Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 973–977

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Rutin determination in pharmaceutical formulations using a carbon paste electrode modified with poly(vinylpyrrolidone)

Ana Cristina Franzoi, Almir Spinelli, Iolanda Cruz Vieira*

Departamento de Química, Universidade Federal de Santa Catarina, CEP 88040-970, Florianópolis-SC, Brazil

ARTICLE INFO

Article history:
Received 17 December 2007
Received in revised form 26 March 2008
Accepted 27 March 2008
Available online 4 April 2008

Keywords:
Carbon paste electrode
Poly(vinylpyrrolidone)
Rutin
Pharmaceutical formulations

ABSTRACT

A carbon paste electrode modified with poly(vinylpyrrolidone) (PVP) was developed for the determination of rutin. PVP enhances 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. The current responses in cyclic and linear sweep voltammetric experiments were investigated and an oxidation peak was observed at +0.87 V vs. Ag/AgCl. Several parameters were investigated to evaluate the performance of the modified electrode. The best analytical response was obtained employing (75:15:10%, w/w/w) graphite powder:Nujol:PVP, with an accumulation time of 10 min, scan rate of 100 mV s^{-1} and 0.1 M phosphate buffer solution (pH 6.0) as supporting electrolyte. The analytical curve was linear for rutin concentrations of 3.9×10^{-7} to 1.3×10^{-5} M ($r = 0.9991$) and the detection limit was 1.5×10^{-7} M. The recovery of rutin from pharmaceutical samples ranged from 98.3 to 101.7% and the relative standard deviation was 3.3% for a solution containing 7.7×10^{-6} M rutin ($n = 5$). This electrode was successfully applied to the determination of rutin in pharmaceutical formulations. The results obtained using the proposed modified carbon paste electrode and those obtained by the standard method are in agreement at the 95% confidence level.

1. Introduction

Flavonoids are benzo-γ-pyrone derivatives containing several hydroxyl groups attached to the C_6–C_3–C_6 ring and are extensively found in nature, in seeds, fruits and vegetables [1,2]. The flavonoid rutin, a natural flavone derivative, has been shown to act as a scavenger of various oxidizing species, such as superoxide anions, hydroxyl radicals and peroxyl radicals. This natural substance presents a wide spectrum of biochemical and pharmacological activities, including antioxidant, antiviral, antitumor, anti-inflammatory, and antiallergic, and is a stimulant of the immune system. The interest in using rutin in cosmetic and pharmaceutical formulations is to enhance their antioxidant and vasoprotective properties, promoting relief of the symptoms of lymphatic and venous insufficiency, and reducing capillary fragility [3,4].

Methods for the determination of rutin include spectrophotometry [5–7], capillary electrophoresis [8,9] and electrochemistry [4,10–21]. Of these methods, the latter is preferred over others because of its low detection limits, fast response time, low cost and simplicity. Oliveira and coworkers [16] have developed a biosensor based on gilo peroxidase immobilized on chitosan chemically crosslinked with epichlorohydrin for determination of rutin in pharmaceutical formulations. Square-wave voltammetric measurements showed that the bioelectrode exhibited a linear response for rutin concentrations from 3.4×10^{-7} to 7.2×10^{-6} M with a detection limit of 2.0×10^{-8} M. An electrochemical approach to the discrimination and determination of predominant flavonoids and phenolic acids using differential pulse voltammetry and a glassy carbon electrode has been reported [4]. The researchers proposed differential pulse voltammetry using a glassy carbon electrode for the analytical characterization of natural phenolic antioxidants in samples containing the phenolic classes cinnamic acids, flavan-3-ols, and flavonols. The relative standard deviation and detection limit were 3.4% and 1.0×10^{-7} M, respectively. Kang et al. [17] have reported the electrochemical determination of rutin in several samples of Chinese medicines using a glassy carbon electrode and an adsorption process was observed. In 0.1 M acetate buffer solution (pH 4.46), a very sensitive polarographic adsorptive wave was observed using differential pulse voltammetry at +0.29 V vs. Ag/AgCl. The electrode had a linear response for rutin concentrations from 3.28×10^{-7} to 3.28×10^{-6} M with a detection limit of 2.51×10^{-8} M. A biosensor using polyphenol oxidase as the bioelement for the detection of rutin has been investigated [18]. This biosensor was constructed by entrapment of the enzyme within a laponite clay film coated onto an electrode surface. The detection of the flavonoid was carried out at −0.1 V via the direct electrochemical reduction of the product of the enzymatic reac-

* Corresponding author. Tel.: +55 48 3721 6844; fax: +55 48 3721 6850.
E-mail address: iolanda@qmc.ufsc.br (I.C. Vieira).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.03.031

tion and the biosensor response was optimized with regard to pH, temperature, applied potential and clay film thickness. Also, an electrochemical procedure for the determination of rutin based on the accumulation of rutin-complex on the surface of a hanging mercury drop electrode has been evaluated [19]. Under the optimum conditions (i.e. pH 6.0, 6.0 ng ml^{-1} copper(II) concentration, accumulation potential of −0.30 V, scan rate of 0.40 V s^{-1} and accumulation time of 80 s), the concentration of rutin was linear in the range of 2.0–85.0 nM. The method was applied to the determination of rutin in tea and pharmaceutical samples with satisfactory results. An electrochemical sensor modified with a film of multiwalled carbon nanotubes/β-cyclodextrin (MWNTs/β-CD) has also been fabricated for the determination of rutin [20]. A linear calibration plot was obtained in the range of 4.0×10^{-7} to 1.0×10^{-3} M with a detection limit of 2.0×10^{-7} M. This electrochemical sensor showed excellent sensitivity, selectivity, stability and recovery for the determination of rutin in urine samples. Zeng et al. [21] have investigated the voltammetric behavior of rutin on a gold electrode modified with single-walled carbon tubes. Under the selected conditions (i.e. pH 5.0 and accumulation time of 210 s with an open circuit), the peak current was linear for rutin concentrations in the range of 2.0×10^{-8} to 5.0×10^{-6} M, with a detection limit of 1.0×10^{-8} M. The method was applied to the determination of rutin in drug tablets and flos sophorae buds and the electrode exhibited good stability and reproducibility.

The poly(vinylpyrrolidone) (PVP) polymer has a high molecular weight and is supplied as a dry, finely ground, white powder. Its remarkable ability to remove phenolic compounds from fruit juices and plant extracts is well documented [22,23]. The strong adsorption of phenolic compounds is attributed to hydrogen bonding between the imide group present in the polymer and hydroxyl groups in phenolic compounds. PVP has been used little in the construction of modified electrodes [24,25]. Nevertheless, rutin determination using a carbon paste electrode modified with this polymer is not found in the literature, because a biosensor [16], glass carbon electrode [4,17] and gold electrode [21,26] are preferred.

This paper describes the construction, performance and application of a PVP-modified carbon paste electrode (CPE) for the determination of rutin. The excellent properties of PVP effectively facilitate the adsorption of rutin on the surface of the modified electrode and improve sensitivity in the determination of this flavonoid in pharmaceutical products. The electrode process was investigated by cyclic and linear sweep voltammetry. The influence of different experimental parameters such as PVP percentage, supporting electrolyte, accumulation time and scan rate was investigated to optimize the proposed electrode, which was used for the determination of rutin in pharmaceutical formulations.

2. Experimental

2.1. Instruments, electrodes and chemicals

Cyclic and linear sweep voltammetry measurements were carried out using a PAR 263A potentiostat/galvanostat (EG&G Princeton Applied Research, Princeton, NJ, USA) interfaced with a microcomputer using the M250/270 software for data acquisition and analysis. A three-electrode cell, containing a PVP-modified CPE as the working electrode, an Ag/AgCl (3.0 M KCl) as the reference and a Pt wire as the counter electrode, was used for the measurements. All electrochemical experiments were carried out using 10.0 ml of supporting electrolyte at room temperature. The CPE was prepared using graphite powder purchased from Fisher and mineral oil purchased from Sigma. The polymer poly(vinylpyrrolidone) (linear, MM = 40,000 g mol^{-1}) and rutin were purchased from Sigma. A 2.0×10^{-4} M rutin stock solution was prepared in 30% (v/v) ethanol and 0.1 M phosphate buffer solution (pH 6.0). Sodium chloride, sucrose, lactose, magnesium stearate, poly(ethylene glycol) and starch were purchased from Sigma. All solutions were prepared with double distilled water and all reagents were of analytical grade. Three Brazilian pharmaceutical formulations containing rutin (A is the Manipulated rutin, B the Novarrutina tablet and C the Novarrutina liquid) were acquired from a local drugstore in Florianópolis (Santa Catarina, Brazil) and analyzed using the proposed PVP-modified CPE and a standard spectroscopic method. A Femto Model 434 UV–vis spectrophotometer with a quartz cell (optical path of 1.0 cm) was used as the comparative method.

2.2. Preparation of PVP-modified CPE

The PVP-modified CPE was prepared by mixing 20 mg of PVP (10%, w/w) and 150 mg of graphite powder (75%, w/w) in a mortar for 20 min to ensure the uniform dispersion of the polymer. Subsequently, 30 mg of Nujol (15%, w/w) were added and mixed for at least 20 min to produce the final paste. This PVP-modified carbon paste was packed in a 1.0 ml plastic syringe and a copper wire was inserted to obtain the external electric contact [27–29]. Prior to measurement, the electrode surface was polished manually to obtain a fresh surface. For comparison, a bare CPE was also constructed.

2.3. Electrochemical measurements

Cyclic and linear sweep voltammetry measurements were scanned in an unstirred, non de-aerated 0.1 M phosphate buffer solution (pH 6.0) at 25.0 ± 0.5 °C and all potentials were measured and reported vs. Ag/AgCl (3.0 M KCl). In a typical run, 10 ml of the supporting electrolyte was transferred to a clean, dry cell and the required volume of the rutin or sample solutions was added by micropipette. After an accumulation time of 10 min and with an open circuit potential, the voltammograms were recorded between +0.5 and +1.2 V at 100 mV s^{-1} after successive additions of rutin.

2.4. Preparation of pharmaceutical samples and determination of the rutin

The content of one tablet (A or B) was dissolved in 30% (v/v) ethanol and added to 0.1 M phosphate buffer solution at pH 6.0. For the liquid sample of rutin (C), 1.0 ml of the pharmaceutical sample was added to ethanol/0.1 M phosphate buffer solution at pH 6.0 (30/70%, v/v) to give 50.0 ml of a solution. Aliquots of pharmaceutical samples were transferred to the cell and quantified after successive additions of rutin standard stock solution. After each addition, linear voltammograms were recorded from +0.5 to +1.2 V at 100 mV s^{-1} scan rate.

A spectrophotometric method for rutin determination available in the Official Methods of Analysis [30] was used to compare the analytical results obtained with the proposed electrode.

3. Results and discussion

3.1. Voltammetric behavior of rutin in PVP-modified CPE

The poly(vinylpyrrolidone) polymer has an interesting property; it facilitates a strong adsorption of phenolic compounds. This is ascribed to the formation of a hydrogen bond between the hydroxyl groups of the phenolic compound and imide group present in the PVP backbone. Fig. 1 shows a schematic representation of the rutin oxidation to the corresponding *o*-quinone on the surface of the PVP-modified carbon paste electrode proposed in this study. Different researchers have studied the mechanism of electrochemical oxidation of rutin [21,31,32] and showed that the oxidation occurs at the

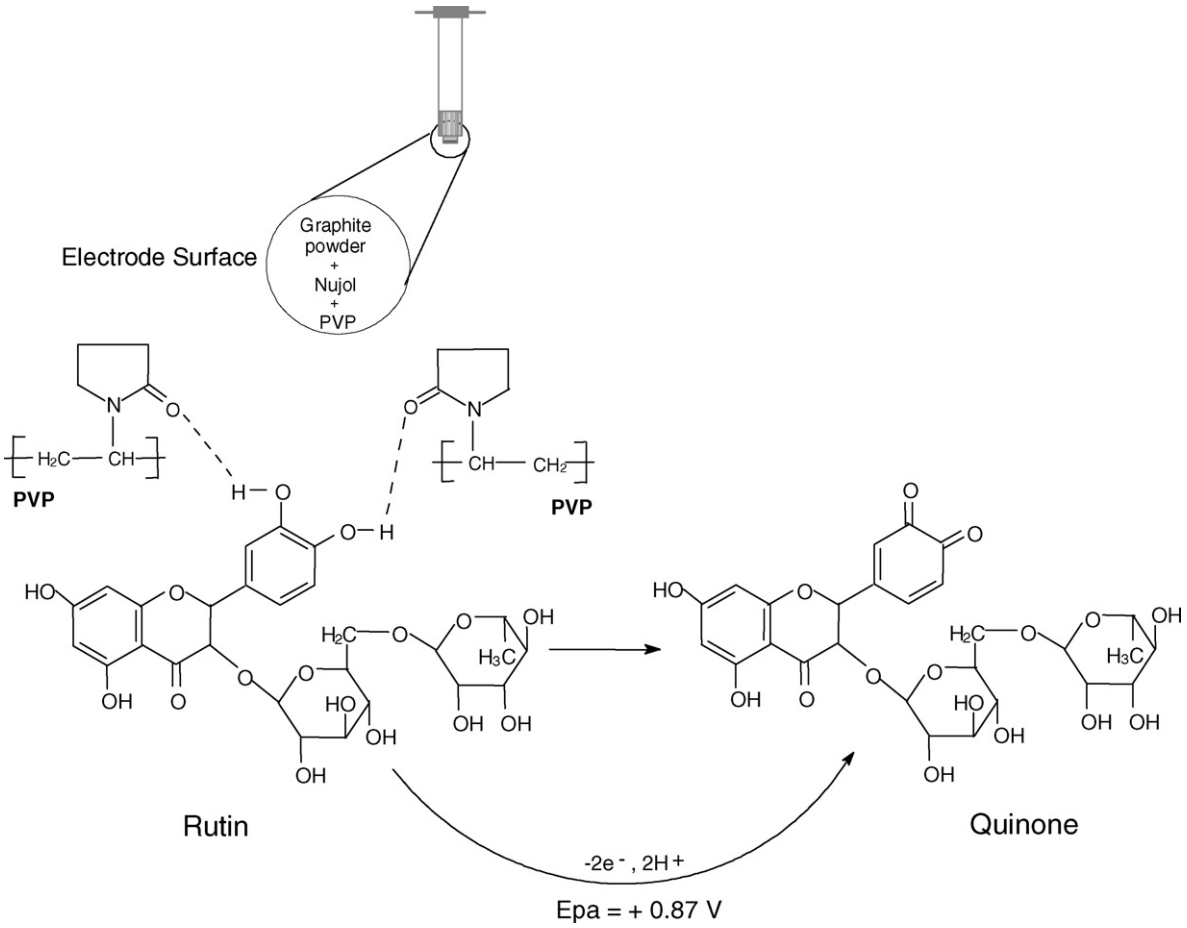

Fig. 1. Schematic representation of rutin oxidation to the corresponding *o*-quinone on the PVP-modified CPE surface.

OH groups, as indicated in Fig. 1, resulting in the formation of the corresponding *o*-quinone.

Preliminary electrochemical measurements were carried out in order to identify the general behavior of rutin in the presence of the PVP-modified CPE. The successive cyclic voltammograms using 7.7×10^{-6} M rutin in 0.1 M phosphate buffer solution (pH 6.0) were recorded between +0.5 and +1.2 V using a scan rate of 100 mV s^{-1}. Fig. 2 shows a well-defined oxidation peak at +0.87 V in the first cycle and no peak was observed in the reverse scan. This behavior suggests that the rutin oxidation is an irreversible process. In addition, the following cycles showed a decreasing current of the oxidation peak due to the fact that the electrode surface was blocked by strong adsorption of the reaction products.

Fig. 3 shows the linear sweep voltammograms obtained with the bare CPE (a and b) and the PVP-modified CPE (c and d) in the potential range of +0.5 to +1.2 V in 0.1 M phosphate buffer solution (pH 6.0) without (Fig. 3a and c) and with (Fig. 3b and d) 7.7×10^{-6} M rutin solution. As can be observed, no peak was

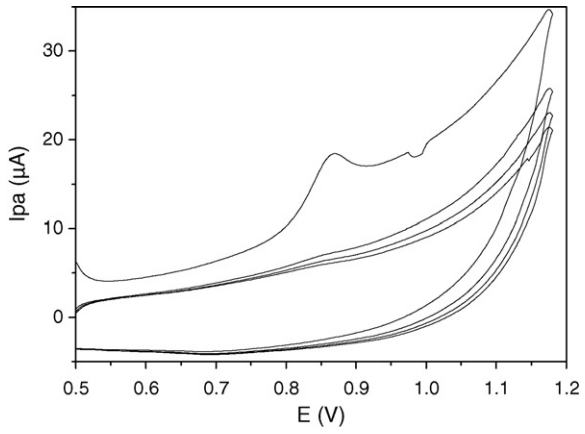

Fig. 2. Successive cyclic voltammograms of rutin at the PVP-modified CPE. Conditions: 7.7×10^{-6} M rutin in 0.1 M phosphate buffer solution (pH 6.0); accumulation time 10 min and scan rate 100 mV s^{-1}.

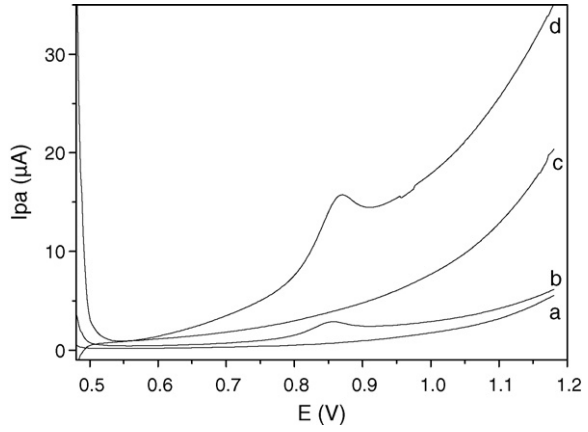

Fig. 3. Linear sweep voltammetric responses of bare CPE (a and b) and PVP-modified CPE (c and d) in the absence of rutin (a and c) and in the presence of 7.7×10^{-6} M rutin (b and d) in 0.1 M phosphate buffer solution. Accumulation time 10 min and scan rate 100 mV s^{-1}.

obtained in the absence of rutin. On other hand, in the presence of the flavonoid compound a well-defined oxidation peak was observed at +0.85 and +0.87 V for the bare CPE and PVP-modified CPE, respectively. When the modified CPE was used, an increase in electrode response and a large difference in the values of the anodic current were observed. The fact that the behaviors of the bare and PVP-modified CPEs are easily distinguishable shows the better efficiency of the latter in terms of rutin oxidation. The well-resolved oxidation peak was used as the analytical response for rutin determination in pharmaceutical products.

3.2. Optimization of measurement parameters

To optimize the response of the proposed PVP-modified CPE, various experimental parameters were investigated, including the PVP percentage (5.0–20.0%, w/w), supporting electrolyte (phosphate buffer, acetate buffer and potassium chloride solution), accumulation time (1–15 min) and scan rate (25.0–100.0 mV s^{-1}).

In the present investigation, the composition of 75:15% (w/w) graphite powder:Nujol was used in the construction of the carbon paste electrode modified by the PVP polymer. As previously mentioned, the PVP polymer facilitates the adsorption of rutin on the electrode surface. Therefore, the effect of the PVP composition, which varied from 5 to 20% (w/w), on the response of the proposed electrode was studied. The analytical signal (anodic peak current) for 7.7×10^{-6} M rutin in 0.1 mol l^{-1} phosphate buffer solution increased with increasing amount of PVP in the carbon paste in the range 5–10% (w/w). For higher PVP content (15 and 20%), a decrease in the value of the anodic current was observed. Thus, the most suitable amount of PVP in CPE was 10%.

The effect of the different supporting electrolytes and pH, such as 0.1 M phosphate buffer solution (pH 6.0–8.0), 0.1 M acetate buffer solution (pH 4.0–5.5) and 0.1 M KCl, on the electrode response to a 7.7×10^{-6} M rutin solution was investigated. The best voltammetric responses were obtained in 0.1 M phosphate buffer solution at pH 6.0. Thus, this supporting electrolyte was selected for further experiments.

The accumulation time directly influences the current responses in the presence of rutin oxidation. Thus, the effect of the accumulation time, varying from 1.0 to 15.0 min, for the proposed electrode response was investigated for 7.7×10^{-6} M of rutin in 0.1 M phosphate buffer solution at pH 6.0, as shown in Fig. 4. As can be observed, the peak current increased significantly up to 10 min of accumulation time and subsequently decreased. The current peak increased with the accumulation time because more rutin was adsorbed on the electrode surface. On the other hand, the decrease in the current peak after 10 min can be explained by the saturation of the electrode surface. Thus, the accumulation time of 10 min was the best condition for quantitative detection of rutin and was selected for further experiments.

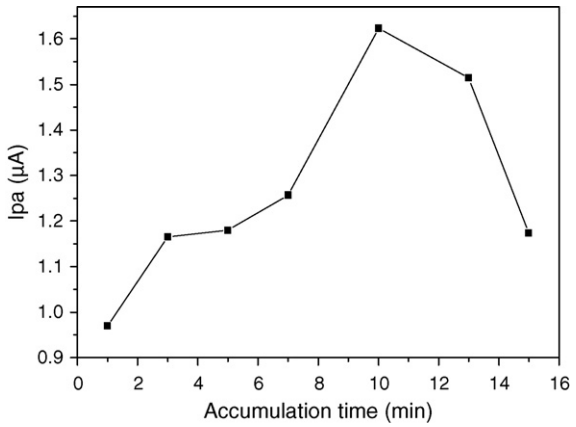

Fig. 4. Effect of PVP percentage (5–20%, w/w) on the carbon paste electrode response in the presence of 7.7×10^{-6} M rutin in 0.1 M phosphate buffer solution. Accumulation time 10 min and scan rate 100 mV s^{-1}.

Electrochemical responses are scan rate dependent. Therefore, the effect of scan rate on current peak in the presence of rutin oxidation was investigated in the range of 25.0–100.0 mV s^{-1} by cyclic voltammetry. The analytical response using 7.7×10^{-6} M rutin in 0.1 M phosphate buffer solution at pH 6.0 increased with scan rate. An angular coefficient close to 1.0, characteristic of an adsorption-controlled process, was obtained.

3.3. Linear sweep voltammograms (LSV) and analytical curve

After establishing the optimal conditions for rutin detection, the analytical curve was constructed using LSV. Fig. 5 shows the linear sweep voltammograms for the PVP-modified CPE using different concentrations of rutin. As can be seen, the anodic current increased with rutin concentration. The inset of Fig. 5 shows the corresponding analytical curve, demonstrating that in the range of 3.9×10^{-7} to 1.3×10^{-5} M, the anodic peak current has a good linear relationship with rutin concentration, with the linear regression equation $I_{pa} = 0.3143 + 0.5867 \times 10^{6}$ [rutin]; $r = 0.9991$, where I_{pa} is the anodic peak current in μA and [rutin] is the rutin concentration in M. From the analytical curve detection limit (three times the signal blank/slope) and quantification limit (10 times the signal blank/slope) were calculated. The values obtained were 1.5×10^{-7} and 5.2×10^{-7} M, respectively.

3.4. Repeatability, reproducibility and interference study

The repeatability of the PVP-modified CPE was evaluated measuring the anodic current at the oxidation potential five times using the same electrode refreshed after each measurement. The experiments were carried out in 7.7×10^{-6} M rutin in phosphate buffer solution (0.1 M; pH 6.0). The relative standard deviation was 3.3%, indicating that the results obtained with the proposed modified electrode have a good repeatability. Reproducibility was investigated considering three modified electrodes prepared independently. An acceptable reproducibility with a relative standard deviation of 4.6% was obtained for measurements carried out in a 7.7×10^{-6} M rutin solution.

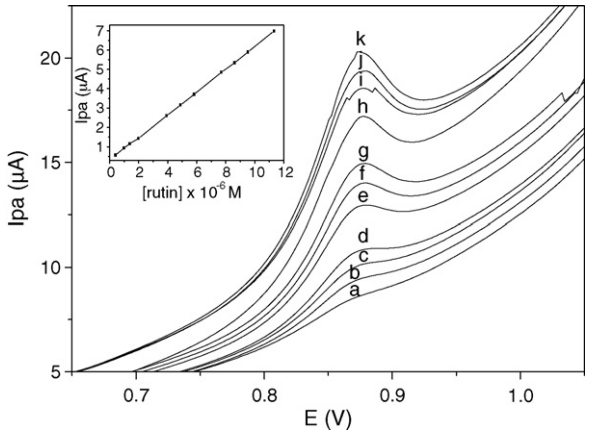

Fig. 5. Linear sweep voltammograms obtained using the proposed electrode in 0.1 mol l^{-1} phosphate buffer solution and rutin solutions at the following concentrations: (a) 3.9×10^{-7}; (b) 9.9×10^{-7}; (c) 1.4×10^{-6}; (d) 2.0×10^{-6}; (e) 3.9×10^{-6}; (f) 4.9×10^{-6}; (g) 5.8×10^{-6}; (h) 7.7×10^{-6}; (i) 8.6×10^{-6}; (j) 9.5×10^{-6}; (k) 1.3×10^{-5} M. Accumulation time 10 min and scan rate 100 mV s^{-1}. Inset: the corresponding calibration curve.

Table 1
Recovery results for rutin standard solution in pharmaceutical formulations using the PVP-modified CPE

Sample	Rutin (mg l^{-1})		Recovery (%)
	Added	Found	
A	0.61	0.6 ± 0.1	98.4
	1.21	1.2 ± 0.2	99.2
	1.81	1.8 ± 0.1	99.4
	2.39	2.4 ± 0.1	100.0
B	0.60	0.6 ± 0.1	101.7
	1.21	1.2 ± 0.2	98.3
	1.81	1.8 ± 0.2	99.4
	2.39	2.4 ± 0.1	98.3
C	0.61	0.6 ± 0.1	98.4
	1.21	1.2 ± 0.1	100.0
	1.81	1.8 ± 0.1	99.4
	2.39	2.4 ± 0.2	99.6

Table 2
Determination of rutin in pharmaceutical formulations using the standard method and PVP-modified CPE

Sample	Stated value	Standard method	PVP/CPE	Relative error (%)	
				R_{e1}	R_{e2}
A[a]	20.0	19.8 ± 0.1	20.9 ± 0.1	+4.3	+5.3
B[a]	20.0	20.8 ± 0.2	21.1 ± 0.1	+5.2	+1.4
C[b]	20.0	20.4 ± 0.1	21.3 ± 0.2	+6.1	+4.2

n = 1, confidence level of 95%. R_{e1} = PVP/CPE vs. stated value; R_{e2} = PVP/CPE vs. standard method value.
[a] mg tablet^{-1}.
[b] mg ml^{-1}.

In order to evaluate the effect of the presence of excipients commonly found in pharmaceutical formulations on the determination of rutin, sodium chloride, sucrose, lactose, magnesium stearate, poly(ethylene glycol) and starch were investigated as potential sources of interference. Ratios of rutin concentration to excipient substances were fixed at 0.1, 1.0 and 10.0. None of these tested substances interfered in the rutin determination with the proposed procedure, that is, the modified electrode was able to determine the amount of rutin in the presence of the potential interferences.

3.5. Recovery and determination of the rutin in pharmaceutical formulations

A recovery study was carried out by adding different amounts of rutin (0.61–2.39 mg l^{-1}) to three samples of pharmaceutical formulations (A–C). The percentage recovery values were calculated by comparing the concentration obtained from the samples with and without the addition of known concentrations of the rutin standard solution. The recoveries of 98.3–101.7% obtained for these samples are shown in Table 1. It can be clearly observed that the recovery results obtained suggest an absence of matrix effects in these determinations.

In order to evaluate the applicability of the developed electrode, the rutin content (mg tablet^{-1} and mg ml^{-1}) in pharmaceutical formulations was determined. The results of the analysis using the PVP-modified CPE are presented in Table 2 and show the comparison with those obtained using the Official Methods of Analysis [30] and values stated by the manufacturers. According to the Student's *t*-test at a 95% confidence level, there was no significant difference between the results obtained with the two methods and with the label values. It can thus be concluded that the proposed method is suitable for rutin determination in commercial pharmaceutical samples. In addition, comparing the proposed method using the PVP-modified CPE with other electrochemical methods described in the literature [4,10–21,26] for rutin determination, the former also combines selectivity with sensitivity, low detection limit and linear dynamic range. High stability, long lifetime, ease of use and quick construction are also advantageous features of the proposed PVP-modified CPE.

4. Conclusions

In this study, a simple and sensitive electroanalytical method using a PVP-modified CPE for rutin determination was developed. This method was based on higher current responses, in the presence of rutin oxidation, of the built electrode compared to the low response obtained with a bare CPE. The PVP on the electrode surface effectively facilitated the adsorption of rutin and induced the enhancement of the anodic current peak. Under optimized conditions, the modified carbon paste electrode exhibited a good performance in terms of sensitivity, detection limit, response and linear calibration range. Moreover, the PVP-modified CPE offers some advantages including low cost, facility of construction and renewal of the surface, and also it demonstrated effectiveness in rutin determination in the selected pharmaceutical samples.

Acknowledgments

Financial support from CNPq (Processes 472169/2004-1 and 472541/2006-4), MCT/CNPq/PADCT, FAPESC and also the scholarship granted by CNPq to ACF are gratefully acknowledged.

References

[1] G. Di Carlo, N. Mascolo, A.A. Izzo, F. Capasso, Life Sci. 65 (1999) 337–353.
[2] J.B. Harbone, C.A. Williams, Phytochemistry 55 (2000) 481–504.
[3] M.L. Calabrò, S. Tommasini, P. Donato, R. Stancanelli, D. Raneri, S. Catania, C. Costa, V. Vilari, P. Ficarra, R. Ficarra, J. Pharm. Biomed. Anal. 36 (2005) 1019–1027.
[4] A.J. Blasco, M.C. Gonzalez, A. Escarpa, Anal. Chim. Acta 511 (2004) 71–81.
[5] H.N.A. Hassan, B.N. Barsoum, I.H.I. Habib, J. Pharm. Biomed. Anal. 20 (1999) 315–320.
[6] K. Lavanya, T.R. Baggi, Microchem. J. 41 (1990) 126–131.
[7] M. Sakamoto, K. Takamura, Microchem. J. 23 (1978) 374–383.
[8] Y. Sun, T. Guo, Y. Sui, F.M. Li, J. Sep. Sci. 26 (2003) 1203–1206.
[9] G. Chen, H.W. Zhang, J.N. Ye, Anal. Chim. Acta 423 (2000) 69–76.
[10] A. Abbaspour, M.A. Mehrgardi, Anal. Chem. 76 (2004) 5690–5696.
[11] P. Tomcik, C.E. Banks, T.J. Davies, R.G. Compton, Anal. Chem. 76 (2004) 161–165.
[12] H.T. Liu, P. He, Z.Y. Li, C.Y. Shi, G.Y. Zhu, J.H. Li, Electrochem. Commun. 7 (2005) 1357–1363.
[13] L. Gorton, Electroanalysis 7 (1995) 23–45.
[14] I. Svancara, K. Vytras, J. Barek, J. Zima, Crit. Rev. Anal. Chem. 31 (2001) 311–345.
[15] K. Kalcher, J.-M. Kauffmann, J. Wang, I. Svancara, K. Vytras, C. Neuhold, Z. Yang, Electroanalysis 7 (1995) 5–22.
[16] I.R.W.Z. Oliveira, S.C. Fernandes, I.C. Vieira, J. Pharm. Biomed. Anal. 41 (2006) 366–372.
[17] J. Kang, X. Lu, H. Zeng, H. Liu, B. Lu, Anal. Lett. 35 (2002) 677–686.
[18] C. Mousty, S. Cosnier, M.S.-P. Lopez, E. Lopez-Cabarcos, B. Lopez-Ruiz, Electroanalysis 19 (2007) 253–258.
[19] A.A. Ensafi, R. Hajian, Electroanalysis 18 (2006) 579–585.
[20] J.-L. He, Y. Yang, X. Yang, Y.-L. Liu, Z.-H. Liu, G.-L. Shen, R.-Q. Yu, Sens. Actuators B 114 (2006) 94–100.
[21] B. Zeng, S. Wei, F. Xiao, F. Zhao, Sens. Actuators B 115 (2006) 240–246.
[22] Z. Borneman, V. Gökmen, H.H. Nijhuis, Sep. Purif. Technol. 22–23 (2001) 53–61.
[23] W.D. Loomis, J. Battaile, Phytochemistry 5 (1966) 423–438.
[24] X. Yang, F. Wang, S. Hu, Colloids Surf. B: Biointerfaces 52 (2006) 8–13.
[25] Q. He, X. Dang, C. Hu, S. Hu, Colloids Surf. B: Biointerfaces 35 (2004) 93–98.
[26] X. Yang, H. Zhang, Food Chem. 102 (2007) 1223–1227.
[27] S.C. Fernandes, I.R.W.Z. Oliveira, I.C. Vieira, Enzyme Microbiol. Technol. 40 (2007) 661–668.
[28] I.R.W.Z. Oliveira, I.C. Vieira, Enzyme Microbiol. Technol. 38 (2006) 449–459.
[29] I.R.W.Z. Oliveira, R.E-H.M.B. Osório, A. Neves, Sens. Actuators B 122 (2007) 89–94.
[30] Official Methods of Analysis of AOAC International, 16th ed., 1995, p. 29.
[31] G.-P. Jin, Q.-Z. Chen, Y.-F. Ding, J.-B. He, Electrochim. Acta 52 (2007) 2535–2541.
[32] A.K. Timbola, C.D. Souza, C. Soldi, M.G. Pizzolatti, A. Spinelli, J. Appl. Electrochem. 37 (2007) 617–624.