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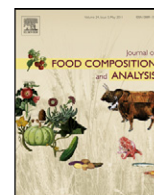

Original Article

Cathodic adsorptive stripping voltammetric determination of rutin in soybean cultivars

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ABSTRACT

A highly sensitive and selective cathodic adsorptive stripping voltammetric method for determination of rutin is presented. The method relies on the accumulation of a Cu(II)–rutin complex at a hanging mercury drop electrode (HMDE), followed by its reduction during a differential pulse voltammetric scan. The electrochemical behavior of the Cu(II)–rutin complex at HMDE was investigated by cyclic voltammetry. Results show that the electrode process is adsorption-controlled and gradually becomes less reversible at high scan rates where peak separation grows. Under the optimized conditions (phosphate buffer pH 6, -1.000 V accumulation potential, 180 s accumulation time, 70 mV pulse amplitude, 50 mV s^{-1} scan rate and 1.6×10^{-6} M Cu(II) concentration), the reduction peak current (I_{pc}) of the Cu(II)–rutin complex is linear (I_{pc} (nA) = 10.070 + 1.9 $\times 10^{8}$ [Rutina]) to rutin concentration in the range from 2.0×10^{-7} to 1.4×10^{-6} M, with a correlation coefficient of 0.999. The detection and quantification limits obtained were 7.0×10^{-9} M and 2.2×10^{-8} M, respectively. The method was successfully applied to the determination of rutin in soybean cultivars, with recoveries of 94–105%.

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1. Introduction

The use of soybeans as food has increased due to the importance of its nutritional properties and beneficial effects on human health (Ribeiro et al., 2007; Kumar et al., 2006). Among these effects, soybeans use can reduce the risk of the major killer diseases, such as breast cancer, cardiovascular disease, osteoporosis, diabetes, and has a role in alleviating menopausal symptoms (Kumar et al., 2006). Several authors have considered flavonoids to be responsible for the beneficial effects of soybeans (Ribeiro et al., 2007; Kumar et al., 2006; Hutabarat et al., 2001; Umphress et al., 2005). The flavonoid constituents of soybeans are responsible for major bioactivities, such as antiphlogistic, antioxidatic, antiallergic and diuretic properties.

Flavonoids are a large family of over 4000 ubiquitous secondary plant metabolites, comprising seven subclasses, anthocyanins (e.g. europinidin), flavones (e.g. apigenin), flavonols (e.g. quercetin), flavanonols (e.g. taxifolin), isoflavones (e.g. genistein), flavanones (e.g. naringenin) and flavonol glycosides (e.g. rutin). Flavonoids are known to possess strong antioxidant properties, which can be due to both their radical-scavenging effects and metal-chelating properties, of which the former may dominate. Owing to the antioxidant properties, they can prevent deoxyribonucleic acid (DNA) and cells from oxidative damage, and thus possess a wide range of pharmacological activities such as antitumor, anti-inflammatory and antiaging (Ündeğer et al., 2004).

Rutin (Scheme 1), one of the most abundant flavonoids in the human diet, also known as vitamin P, has been shown to act as a scavenger of various oxidizing species, i.e. hydroxyl and peroxyl radicals. As a result of these biological effects, its pharmacological activities have been widely explored including antibacterial, anti-inflammatory, antitumor, antiallergic, antiviral and antiprotozoal properties (Hasumura et al., 2004; Blasco et al., 2004).

The determination of rutin in different matrices (herbs, pharmaceutical preparations, fruits and cereals) may be performed using various analytical methods, such as high performance liquid chromatography with various detectors (Danila et al., 2007; Kurzawa, 2010; Lu et al., 2006; Novak et al., 2008), spectrophotometric (Kreft et al., 2002; Kurzawa, 2010), capillary electrophoretic (Chen et al., 2001) and electrochemical (Zoulis and Efstahiuo, 1996). The detection limits achieved by these methods ranged from 1.5×10^{-13} M to 1.9×10^{-6} M.

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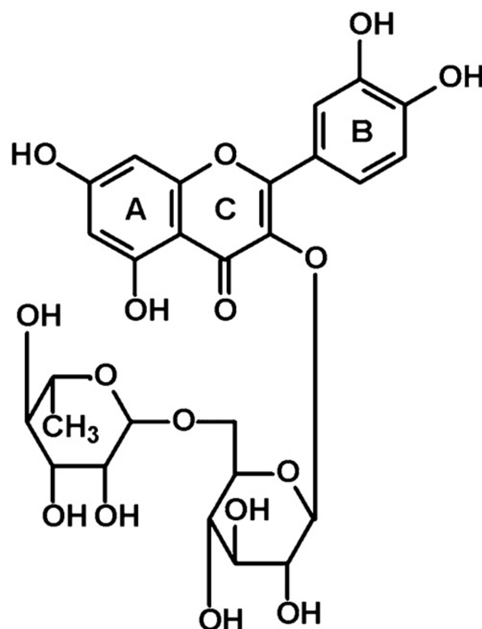


Scheme 1. The chemical structure of rutin.

Since most flavonoids are electrochemically active at moderate oxidation potentials, electrochemical methods of analysis are preferable due to the advantages of high sensitivity, simplicity, good stability, inexpensive instrumentation and less interference from non-electroactive substances. Many publications concerning this topic can be found in the literature. A carbon composite electrode modified with Cu(II)–resin was used in the voltammetric determination of rutin in Brazilian pharmaceutical formulations (Freitas et al., 2009). Rutin has also been determined in pharmaceutical samples by electrochemical methods based on many modified glassy carbon electrodes. Chen et al. (2010) prepared a poly(*p*-aminobenzene sulfonic acid) glassy carbon modified electrode to determine rutin with no interference from the coexisting ascorbic acid. A gold nanoparticle/ethylenediamine/carbon nanotube modified glassy carbon electrode and anodic stripping voltammetry were successfully used in the determination of rutin in the presence of ascorbic acid (Yang et al., 2010). Santos et al. (2007) demonstrated that the determination of rutin by square wave voltammetry at a poly glutamic acid modified glassy carbon electrode could be done with good sensitivity. Acetylene black nanoparticles were used to modify the surface of a glassy carbon electrode and this electrochemical sensor was successfully employed for rapid determination of rutin (Song et al., 2010). Du et al. (2010) designed a graphene nanosheets modified glassy carbon electrode to determine rutin with satisfied recovery. A voltammetric procedure based on a lead film modified glassy carbon electrode was applied to rutin determination without any matrix effects (Tyszczuk, 2009). Ionic liquid modified electrodes have also been employed to investigate the electrochemical behavior of rutin (Liu et al., 2010) as well as in the determination of rutin in pharmaceutical and biological samples (Zhang and Zheng, 2008; Zhan et al., 2010; Sun et al., 2008; Wang et al., 2010). Wu et al. (2008) developed an electrochemical method based on adsorptive stripping analysis at single-sided heated graphite cylindrical electrodes with direct current heating to determine nanomolar concentrations of rutin. The detection limits achieved by these electrochemical methods ranged from 2.5×10^{-10} M to 3.6×10^{-7} M.

However, the modified electrodes in many cases are expensive, difficult and tedious to be prepared and have low stability. In comparison to solid electrodes, mercury electrodes offer a clean electrode surface and more favorable electrode kinetics in many redox couples. Rutin is known to complex with various metal cations to form stable compounds, which have demonstrable anti-inflammatory and anti-cancer activities. In this context, some studies have shown the interaction between rutin and Cu(II) (Le Nest et al., 2004a,b). Esparza et al. (2005) reported a 1:1 stoichiometry ratio for the complex formed between copper and rutin.

In recent years, the Cu(II)–rutin complex has been used for rutin determination by cathodic adsorptive stripping voltammetry (CAdSV) with a hanging mercury drop electrode (HMDE) (Temerk et al., 2006; Ensafi and Hajian, 2006). This methodology, which involves the adsorption of the Cu(II)–rutin complex on a HMDE surface and its reduction, has been employed in the quantification of rutin in pharmaceutical formulations, human urine, blood serum and tea (Temerk et al., 2006; Ensafi and Hajian, 2006) with detection limits ranging from 0.5×10^{-9} M to 4.9×10^{-9} M. The CAdSV at HMDE has also been used in naringin and hesperidin determinations in fruit juices and pharmaceutical preparations (Reichart and Obendorf, 1998; Obendorf and Reichart, 1995).

However, to the best of our knowledge, this methodology has not yet been applied for rutin determination in soybean samples. This work describes a highly sensitive and selective CAdSV method for the determination of rutin in soybean cultivars. Controlled adsorptive accumulation of the rutin–Cu(II) complex on the HMDE provides the basis for the direct stripping measurement of rutin. Thus, different parameters were evaluated and optimized for its quantification. Compared to other analytical techniques, the proposed method represents good selectivity, high sensitivity, rapid responses, low-cost instrumentation and reduced sample size for the determination of rutin. Moreover, the study presents additional data on the interfering effect of other phenolic compounds to the measured analytical signal.

2. Materials and methods

2.1. Apparatus

CAdSV and cyclic voltammetric (CV) measurements were carried out on a 797 Voltammetric Analyzer (VA) Computrace (Metrohm, Switzerland) with an electrochemical cell composed of a HMDE as working electrode, Ag/AgCl (3 M KCl) electrode as reference and a platinum wire as auxiliary electrode. CAdSV measurements were performed in the potential range of 0.100 V (initial potential, E_i) to -1.100 V (final potential, E_f) at the following settings: $E_{acc} = -1.000$ V accumulation potential, $t_{acc} = 180$ s accumulation time, $t_e = 10$ s equilibration time, 0.3 mm^2 surface area of the mercury drop, 70 mV pulse amplitude and $\nu = 50$ mV s^{-1} scan rate. CV measurements were performed at the following settings: $E_i = 0.200$ V, $E_w = -1.500$ V switching potential, $\nu = 10$–600 mV s^{-1}. The hydrogen-ion potential (pH) of the solutions was determined using a 3030 pH-meter (Jenway, United Kingdom) with a DME-CV1 combination pH electrode (Digimed, Brazil). Ultraviolet (UV) Spectrophotometric measurements at 352.5 nm were carried out on a UV mini-1240 spectrophotometer (Shimadzu, Japan) with 1 cm quartz cuvettes.

2.2. Chemicals and samples

Analytical-reagent grade chemicals and ultrapure water (Millipore, United States) were used to prepare all solutions. Rutin hydrate (95% purity) was purchased from Sigma–Aldrich (Brazil) and used without further purification. Cooper stock standard 1000 mg L^{-1} (>99.9% purity) solution was purchased from Fluka (Switzerland). Nitric acid and ethanol were purchased from

Quimex (Brazil) and Vetec Química Fina (Brazil), respectively. Rutin Standard Stock solution (1.0×10^{-3} M) was prepared by dissolving 6.1 mg of rutin hydrate in 5 mL of ethanol, aided by 5 min sonication, and then diluting with water to 10 mL. Britton–Robinson (BR) buffers in pH range of 3–12 were prepared using phosphoric, acetic and boric acids purchased from Sigma–Aldrich (Brazil). Acetic acid was also used to extract rutin from soybean seeds and leaves. Phosphate buffers in the pH range of 3–12 were prepared using dibasic sodium phosphate, monobasic potassium phosphate and phosphoric acid purchased from Sigma–Aldrich (United States, Brazil). Sodium hydroxide and hydrochloric acid (Sigma–Aldrich, Germany) were used for pH adjustment. Hexane (Sigma–Aldrich, Germany) was used to degrease the soybean flour. A pharmaceutical formulation (Zurita, Brazil) labeled to contain 20 mg mL^{-1} of rutin was purchased in a local drugstore of Brasília (Brazil) and was prepared for voltammetric analyses by diluting it (10 μL) in 10 mL of utltrapure water. Soybean leaves of the genotype IAC-100 and Dowling (Embrapa Cerrados, Brazil), produced under greenhouse conditions (temperature (T) = 27 $\pm$ 2 °C, relative humidity (RH) = 70%), were harvested at growth Vegetative 6 (V6) (Fehr and Caviness, 1977). Soybean seeds of the genotypes P98R6 (Pioneer Sementes, Brazil) and BRQ 96-3065 (Embrapa Cerrados, Brazil) were collected from a central-west Brazilian soybean production region (15°56′20.06″S latitude, 47°34′54.14″O longitude). Rutin was extracted from 100 mg samples (three) of dried macerated soybean leaves or defatted soybean flour with 4.0 mL of 70% aqueous ethanol containing 0.1% acetic acid, at room temperature, for 1 h, with constant agitation, according to the optimized isoflavone (daidzein, genistein, glycitein, genistin, daidzina, glycitin, 6″-O-malonyldaidzin, 6″-malonyl-genistin, 6″-O-malonylglycitin, 6″-acethyldaidzin, 6″-O-acethylgenistin and 6″-O-acethylglicitin) extraction procedure described by Carrão-Panizzi et al. (2002) and Park et al. (2001). The defatted soybean flour was obtained by drying and grinding 5 g of soybean seeds with subsequent degreasing of the resultant flour (2 g) with 200 mL of hexane for 6 h.

2.3. Procedure

The laboratory glassware was kept in a 20% (by volume) nitric acid solution overnight. Afterwards, it was kept in an ultra pure water bath overnight, rinsed thoroughly with ultra pure water and air-dried.

In order to avoid interferences due to rutin and copper adsorption on the working surfaces of the electrode system, electrodes were submitted to periodic cleaning with 20% HNO_3 (by volume) followed by a generous wash with ultra pure water. Experiments were performed at room temperature in three replicates and preceded by a gentle N_2 bubbling to prevent oxygen diffusion into the electrochemical cell (5 min for the supporting electrolyte and 1 min after copper and rutin addition).

The general information about the electroactivity and possible surface activity of Cu(II)–rutin complex were obtained by CV through additions of 100 μL of 1.0×10^{-3} M Cu(II) and 100 μL of 1.0×10^{-3} M rutin to the electrochemical cell containing 10 mL of phosphate buffer pH 6 with scan rate ranging from 10 to 600 mV s^{-1}.

In order to optimize the experimental conditions and gain the highest sensitivity for the CAdSV method, the influence of various operational parameters including composition of the supporting electrolyte, pH, accumulation potential, accumulation time, Cu(II) concentration, ν and pulse amplitude on the reduction peak current of Cu(II)–rutin (I_{pc}) complex were studied.

The influence of the supporting electrolyte (phosphate, BR, borate and Mclavaine buffer solutions) and pH on the peak current of Cu(II)–rutin complex by using CAdSV was studied through additions of 100 μL of 1.0×10^{-4} M Cu(II) and 100 μL of 1.0×10^{-4} M rutin to the electrochemical cell containing 10 mL of BR (4.0×10^{-2} M H_3PO_4, 4.0×10^{-2} M CH_3COOH and 4.0×10^{-2} M H_3BO_3) or phosphate (2.0×10^{-2} M H_3PO_4; 1.2×10^{-3} to 5.7×10^{-2} M Na_2HPO_4; 1.2×10^{-1} to 4.5×10^{-3} M KH_2PO_4; 4.6×10^{-3} to 7.7×10^{-2} M Na_3PO_4) buffers in the pH range of 3–12.

The study of the influence of accumulation potential (range of −1.200 to −0.500 V), pulse amplitude (range of 10–100 mV), scan rate (rage of 5–600 mV s^{-1}) and accumulation time (range of 30–300 s) on the peak current of Cu(II)–rutin complex by using CAdSV was accomplished through additions of 100 μL of 1.0×10^{-4} M Cu(II) and 100 μL of 1.0×10^{-4} M rutin to the electrochemical cell containing 10 mL of phosphate buffer pH 6.

The effect of the copper concentration on the peak current of Cu(II)–rutin complex by using CAdSV was evaluated through additions of 100 μL of 1.0×10^{-4} M rutin and successive additions of 20 μL of 1.0×10^{-4} M Cu(II) to the electrochemical cell containing 10 mL of phosphate buffer pH 6.

In order to evaluate the selectivity of the proposed method, the interfering effect of the main isoflavones (daidzein, daidzin, genistein, genistin, glycitein and glycitin) and phenolic acids (galic, *p*-hydroxibenzoic, vanilic, chlorogenic, caffeic and ferulic acids) found in soybeans on the determination of rutin by CAdSV was investigated. This interference study was accomplished through successive additions of 20 μL of 1.0×10^{-4} M rutin to the electrochemical cell containing 10 mL of phosphate buffer pH 6, 160 μL of 1.0×10^{-4} M Cu(II), 50 μL of 1.0×10^{-4} M rutin and 5–500 μL of 1.0×10^{-4} M isoflavone or phenolic acid. Tolerance limit was defined as the concentration which gives an error of $\leq$10% in the determination of 4.9×10^{-7} M rutin.

The calibration curve was obtained by using CAdSV through successive additions of 20 μL of 1.0×10^{-4} M rutin to the electrochemical cell containing 10 mL of phosphate buffer pH 6 and 160 μL of 1.0×10^{-4} M Cu(II). The practical feasibility of the CAdSV method was tested by the analysis of a real sample (pharmaceutical formulation) in order to compare the results obtained by this new procedure with those acquired by a standard spectrophotometric method [AOAC (The Scientific Association Dedicated to Analytical Excellence) International, 1995]. The determination of rutin in the pharmaceutical formulation by the standard addition method was accomplished through successive additions of 20 μL of 1.0×10^{-4} M rutin to the electrochemical cell containing 10 mL of phosphate buffer pH 6, 160 μL of 1.0×10^{-4} M Cu(II) and 100 μL of the pharmaceutical formulation.

The proposed method was applied to the determination of rutin in four soybean cultivars. Rutin was determined in soybean samples using the standard addition method through successive additions of 20 or 50 μL of 1.0×10^{-4} M rutin to the electrochemical cell containing 10 mL of phosphate buffer pH 6, 160 μL of 1.0×10^{-4} M Cu(II) and 40 or 50 μL of soybean sample.

3. Results and discussion

3.1. Cyclic voltammetry

Fig. 1 shows the cyclic voltammograms obtained for 4.5×10^{-5} M Cu(II) in the absence and in the presence of 4.5×10^{-5} M rutin in 10 mL of phosphate buffer pH 6 at ν = 100 mV s^{-1}. Curve A represents the oxidation-reduction process of the supporting electrolyte (phosphate buffer pH 6). Curve B shows the oxidation (peak 2) and reduction (peak 1) process of Cu(II) in the absence of rutin. The addition of rutin to a solution of Cu(II) results in two well defined peaks: one at −0.250 V (peak 3, Curve C) and the other at −0.180 V (peak 4, Curve C) that can be assigned to the reduction and oxidation of Cu(II)–rutin complex, respectively. In the range of 10–600 mV s^{-1}, the reduction peak current of Cu(II)–rutin complex is linear to the

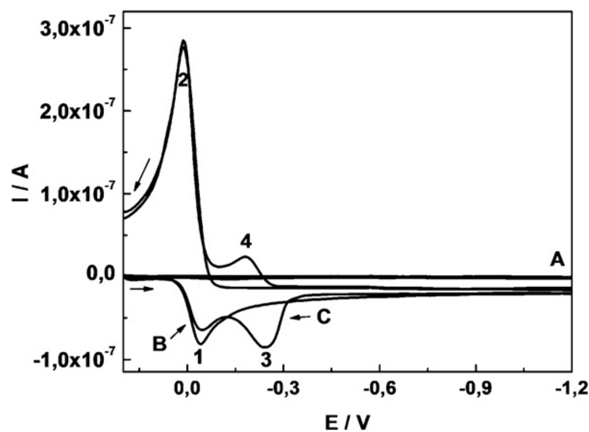


Fig. 1. Cyclic voltammograms obtained for 4.5×10^{-5} M Cu(II) in the absence (curve B) and in the presence of 4.5×10^{-5} M rutin (curve C) in 10 mL of phosphate buffer pH 6 (curve A). Initial potential (E_i) = 0.200 V, switching potential (E_w) = −1.200 V, scan rate (ν) = 100 mV s^{-1}, working electrode: hanging mercury drop electrode (HMDE), reference electrode: Ag/AgCl (KCl 3 M).

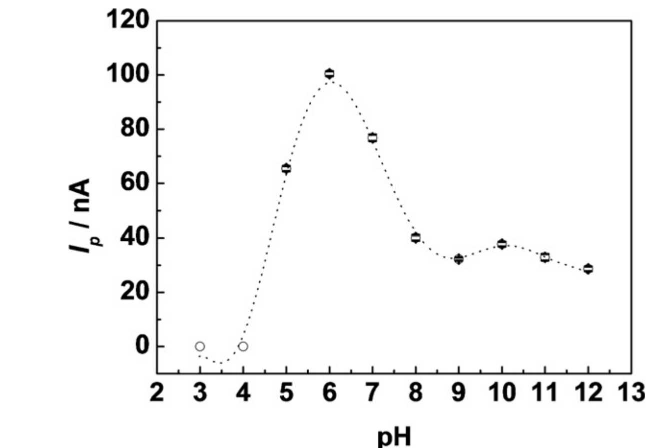


Fig. 2. Effect of the pH on the adsorptive cathodic stripping peak currents of 1.0×10^{-6} M of rutin in the presence of 1.0×10^{-6} M Cu(II). Accumulation potential (E_{acc}) = −1.100 V, accumulation time (t_{acc}) = 180 s, initial potential (E_i) = 0.100 V; final potential (E_f) = −1.100 V, pulse amplitude = 70 mV, scan rate = 50 mV s^{-1}, working electrode: hanging mercury drop electrode (HMDE), reference electrode: Ag/AgCl (KCl 3 M).

scan rate, and the regression equation is $I_{pc} = -1.491 + 0.529\nu$ (I_{pc}: nA, ν: mV s^{-1}, $r = 0.999$), indicating that the electrode process is adsorption-controlled. The reduction peak potential of Cu(II)–rutin complex (E_{pc}) shifts to negative potential with increasing scan rate and it is linear to the logarithm of the scan rate (log ν) for scan rates in the range from 50 to 600 mV, following the regression equation: $E_{pc} = -0.285 + 0.035 \log \nu$ (E_{pc}: V, ν: V s^{-1}, $r = 0.993$). Considering that two electrons are involved in the reduction process (Ensafi and Hajian, 2006), the electron-transfer coefficient ($\alpha = 0.420$) was calculated from the slope ($b/2$ where b is the Tafel slope ($-2.3RT/\alpha nF$)) of E_{pc} versus log ν plot (Laviron, 1979). This value is in close agreement with that determined by Ensafi and Hajian (2006). The peak separation ($\Delta E_p = |E_{pc} - E_{pa}|$) is about 60 mV at ν = 100 mV S^{-1}. At high scan rates, ΔE_p grows with increasing scan rates, indicating that the electron-transfer rate is not very fast and the electrochemical reaction gradually becomes less reversible.

The formation of this Cu(II)–rutin complex at the surface of HMDE provides the possibility of accumulation and indirect determination of rutin by CAdSV.

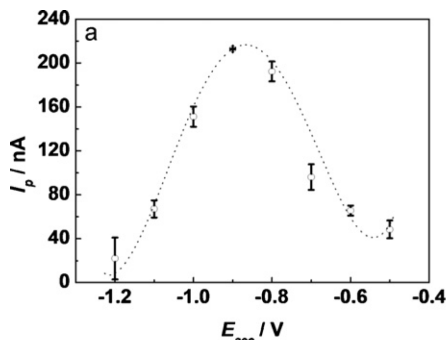
3.2. Influence of operational parameters

3.2.1. Influence of composition of the supporting electrolyte and pH

The best results concerning signal enhancement and shape of Cu(II)–rutin complex reduction peak current were obtained in a phosphate buffer at pH 7 and a rutin concentration of 1.0×10^{-6} M. Thus, for all further measurements, phosphate buffer was chosen as the supporting electrolyte. As expected, the reduction peak current of the Cu(II)–rutin complex is affected by the pH of the phosphate buffer solution (Fig. 2). With increasing pH of the solution, the Cu(II)–rutin complex reduction peak current increases and reaches a maximum at pH 6, then decreases. This phenomenon can be assigned to the reaction of hydroxide ions with copper ions that occurs at pH higher than 6, preventing the formation of the Cu(II)–rutin complex. This is in accordance with that reported in the literature (Ensafi and Hajian, 2006). Hence, the phosphate buffer pH 6 was chosen for further measurements.

3.2.2. Accumulation potential and time

Fig. 3a shows that the reduction peak current of the Cu(II)–rutin complex increases when the accumulation potential is changed from −1.200 to −0.900 V and decreases when the accumulation potential is changed from −0.900 to −0.500 V. This means that a more negative potential is favorable to the accumulation of the Cu(II)–rutin complex on the HMDE surface. These results are in agreement with Esparza et al. (2005), who found that a very negative accumulation potential (−1.200 V) is necessary to favor the reduction of rutin and the formation of Cu(II)–rutin complex on the electrode surface. Although the best results concerning signal



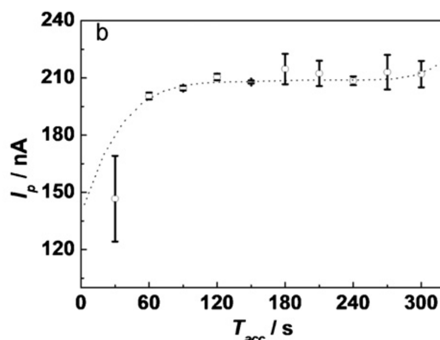


Fig. 3. (a) Effect of the accumulation potential (E_{acc}) on the adsorptive cathodic stripping peak currents of 1.0×10^{-6} M of rutin in the presence of 1.0×10^{-6} M Cu(II) in 10 mL of phosphate buffer pH 6. E_{acc} = −1.200 to −0.500 V, accumulation time (t_{acc}) = 180 s, initial potential (E_i) = 0.100 V, final potential (E_f) = −1.100 V, pulse amplitude = 70 mV, (ν) scan rate = 50 mV s^{-1}; (b) effect of the accumulation time (t_{acc}) on the adsorptive cathodic stripping peak currents of 1.0×10^{-6} M of rutin in the presence of 1.0×10^{-6} M Cu(II) in 10 mL of phosphate buffer pH 6. E_{acc} = −1.000 V, t_{acc} = 30–300 s, initial potential (E_i) = 0.050 V, final potential (E_f) = −0.400 V, pulse amplitude = 70 mV, (ν) scan rate = 50 mV s^{-1}, working electrode: hanging mercury drop electrode (HMDE), reference electrode: Ag/AgCl (KCl 3 M).

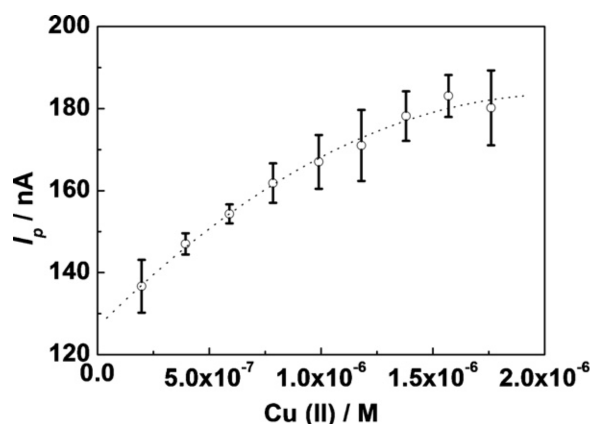


Fig. 4. Effect of the copper(II) concentration on the adsorptive cathodic stripping peak currents of 1.0×10^{-6} M of rutin in the presence of 2.0×10^{-7} to 1.8×10^{-6} M Cu(II) in 10 mL of phosphate buffer pH 6. Accumulation potential (E_{acc}) = −1.000 V, accumulation time (t_{acc}) = 180 s, initial potential (E_i) = 0.050 V, final potential (E_f) = −0.400 V, pulse amplitude = 70 mV, (ν) scan rate = 50 mV s^{-1}, working electrode: hanging mercury drop electrode (HMDE), reference electrode: Ag/AgCl (KCl 3 M).

enhancement of the reduction peak current of Cu(II)–rutin complex was obtained with an accumulation potential of −0.900 V, the best linear relationship between Cu(II)–rutin complex reduction peak current and concentration of rutin was observed with an accumulation potential of −1.000 V. Hence, in subsequent measurements, an accumulation potential of −1.000 V was used.

Fig. 3b shows that the reduction peak current of Cu(II)–rutin complex grows with increasing accumulation time and saturates after 120 s. A similar behavior was observed by Temerk et al. (2006) and Ensafi and Hajian (2006). The best results concerning signal enhancement and format of reduction peak current of Cu(II)–rutin complex was obtained with an accumulation time of 180 s. Thus, a 180 s accumulation time was chosen for further measurements.

3.2.3. Copper(II) concentration

Fig. 4 shows that in the presence of 1.0×10^{-6} M rutin, the reduction peak current of the Cu(II)–rutin complex increases by increasing the copper(II) concentration from 2.0×10^{-7} to 1.6×10^{-6} M until it becomes constant at a concentration that is close to the stoichiometric ration of 1 Cu(II) per 1 rutin. These results show strong coherence with CAdSV data obtained by Temerk et al. (2006). An optimum copper(II) concentration of 1.6×10^{-6} M was selected for further experiments.

3.2.4. Scan rate and pulse amplitude

Fig. 5 shows that the reduction peak current of the Cu(II)–rutin complex increases with increasing scan rate and pulse amplitude, respectively. It was observed that these two parameters do not affect the Cu(II)–rutin complex reduction peak current for scan rate values below 100 mV s^{-1} and for pulse amplitude values above 70 mV, which is agreement with the literature data (Laviron, 1979). For further experiments, a 50 mV s^{-1} scan rate and 70 mV pulse amplitude were applied.

3.3. Interferences

The proposed method is selective for rutin determination in the presence of each of the tested isoflavones (daidzein, daidzin, genistein, genistin, glycitein and glycitin) or phenolic acids (galic, *p*-hydroxibenzoic, vanilic, chlorogenic, caffeic and ferulic acids) in the concentration range from 4.9×10^{-8} to 4.9×10^{-7} M. At a 1:1 concentration ratio, none of these phenolic compounds caused any interference and the reduction peak of the Cu(II)–rutin Complex at −0.152 V remained unchanged. This behavior can be ascribed to the differences of peak potential values and formation constants of the Cu(II)–rutin, Cu(II)–isoflavones and Cu(II)–phenolic acids complexes, as has already been proposed by Ensafi and Hajian (2006). Thus, the selectivity of the proposed method can be compared to those observed by other analytical methods in which rutin was determined in the presence of interferences usually found in pharmaceutical formulations, tea, blood serum and urine.

3.4. Calibration curve and figures of merit

Fig. 6 shows the cathodic adsorptive stripping voltammograms obtained for 1.6×10^{-6} M Cu(II) in 10 mL of phosphate buffer pH 6.0 with different rutin concentrations. Under the optimized conditions, a linear response of Cu(II)–rutin reduction peak current as a function of rutin concentration (I_{pc} (nA) = 10.070 + 1.9×10^8 [Rutina]) was obtained in the range of 2.0×10^{-7} to 1.4×10^{-6} M, with a correlation coefficient (R) of 0.999 (insert Fig. 6). A detection limit (DL) of 7.0×10^{-9} M and a quantification limit (QL) of 2.2×10^{-8} M were obtained by using the relations $3S_b/b$ and $10S_b/b$, respectively, where S_b is the blank standard deviation and b the slope of the calibration curve (Miller and Miller, 1993). DL is in the same order of magnitude of those obtained by Ensafi and Hajian (2006) and Temerk et al. (2006) in which rutin was determined in tea, pharmaceuticals, human urine and blood serum by CAdSV. Moreover, DL is 1000 times lower than those obtained by spectrophotometric (Kurzawa, 2010), capillary electrophoretic (Chen et al., 2001) and high performance liquid chromatographic with UV detection (Kurzawa, 2010) methods and 100 to 10 times

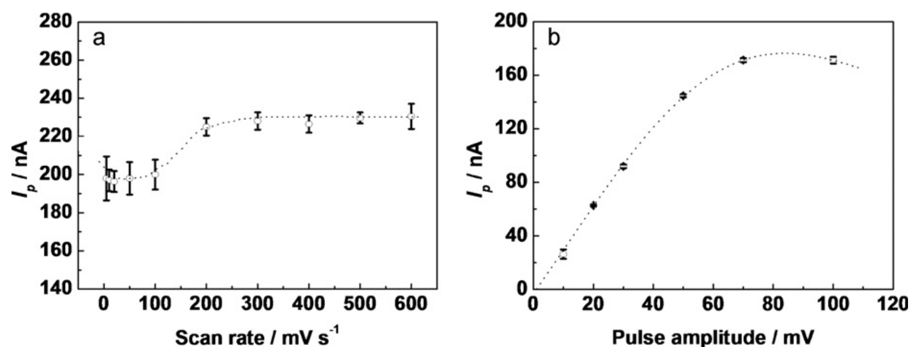


Fig. 5. (a) Effect of the scan rate (ν) on the adsorptive cathodic stripping peak currents of 1.0×10^{-6} M of rutin in the presence of 1.0×10^{-6} M Cu(II) in 10 mL of phosphate buffer pH 6; (b) effect of the pulse amplitude on the adsorptive cathodic stripping peak currents of 1.0×10^{-6} M of rutin in the presence of 2.0×10^{-7} to 1.8×10^{-6} M Cu(II) in 10 mL of phosphate buffer pH 6. Accumulation potential (E_{acc}) = −1.000 V, accumulation time (t_{acc}) = 180 s, initial potential (E_i) = 0.050 V, final potential (E_f) = −0.400 V, pulse amplitude = 10–100 mV, ν = 5–600 mV s^{-1}, working electrode: hanging mercury drop electrode (HMDE), reference electrode: Ag/AgCl (KCl 3 M).

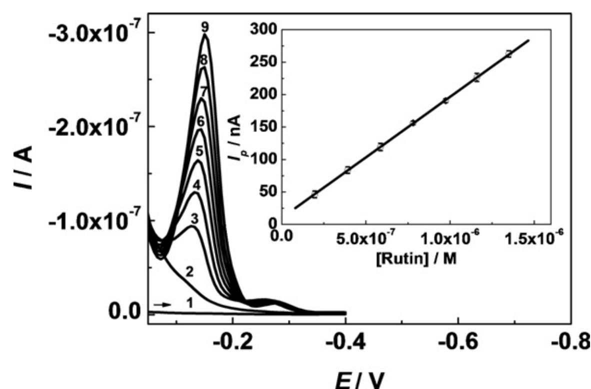


Fig. 6. Cathodic adsorptive stripping voltammograms obtained for 1.6×10^{-6} M Cu(II) (2) in 10 mL of phosphate buffer pH 6 (1) in different rutin concentrations: (3) 2.0×10^{-7}, (4) 3.9×10^{-7}, (5) 5.9×10^{-7}, (6) 7.8×10^{-7}, (7) 9.7×10^{-7}, (8) 1.2×10^{-6}, and (9) 1.4×10^{-6} M. Insert: The calibration curve (correlation coefficient (R) = 0.999; probability (P) < 1.0×10^{-4}). Accumulation potential (E_{acc}) = −1.000 V, accumulation time (t_{acc}) = 180 s, initial potential (E_i) = 0.050 V, final potential (E_f) = −0.400 V, pulse amplitude = 70 mV, scan rate = 50 mV s^{-1}, working electrode: hanging mercury drop electrode (HMDE), reference electrode: Ag/AgCl (KCl 3 M).

lower than those obtained by other electrochemical methods (Chen et al., 2010; Du et al., 2010; Freitas et al., 2009; Liu et al., 2010; Santos et al., 2007; Song et al., 2010; Sun et al., 2008; Yang et al., 2010; Zeng et al., 2006; Zhan et al., 2010; Zhang and Zheng, 2008). The sensitivity of the proposed method can also be compared to those observed by high performance liquid chromatographic with electrochemical (Danila et al., 2007) and photodiode array (Lu et al., 2006) detections and by other electrochemical (Malagutti et al., 2006; Wang et al., 2010; Wu et al., 2008) methods in which DL values were found to be in the order of 10^{-9} M. Only in two cases is the DL determined in this work higher than those obtained by other methods (Novak et al., 2008; Tyszczuk, 2009). However, these methods, and the ones in which the sensitivities are similar, present some disadvantages, such as high cost, complicated operations, multi-solvent extractions, time-consuming procedures, more specialized technicians and low stability of modified electrodes.

3.5. Analytical applications

3.5.1. Determination of rutin in pharmaceutical formulations

Table 1 presents the results obtained in the determination of rutin in a pharmaceutical sample by using the CAdSV method and Method 952.28 Rutin in Drugs (AOAC International, 1995). According to the t-test $|t_{exp}| \leq t_{label}$ (t_{exp} = 1.223 and t_{label} = 2.920), there are no significant differences between the results obtained by both methods within 95% of confidence level, indicating the practical feasibility of the proposed method. The absence of the matrix effect on this determination was attested by single point standard addition-recovery values that varied from 94 to 103%.

Table 1
Rutin determination in the pharmaceutical formulation.

Rutin[a] (mg mL^{-1})				
Label value	CAdSV	AOAC method	Relative error (%)[b]	Relative error (%)[c]
20	26.370 ± 1.190	25.530 ± 0.060	32	3

Detection limit (DL) = 4.27 ng mL^{-1}.
[a] Mean values from three independent determinations.
[b] CAdSV (cathodic adsorptive stripping voltammetric) versus label value.
[c] CAdSV versus AOAC method (The Scientific Association Dedicated to Analytical Excellence) method (AOAC International, 1995).

Table 2
Rutin determination in soybean samples.

Soybean samples	Rutin found[a] (mg g^{-1})	Rutin reported value (mg g^{-1})
P98R62 (seeds)	n.d[b]	n.r.[c]
BRQ 96-3065 (seeds)	n.d.	n.r.
Dowling (seeds)	n.d.	n.r.
Dowling (leaves)	n.d.	n.r.
IAC 100 (leaves)	0.197 ± 0.038	0.972 ± 0.082[d]

Detection limit (DL) = 4.27 ng mL^{-1}.
[a] Mean values from three independent determinations.
[b] Not detected.
[c] Not reported.
[d] Piubelli et al.

3.5.2. Determination of rutin in soybean samples

Table 2 presents the results obtained in the determination of rutin in four soybean cultivars by the CAdSV method. Rutin was not detected in P98R62, BRQ 96-3065 and Dowling soybean seeds. These results were expected and are supported by published data where genistin, daidzin and their aglycones were identified as the main isoflavone compounds in Brazilian soybean seeds (Carrão-Panizzi et al., 2002). Rutin was not detected in the leaves of Dowling soybean sample either. This result was expected and is supported by published data where changes in chemical composition of midgut of *Anticarsia gemmatallis* were observed after the ingestion of Dowling, a soybean genotype resistant to insects which contains 0.0429 mg g^{-1} of genistin (Levy et al., 2010). However, for the IAC 100 soybean cultivar, the result of the analysis was positive for this flavonoid. The mean concentration of rutin in IAC 100 was much lower than the value reported by Piubelli et al. (2005), using HPLC to determine rutin in soybean leaves of the same genotype (IAC-100), produced under greenhouse conditions (T = 23 ± 2 °C, RH = 78%) in the southern region of Brazil (23 °C average local temperature). However, this difference can be due to the higher temperatures of the central-west region of Brazil (27 °C average local temperature, which was reproduced in the greenhouse) during seed development, which was reported by Tsukamoto et al. (1995) as being the major factor in the determining the levels of flavonoids in seeds. This variability on flavonoid concentration was also observed among Brazilian soybean cultivars grown in different locations by Carrão-Panizzi and Kitamura (1995) and Carrão-Panizzi et al. (1999). The absence of the matrix effect on this determination was attested by single point standard addition-recovery values that varied from 94 to 105%.

4. Conclusions

A cathodic adsorptive stripping voltammetric method based on the accumulation of a Cu(II)–rutin complex on the HMDE was successfully applied to the determination of rutin in a pharmaceutical formulation and in soybean leaves and seeds. The analytical results obtained by CAdSV method in the determination of rutin in the pharmaceutical formulation sample are in close agreement with those obtained by AOAC procedure within 95% of confidence level, demonstrating its practical feasibility. The difference between rutin concentration value determined in the IAC-100 soybean sample grown in the central-west of Brazil using the proposed method and the value reported in the literature for the same cultivar, but grown in southern Brazil, suggests that the temperature during seed development is one of the major factors that influence the flavonoid content in soybean samples. The data obtained for soybean cultivars in the present work are important elements for future studies on soybean nutritional properties and their beneficial effects on

human health. The knowledge that IAC-100 cultivar possesses rutin in its leaves has many applications in Integrated Pest Management and breeding programs, contributing to the sustainability of soybean-based agricultural systems. The CAdSV method represents an alternative tool for rutin determination considering that it has the advantages of high speed and sensitivity (lower detection limits), low cost, easy operation and good selectivity when compared to other analytical methods. The main advantage of the proposed method over the existing CAdSV methodologies for determination of rutin is its novel application in the agricultural and nutritional areas, providing data about rutin concentration in soybean cultivars, which are important information for future investigations on using soybean as a source of flavonoids in the human diet. The only disadvantage of the CAdSV method is the impossibility of determining other phenolic compounds found in soybeans, such as the isoflavones. This limitation can be assigned to the inability of glycitin and daidzin to complex with Cu(II) or the formation of different species among glycitein, genistein, genistin and daidzein and Cu(II).

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