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Analytical Methods

Sensor-containing microspheres of chitosan crosslinked with 8-hydroxyquinoline-5-sulphonic acid for determination of Cu(II) in instant coffee

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ABSTRACT

A novel carbon paste electrode containing chitosan microspheres for the determination of Cu(II) in instant coffee by anodic stripping voltammetry was developed. Chitosan was crosslinked with the chelating agent 8-hydroxyquinoline-5-sulphonic acid and glutaraldehyde. The microspheres of the crosslinked chitosan biopolymer were obtained by the spray drying technique and employed in the construction of the sensor. In acetate buffer solution (0.1 mol L^{-1}, pH 6.0), the calibration curve obtained was linear for concentrations of 5.0×10^{-7} to 1.4×10^{-5} mol L^{-1} ($r = 0.9990$); the detection limit was 5.5×10^{-8} mol L^{-1}. The relative standard deviation ($n = 8$) was lower than 3.0% for solutions containing 6.0×10^{-6}, 5.0×10^{-5} and 1.5×10^{-4} mol L^{-1} of Cu(II). The method was successfully employed for determination of Cu(II) in instant coffee and the results obtained showed good agreement when compared with those using electrothermal atomic absorption spectrometry.

1. Introduction

In recent years, several studies employing the biopolymer chitosan have been developed in the areas of science and technology. This polysaccharide is obtained from renewable resources and currently chitosan is intensively studied due to its application in the pharmaceutical, cosmetics, biomedical, biotechnological, agricultural, and food industries (Mourya & Inamdar, 2008). Also, it is widely used for the removal of toxic metals through adsorption processes for environmental purposes, recovery of metals by separation processes in hydrometallurgy, and in analytical methods in a pre-concentration step before the use of conventional spectroscopic analysis. Chitosan is obtained by the alkaline deacetylation of chitin, one of the most abundant biopolymers in nature, present in the exoskeletons of crustaceans and also the cell walls of fungi and insects (Kumar, 2000). One of the advantages of chitosan which attracts greatest interest is its versatility. This polymer can be easily modified by chemical or physical processes to prepare chitosan derivatives. The material can be quickly modified physically and obtained in different forms including powder, nano particles, gel beads, membranes, sponge, honeycomb, fibres or hollow fibres. The presence of a high percentage of reactive amino groups, generally higher than 80%, distributed in its polymeric matrix, allows chemical changes to be carried out. The chemical modification of chitosan may be necessary to prevent the dissolution of the polymer when the reactions are performed in acidic solutions, and/or to change its properties, such as improving its ability to adsorb metals (Guibal, 2004). This biopolymer has been crosslinked with different substances including glutaraldehyde, 1,1,3,3-tetramethoxypropane, ethyleneglycol diglycidyl ether, epichlorohydrin, glyoxal, carbodiimide, and tripolyphosphate and has been used in many different fields (Osifo et al., 2008).

Spray drying is a technique for the formation of microparticles which has been used by researchers for different ends. It is employed in a wide variety of processes ranging from the manufacture of food products to pharmaceuticals (Tonon, Brabet, & Hubinger, 2008). This well-established technique has been around for over a century, but it remains an active field of innovation, driven by the ever increasing demand for more sophisticated particles. It has many advantages over other techniques for the preparation of particles, such as excellent reproducibility and speed in obtaining the microspheres (Vehring, Foss, & Lechuga-Ballesteros, 2007). It is used to produce dry powder from solutions or suspensions in three steps of operation: atomization of the liquid feed, drying of the droplets once they are formed, and motion of the droplets to model the spray drying process (Shabde & Hoo, 2008). The atomization of the biopolymer chitosan by this technique is generally used in pharmacological processes, especially in controlled-drug delivery systems, and produces good results. Recently, the spray drying technique has been employed to obtain microspheres of chitosan crosslinked with 8-hydroxyquinoline-5-sulphonic acid and glutaraldehyde, as a new adsorbent for metallic ions (Vitali, Laranjeira, Gonçalves, & Favere, 2008). This new

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adsorbent showed a maximum adsorption capacity for Cd(II) and Zn(II) ions higher than that of chitosan microspheres crosslinked with glutaraldehyde without the chelating agent 8-hydroxyquinoline-5-sulphonic acid.

Environment pollution, particularly by metals, is a serious problem confronting society today, because some metals are toxic even at low concentrations (Galaris & Evangelou, 2002). Copper is one of the most used metals, mainly in electrical industries, galvanisation, fertilizers, mining activities, smelting and refining of metals, and is present in pigments, pesticides and fungicides. Since it is a widely employed element, it has a high potential for contamination and may be present in different types of samples including water and sediments from rivers and lakes, beverages, and food (Ngah, Endud, & Mayanar, 2002). Due to its technological importance, many sensors have been developed for copper determination, such as ion-selective electrodes (Gismera, Hueso, Procopio, & Sevilla, 2004), self-assembled monolayer modified gold electrodes (Mohadesi & Taher, 2007), bismuth film electrodes (Pacheco et al., 2008) and modified carbon paste electrodes (MCPE) (Cesarino, Marino, Matos, & Cavalheiro, 2008). The use of these sensors with voltammetric and chronoamperometric techniques shows some advantages, such as high sensitivity and precision and low cost of the equipment, when compared to spectroscopic techniques, for instance, graphite furnace atomic adsorption spectroscopy and inductively coupled plasma mass spectroscopy (Mhammedi, Achak, & Chtaini, 2009).

The application of MCPE in metal analysis by stripping voltammetry has attracted considerable attention, mainly because the introduction of a chemical modifier allows the concentration of metallic ions at the electrode surface, either by complexation or electrostatic attraction, which leads to more sensitive electroanalytical procedures with lower detection limit values (Takeuchi, Santos, Padilha, & Stradiotto, 2007). Different chemical modifiers have been used in the construction of carbon paste electrodes, such as aminopropyl-grafted silica gel (Etienne, Bessiere, & Walcarius, 2001), 3,4-dihydro-4,4,6-trimethyl-2(1*H*)-pyrimidine thione (Abbaspour & Moosavi, 2002), carbamoylphosphonic acid self-assembled monolayer on mesoporous silica (Yantasee, Lin, Fryxell, & Busche, 2004), natural zeolite (Alpat, Yuksel, & Akcay, 2005), and 2-aminothiazole organofunctionalized silica (Takeuchi et al., 2007). These electrodes show several advantages including easy fabrication and rapid renewal, low background current, low cost, no toxicity, stability in various solvents and a wide electrochemical window (Estévez-Hernández, Naranjo-Rodriguez, Hidalgo-Hidalgo de Cisneros, & Reguera, 2007). Moreover, the use of these electrodes combined with electroanalytical techniques, such as stripping voltammetry, allows different experimental conditions to be set, enhancing the electrode performance. For example, an accumulation step preceding the measurement can be performed with controlled potential or under open circuit potential conditions, depending on the nature of the pre-concentration. This preliminary step increases the metal concentration at the electrode surface and enhances the sensitivity of the stripping step (Mohadesi & Taher, 2007; Yantasee et al., 2004).

In this study, a novel carbon paste electrode (CPE), containing microspheres of chitosan crosslinked with 8-hydroxyquinoline-5-sulphonic acid and glutaraldehyde (CPE-CTS) for determination of Cu(II) by square wave anodic stripping voltammetry, was constructed. Experimental conditions affecting the pre-concentration step, including the solution pH, potential and time of pre-concentration, were evaluated. Although the proposed sensor can be used to determine other heavy metals, since microspheres of chitosan crosslinked with 8-hydroxyquinoline-5-sulphonic acid can act as an adsorbent for several metallic ions (Vitali et al., 2008), the performance of the proposed sensor was examined using optimised operating parameters for Cu(II) determination in instant coffee samples. Copper was chosen as the test element because it has been found to be best suited to identifying the geographical growing origin of coffee, together with manganese and cobalt (Oleszczuk et al., 2007). Therefore, the main goal of this work is to show that the proposed CPE-CTS sensor can be successfully used to determine Cu(II) in instant coffee samples. The determination of metals in coffee is commonly carried out in green coffee. To the best of our knowledge, the determination of Cu(II) in instant coffee is shown here for the first time.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and all the solutions were prepared with ultrapure water obtained from a Millipore Milli-Q system (18.2 MΩ cm). Chitosan (deacetylation degree of 80%), 8-hydroxyquinoline-5-sulphonic acid, glutaraldehyde and copper nitrate were acquired from Sigma. Graphite powder and Nujol were purchased from Fischer Scientific and Aldrich, respectively. Acetate buffer (0.1 mol L^{-1}, pH 4.0, 5.0 and 6.0); tris(hydroxymethyl)aminomethane (0.1 mol L^{-1}, pH 7.0 and 8.0) and ammonia (0.1 mol L^{-1}, pH 9.0 and 10.0) solutions were tested as supporting electrolytes.

2.2. Apparatus

Square wave and cyclic voltammetry experiments were performed on an electrochemical detector, a Voltalab PGZ-100 potentiostat/galvanostat (Radiometer, Copenhagen, Denmark), equipped with a three-electrode system: a carbon paste electrode containing crosslinked chitosan (CPE-CTS) as the working electrode, a platinum wire as the auxiliary electrode, and an Ag/AgCl electrode as the reference electrode. The system was coupled to a microcomputer and controlled by VoltaMaster 4.0 software (Radiometer, Copenhagen, Denmark), for data acquisition and subsequent analysis. The weighing of reagents and mineralisation of coffee samples were carried out on a Shimadzu analytical balance, model AY-220, and in a Jung muffle, model BTC-9090, respectively. The morphology of the surfaces of the CTS microspheres, CP and CP-CTS was analyzed using a Philips XL 30 Scanning Electron Microscope (SEM) with an accelerating voltage of 20 keV. Samples were then fixed on stubs and coated with a thin gold layer using a cool sputter-coater (Blazers SCD 005, Liechtenstein).

2.3. CPE-CTS construction and electrochemical measurements

The chitosan crosslinked with the chelating agent 8-hydroxyquinoline-5-sulphonic acid and glutaraldehyde and the microspheres were obtained according to a procedure described in the literature (Vitali et al., 2008). The sensor was constructed as follows: 30 mg (15% w/w) of chitosan microspheres and 130 mg (65% w/w) of graphite powder were mixed in a small mortar for 20 min to form a homogeneous mixture and 40 mg (20% w/w) of Nujol was then added followed by mixing for another 20 min. The resulting modified carbon paste was tightly packed into a syringe and a copper wire was introduced into the other end for electrical contact. A bare carbon paste electrode (CPE), used for comparison purposes, was prepared as previously described, using only graphite powder and Nujol in the proportion of 65:35% w/w (Oliveira, Fernandes, & Vieira, 2006).

In a typical procedure for electrochemical measurements, 10.0 mL of the acetate buffer solution (pH 6.0) was transferred to a clean dry cell and successive additions of standard or sample solutions of Cu(II) were added by micropipette. The voltammetric

procedure consisted of pre-concentration (accumulation), stripping (detection), and electrode regeneration steps. During the pre-concentration step, the electrode was immersed in the cell containing the supporting electrolyte and the metallic ion solution. A negative controlled potential (−0.1 to −0.7 V) was applied to the sensor for a specified time (0–300 s). The solution was stirred using a magnetic stirring bar. Stripping voltammetry was then performed in the same cell with a sweeping square wave potential toward the positive direction (from −0.3 to 0.1 V), at frequencies of (f) 1.0–50 Hz, pulse amplitudes (a) of 10–50 mV and scan increments (ΔE_s) of 1.0–10 mV, after successive additions of the analyte. The electrode cleaning step was performed by applying a positive potential under stirring. No de-aeration of solutions was required in any step.

2.4. Sample preparation

The sample and blank solutions were prepared following previously described procedures (Onianwa, Adetola, Iwegbue, Ojo, & Tella, 1999). Briefly, three samples of instant coffee (A, B and C) were obtained from local supermarkets in Florianópolis (Santa Catarina, Brazil). For the sample preparation, 1.0 g of instant coffee was weighed in triplicate in porcelain crucibles and mineralised in a muffle furnace at 550 °C for 20 h. The mineralisation step is necessary in order to eliminate the organic compounds present in the coffee sample, which can act as complexing agents for many metals (including Cu(II)) and can thus affect the results if present in the sample. The residue was dissolved with 0.2 mL of 1.0 mol L^{-1} nitric acid and diluted to 3.0 mL with acetate buffer solution (0.1 mol L^{-1}, pH 6.0). For the electrochemical measurements using the proposed sensor, 1.0 mL of each coffee sample was transferred independently to the cell containing 10 mL of acetate buffer solution (0.1 mol L^{-1}, pH 6.0) and successive additions of Cu(II) standard solution were performed. After each addition, square wave voltammograms were recorded, also in triplicate, using the optimised experimental conditions.

2.5. Comparative method

The electrothermal atomic absorption spectrometry (ET AAS) measurements for the validation of the present procedure were carried out with a Perkin–Elmer AAnalyst 100 atomic absorption spectrometer (Norwalk, CT, USA) interfaced with a PC. Unspecific light absorption was corrected by continuum light source (deuterium lamp) background correction. A hollow cathode lamp (Perkin–Elmer, USA) was used as the radiation source of the 324.8 nm copper line. The pyrolysis and atomization temperature employed in the determinations were 900 and 2200 °C, respectively.

2.6. Statistical analysis

The t-values and F-values of the statistical tests were used to evaluate the results for the determination of Cu(II) in coffee samples by CPE-CTS and ET AAS. The robustness of the proposed method was evaluated by examining the data through one-way analysis of variance (ANOVA). All results of the statistical analysis were obtained using the GraphPad InStat® software, version 3.05.

3. Results and discussion

3.1. Sensor characterisation

Fig. 1 shows the scanning electron microscopy (SEM) micrographs of the (a) chitosan microspheres crosslinked with 8-hydroxyquinoline-5-sulphonic acid and glutaraldehyde (CTS) obtained by spray drying, (b) carbon paste (CP), (c) CP containing crosslinked chitosan microspheres (CP-CTS) and (d) the proposed chemical structure of chitosan crosslinked with 8-hydroxyquinoline-5-sulphonic acid and glutaraldehyde. As can be seen in the CP-CTS micrograph, the spherical form and regular shape of the CTS microspheres in the modified carbon paste were maintained even after the maceration process during the electrode preparation. This is attributed to the interaction of the amine groups of the chitosan crosslinked with glutaraldehyde through the Schiff's base reaction and to the ionic interaction with the 8-hydroxyquinoline-5-sulphonic acid, providing a good mechanical stability of microspheres (Guibal, 2004).

3.2. Voltammetric study of the sensor and reaction with Cu(II)

The electrochemical behaviour of the bare CPE and CPE-CTS in aqueous solution was investigated by cyclic voltammetry. Firstly, the potential was positively swept from −0.2 to +0.1 V, at which point the scan direction was reversed, causing a negative potential sweep back to the initial condition. In Fig. 2a, for comparison, a cyclic voltammogram of the CPE-CTS immersed in the supporting electrolyte without copper ions in solution is shown. As expected, no peak was observed. Fig. 2b shows the cyclic voltammogram obtained with the bare CPE immersed in the supporting electrolyte containing a 5.0×10^{-5} mol L^{-1} Cu(II) solution after a pre-concentration step at $E_{pc} = -0.4$ V for $t_{pc} = 20$ s. In the pre-concentration step, Cu(II) is reduced to Cu^0. In the cyclic voltammogram an oxidation peak was present at 0.0 V due to the oxidation of Cu^0 to Cu(II) in the forward scan. In the reverse scan, the reduction peak was not observed, indicating that the system is irreversible. With the CPE-CTS (Fig. 2c), the voltammogram obtained under the same conditions as those used for the bare CPE shows a considerable increase in the anodic current peak. The increase in the anodic current can be attributed to the pyridinic nitrogen and phenolic group present in the structure of the chelating agent anchored in the biopolymer chitosan, improving the sensitivity of the electrode for copper determination. When the potential was negatively swept, a broad signal of low intensity centred around −0.10 V was observed. This signal is probably due to the reduction of Cu(II) present in solution or at the electrode surface.

The properties of the oxidation peak observed in the stripping step with the CPE-CTS were also investigated as a function of the scan rate. The experimental data indicate that the relationship between the potential peak and the scan rate is characteristic of adsorbed species (Lu, He, Zeng, Wan, & Zhang, 2003). Likewise, the plot of log $i_p \times \log v$ (where i_p is the anodic current peak and v the scan rate) showed a linear relationship: $\log i_p = 1.57 + 0.741 \log v$ ($r = 0.99$), in which the slope observed between 0.5 and 1.0 suggests that the oxidation process is simultaneously controlled by adsorption and diffusion (Garay & Solis, 2003).

Fig. 3 shows a proposed mechanism for the reactions of Cu(II) on the surface of the CPE-CTS. A similar mechanism has previously been reported (Lu et al., 2003). In the first step (A), the accumulation of copper ions at the modified electrode surface occurs by complexation; in the second step (B), the copper ions in the complexed form are reduced to metallic copper at a controlled-potential E_{pc}; and in the final step (C), the copper is oxidised back to copper ions in the stripping step and the resulting oxidation current peak constitutes the analytical signal. The complexation of copper ions on the electrode surface in the first step occurs due the presence of chelating groups in the molecular structure of the material inserted in the modified carbon paste. The application of $E_{pc} = -0.4$ V causes the reduction of complexed Cu(II) to Cu^0 (step B) and, subsequently, in the anodic stripping voltammetry a

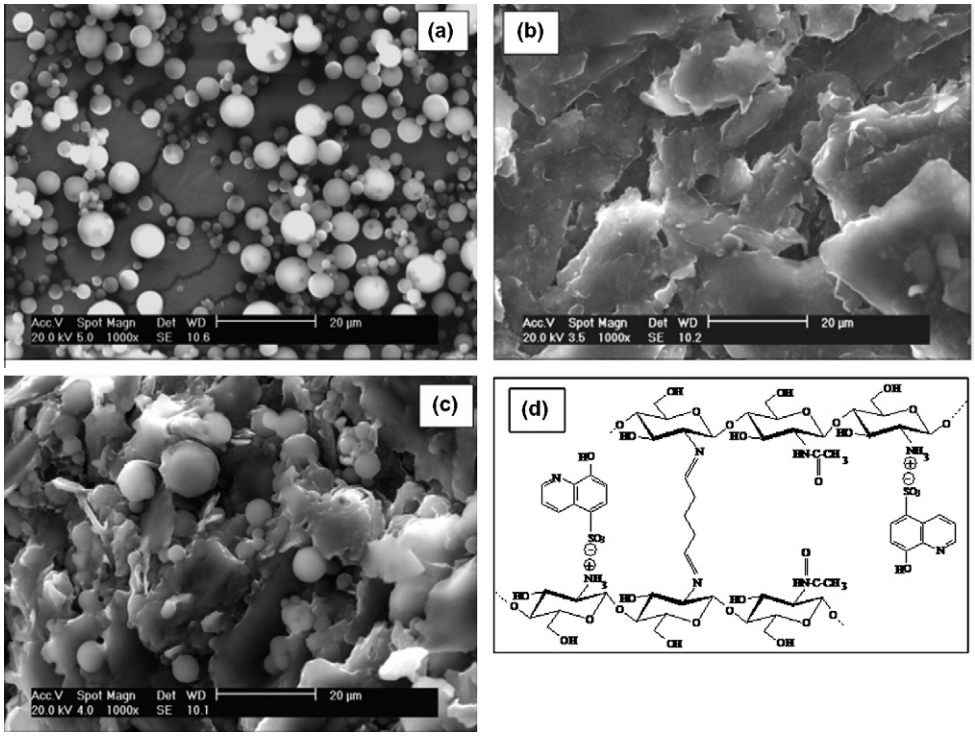

Fig. 1. SEM images of (a) microspheres of crosslinked chitosan, (b) carbon paste, (c) carbon paste containing microspheres of chitosan, and (d) chitosan crosslinked with 8-hydroxy-5-quinoline sulphonic acid and glutaraldehyde.

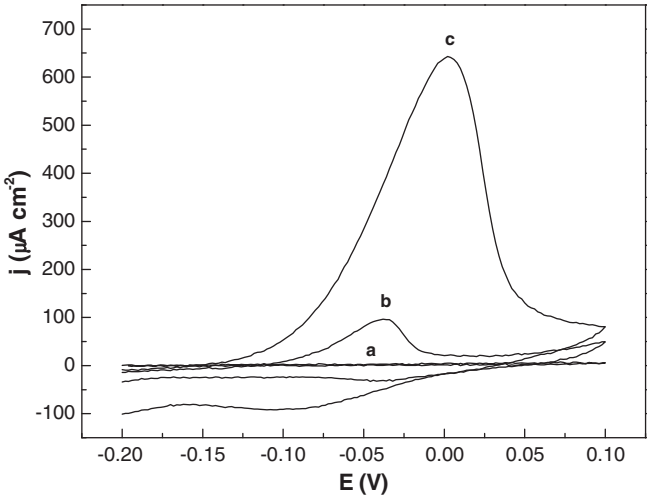

Fig. 2. Cyclic voltammograms obtained using the (a) CPE-CTS for blank in acetate buffer solution (0.1 mol L^{-1}, pH 6.0), (b) CPE and (c) CPE-CTS in 5.0×10^{-5} mol L^{-1} Cu(II) solution; $E_{pc} = -0.4$ V, $t_{pc} = 20$ s and $\nu = 100$ mV s^{-1}.

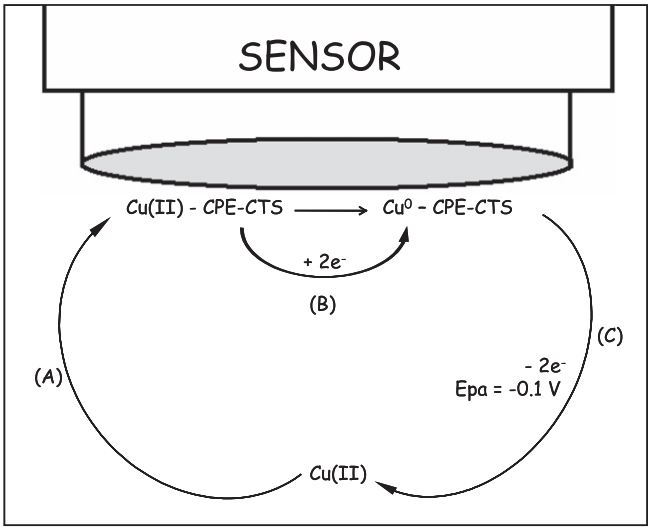

Fig. 3. Schematic representation of the Cu(II) reactions on the CPE-CTS surface.

current peak appears at potentials between −0.1 and 0.0 V, depending of the Cu(II) concentration.

3.3. Optimisation of the experimental conditions

The effect of the pH (4.0–10.0) on the anodic current peak employing the CPE-CTS in a 5.0×10^{-5} mol L^{-1} Cu(II) solution was investigated. The maximum current was observed at pH 6.0. For solutions with pH higher than 6.0, the current measured was almost zero. The low analytical signal in alkaline solutions can be ascribed to the formation of a complex ion in a competitive parallel reaction between Cu(II) and the complexing agent constituents of the buffer solution used to avoid the metal precipitation (Justi, Laranjeira, Neves, Mangrich, & Favere, 2004). In acidic solutions, the current decreased with a decrease in the pH solution. This behaviour can be attributed to the protonation of complexation sites present in the modified material, preventing the Cu(II) accumulation at the CPE-CTS. Thus, acetate buffer solution (0.1 mol L^{-1}, pH 6.0) was selected as the supporting electrolyte in further studies.

The effect of the CTS percentage (10–30% w/w) in the CPE on the voltammetric response of the sensor was evaluated. The maximum anodic current peak was obtained with 15% (w/w) of CTS in the paste and a 5.0×10^{-5} mol L^{-1} Cu(II) solution. For lower Cu(II) concentrations, a decrease in the current was observed, which can be attributed to the low amount of CTS available for the Cu(II)

complexation. On the other hand, the current decrease observed when the CTS concentration in the paste was higher than 15% can be explained by the decrease in the electronic conductivity of the modified CPE, since CTS shows poor conductivity which can not be supplied by the low concentration of graphite. Consequently, the composition of 15:20:65% (w/w/w) CTS:Nujol:graphite powder, respectively, was used in the construction of the CPE-CTS.

In stripping voltammetry the analyte pre-concentration from the solution to the electrode surface is a critical step. In most cases, a pre-concentration potential (E_{pc}) is applied for a preset time (t_{pc}) and both of these parameters exert a strong influence on the electrode voltammetric response. The effect of the E_{pc} from −0.1 to −0.7 V and a pre-concentration step carried out at open circuit potential on the anodic current peak obtained by cyclic voltammetry employing the CPE-CTS in a 5.0×10^{-5} mol L^{-1} Cu(II) solution were evaluated. At open circuit potential the pre-concentration was poor. Better results were obtained at controlled-potential, particularly at −0.4 V, which was the potential chosen to be employed in the subsequent tests.

Another important parameter that must be precisely controlled in the experiments is the pre-concentration time. Increased t_{pc} resulted in increasing anodic currents. A linear relationship was observed over 90 s, but with increasing wideness in the anodic current peak, causing a considerable loss of resolution. Therefore, the t_{pc} that provided the best relationship between voltammetric profile and current magnitude was 180 s, which was used in further experiments.

Ensuring a clean electrode after the stripping is important in order to achieve reproducible results. Thus, the conditioning potential (0.1–0.7 V) and time (0–120 s) of the anodic current supplied by the CPE-CTS were studied. The cleaning step removes adsorbed impurities and copper that remain on the electrode surface after the stripping. The studies showed that a potential of 0.5 V applied for 30 s after each experiment is sufficient to clean the electrode surface. These conditioning parameters were therefore used in all subsequent experiments.

In order to improve the sensitivity and enhance the performance of the proposed CPE-CTS, after the pre-concentration step anodic stripping was carried out by square wave voltammetry. To apply this pulse technique, experimental variables such as frequency, pulse amplitude and scan increment need to be adjusted to achieve the best relationship between current intensity and the voltammetric profile. The parameters scan increment (1–10 mV), frequency (1–50 Hz) and pulse amplitude (10–50 mV) were investigated for the CPE-CTS in 5.0×10^{-5} mol L^{-1} Cu(II) in acetate buffer solution (0.1 mol L^{-1}, pH 6.0). The measurements showed that the anodic current increased linearly with increasing scan increment up to 5 mV, remaining constant thereafter. The highest anodic current was obtained with a scan increment of 5 mV and frequency of 30 Hz, but a wide peak was generated. A peak with good resolution and current intensity was obtained with a scan increment of 3 mV and frequency of 10 Hz. The pulse amplitude did not change significantly the profile of the above-described square wave voltammogram. A pulse amplitude of 50 mV was thus used in all subsequent experiments.

3.4. Method validation

3.4.1. Robustness

After optimisation of the experimental conditions, a robustness study of the proposed method was carried out. The factors (González & Herrador, 2007) chosen arbitrarily to be evaluated were solution pH and CTS percentage in the modified carbon paste electrode. One of the fundamental differences between optimisation and robustness studies is the interval under investigation (González & Herrador, 2007). While in the latter the interval is very narrow, in the former it is wider. For this reason, the solution pH was varied between 5.7 and 6.3 (around 6.0, the optimised solution pH) and the CTS percentage between 14.7% and 15.3% (around 15%, the optimised CTS percentage) to carrying out the robustness study. The anodic current peak employing the CPE-CTS in a 5.0×10^{-5} mol L^{-1} Cu(II) did not change significantly (according to the statistical analysis by ANOVA) when the pH solution was modified between 5.7 and 6.3, or when the CTS percentage used for electrode preparation was between 14.7% and 15.3%. Therefore, the proposed method offers an acceptable level of robustness.

3.4.2. Interferences

The Cu(II) determination employing the CPE-CTS can be influenced by interfering species such as transition metal ions, which form stable complexes with 8-hydroxyquinoline-5-sulphonic acid (Martins et al., 2004) present in the modified material. Thus, the interference of Ni(II), Pb(II), Zn(II), Cd(II) and Fe(III) ions in the stripping voltammogram of Cu(II) was studied for molar ratios of interferent ion/Cu(II) of 0.1, 1.0 and 10. All steps in the Cu(II) determination were carried out in the presence of the potential interferents. Only Fe(III) caused interference, generating oxidation peaks that partially hindered the determination of Cu(II) when Fe(III) was present in a 10-fold molar excess with respect to Cu(II). For other ratios and other potential interferents, the change in the anodic current of the stripping voltammogram of Cu(II) was less than 12%. Although microspheres of chitosan crosslinked with 8-hydroxyquinoline-5-sulphonic acid can act as an adsorbent for several metallic ions (Vitali et al., 2008), the interference was greatly minimised by the application of the pre-concentration potential. At −0.4 V, the potential chosen to pre-concentrate Cu(II), the metallic ions with a reduction potential more negative than −0.4 V are not reduced (pre-concentrated) at the electrode surface. These results show that the proposed sensor can be used for Cu(II) determination in solutions containing the tested ions without a notable loss in the analytical response.

3.4.3. Square wave voltammograms and calibration curve

The anodic stripping voltammograms for different Cu(II) concentrations under the optimised conditions are shown in Fig. 4. In the inset, the respective calibration curve obtained is represented, while the validation parameters obtained for Cu(II) determination employing the CPE-CTS are given in Table 1. From these data it can be seen that the current peak increases linearly with increasing Cu(II) concentration in the range of 5.0×10^{-7} to 1.4×10^{-5} mol L^{-1} ($\Delta i_p = -0.70 + 0.12 \times 10^7$ [Cu(II)], $r = 0.9990$). However, for higher Cu(II) concentrations a negative deviation from linearity was observed due to the electrode surface saturation. Also, a slight shift toward more positive potentials is observed in the peak potential with increasing Cu(II) concentration. The detection and quantification limits calculated (Table 1) show that the proposed sensor has a high sensitivity toward Cu(II) detection. The relative standard deviation ($n = 8$) was lower than 3.0% for the determination of Cu(II) in solutions with concentrations of 6.0×10^{-6}, 5.0×10^{-5} and 1.5×10^{-4} mol L^{-1} indicating that the electrode provides reliable data with excellent precision. In this study, the concentration of 1.5×10^{-4} mol L^{-1} Cu(II) is out of the linear range of the calibration curve, however, the relative standard deviation was practically the same as those observed for the other concentrations lying within the calibration curve. This behaviour indicates that the electrode provides reliable data even for solutions with concentrations slightly higher than those of the calibration curve. The repeatability for ten measurements of the current peak for solutions of 5.0×10^{-5} mol L^{-1} Cu(II) under optimised conditions was excellent, with relative standard deviations of 1.31%. The reproducibility of the current peak was tested over four

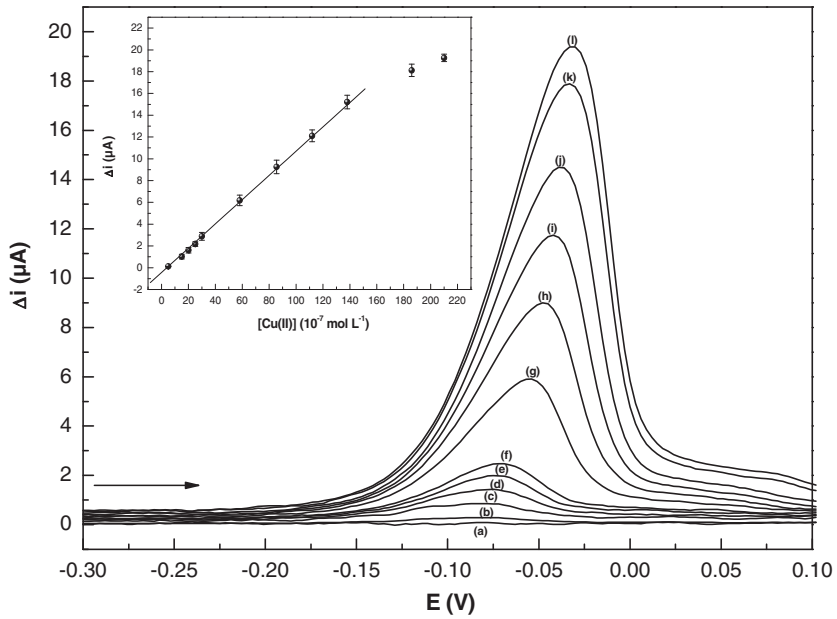

Fig. 4. Square wave voltammograms obtained using the proposed CPE-CTS for (a) blank acetate buffer solution (0.1 mol L^{-1}, pH 6.0) and Cu(II) solutions at the following concentrations (mol L^{-1}): (b) 5.02×10^{-7}; (c) 1.51×10^{-6}; (d) 2.01×10^{-6}; (e) 2.51×10^{-6}; (f) 3.00×10^{-6}; (g) 5.90×10^{-6}; (h) 8.60×10^{-6}; (i) 1.10×10^{-5}; (j) 1.4×10^{-5}; (k) 1.9×10^{-5}; (l) 2.1×10^{-5} mol L^{-1} at $E_{pc} = -0.4$ V; $t_{pc} = 180$ s; $f = 30$ Hz; $a = 50$ mV; $\Delta E_s = 3$ mV. Inset: the calibration curve. The error bars indicate the uncertainty of three measurements ($n = 3$).

Table 1
Validation parameters for Cu(II) determination with the CPE-CTS using anodic stripping square wave voltammetry.

Parameter	Value
Linear range (mol L^{-1})	5.0×10^{-7} to 1.4×10^{-5}
Slope (μA L mol^{-1})	0.12×10^{7}
Correlation coefficient (r)	0.9990
Detection limit (mol L^{-1})[a]	5.48×10^{-8}
Quantification limit (mol L^{-1})[b]	1.83×10^{-7}
Relative standard deviation (%)[c]	0.72–2.9
Repeatability of peak current[d]	1.31
Reproducibility of peak current[d]	2.73

[a] DL = 3S/slope (S = standard deviation of 10 successive measurements of the blank) (Marino, Bergamini, Teixeira, & Cavalheiro, 2003).
[b] QL = 10S/slope.
[c] Values obtained for eight measurements carried out in 6.0×10^{-6}, 5.0×10^{-5} and 1.5×10^{-4} mol L^{-1} Cu(II) solutions.
[d] Relative standard deviation, %. Values obtained for 10 measurements carried out in 5.0×10^{-5} mol L^{-1} Cu(II) solutions.

days using different solutions prepared in the concentration of 5.0×10^{-5} mol L^{-1} Cu(II). The relative standard deviation was 2.73%. When compared to other modified carbon paste electrodes employed for Cu(II) determination, the CPE-CTS sensor also showed good performance. For example, a carbon paste electrode modified with 3,4-dihydro-4,4,6-trimethyl-2(1*H*)-pyrimidine thione for use in potentiometry showed a linear range of 9.8×10^{-7} to 7.6×10^{-2} mol L^{-1} with a detection limit of 7.0×10^{-7} mol L^{-1} (Abbaspour & Moosavi, 2002). A linear range of 8.0×10^{-7} to 1.0×10^{-5} mol L^{-1} and a detection limit of 2.0×10^{-7} mol L^{-1} was obtained with a carbon paste electrode modified with SBA-15 nanostructured silica organofunctionalized with 2-benzothiazolethiol for determination of Cu(II) by differential pulse anodic stripping voltammetry (Cesarino et al., 2008). Values of 1.0×10^{-7} to 1.0×10^{-2} mol L^{-1} and 8.0×10^{-8} mol L^{-1} were obtained for the linear range and detection limit, respectively, for a carbon paste electrode modified with *N*-(2-aminoethyl)-3-aminopropyl-trimethoxy silane and 2,2-dipyridyl ketone for use in potentiometric determinations of Cu(II) (Javanbakht et al., 2007). Lower detection limits were obtained only when the carbon paste electrode was modified with 2-aminothiazole organofunctionalized silica (Takeuchi et al., 2007) and aminopropyl-grafted silica gel (Etienne et al., 2001) for use with anodic stripping voltammetry. The values obtained were 3.1×10^{-8} and 3.0×10^{-9} mol L^{-1}, respectively. The above-described methods for modification of the carbon paste electrode were based on synthesis, while in the method used to prepare the CPE-CTS electrode the chitosan obtained by the spray drying technique was crosslinked with the chelating agent 8-hydroxyquinoline-5-sulphonic acid and glutaraldehyde. The main advantages of the method using the spray drying technique are simplicity, versatility and fast obtainment of microspheres. On the other hand, the methods using synthesized materials are time consuming and make use of toxic reagents. In conclusion, the results obtained demonstrate that the method developed employing the CPE-CTS sensor shows excellent accuracy, precision, reproducibility and sensitivity. In addition, the proposed sensor shows excellent performance for Cu(II) determination when compared to other modified carbon paste electrodes.

3.4.4. Analytical application and recovery

In order to evaluate the performance of the CPE-CTS in practical analytical determinations, quantitation of Cu(II) was carried out experimentally in instant coffee samples. The procedures used to evaluate the accuracy of a method include the use of certified reference materials, comparison of methods, standard addition and recovery experiments. As no certified reference material for instant coffee is available, in this study the proposed sensor and analytical method were evaluated for Cu(II) determination employing the comparative and standard addition methods in three samples of instant coffee (A, B and C) and calculating the recovery factor. The results obtained using the proposed sensor were close to those obtained using the ET AAS method (Table 2). The *t*-test was carried out in order to check the validity of the data obtained. At the 95% confidence level, the statistical treatment showed that there is no difference between the data obtained using the two methods, except for one sample. Also, similar performance of the two methods was verified through statistical examination of the values obtained

Table 2
Determination of Cu(II) in instant coffee by ET AAS and CPE-CTS sensor and recovery experiments using the electroanalytical method.

Sample	ET AAS ($\mu g\ g^{-1}$)[a]	CPE-CTS ($\mu g\ g^{-1}$)[a]	t[b]	F[c]	Added (μg)[d]	Found (μg)[d]	Recovery (%)[d]
A	0.134 ± 0.012	0.145 ± 0.074	0.73	39	0.5	0.45 ± 0.04	90.0
					1.0	1.10 ± 0.06	110.0
B	0.184 ± 0.014	0.211 ± 0.022	4.7	2.7	0.5	0.56 ± 0.01	112.0
					1.0	1.20 ± 0.01	120.0
C	0.115 ± 0.013	0.125 ± 0.011	1.8	1.4	0.5	0.59 ± 0.03	118.0
					1.0	1.20 ± 0.05	120.0

[a] Mean ± SD (standard deviation), confidence level of 95%; $n = 3$.
[b] $t_{critical} = 4.3$ ($P = 0.05$ with 2° of freedom).
[c] $F_{2/2\ critical} = 19.0$ ($P = 0.05$).
[d] Data obtained using CPE-CTS sensor.

in *F*-tests. The fact that different concentrations of Cu(II) were found using both methods in the samples analyzed is not surprising since the coffee samples were produced in areas distant from one another. As a consequence, the mineral soil composition, as well as the fertilizers used, could influence the results. Similar results were found by other authors (Oleszczuk et al., 2007; Onianwa et al., 1999) for the content of copper in solid coffee samples from different areas around the world, however, no results could be found in the literature concerning the content of copper in samples of instant coffee. The standard addition method and the recovery experiments were carried out using the electroanalytical sensor. The recovery values ranged from 90.0% to 110.0% for sample A, 112.0% to 120.0% for sample B, and 118.0% to 120.0% for sample C. According to the literature (Ribani, Bottoli, Collins, Jardim, & Melo, 2004), the acceptable range of recovery values is generally between 70% and 120% and, depending on the analytic complexity of the sample, may be extended to 50%–120%. The results obtained indicate that the accuracy of the proposed method using the CPE-CTS is not affected by the matrix complexity. Taking into consideration these results we can conclude that the sensor is suitable for Cu(II) determination in instant coffee samples.

4. Conclusions

A novel carbon paste electrode containing chitosan crosslinked with the chelating agent 8-hydroxyquinoline-5-sulphonic acid and glutaraldehyde was developed for determination of Cu(II). The analysis was carried out employing a pre-concentration step at controlled-potential and detection by square wave voltammetry. The results showed that the response of the proposed modified electrode was more than six times better than that of the bare carbon paste electrode. The optimisation of experimental conditions showed that the pH of the solution strongly affects the voltammetric response and pH 6.0 was the optimal value found. The validation parameters determined using the optimal experimental conditions showed a linear range for quantitative determination of Cu(II) from 5.0×10^{-7} to 1.4×10^{-5} mol L^{-1} and good detection limit with a pre-concentration time of 180 s. The analytical application of the method employing standard addition showed a recovery that was only slightly dependent on the matrix complexity, verifying the viability of the proposed sensor for Cu(II) determination. The use of the spray drying technique in the preparation of CPE-CTS highlighted the great potential of this technique as an alternative for developing new compounds for further use in the construction of modified carbon paste electrodes and for application in various electroanalytical processes.

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