Analysis of Cu in Mezcal Commercial Samples using Square Wave Anodic Stripping Voltammetry

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ABSTRACT

High concentration of copper in mezcal, a representative Mexican spirituous alcoholic beverage, is a serious problem due to the damage that it may cause to human health. A cyclic voltammetry and square wave anodic stripping voltammetry study of copper (II) in three commercial mezcal samples based on glassy carbon electrode response was undertaken. The analysis was developed using a simulated matrix solution (EtOH/H_2O (1:1), 0.1 M $LiClO_4$ and AcOH/AcONa 0.05 M/ 0.008 M), with Cu (II) concentrations in the range 0 - 1 ppm. Direct electrochemical analysis of mezcal samples was complicated by the presence of different organic compounds in the matrix. The analytical signal of Cu (II) in the spirituous was notably improved and the interferences caused by organic compounds were minimized, by diluting the mezcal samples 10% with EtOH/H_2O (1:1) solution. An efficient quantification of Cu (II) was obtained from the calibration curve by the SWASV and using the internal standard method (Cd (II)) in commercial samples (1.2-6.7 ppm); the results were correlated satisfactorily with the values obtained by AAS.

Keywords : Copper analysis, Electroanalysis, SWASV, Mezcal samples, AAS

1. Introduction

The presence of metallic ions in alcoholic beverages is a common occurrence caused mainly by the use of metal equipment during the distillation or fermentation processes [1,2]. For example, copper is ubiquitous in tequila and mezcal (representative Mexican alcoholic beverages) mainly due to the corrosion of the copper pot distillation stills [3]. Some producers consider that distillation must be performed in stainless steel equipment fitted with a copper coil or else in pure copper stills, because the presence of copper is necessary to guarantee different organoleptic properties [4,5]. Previous studies show that Cu concentration in tequila is in the 0.011 - 11.6 ppm range [6], even when the Mexican legislation limits this concentration to 2 ppm [7]. Since high Cu concentrations may cause serious damage to human health involving liver and brain damage, a rapid, low cost and effective quantification is needed [8] to monitor the Cu concentration during the production process. This is currently performed with spectroscopic techniques such as atomic absorption spectroscopy (AAS) or inductively coupled plasma spectroscopy (ICP) with high acquisition, operation and maintenance costs [9] and they are not available to afford with all the spirits producers. In addition, samples require pre-treatment before injection, making slow the analysis and with several additional error possibilities. Electrochemical techniques, on the other hand, can be performed at low cost with easy sample manipulation and therefore they represent a promising option for the artisan producers [10,11]. These have been used to quantify metals in beer, red, and white wines, rum, and whiskey [12-17]. Cu, Pb,

Cd and Zn have been analyzed in tequila, raicilla, sotól, and mezcal (spirit beverages derived from *agave sp.*) using differential pulse polarography [18], anodic stripping voltammetry [19], and differential pulse adsorptive voltammetry [20] without any pre-treatment. Disadvantages in the previous reports include the use of toxic electrodes such as mercury, or expensive electrodes such as platinum and the requirement of relatively long pre-concentration times. Reports of direct electrochemical analyses of commercial mezcal samples are scarce [18]. A voltammetric study of commercial mezcal samples and their copper analysis by SWASV is hereby reported.

2. Experimental

2.1 Apparatus and reagents

A single compartment, three-electrode cell equipped with a glassy carbon working electrode (geometric area = 0.07 cm^2, BASi®), a Pt wire as the auxiliary electrode, and an Ag/AgCl reference electrode (BASi®) was used. All experiments were performed with a BAS100B/W potentiostat (West Lafayette, USA). LiClO$_4$ (Aldrich, 99.9%), absolute ethanol (Aldrich, 99.8%), glacial acetic acid (Aldrich, 99.7%), sodium acetate (Aldrich, ACS reagent), EDTA (Adrich, 98%), and 3CdSO$_4$·8H$_2$O (Baker, analytical reagent) were used as received. The Cu standard stock solution (1000 ppm) was prepared by dissolving pure metal commercial wires in nitric acid and diluting as required. All solutions were prepared with MilliQ grade water.

2.2 Electrochemical studies

A mezcal matrix was simulated using a 0.1 M LiClO$_4$, acetic acid/sodium acetate buffer (0.05 M/ 0.008 M), EtOH/H$_2$O (1:1) solution. SWASV was performed on this medium before each Cu quantitation. After each experiment, the working electrode was cleaned by subjecting it to an oxidation potential of 0.6 V for 180 s in a 0.1 M EDTA, 0.1 M LiClO$_4$ solution, then polished with 0.3 mm alumina, and thoroughly rinsed with distilled water and ethanol. To ensure reproducibility, all the experiments were triplicated, and the average and the standard deviation bars are reported.

The Cu content was determined in three commercial mezcal samples of 100% *Angustifolia* agave from the region of Oaxaca (Mexico) by placing 5 mL aliquots in the electrochemical cell and adding 53 mg

of LiClO$_4$ as electrolyte to each one. The samples were diluted to a 10% of the original concentration with the simulated matrix solution (10% dilution with EtOH/H$_2$O (1:1) solution). Cu was analyzed in each sample by AAS as the control analytical technique according to the corresponding standards [9] that indicate the use of 20 mL samples with the addition of 5 mL of concentrated HNO$_3$. Then, the samples were digested by heating to a final volume of approximately 10 mL, allowed to cool, and diluted to 25 mL with the simulated matrix solution. After this pretreatment the samples were analyzed with the AAS instrument.

3. Results and Discussion

Cyclic voltammograms on glassy carbon electrode (GC) of EtOH/H$_2$O (1:1) in a 0.1 M LiClO$_4$, AcOH/AcONa (0.05 M/0.008 M) buffer solution, as a simulated medium, with and without 3.4 ppm of Cu (II) are shown in the Fig. 1. Solutions were not N$_2$ saturated to simplify the operative procedure. In the presence of Cu (II), the voltammogram shows two current waves in the cathodic sweep: the reduction of Cu (II) to Cu (0) (peak Ic, $E_{(Ic)} \approx$ -0.297 V vs. Ag/AgCl) and the reduction of O$_2$ to H$_2$O$_2$ (peak IIc, $E_{(IIc)} \approx$ - 0.68 V vs. Ag/AgCl) [21]. In addition, it can be seen a decrease in the cathodic barrier due to the
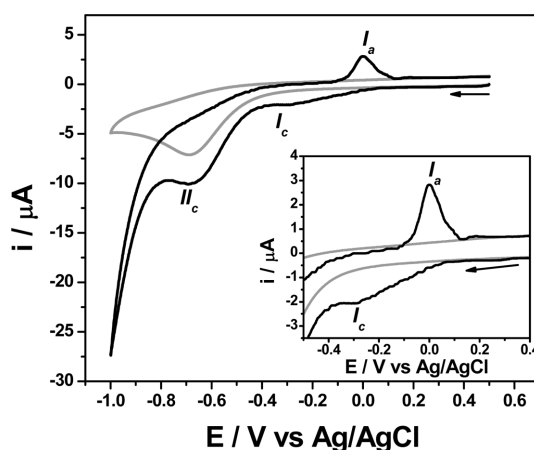

Fig. 1. Potentiodynamic reduction at GC electrode of EtOH/H$_2$O (1:1) with 0.1 M LiClO$_4$ and AcOH/AcONa (0.05 M/0.008 M) solution with 3.4 ppm Cu (II) (black line) and without Cu (II) (grey line). Inset: enlargement of the potential range where the copper deposit and stripping takes place.

facilitated reduction of protons caused by the presence of copper particles on the surface of the electrode [22]. An oxidative stripping peak of Cu (0) to Cu (II) (peak Ia, $E_{(Ia)} \approx 0$ V vs. Ag/AgCl) with a half width peak (evaluated at the half maximum of each peak, $W_{1/2}$) of 0.102 V is obtained in the anodic sweep. The peak potential remained invariant with or without ethanol, and therefore the possible formation of ethanol-Cu (II) complexes is ruled out. Another organic compound present in this hydro-alcoholic mixture that could cause the formation of Cu (II) complexes is acetic acid. The corresponding predominance-zone diagrams show the predominat system under the present experimental conditions is that of Cu (II)/Cu (0).

Cyclic voltammograms of three mezcal samples in the presence of 0.1 M of LiClO$_4$ as supporting electrolyte were obtained (Figure 2). All of them show the reduction of O$_2$ to H$_2$O$_2$ between -0.65 and -0.60 V vs. Ag/AgCl (peak IIc) and a peak consistent with the oxidative stripping of Cu (0) to Cu (II) between 0.01 and 0.07 V vs. Ag/AgCl (peak Ia). Both peaks are not related, and their current intensity is function of the concentration of Cu (II) (Peak Ia) and O$_2$ present on the mezcal samples (peak IIc) respectively. Depending on the sample, a slight change in $E_{(IIc)}$ and $E_{(Ia)}$ towards anodic values and changes in the $W_{1/2}$ were observed. This was attributed to the presence of organic molecules that can adsorb on the glassy carbon electrode, affecting the signals of the redox processes. Thus, the oxidative Cu stripping signal changes its half-width peak and peak potential shifts depending on the sample matrix [11]. Chromatographic techniques have shown that the most common types of organic compounds present in mezcal include: saturated alcohols, esters, aldehydes, ketones, organic acids, furans, terpenes, alkenes, and alkynes [23], some of them are electroreducible. The possible reduction of some organic molecules present in the mezcal matrix is observed around -0.8 V vs Ag/AgCl (peak IIIc). For our samples, no other metal oxidative stripping signals were observed using cyclic voltammetry. Carreon-Alvarez et al. reported the possible presence of Zn and Cd in mezcal samples [19], which were not observed in our samples probably due to the low sensitivity of the cyclic voltammetry technique.

For an effective elimination of the capacitive current and the minimization of problems associated
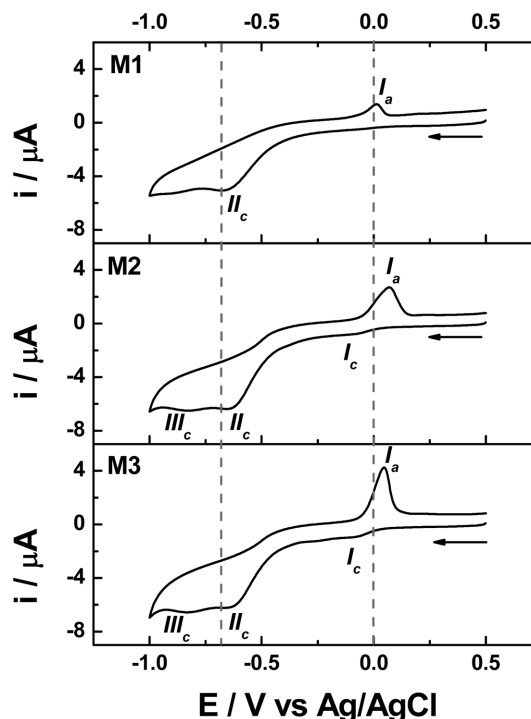

Fig. 2. Direct (non-diluted) potentiodynamic reduction of three commercial mezcal samples in the presence of 0.1 M of LiClO$_4$, $v = 25$ mV/s. The first cycle is shown.

with the adsorption of organic molecules onto the electrodes, the Cu (II) quantitative analysis was carried out using square wave anodic stripping voltammetry (SWASV) [24-25]. SWASV of a simulated matrix solution containing 0.5 ppm of Cu (II) showed the characteristic Cu oxidation wave at potentials between -0.10 and 0.15 V vs. Ag/AgCl; also can be observed the oxygen reduction as a broad peak ca. -0.6 V vs. Ag/AgCl and the protons reduction at -1.2 - 1.0 V vs. Ag/AgCl. This last signal increases with the amount of copper present in the sample, which agrees with the faster reduction of protons observed in cyclic voltammetry. After several tests, the following parameters were selected: $E_d = -1.1$ V, $t_d = 180$ s, $\Delta E_S = 4$ mV, E_{SW}, and $f = 15$ Hz. With these parameters, a $W_{1/2} = 80.9$ mV was obtained at a scan rate of 60 mV/s, which allowed sample analysis in ≈ 4 min. Direct (non-diluted) SWASV analysis of the three-different commercial mezcal samples showed the characteristic Cu oxidation wave in a potential interval of -0.1 to 0.07 V vs. Ag/AgCl. No other metal oxidative stripping was observed, which may be caused by the formation of
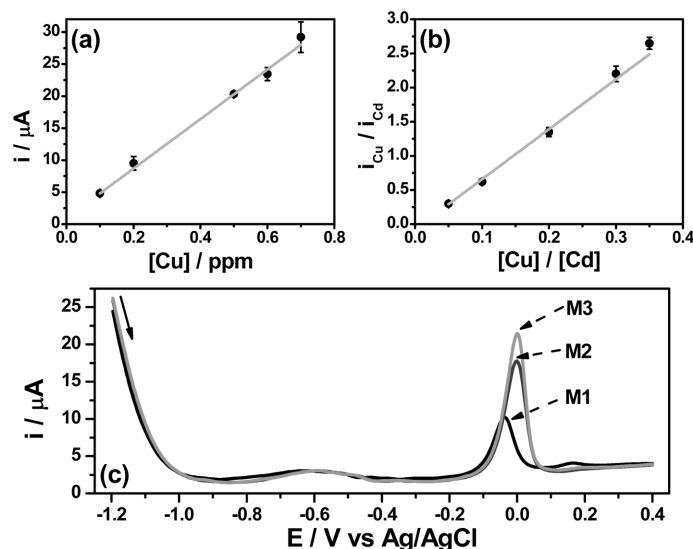
Fig. 3. (a) Calibration curve of copper concentration from 0.1 ppm to 0.7 ppm, (b) internal standard method plot of Cu (II) concentration from 0.05 ppm to 0.35 ppm using a constant Cd (II) concentration of 1 ppm. (c) SWASV of three commercial mezcal samples 10% diluted with with EtOH/H$_2$O (1:1) solution. For all the experiments, E_d = -1.1 V, t_d = 180 s, E_{SW} = 30 mV, ΔE_S = 4 mV and f = 15 Hz as analytical parameters.

intermetallic alloys with the Cu (0) surface which enables the detection of metal ions at trace-level concentrations. The positive current obtained in both current pulses (inverse and direct) causes a decrease in the square wave current and a saturation of the electrode's interface.

To improve the analytical signal of the SWASV and reduce the interferences caused by the organic compounds present in the mezcal matrix, the mezcal samples were diluted (10% dilution with EtOH/H$_2$O (1:1) solution). The quantification of Cu (II) by the calibration curve method and the internal standard method using 1 ppm of Cd (II) as internal standard were compared (Fig. 3a and 3b). The use of the internal standard improves the quantification of copper and minimizes the interferences due to mezcal matrix; Cd (II) fitted the requirements of potential and response and fortunately it was used in very low quantity. A calibration curve was obtained by changing the Cu (II) concentration from 0.1 to 0.7 ppm in our simulated matrix solutions (Fig. 3a). The calibration curve shows a linear trend for the peak current vs. metal ion concentration. The regression equation and correlation coefficient are, respectively; i = 0.937 + 38.7C, and r^2 = 0.999, with a calculated detection and a quantification limit of 0.066 and 0.222 ppm.

For the internal standard method (Fig. 3b), the plot of i_{Cu}/i_{Cd} vs. [Cu]/[Cd] was obtained by changing the Cu (II) concentration from 0.05 to 0.35 ppm and showed also a linear trend for the internal standard method. The regression equation and correlation coefficient were, respectively: i_{Cu}/i_{Cd} = - 0.075 + 7.32[Cu]/[Cd], and r^2 = 0.99, with a calculated detection and a quantification limit of 0.025 and 0.085 ppm.

Once the curves were prepared and the SWASV method assessed, the Cu (II) content of the diluted commercial mezcal samples was analyzed (Fig. 3c). The Cu (0) oxidation wave using a glassy carbon electrode was obtained in the potential interval between -60 to 4 mV vs. Ag/AgCl, indicating a dependence of the Cu(0) oxidation potential with the sample matrix. The obtained concentration (Table 1) was comparable to the analysis of alcoholic distillated beverages obtained in previous works by Barbeira et al. using the hanging mercury drop electrode [10]. The use of a GCE allows the efficient quantification of Cu (II) in distillated beverages without the use of the toxic electrode material (Hg) used in these previous determinations.

The Cu (II) content obtained by SWASV (calibration curve method) was compared to that obtained by AAS (Table 1); in some cases a considerable differ-

Table 1. Cu content as determined for three commercial mezcal samples using SWASV and AAS. The average of the independent experiments is shown.

Sample	AAS	SWASV [a]	SWASV [b]
M1	1.51 +/- 0.10	2.44 +/- 0.06	1.26 +/- 0.03
M2	4.77 +/- 0.11	4.42 +/- 0.29	4.23 +/- 0.18
M3	6.78 +/- 0.13	5.39 +/- 0.08	6.72 +/- 0.22
M4	4.84 +/- 0.14	4.34 +/- 0.24	
M5	3.71 +/- 0.16	3.31 +/- 0.27	
M6	4.78 +/- 0.10	4.11 +/- 0.04	
M7	7.00 +/- 0.11	5.60 +/- 0.31	

[a] Standard additions method. [b] Internal standard method.

ence between the two sets of values was observed. Nevertheless, the internal standard method was more reliable and the obtained values were in better agreement with those of AAS technique. This may be due to interaction among the organic present in the mezcal matrix (like aminoacids, quinones or catechols which show electrochemical activity [26]) and the GCE; in the internal standard method these interactions are present for both metals in analysis. Even though the three samples used in this work have the same classification (i.e., young mezcal, 100% agave), come from the same region in México (i.e., Oaxaca) and were produced using the same type of agave (i.e., *Angustifolia*), their organic compounds content should be different in each sample and therefore modify slightly the current response [5]. The standard deviations obtained from SWASV using a GC electrode are similar or in some cases even better than those obtained by AAS, which indicate the high reproducibility of the electrochemical technique. As can be seen from these results the increase in the copper concentration in the sample causes a larger difference between the SWASV and the AAS values, therefore samples containing a high content of copper must be diluted. This behavior was attributed to a possible interaction between the organic compounds of the mezcal matrix with the copper ions which does not occur in AAS because of the acidic treatment of the sample. The Cu (II) values obtained by SWASV are comparable to those obtained in other distilled alcoholic beverages such as vodka, cachaça, gin, and tequila using carbon paste modified electrodes [20]. In spite of the complicated matrix present in mezcal,

the electroanalytical results showed here using SWASV, indicate that this technique can be very useful in Cu (II) determination in real mezcal samples, using a fast method and low-cost electrochemical technique with a non-toxic electrode material. These results stimulated the study other metallic ions (*e.g.* Zn^{+2}) as alternative for internal standard for this complex matrix, study which is under way in our laboratory.

4. Conclusions

Direct analysis of copper in mezcal commercial samples using SWASV technique on a glassy carbon electrode showed problems due to the adsorption of different organic compounds on the surface of the glassy carbon electrode, leading to a broader peak and peak potential shifts. Therefore, the analysis of Cu in mezcal samples required a 10% dilution with with EtOH/H_2O (1:1) solution. Characteristic oxidation waves for Cu with high current responses and with the low $W_{1/2}$ = 80.9 mV were obtained using E_d = -1.1 V, t_d = 180 s, E_{SW} = 30 mV, ΔE_S = 4 mV and f = 15 Hz as analytical parameters. These conditions allow a fast, easy-handling and low cost analytical technique in contrast to the typical spectroscopic techniques. The quantification of Cu (II) by the calibration curve method and the internal standard method were compared. Both methods show a linear trend with a calculated detection limit of 0.066 ppm and 0.025 ppm, and a quantification limit of 0.222 ppm and 0.085 ppm for the standard additions method and the internal standard method respectively. The use of the internal standard improves the quantification of copper and minimizes the interferences due to the complex mezcal matrix. Cu (II) content in six samples of commercial mezcal was found in the 1.5 to 7 ppm range, showing good correlation with the corresponding values obtained by AAS and previous reported values.

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