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Determination of Copper in Wine by Anodic Stripping Voltammetry with Rotating Glassy Carbon and Microfiber Carbon Electrode

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THIS PAPER IS DEDICATED TO PROF. MIRJANA METIKOŠ-HUKOVIĆ ON THE OCCASION OF HER BIRTHDAY

Abstract: Anodic stripping voltammetry (ASV) with rotating glassy carbon electrode (RGCE) and microfiber carbon electrode (μFCE), both filmed with mercury, were used to determine copper content in wine. Influence of phenolics on *in situ* mercury film formation was studied. Wine and quercetin were added gradually into solution of Hg(II) in acetic acid-sodium acetate buffer (pH = 4.60). Reduction of Hg(II) was observed in both cases. *In situ* filmed electrode was found unsuitable. Results obtained using *ex situ* filmed RGCE and μFCE were found in good agreement with ASV using hanging mercury drop electrode. Thus, ASV with *ex situ* mercury filmed electrodes can be recommended for accurate quantification of copper in complex samples containing phenolics, without any special pretreatments.

Keywords: anodic stripping voltammetry, glassy carbon electrode, carbon microfiber electrode, mercury film electrode, wine, copper.

INTRODUCTION

WIDE variety of phenolics is present in natural products, including grape and wine. They act as antioxidants through scavenging of reactive oxygen species (ROS) or chelate the metal ions responsible for the generation of ROS. Until now, various electrochemical techniques have been applied to determine wine phenolics and antioxidant (AO) activity. Cyclic voltammetry with glassy carbon electrode (GCE) was often used.[1–3] Wine AO capacity was measured also by potentiometric titration,[4] differential pulse polarography (DPP) at GCE[5,6] and direct current polarography (DCP) at dropping mercury electrode (DME).[7,8] Two recently developed DCP assays based on Hg(II) reduction were applied on individual compounds and various natural products including wine.[7–9] Investigation of polarographic behavior of various phenolics and their interaction with metal ions was conducted by DCP and DPP. In presence of phenolics both Hg(II) reduction and Cu(II) complexation were observed.[10] Interaction of phenolics present in complex samples, such as wine, with metal ions can influence their direct electrochemical determination, making sample preparation or pretreatment necessary.

The analysis of metal-ions content in natural products, including grape and wine, was subject of numerous electrochemical studies.[11–15] The anodic stripping voltammetry (ASV) at various working electrodes, such as dental amalgam[11], thick-film modified[12] and DME,[16] was widely applied.

Presence of Cu(II) in wines, usually associated with copper-based vineyard spraying or the addition of Cu(II) sulfate to remove sulfidic-of odors, is of particular interest due to the spoilage occurrence. Medium exchange stripping potentiometry at mercury filmed GCE was efficiently used to determine labile copper in wine.[13] Anodic stripping voltammetry at hanging mercury drop electrode (HMDE) was successfully applied to quantify heavy metals including copper in wines, either subjected to digestion or not.[14–16]

The purpose of the present study was to determine Cu(II) content in chosen red wine using mercury filmed rotating glassy carbon electrode (RGCE) and microfiber carbon electrode (μFCE), considered environmentally friendlier than HMDE. Either *in situ* or *ex situ* mercury plated carbon electrodes have been used in parallel. Finding that Hg(II) reduction occurs in presence of phenolics[7–9] lead to assumption that presence of phenolics could prevent formation of mercury film *in situ*. The assumption has been checked by gradual addition of wine and quercetin into buffered Hg(II) solution. Procedure with externally formed electrodes has been recommended as suitable for accurate determination of copper in wine, without any pretreatment.

MATERIALS AND METHODS

Samples and Chemicals

The wine samples, red wine Traminac and white wine Alexandria, were produced by winery "Vršački vinogradi" (Serbia) (vintage 1995). Quercetin (anhydrous) was obtained from Sigma (USA). The supporting electrolyte was acetic acid-sodium acetate buffer (pH = 4.60). Standard solution of 1.00×10^{-2} mol L^{-1} Hg(II) was prepared from $HgCl_2$ (Merck, Germany). Cu(II) solution (5.0×10^{-3} mol L^{-1}) was prepared daily from the 0.100 mol L^{-1} stock solution obtained by Cu (99.999 %, RTB Bor, Serbia) dissolved in HNO_3 and neutralized with NaOH (Merck, Germany). The ultra pure water was used for all solution preparation. All chemicals used were of analytical grade quality.

Instrumentation and Electrodes

Anodic stripping voltammograms were recorded using either Polarographic analyzer PO4 Praha přistroje (Prague, Czech Rep.) equipped with X-Y recorder (Hewlett Packard, model 70158) or PAR (Princeton Applied Research, USA) Polarographic analyzer 174A equipped with X-Y recorder Omnigraphic 2000 (Huston USA). The three electrode electrolytic cell was used. As a working electrode either rotating glassy carbon electrode (RGCE) with surface area of 1 mm^2 or microfiber carbon electrode (μFCE) prepared from carbon fiber having diameter of about 10 μm (TUP Dubrovnik, former Yugoslavia) were used. Carbon fiber 10 cm in length is fixed between two plastic bands. A reference electrode was saturated calomel electrode (SCE) while counter electrode was Pt-wire. Rotating speed of RGCE was 1500 rpm. When μFCE was used the solution was stirred by magnetic stirrer set at 600 rpm.

The working RGCE electrode surface was cleaned before each experiment combining several cleaning procedures from the literature. After polishing with alumina powder (φ = 0.3 μm) electrode was rinsed with distilled water, rotated for 3 min in hot 20 % (*v*/*v*) H_2SO_4, again rinsed with distilled water, and finally ultrasonicated for 5 min.

The working μFCE surface renewal was enabled by cutting approximately half of mm before each experiment.

Experimental Procedure

In order to film RGCE with mercury, Hg(II) standard solution was added in electrolytic cell containing 20 mL of acetic acid-sodium acetate buffer (pH = 4.60).

The dependence of ASV current peak of mercury dissolution after deposition at RGCE: i) on Hg(II) concentration in buffered solution with constant Cu(II) concentration (0.05 mmol L^{-1}), ii) on wine amount in buffered solution containing 0.15 mmol L^{-1} of Hg(II) and 0.05 mmol L^{-1} Cu(II) and iii) on quercetin concentration in the presence of 0.10 mmol L^{-1} Hg(II) and 0.01 mmol L^{-1} Cu(II) was followed. Electrolysis was performed at cathodic potential of −1.0 V during 60 s. The potential scan rate was 200 mVs^{-1} and final anodic potential was from 0.5 to 1.0 V *vs* SCE.

Determination of Cu(II) concentration in red wine Traminac by ASV with working RGCE and μFCE electrodes plated *in situ* with mercury was performed from 20 mL of buffered solution containing 0.10 mmol L^{-1} Hg(II) and 2 mL of red wine. Electrolysis was performed at cathodic potential −1.2 V *vs* SCE during 60 s (RGCE) or 240 s (μFCE). After rotation of RGCE as well as magnetic stirring in case of μFCE and rest time of 5 s ASV curves were recorded to the final potential 0.7 V, with potential scan rate 500 mVs^{-1}.

Working electrodes (RGCE and μFCE) were mercury plated externally (*ex situ)* from 20 mL of buffered solution containing 0.10 mmol L^{-1} Hg(II). After 60 s (RGCE) or 240 s (μFCE) of electrolysis at −1.2 V electrodes were removed from cell solution, washed with distilled water and immersed into buffered solution containing 2 mL of wine Traminac. The same conditions were used as when plating was performed *in situ*.

Determination of Cu(II) concentration in wine Traminac by ASV method with HMDE electrode, was performed in parallel in Oenological Station (Vršac, Serbia), using HMDE and Polarograph PO4 Praha přistroje (Czech Rep.).

All experiments were performed at the room temperature.

RESULTS AND DISCUSSION

Influences of Phenolics on Mercury Film Formation at Carbon Electrodes

The influence of wine phenolics on mercury film formation at RGCE has been investigated. The effect of wine Alexandria as well as individual phenolic compound quercetin presence in plating solution has been followed.

The dependence of ASV current peak of mercury dissolution on its concentration, after deposition at RGCE from buffered solution (acetate buffer, pH = 4.60), in the presence

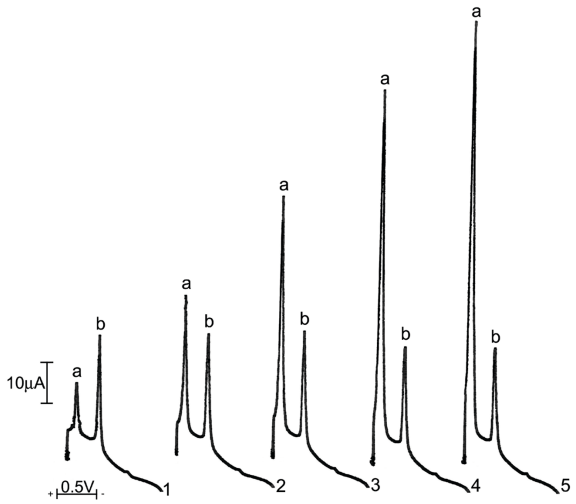

Figure 1. ASV curves of mercury (a) and copper (b) dissolution, deposited at RGCE from acetate buffer (pH = 4.6), upon gradual addition of 0.10 mol L^{-1} Hg(II) standard solution in aliquots of 10 μL, in the presence of 0.05 mmol L^{-1} Cu(II).

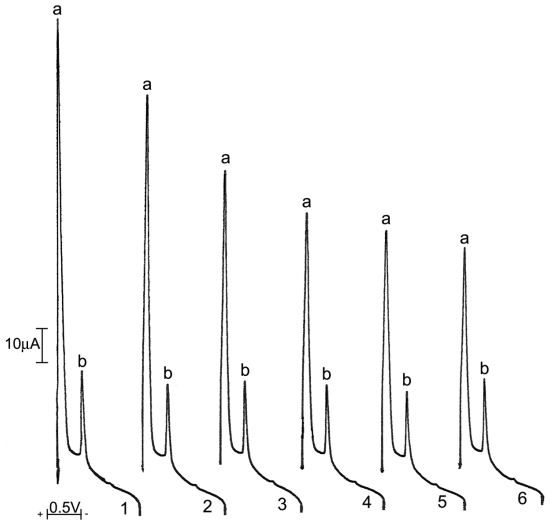

Figure 2. ASV curves of 0.15 mmol L^{-1} Hg(II) and 0.05 mmol L^{-1} Cu(II) in acetate buffer (pH = 4.6) before (1) and after addition of white wine Alexandria in aliquots of 100 μL (2–6).

of increasing Hg(II) and constant Cu(II) concentration (0.05 mmol L^{-1}), has been shown on Figure 1. Final anodic potential was 0.5 V. As seen, dependence of current intensity of ASV mercury peak (Figure 1, peak a) on Hg(II) concentrations is linear while intensity of current peak attributed to copper (Figure 1, peak b) dissolution has been almost constant.

The behavior of mercury dissolution ASV current peak recorded on *in situ* filmed RGCE in presence of constant Hg(II) (0.15 mmol L^{-1}) and Cu(II) (0.05 mmol L^{-1}) concentrations, upon gradually added wine, is shown on Figure 2. Final anodic potential was 0.7 V. As seen, ASV peak of mercury dissolution (Figure 2, peak a) becomes significantly lower upon gradual addition of wine. Its decrease is linearly dependent on volume of wine added. Change of ASV current peak of amalgamated copper dissolution (Figure 2, peak b) in the presence of wine has been found negligible, probably due to low content of copper in white wines (≈ 0.001 mg L^{-1}).

The ASV peak of mercury dissolution (Figure 3, peak a) recorded on *in situ* filmed RGCE from buffered 0.1 mmol L^{-1} Hg(II) and 0.01 mmol L^{-1} Cu(II) solution also linearly decreases with increase of amount of quercetin added, while copper dissolution peak (Figure 3, peak b) is almost constant. Final anodic potential was 1.0 V.

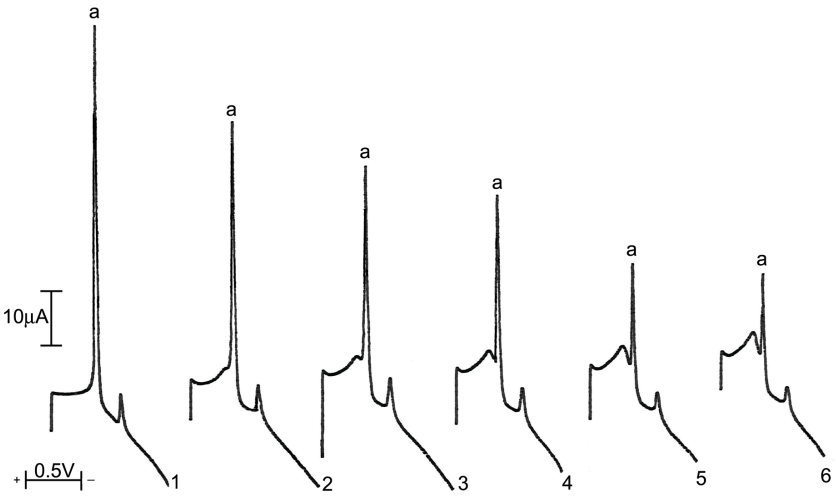

Figure 3. ASV curves of 0.10 mmol L^{-1} Hg(II) and 0.01 mmol L^{-1} Cu(II) in acetate buffer (pH = 4.6) before (1) and after addition of 0.2 mmol L^{-1} of quercetin in aliquots of 100 μL (2–6).

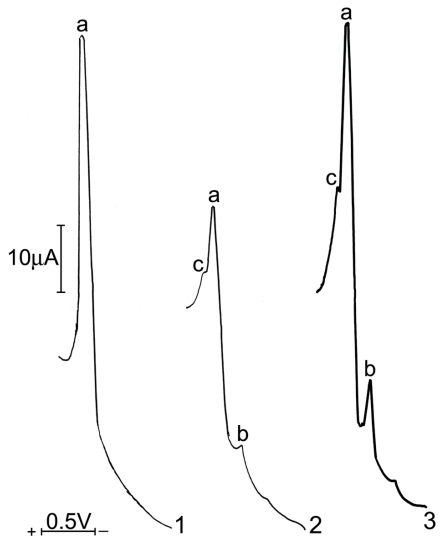

Figure 4. ASV curves obtained before (1) and after (2) addition of 2.0 mL of red wine Traminac, on *in situ* plated RGCE from 20 mL of acetate buffer (pH =4.6) in the presence of 0.1 mmol L^{-1} of Hg(II), and (3) after addition of 2 mL of Traminac on *ex situ* mercury plated electrode.

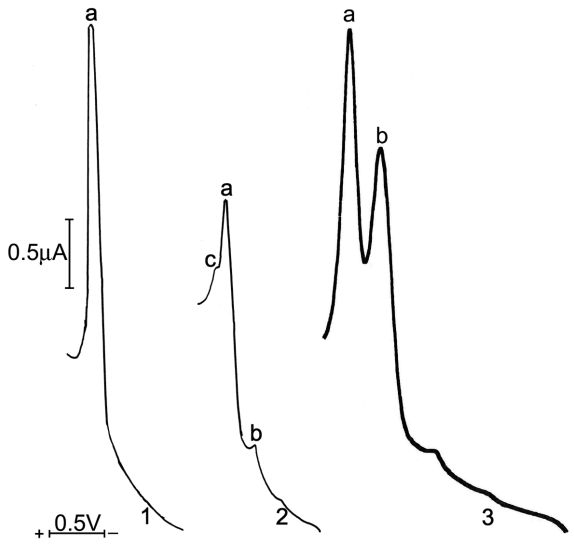

Figure 5. ASV curves obtained before (1) and after addition (2) of 2.0 mL of red wine Traminac, on *in situ* plated μFCE from 20 mL of acetate buffer (pH =4.6) in the presence 0.1 mmol L^{-1} of Hg(II) and (3) after addition of 2 mL of Traminac on *ex situ* mercury plated microelectrode.

Similar behavior of ASV of mercury dissolution peak, noticed on Figures 2 and 3, indicate occurrence of Hg(II) reduction, both in presence of phenolics from wine and individual ones. Based on this finding, as well as on previous studies on Hg(II) reduction,[7–9] it can be supposed that accurate *ASV* determinations of copper as well as other heavy metal-ions amalgamated with mercury, is not possible using carbon electrodes plated with mercury *in situ*.

Possibility of Copper Determination in Red Wine by ASV with *in Situ* and *ex Situ* Mercury Plated Carbon Electrodes

In order to check effect of phenolic presence on mercury film formation and to develop method for accurate copper determination in wine, ASV with *in situ* and *ex situ* mercury film formation at RGCE and μFCE has been applied in the analysis of red wine Traminac. ASV curves obtained have been compared.

When RGCE is filmed with mercury *in situ* from buffered 0.1 mmol L^{-1} Hg(II) solution, a symmetric ASV peak of mercury dissolution at 0.4 V has been observed (Figure 4, curve 1, peak a). Upon addition of wine Traminac, significant decrease of peak a has been noticed (Figure 4, curve 2, peak a). Low intensity current (Figure 4, curve 2, peak b), at about −0.15 V, has been attributed to copper dissolution. Additional current of low intensity (Figure 4, curve 2, peak c) that probably appears from the oxidation of some mercury organic compound becomes higher in the presence of higher amount of wine (not shown).

The intensity of mercury ASV dissolution peak obtained upon wine addition on *ex situ* formed mercury film at RGCE (Figure 4, curve 3, peak a) has been found the same as on *in situ* formed mercury film before the wine addition (Figure 4., curve 1, peak a). However, the copper dissolution current peak recorded upon red wine addition at *ex situ* formed mercury film (Figure 4, curve 3, peak b) has been found several folds higher than the same peak obtained at *in situ* formed mercury film (Figure 4., curve 2, peak b).

The same behavior of mercury and copper dissolution peaks obtained by ASV at RGCE has been noticed when μFCE has been used (Figure 5, curves 1–3, peaks a and b).

Obviously, in presence of complex sample such as wine, formation of mercury film *in situ* is not regular due to reduction of Hg(II) with present phenolics, observed also previously.[7–9] Results presented here confirm assumptions that ASV determination of Cu(II) in complex samples containing phenolics requires mercury film to be prepared *ex situ*.

Determination of Copper in Red Wine by ASV with *ex Situ* Mercury Plated RGCE and μFCE

The concentration of Cu(II) in red wine Traminac has been determined by ASV with RGCE and μFCE. The calibration curve for each electrode has been constructed for Cu(II) concentration from 0.05 to 0.25 mg L^{-1}. Value of Cu(II) determined from three successive ASV measurements on

ex situ formed mercury film RGCE (0.15±0.03 mg L^{-1}) and µFCE (0.13±0.02 mg L^{-1}) has been found in good agreement. Also, obtained results corroborated well with the result obtained by ASV with HMDE electrode (0.11±0.03 mg L^{-1}).

CONCLUSION

Application of anodic stripping voltammetry (ASV) with working rotating glassy carbon electrode (RGCE) and microfiber carbon electrode (µFCE) mercury plated *in situ* has not been found useful in determination of Cu(II) present in complex samples containing phenolics, such as wine. Occurrence of Hg(II) reduction in presence of individual phenolics and complex samples containing them has been supposed to be responsible for irregular mercury film formation at electrodes plated *in situ*. It has been proved that phenolics from wine prevent regular formation of mercury deposit as well as amalgam of the investigated metal. Concentration of copper in wine Traminac has been successfully determined applying ASV with externally mercury filmed electrodes. Quantity of copper obtained by ASV with both RGCE and µFCE, has been found in good agreement with result obtained by applying ASV with hanging mercury drop electrode (HMDE).

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