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Direct Quantification of Cu^{2+} in *Cachaça* Using a Solid Paraffin-Based Carbon Paste Electrode Chemically Modified with 2-Aminothiazole-Silica-Gel

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A solid paraffin-based carbon paste electrode modified with 2-aminothiazole-silica-gel (SPCPE-SiAt) was used for direct quantification of Cu^{2+} in sugarcane spirits (*cachaça*) samples by anodic stripping voltammetry. The composite formed between carbon powder and solid paraffin was totally stable in ethanol/water mixtures enabling the quantification of Cu^{2+} directly in the samples without any pretreatment procedure. Under optimized conditions, LOD and LOQ were, respectively 1.8 µmol L^{-1} (0.11 mg L^{-1}) and 5.4 µmol L^{-1} (0.33 mg L^{-1}). These values are remarkably below the maximum Cu^{2+} content allowed by Brazilian (5 mg L^{-1}) and European legislation (2 mg L^{-1}). This fact strongly suggests that the proposed method can be adopted for *cachaça* quality control regarding Cu^{2+} content. Four commercial samples were analyzed by using the proposed method and the results were compared with those obtained from FAAS official method. t-test has shown that there are no statistical differences between the results provided by both methods at 95% confidence level. In addition, F-test showed that the precision of these methods are statistically equivalent. These results are a clear demonstration of the good accuracy of the proposed electroanalytical method.

Keywords: *Cachaça*, Copper determination, Anodic Stripping Voltammetry, Carbon Paste electrode, 2-Aminothiazole silica gel

1. INTRODUCTION

Brazilian sugarcane spirits, whose name is established by Brazilian legislation as *cachaça*, is an alcoholic beverage containing from 38 to 48% ethanol by volume [1,2]. *Cachaça* industry plays an important role on Brazilian economy since it is responsible for generation of almost 0.5 million direct jobs producing more than US$ 2 billion per year [1,3]. *Cachaça* is produced by distillation of previously fermented sugarcane juice and this process is usually conducted in distillers fabricated with copper [4]. Copper stills are preferred because this metal is able to improve sensorial properties of *cachaça* producing a beverage with better flavor and fragrance [4]. Despite the advantages of copper stills for the final quality of *cachaça*, the contamination of this beverage with Cu^{2+} represents a serious risk to consumers, since excess of Cu^{2+} is able to cause deleterious effects on human health, such as Wilson's disease [4,5]. Moreover, Cu^{2+} can catalyze the formation of ethyl carbamate [6], which is a potential carcinogenic specie [1]. *Cachaça* is contaminated with Cu^{2+} during distillation processes in which a basic copper carbonate [$Cu_2CO_3(OH)_2$] is formed on distiller walls [4,5]. Acidic alcoholic vapors produced from distillation process are able to dissolve this basic copper salt, introducing significant amounts of Cu^{2+} in the produced *cachaça*. For this reason, Cu^{2+} content is under regulation by Brazilian and international legislation. In Brazil, the maximum concentration of Cu^{2+} allowed in *cachaça* is 5 mg L^{-1} [3,7], however some countries (mainly European) are even more restrictive limiting Cu^{2+} concentration in sugarcane spirits at 2 mg L^{-1} [7]. Therefore, the development of analytical methods able to quantify Cu^{2+} in *cachaça* in a fast, inexpensive and reliable way is very important in order to guarantee the quality of this beverage and to protect consumers' health. Moreover, rigorous control of the content of Cu^{2+} in *cachaça* is indispensable for the successful introduction of this beverage in the global market.

Some methods for Cu^{2+} quantification in *cachaça* are found in literature, most of them are based on spectroscopic or electroanalytical techniques. Among spectroscopic techniques, visible spectrophotometry has been used after Cu^{2+} chemical derivatization with biquinoline [5]. The use of multivariate optimization combined with visible spectrophotometry has been reported as well [8]. Flame Atomic Absorption Spectrophotometry (FAAS) [4] and Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) [9] coupled with internal standardization have also been successfully used. Electroanalytical methods found in literature for Cu^{2+} quantification in *cachaça* are based on potentiometric titration using a copper membrane electrode [10] and anodic stripping voltammetry at a Hanging Drop Mercury Electrode (HDME) [11]. Despite the success of HDME for Cu^{2+} quantification in *cachaça* samples, currently there is a growing interest in mercury-free electroanalytical methods mainly due environmental concerns. Thus, HDME has been gradually replaced by solid electrodes and chemically modified solid electrodes which have allowed the quantification of several metal ions in different samples including As^{3+} in water samples [12], Cu^{2+} in water samples [13,14], Pb^{2+} in tap water [15], Cu^{2+} and Pb^{2+} in biofuels [16-19] and gasoline [20], Cd^{2+} and Zn^{2+} in seawater [21,22], etc.

Among solid electrodes, Carbon Paste Electrodes (CPEs) have received great attention due several attractive properties such as low residual current, wide useful potential window, chemical inertness, ease surface renewal, low cost and easiness of prepare. Thus, CPEs have brought impressive advances in electroanalysis which are discussed in several review articles [23-25] including a celebrative review on the occasion of the 50th anniversary of discovering carbon paste by Professor Ralph Norman Adams celebrated in 2008 [26]. Despite the success of CPEs in electroanalysis the use of this kind of electrode in solutions with high content of organic solvents is strongly limited. This limitation is associated with the instability of the binder agent (usually mineral oil) in presence of organic solvents leading to desegregation of the paste and therefore deterioration of the electrode. To overcome this problem, several alternative binders able to produce more rigid and stable composites have been proposed in literature [27-31]. Among them, solid paraffin, a binder agent introduced by

Kauffman and Petit [32,33] has attracted attention because it presents low cost and allows preparing electrodes in a fast and simple way.

Our group has demonstrated that Solid Paraffin-based Carbon Paste Electrodes chemically modified with 2-Aminothiazole-Silica gel (SPCPE-SiAt) are totally stable in ethanol media, which has allowed the use of these electrodes for quantification of Cu^{2+} [34] and Ni^{2+} [35] directly in commercial ethanol fuel samples. SPCPE-SiAt was very efficient to extract metal species from ethanol fuel samples by chelation at 2-aminothiazole groups. After extraction, the electrode was transferred to an electrochemical cell containing a supporting electrolyte in which the metallic specie accumulated at electrode surface was detected by stripping voltammetry. This strategy was very efficient to prevent interference effects. However, extraction step required a very long preconcentration time (20 min) to achieve the desired sensitivity which led to very low analytical frequency. Cesarino *et al.* [36,37] have demonstrated that the combination between chelating properties of organofunctionalized silica and electrodeposition process is very efficient to preconcentrate metal ions at electrode surface in a fast way. This strategy has allowed developing high analytical frequency and sensitive stripping voltammetric methods for simultaneous determination of several metal ions in natural water and ethanol fuel samples [36,37]. Recently, our group has demonstrated that this strategy is also efficient in order to achieve fast and simultaneous anodic stripping voltammetric determination of Cu^{2+} and Pb^{2+} in water samples using a SPCPE-SiAt [38]. This work has also showed that the use of solid paraffin as binder agent presents some advantages over mineral oil because solid paraffin produces a more robust composite which presents lower background currents providing higher sensitivity and lower detection limits (LOD).

Despite the highly attractive features of CPEs chemically modified with organofunctionalized silica for Cu^{2+} quantification in sugarcane spirits samples, there is only one report about it in literature using conventional CPEs prepared with mineral oil as binder agent [39]. To the best of our knowledgement, the literature does not present works describing the use of a composite totally stable in ethanolic solutions for Cu^{2+} determination directly in sugarcane spirits samples. This stability is very important in order to develop a sensor with high lifetime. In this context this is the first work describing de use of a SPCPE-SiAt for direct determination of Cu^{2+} in *cachaça* samples in a very fast and sensitive way without any sample pretreatment procedure.

2. EXPERIMENTAL

2.1. Reagents

Stock solutions of copper were prepared from Cu^{2+} Atomic Absorption 1000 mg L^{-1} standard solution (Fluka). 0.1 mol L^{-1} acetate buffer solutions (pH = 4.7) were used as supporting electrolyte in all electrochemical experiments. All used solutions were prepared with ultrapure water (ASTM type I, resistivity >18 MΩ cm) produced from a Megapurity® water purification system. Absolute ethanol (Vetec) was used to perform studies in ethanol:water mixtures.

Silica gel (Merck) with specific surface area between 486-520 m^2 g^{-1} and average pore diameter of 0.6 nm was used to construct SPCPE-SiAt. 2-aminothiazole (Aldrich) was used to achieve silica functionalization. The 2-aminothiazole organofunctionalized silica gel was prepared according to the procedure previously described by Roldan *et al.*[40] Spectroscopic carbon powder with 1-2 µm particle size from Merck was used to obtain the SPCPEs. Solid paraffin from Synth was used as the binder agent.

2.2. Apparatus

Differential pulse anodic stripping voltammetry was performed using a µAUTOLAB type III potentiostat/galvanostat coupled to a microcomputer and controlled by GPES 4.9 software. Electrochemical experiments were performed at room temperature in an one-compartment electrochemical cell filled with 10 mL of analyzed solution. A three electrode system comprising a platinum wire auxiliary electrode, a KCl saturated-Ag/AgCl ($AgCl_{sat}$) reference electrode and a homemade SPCPE working electrode was used in all electrochemical experiments. FAAS experiments carried out for comparative purposes were performed at a SHIMADZU-FAAS spectrophotometer model AA-680.

2.3. Electrode preparation

SPCPEs-SiAt were prepared by hand-mixing 2-aminothiazole functionalized silica and carbon powder at the ratio 20%:40% (w:w). This mixture was homogenized in a mortar and pestle for 20 min and subsequently it was added to melted paraffin whose mass percentage was always kept at 40%. This new mixture was again homogenized in a thermostated bath (65-75^{o}C) for 10 min. This final mixture was placed in an insulin syringe (internal diameter of 5 mm) containing a copper piston to provide electric contact. Appropriate packing was achieved by pressing the composite against a weighing paper placed on a flat piece of glass. Before their first use, all electrodes were hand-polished on a weighing paper until a smooth surface was obtained.

2.4. Analytical procedure

Before their utilization, SPCPEs-SiAt were preconditioned by applying +0.35 V *vs*. Ag/$AgCl_{sat}$ for 60 s. This electrochemical procedure was adopted after each measurement.

The analytical procedure comprised three steps: 1) metallic ions electrodeposition at SPCPE-SiAt, 2) differential pulse anodic stripping voltammograms recording, 3) electrochemical surface regeneration by applying +0.35 V *vs*. Ag/$AgCl_{sat}$ for 60 s. All these steps were conducted directly in *cachaça* samples containing supporting electrolyte (acetate buffer 0.1 mol L^{-1}). Analytical parameters involved in the proposed method were submitted to optimization studies which were conducted in a spiked *cachaça* sample. This procedure was adopted in order to keep the conditions in optimization studies as close as possible to real analysis conditions. Four *cachaça* samples were analyzed by the proposed method and the obtained results were compared with those obtained by official method according Brazilian legislation (NBR-13921 [41]). In order to keep sample properties almost unchanged for voltammetric analyses, acetate buffer was prepared by adding the reagents directly in the sample. This procedure avoids drastic sample dilution because just 58 µL of glacial acetic acid and 136 mg of $H_3CCOONa.3H_2O$ were added to 10 mL of the sample. No additional sample pretreatment was adopted.

3. RESULTS AND DISCUSSION

3.1. Preliminary studies

In a previous work [34] we have demonstrated that the best composition of SPCPEs-SiAt is 40% (m/m) of solid paraffin, 40% (m/m) of carbon powder and 20% (m/m) of SiAt. We have observed that higher contents of SiAt lead to a decrease in peak currents for copper and provide poor voltammetric profiles.

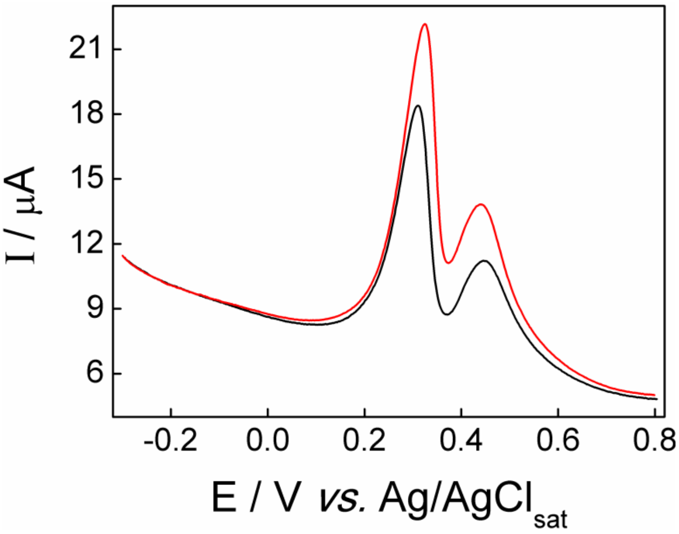

Figure 1. Differential pulse anodic stripping voltammograms recorded in a mixture ethanol:water (40:60 v/v) containing 0.1 mol L^{-1} acetate buffer solution (pH = 4.7) spiked with 25 µmol L^{-1} Cu^{2+}. Accumulation potential: −0.3 V; accumulation time: 180 s. Scan rate: 10 mV s^{-1}; pulse amplitude: 50 mV; pulse width: 25 ms. (—) SPCPE-Si(20%). (—) SPCPE-SiAt(20%).

These observations are probably associated to an increase in electrical resistance of the electrode promoted by the replacement of conductive carbon powder by non-conductive SiAt. On the other hand, SiAt contents lower than 20% (m/m) led to lower sensitivity. Solid paraffin contents have a strong influence on the mechanical strength of the composite. It was observed that contents lower than 40% (m/m) lead to unacceptably fragile composites. Higher solid paraffin contents have produced highly resistive composites which forbid their practical application as electrodes. Therefore, based on our previous experience, SPCPEs-SiAt containing 40% (m/m) of solid paraffin, 40% (m/m) of carbon powder and 20% (m/m) of SiAt were also used in the present work.

Initially, the voltammetric response of copper at SPCPE-SiAt(20%) was compared with that observed at a non-functionalized SPCPE-silica (SPCPE-Si). This study was performed in a mixture ethanol:water (40:60 v/v) containing acetate buffer and spiked with 25 µmol L^{-1} of Cu^{2+}. Differential pulse anodic stripping voltammograms obtained in this study are presented in Figure 1.

Figure 1 shows that copper anodic stripping voltammetric peaks are observed not only at SPCPE-SiAt(20%) but also at SPCPE-Si(20%) indicating that both electrodes are able to detect copper. These peaks are not observed when the same experiments are performed in absence of Cu^{2+} (data not shown). From the voltammograms presented at Figure 1 it can be observed double peaks associated with the anodic stripping of copper. These double peaks are observed at both electrodes at +0.3 V (main peak) and at +0.44 V (secondary peak). It is important to mention that in a previous work we have observed that this secondary peak is absent at a non modified SPCPE when the preconcentration step is performed in a totally aqueous solution [38]. Moreover we have observed that if preconcentration step is performed at open circuit conditions in ethanol media the secondary peak is also absent [34]. Thus, although the origin and nature of these double peaks are not completely understood we can conclude that they are dependent on both the presence of silica or organofunctionalized silica at electrode surface and the procedure adopted for copper accumulation. The secondary peak is probably a copper adsorption post-peak which is present only when the experimental conditions enable very strong adsorption, i.e. electrodeposition at SPCPE-SiAt/Si.

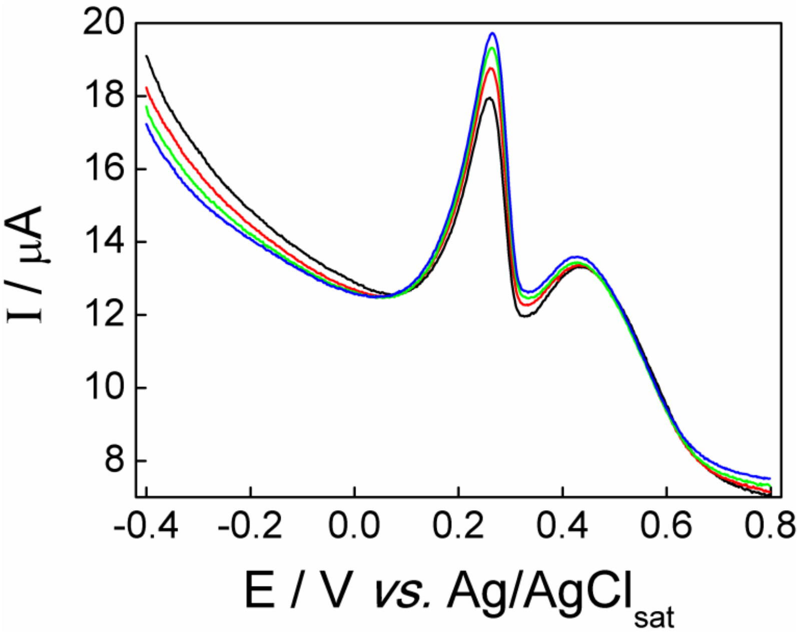

Figure 2. Successive differential pulse anodic stripping voltammograms recorded in a *cachaça* sample containing acetate buffer 0.1 mol L^{-1} (pH = 4.7) and spiked with 2 µmol L^{-1} Cu^{2+}. Accumulation potential: −0.4 V; accumulation time: 180 s. Scan rate: 10 mV s^{-1}; pulse amplitude: 50 mV; pulse width: 25 ms. (—) 1st (—) 2nd (—) 3rd (—) 4th voltammetric scan.

Only the main peak at +0.3 V was used for analytical studies due its higher intensity which allows the development of a more sensitive electroanalytical method. Peak currents (i_p) for this peak were calculated from a linear extrapolation of its pre-peak baseline. Figure 1 clearly shows that SPCPE-SiAt provides higher sensitivity than SPCPE-Si and therefore the organofunctionalized silica presents analytical advantages over non-functionalized silica for copper quantification. It was observed that i_p provided by SPCPE-SiAt is approximately 34% higher than that obtained with Si-SPCPE which is a considerable gain in sensitivity. This increase of i_p is a demonstration that the combination between electrodeposition and chelation at 2-aminothiazole is efficient to improve the sensitivity.

After the demonstration that SPCPE-SiAt presents better analytical performance than SPCPE-Si, the repeatability of the voltammetric measurements was evaluated. This study was performed in a *cachaça* sample containing acetate buffer 0.1 mol L^{-1} and spiked with 2 µmol L^{-1} Cu^{2+}. Figure 2 presents the successive differential pulse anodic stripping voltammograms obtained in this study. From Figure 2 it can be observed a continuous increase on i_p values for successive cycles, indicating that copper previously accumulated at electrode surface is not completely removed during the voltammetric scan. This behavior can be explained regarding copper is oxidized to Cu^{2+} during the voltammetric measure and this ionic form can partially remain adsorbed onto electrode surface via chelation at S and N atoms of 2-aminothiazole groups leading to the observed memory effect. The results presented in Figure 2 clearly show that these experimental conditions lead to unacceptably low repeatability. In order to overcome this limitation, an electrochemical regeneration step was introduced which consists in the application of +0.35 V during 60 s under constant stirring. This procedure has considerably improved the repeatability of the measurements, as shown in Figure 3.

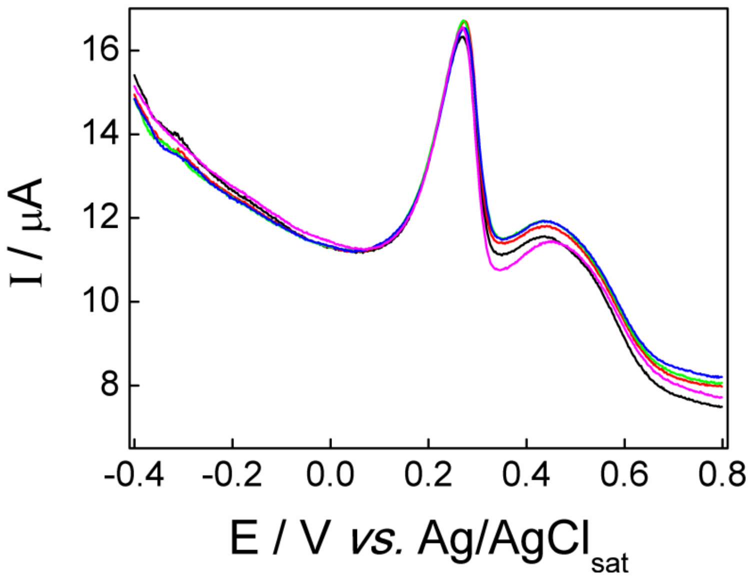

Figure 3. Successive differential pulse anodic stripping voltammograms recorded in a *cachaça* sample containing acetate buffer 0.1 mol L^{-1} (pH = 4.7) and spiked with 2 µmol L^{-1} Cu^{2+}. Accumulation potential: −0.4 V; accumulation time: 180 s. Scan rate: 10 mV s^{-1}; pulse amplitude: 50 mV; pulse width: 25 ms. Electrochemical regeneration adopted after each scan: +0.35 V for 60 s. (—) 1st (—) 2nd (—) 3rd (—) 4th (—) 5th voltammetric scan.

The objective of the electrochemical regeneration is to keep the electrode positively charged removing Cu^{2+} from electrode surface by electrostatic repulsion. The electrochemical regeneration was very efficient to remove copper from electrode surface which was demonstrated by the good repeatability observed when this procedure was adopted. Adopting this electrochemical regeneration procedure and using the same electrode surface, it was observed a relative standard deviation (RSD) for i_p values of 1.9% (n = 5). Besides its efficiency, the electrochemical regeneration is very practical, because it avoids excessive electrode manipulation and polishing after each measure, contributing to decrease the time consumed in the analyses.

3.2. Optimization of operational parameters

As above mentioned, the composition of SPCPE-SiAt was not submitted to optimization studies, based on our previous experience with this kind of electrode the following composition was chosen for analytical applications: 40% (m/m) of solid paraffin, 40% (m/m) of carbon powder and 20% (m/m) of SiAt. Supporting electrolyte was also not submitted to optimization studies. It is well known that a slight acidic media is the most appropriated for stripping analysis of metals because this media prevents metal ion hydrolysis. Extremely low pH values must be avoided because they promote intensive hydrogen evolution during electrodeposition step leading to poor sensitivity. In addition, intensive hydrogen evolution could promote composite desegregation damaging electrode surface and comprising the repeatability. Finally extremely acidic medium should be avoided because in this condition N and S atoms from 2-aminothiazole would be protonated making impossible the preconcentration of metallic cations. Thus, a pH close to 5 seems to be the most appropriate to perform metal voltammetric stripping analysis. Buffer acetate is very effective in this region of pH, therefore it was selected as supporting electrolyte in this work.

All other operational parameters involved in the analytical procedure were optimized. The first optimized parameter was preconcentration time (t_{pc}). This study was conducted in a *cachaça* sample containing acetate buffer 0.1 mol L^{-1} and spiked with 2 μmol L^{-1} Cu^{2+}. Preconcentration potential (E_{pc}) was kept at −0.2 V. The effect of preconcentration time was evaluated from 60 to 300 s, higher preconcentration time were not evaluated because the objective of this work is to develop an analytical method with high analytical frequency. Moreover, the content of copper in commercial samples is relatively high making unnecessary long preconcentration times. In the studied range, a linear relationship between i_p and preconcentration time was observed according to the equation: $i_p(\mu A) = -0.02 + 1.41\ t_{pc}$ (min) with linear correlation coefficient (R) equal to 0.9986. In order to obtain the best compromise between sensitivity and analytical frequency, t_{pc} = 180 s was adopted in subsequent studies.

E_{pc} was the second operational parameter optimized. This study was performed using the same experimental conditions described above and keeping preconcentration time at 180 s. The dependence of i_p on E_{pc} is shown in Figure 4.

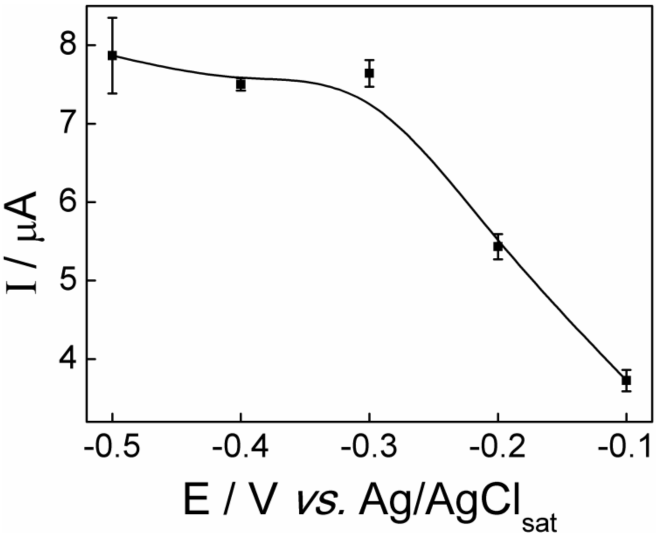

Figure 4. Dependence of i_p on E_{pc}. I_p values obtained from differential pulse anodic stripping voltammograms recorded in a *cachaça* sample containing acetate buffer 0.1 mol L^{-1} (pH = 4.7) and spiked with 2 μmol L^{-1} Cu^{2+}. Accumulation time: 180 s. Scan rate: 10 mV s^{-1}; pulse amplitude: 50 mV; pulse width: 25 ms. Electrochemical regeneration adopted after each scan: +0.35 V for 60 s.

From Figure 4 it can be observed that E_{pc} more negative than −0.3 V are unable to improve sensitivity. On the other hand potentials less negative than −0.3 V have caused a remarkable decrease on i_p values, compromising the sensitivity. Regarding that excessively negative potentials can compromise selectivity in voltammetric stripping analysis, E_{pc} = −0.3 V was adopted in subsequent experiments.

The parameters involved in DPV were also submitted to optimization studies, keeping the same experimental conditions above described and the other operational parameters were kept at the previously optimized values. These optimization studies were conducted in order to achieve the best compromise between peak width and peak intensity. Following this criteria the best voltammetric conditions found were: pulse amplitude of 50 mV, pulse width of 25 ms and scan rate equal 10 mV s^{-1}.

3.3. Analytical studies

Under the previously optimized conditions, differential pulse anodic stripping voltammograms were recorded at different Cu^{2+} concentrations in a mixture ethanol:water (40:60 v:v) containing acetate buffer 0.1 mol L^{-1}. These voltammograms were used to construct an analytical curve and the results are presented in Figure 5.

It was observed that analytical curve presented a linear relationship between i_p and Cu^{2+} concentration from 0.3 to 5.0 mg L^{-1} (5 to 75 μmol L^{-1}) according to the equation: $i_p(\mu A) = -0{,}14 + 5.6\ C_{Cu2+}$ (mg L^{-1}) with R = 0.9997. The obtained LOD was 0.11 mg L^{-1} (1.8 μmol L^{-1}) and the quantification limit (LOQ) was equal to 0.33 mg L^{-1} (5.4 μmol L^{-1}). LOD and LOQ were determined by the equations: LOD = 3 sd/(slope of analytical curve) and

LOQ = 10 sd/(slope of analytical curve) [42]. sd corresponds to standard deviation of the blank. The error of the intercept of analytical curve was used to determine sd. The obtained LOD and LOQ were significantly higher than those obtained when SPCPE-SiAt was used to determine copper in water samples [38]. This loss of sensitivity can be attributed to the presence of ethanol. Several works have demonstrated that ethanol causes a remarkable decrease on the intensity of anodic stripping voltammetric peaks for many metal ions [17,19,43]. Despite these higher LOD and LOQ compared with those obtained in totally aqueous medium [38], their values are considerably below the maximum copper amount allowed in *cachaça* by Brazilian (5 mg L^{-1}) and international (2 mg L^{-1}) legislation. This fact suggests that the proposed method can be successfully used for reliable quantification of copper in *cachaça* samples.

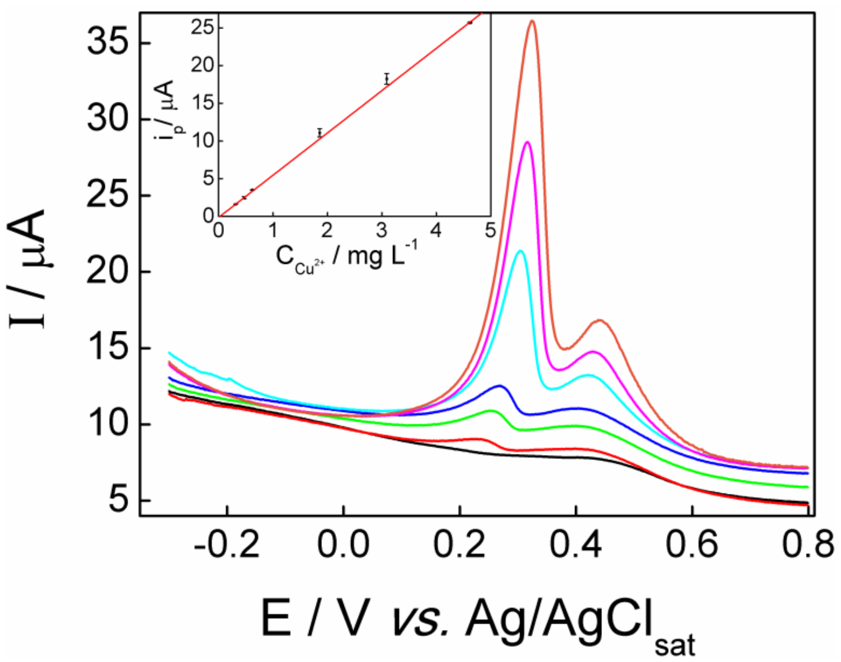

Figure 5. Differential pulse anodic stripping voltammograms recorded in a mixture ethanol:water (40:60 v:v) containing 0.1 mol L^{-1} acetate buffer solution spiked with: (—) 0, (—) 0.32; (—) 0.48; (—) 0.63; (—) 1.9; (—) 3.2 and (—) 4.8 mg L^{-1} de Cu^{2+}. $E_{pc} = -0.3$ V; $t_{pc} = 180$ s. Pulse amplitude: 50 mV; Scan rate: 10 mV s^{-1}; pulse width: 25 ms. Inset: analytical curve. Electrochemical regeneration adopted after each scan: +0.35 V for 60 s.

Compared with AAS-based methods described in literature for Cu^{2+} quantification in *cachaça* samples, the proposed method has the advantage of using a simple and relatively inexpensive instrumentation. In addition, the proposed voltammetric method does not require any chemical Cu^{2+} derivation step which is an advantage over visible spectrophotometric methods previously reported in literature. Compared with other electroanalytical methods described in literature for Cu^{2+} quantification in *cachaça* samples, the present method is faster than potentiometric titration and it is mercury-free. Moreover, the composite used in this work is totally stable in ethanol:water mixtures which means that the proposed electrode presents a very long lifetime. Therefore, the main advance for

Cu^{2+} determination in *cachaça* samples introduced by this method is provided by the high stability of the electrode which allows Cu^{2+} determination directly in the samples in a very simple, fast and inexpensive way with no needing of chemical derivation step or sample pretreatment procedures.

3.4. Real samples analyses

SPCPEs-SiAt were used to determine Cu^{2+} in four commercial *cachaça* samples acquired from local market. Two of these samples were produced by industrial processes and the others were home-made produced. Each one of these samples was submitted to three replicate determinations by standard addition method. Figure 6 shows differential pulse anodic voltammograms and the respective standard addition curve obtained for one of the analyzed samples.

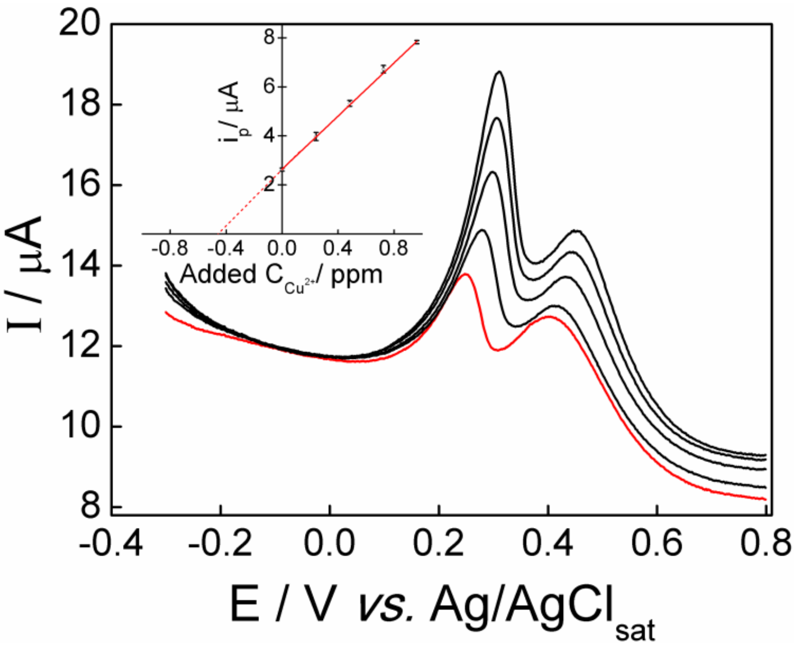

Figure 6. Differential pulse anodic stripping voltammograms recorded in a industrialized *cachaça* sample containing acetate buffer 0.1 mol L^{-1} (pH = 4.7). (—) Original sample. (—) Successive standard additions of 0.24 mg L^{-1} of Cu^{2+}. $E_{pc} = -0.3$ V; $t_{pc} = 180$ s. Pulse amplitude: 50 mV; Scan rate: 10 mV s^{-1}; pulse width: 25 ms. Inset: standard addition curve. Electrochemical regeneration adopted after each scan: +0.35 V for 60 s.

The slope of the standard addition curve shown in Figure 6 was 5.4 µA mg^{-1} L. This value is only 3.6% lower than that observed for analytical curve constructed in a mixture ethanol:water (40:60 v:v). This result suggests that for this particular sample (industrialized I in Table I) matrix effects are minimal. This was not true for the other analyzed samples for which slopes of standard addition curves ranged from 5.0 to 10.4 µA mg^{-1} L. This variability indicates that samples are not homogeneous in their composition which is expected regarding the high variability of raw material, production processes, etc. This inhomogeneity demonstrates that standard addition method must always be used for Cu^{2+} determination in *cachaça* samples in order to avoid errors caused by matrix effects whose nature and intensity are strongly depend on the sample. The slope of the standard addition curve obtained for one of the analyzed sample (home-made II in Table I) was very high (10.4 µA mg^{-1} L) which suggests that the ethanol content in this sample is exceptionally low.

The contents of copper found in the analyzed samples by the proposed method were compared with those found by official FAAS method. FAAS analyses were performed according to the procedure specified in Brazilian Technical Norm NBR 13921 [41] which establish the official procedure for Cu^{2+} quantification in *cachaça* samples. The results obtained in this comparative study are summarized in Table I.

Table I. Copper contents in *cachaça* samples found by the proposed method and FAAS method.

Sample	Official/mg L^{-1a}	Proposed/mg L^{-1a}	RSD/%	$F_{2/2}$ [b]	t [c]
Industrialized I	0.51 ± 0.01	0.52 ± 0.01	1.6	1	0.11
Industrialized II	0.73 ± 0.01	0.73 ± 0.01	1.5	1	0.15
Home-made I	3.10 ± 0.02	3.17 ± 0.07	2.2	12	0.83
Home-made II	1.17 ± 0.02	1.09 ± 0.10	9.2	25	0.68

[a] Results shown in the form: mean value ± sd (n=3). [b] $F_{2/2}$ = 39. [c] t = 2.78 (P = 0.05 with 4 degrees of freedom).

Table 1 shows that F values for all analyzed samples were always smaller than the critical one indicating that both analytical methods present statistically equivalent precision. The t-values obtained for all analyzed samples have also not exceeded the critical value indicating that there are not statistical differences between the results provided by both methods at 95% confidence level. These results are a clear demonstration of the good accuracy of the proposed method. In addition, the agreement between the results provided by both methods shows that matrix effects are efficiently overcome by the use of standard addition method. Finally, Table 1 shows that all analyzed samples satisfy Brazilian legislation regarding Cu^{2+} content and three of them also satisfy international legislation which establishes the maximum content of Cu^{2+} at 2 mg L^{-1}. This observation indicates a continuous improvement in the quality of *cachaça* since in the past it was very common to find *cachaça* samples with excessive amount of copper [7]. This improvement in quality is very important in order to preserve consumers' health and to make *cachaça* a Brazilian product internationally appreciated.

4. CONCLUSIONS

This work have demonstrated that SPCPEs-SiAt are totally stable in ethanol:water mixtures which has allowed Cu^{2+} determination directly in *cachaça* samples. It was observed that electrochemical regeneration of electrode surface is essential in order to achieve a satisfactory repeatability. Despite some matrix effects presented by *cachaça* samples, Cu^{2+} can be reliably quantified in these samples if standard addition method is used. The use of an accumulation potential directly in the analyzed sample enables using very short preconcentration time significantly improving the sensitivity and analytical frequency which has allowed Cu^{2+} determination directly in *cachaça* in a simple and fast way with no needing of any sample pretreatment procedure.

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