method for the determination of rutin in real samples. The use of less positive electrode potential improved the applicability of the proposed method.

The proposed method is versatile, robust and environmentally-friendly since it requires the use of immobilized material. It is an interesting strategy for avoiding or minimizing environmental contamination. In view of the low-cost of the proposed method, it is feasible for large-scale analysis and/or for quality assurance of pharmaceutical formulations, drugs, food, and other samples.

The results obtained using the proposed MCCE-Cu$_3$(PO$_4$)$_2$ electrode for determination of rutin in pharmaceutical samples are in close agreement with those obtained using the HPLC method at a 95% of confidence level, showing its practical feasibility.

Moreover, the proposed method offers the advantages of reliability, simplicity of the fabrication of modified electrode, good precision, and low cost.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from Brazilian foundations (FAPESP, CAPES, CNPq and Mobility Academic Program-Teachers scale (AUGM).

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Received for publication on 13th January 2012
Accepted for publication on 20th July 2012