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Dedicated to prof. dr. I. C. Popescu
on the occasion of his 70th anniversary

DETERMINATION OF COPPER IN ORANGE JUICE BY VOLTAMMETRIC TECHNIQUE: A COMPARATIVE STUDY OF SAMPLE PREPARATION METHODS

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ABSTRACT. In the present work, carbon paste electrodes (CPE) modified with conducting polymers (1,8-diaminonaphthalene, 1,8-DAN) combined with rotating disk electrode (RDE) was applied to determine copper in orange juice samples. Copper (II) was preconcentrated at open circuit potential on the modified electrode. Then, the preconcentrated metals were oxidized by scanning the potential of the electrode from -0.4 to 0 V using differential pulse anodic stripping voltammetry (DPASV). Samples were analyzed with and without previous treatment. The current peak was high in orange juice sample digested with wet ashing and dry ashing procedures compared to those in sample without pretreatment. The wet ashing procedure was chosen for the digestion of orange samples because it required shorter time. The results obtained were validated by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Keywords: *carbon paste electrode, 1,8-diaminonaphtalene, differential pulse anodic stripping voltammetry, rotating disk electrode, orange juice*

INTRODUCTION

Fruits are known as good for health because they are sources of vitamin C, carotenoids, minerals and various kinds of antioxidants. However in production process, the quality of fruit products (juice) can be affected with increasing concentration of toxic compounds and environmental pollutants especially heavy metals.

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Copper is important as an essential trace element in biological systems [1]. Acute copper poisoning occurs when several grams of copper salt are ingested. The maximum tolerable daily intake for copper is 0.5 mg per kg body weight [2]. Wilson's disease is a prototypical disease caused by copper toxicosis, affecting first the liver and later the central nervous system, kidneys and eyes. Monitoring trace or ultra trace copper in canned juice is very important. For this reason, a sufficiently selective and sensitive method for reliable determination of copper would be of great interest. Commonly, the analytical methods for the quantification of heavy metals are neutron activation analysis (NAA), atomic absorption spectrometry (AAS), inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES), which are also used for the analysis of copper in juices [3,4].

The electrochemical technique, especially stripping voltammetry for the trace analysis of metal ions, obtained considerable interest because of its low cost, easy operation, good sensitivity, high selectivity and accuracy [5]. It is well known that voltammetric methods coupled with hydrodynamic sensors is very advantageous. The rotating-disk electrode technique allows mass transport control, providing thus higher levels of sensitivity and very low detection limits [6].

The use of carbon paste electrodes CPEs in analytical chemistry has attracted considerable attention in recent years especially, chemically modified carbon paste electrodes (MCPE) [7-9]. Complexation reactions with organic or inorganic reagents in carbon paste electrodes (CPE) have been reported [10–12]. The poly (1,8-DAN) incorporated in carbon paste electrode leads to a conducting polymer able to form complex with copper [13]. This polymer has been used for detection of lead and copper in water [13, 14]. In addition, it was showed that metal ions, which can compete for complexation at binding sites on the poly (1,8-DAN) film, are lead, mercury, zinc or iron. The interference can be eliminated using appropriate masking agent.

The aim of the present study was to apply carbon paste electrode modified with poly (1,8-DAN) for the determination of copper in orange juice samples, with and without pretreatment of the juice. In this article, we address the detection issues of copper in food samples that occur when MCPE is used after different treatment methods.

RESULTS AND DISCUSSION

Synthetic Cu^{2+} solutions analysis

The surfaces of fresh MCPEs were immersed in aqueous solution under rotation of the electrode. The preconcentration of Cu^{2+} was carried out under open circuit. Following the preconcentration step at open circuit potential, the rotation of electrode was stopped and the solution was left to equilibrate for 30 s. An initial potential of –0.6 V was applied for an appropriate time and then scanned from –0.4 V to 0 V using differential pulse anodic stripping voltammetry. An anodic peak at –0.2 V (*vs.* ECS) was observed (see figure 1). No deaeration of the solution was required. The preliminary studies of copper accumulation have been performed on bare electrode and on modified electrode. In the same solution and under the same conditions, the results show that an anodic stripping peak at –0.2 V was observed for CPE (1.3 µA) and a well defined stripping peak was observed at the same potential on MCPE (11.5 µA) [14]. It is important to mention that the bare carbon paste electrodes were found to be less efficient and less reproducible compared to the modified electrode. Because of complexity of matrix, the copper content was always determined by the multiple standard addition method, each data point used was the average of at least five distinct measurements.

Calibration curve

Using the chosen optimized condition a calibration graph for the determination of Cu (II) was constructed for a concentration range 0.1 – 250 ppb. The calibration plot in Figure 1 is linear from 0.1 – 250 ppb with R^2= 0.9998.

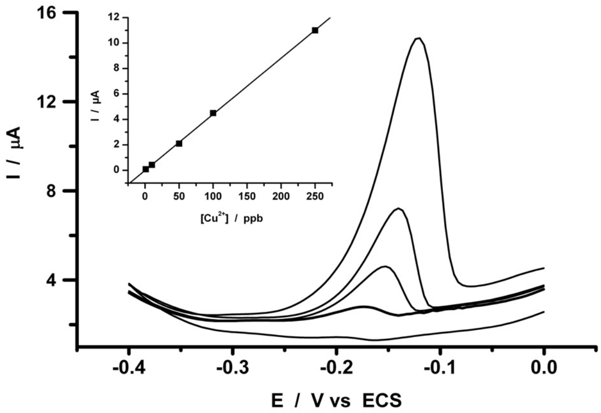

Figure 1: Calibration plot for copper (II) in 0.1 M L^{-1} HCl, preconcentration time 180 s, initial potential –0.6 V, potential step height 10 mV, pulse amplitude 50 mV, duration of 50 ms, speed of rotation 300 rpm. Concentration range 0.1 to 250 ppb.

The detection limit of Cu (II) was 0.05 ppb based on three times blank noise (3σ). The relative standard deviation of six determinations was 4.7% at 100 ppb.

Determination of copper in orange juice samples without pretreatment

The method proposed was applied to the determination of copper in orange juice samples, where possible interferences due to the complexity of the matrix could appear. No copper traces were found when the proposed procedure was used in the range of 0.1 to 250 ppb. Samples don't contain copper or, matrix complexity does not allow copper detection. Fruits contain acids such as: citric, tartaric, and malic acids that have the power of chelating heavy metals. In general, the concentration of metals in fruit juice and orange juice in particular depends on several factors related to the geographical origin of oranges [15], contamination by fertilizers, pesticides, the industrial process or the acidity. Tufuor et al. [16] have detected any trace of copper in orange juice samples by ICP-AES. However, the concentration of copper in orange juices available in the Polish market was determined using AAS by Krejpcio et al., the results show of copper concentrations ranging from to 47 to 1750 ppb [17].

Then, a new calibration graph was constructed in the concentration range 100 ppb to 1 ppm. The equation of calibration plot was $I/A = -19.02 + 30.76[Cu^{2+}]/\mu g\ L^{-1}$ with a regression coefficient of 0.996. Then, five replicate determinations of Cu (II) in spiked orange juice samples were carried out using the standard addition method. Table 1 summaries the recoveries of the results obtained from five replicate determinations of 500 ppb in an orange juice sample. A recovery between 98% and 118% were obtained and show that the procedure can be applied for the determination of Cu (II) in orange juice with a concentration above 500 $\mu g\ L^{-1}$. The minimum concentration of copper determined is 500 $\mu g\ L^{-1}$, which is within the safe limit set by World Health Organization (WHO), *i.e.* 3000 $\mu g\ L^{-1}$ [18]. This method based on stripping voltammetry could be used to detect copper in assessment of orange juices quality.

Table 1. Recovery data for copper (II) ($\mu g\ L^{-1}$) obtained for orange juice samples without pretreatment.

Sample	**[Cu^{2+}] add /** $\mu g\ L^{-1}$	**[Cu^{2+}] found /** $\mu g\ L^{-1}$	**Recovery / %**
S_1	500	590	118
S_2	500	490	98
S_3	500	535	107
S_4	500	~500	100
S_5	500	~500	100

Detection of copper in orange juice after pretreatment

Under the conditions reported in calibration section, MCPEs were applied to the analysis of orange juice samples after pretreatment (wet ashing and dry ashing).

Considering the safe limit set by WHO, *i.e.* 3 mg L^{-1} and the limit of detection of DPV method developed 0.05 µg L^{-1} we determined the concentration of copper recovered from five samples of orange juice spiked with 0.2 mg L^{-1} of copper treated by wet-ashing method analyzed by DPASV and by ICP-AES. In the Table 2, the results of copper determination in five juice samples obtained by DPASV and validated by ICP-AES method have been presented; the obtained recoveries were ranging from 95 to 98%.

Table 2. Detection of Cu (II) concentration in five orange samples treated by wet-ashing method, analyzed by DPASV and by ICP-AES.

amples	$[Cu^{2+}]$ by DPASV µg L^{-1}	$[Cu^{2+}]$ by ICP-AES µg L^{-1}	Recovery %
S1	186 ± 4.7	198	94
S2	182 ± 5.6	192	95
S3	192 ± 4.5	197	97
S4	208 ± 4.2	212	98
S5	205 ± 6.3	218	94

These results show that it was possible to use DPASV on CPE modified by poly (1,8-DAN) film in order to determine low concentration of copper by a judicious choice of the experimental conditions and after pretreatment. The comparison between DPASV method and ICP-AES method shows that there is satisfactory agreement between the two techniques.

Table 3 presents the results of copper concentrations in five orange juice samples obtained using wet ashing and dry ashing digestion methods. The simple comparison between these two pretreatment methods shows that the wet ashing treatment provides the same recoveries obtained for dry ashing method. However, the wet ashing treatment requires a very short time than the dry ashing method.

Table 3. Results for the determination of Cu (II) concentration in orange juice samples treated by dry ashing and wet ashing digestions methods.

Samples	$[Cu^{2+}]$ by wet ashing / µg L^{-1}	$[Cu^{2+}]$ by dry ashing / µg L^{-1}
S1	186 ± 4.7	182 ± 2.7
S2	182 ± 5.6	175 ± 4.5
S3	192 ± 4.5	178 ± 5.2
S4	208 ± 4.2	205 ± 4.6
S5	205 ± 6.3	198 ± 3.4

CONCLUSIONS

In this paper, we applied a novel method to determine copper in juice; it has been proved that carbon paste electrode modified with poly (1,8-DAN) can be used for the determination of copper in orange juice samples. However for the determination of low concentration, the sample pretreatment is necessary. Moreover, DPASV can be certainly a good alternative to spectroscopic methods owing to its inexpensive instrumentation and the simple operation. In addition, Copper levels in the samples analyzed were within the limit set by WHO and therefore pose no threat to public health.

EXPERIMENTAL SECTION

Reagents

All chemicals were of analytical grade. 1,8-DAN was purchased from Sigma. Copper sulphate was obtained from Riedel-de Héan. Solutions were prepared with distilled water. Standard copper (II) stock solution was prepared by dissolving $CuSO_4$ in water. Diluted copper standards for optimization studies were prepared from this solution. All experiments were carried out at room temperature (25°C). A solution of 0.1 M HCl served as supporting electrolyte.

Electrochemical measurements

Electrochemical experiments were carried out with an Autolab PGSTAT 10 potentiostat (Ecochemie, Utrecht Netherlands) controlled by 4.8 software. A rotating disk carbon paste and a rotating disk modified carbon paste electrode were used as working electrode (WE, $\Phi = 3$ mm). A rotating disk electrode was used during the preconcentration step. A saturated calomel electrode (SCE) was used as the reference electrode (RE) and a platinum wire was used as the counter electrode (CE). The pH was adjusted using a Fisher Scientific Accumet AB15 BASIC pH meter. Analysis ICP-AES were carried out using an Ultima 2 Jobin Yvon Model spectrometer.

Preparation of electrodes

The modified carbon paste electrode (MCPE) was prepared by thoroughly mixing weighed amounts of monomer (0.1 g of 1,8-DAN dissolved in a small quantity of acetonitrile) with 1 g of graphite powder and 0.3 mL of paraffin oil until a uniformly wetted paste was obtained. The mixture was kept at room temperature for one day to let the solvent evaporate. After that, the paste was inserted into the cavity of the electrode. The electrode surface could be renewed by simple extrusion of a small amount of paste from the tip of the electrode. Otherwise, before each use the electrode surface, it was rubbed with a piece of paper until a smooth surface was observed.

Sample preparation

Five samples of orange juice from Morocco were purchased on the market of Mohammedia. For the determination of copper by the proposed method in juice, three ways were used.

Procedure a: The sample were filtered through a membrane (0.45 µm), diluted and adjusted to pH < 2, without any other pretreatment.

Procedure b: Pretreatment of orange juice was performed using an oxi-acidic mixture of 5ml HNO_3 (67%) and 5 ml of H_2O_2 (37%) for 25 ml of sample. This mixture was heated until all the organic matter was destroyed. The final solution was transferred to a 50 ml volumetric flask. After that, 5 ml of acetate buffer (1 M) was added. The pH of mixture was adjusted at 2 and diluted with distilled water until 50 mL.

Procedure c: orange juice samples were submitted to degasification by heating reflux during 40 min under 120 °C. After that, 5 ± 0.5 g of sample was placed in a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 550 °C in 1 h. The samples were ashed for about 24 h until a white ash residue was obtained. The residue was dissolved in 5 ml of HNO_3 (25%, v/v) and the mixture, when necessary, was heated slowly to dissolve the residue. The solution was transferred to a 50 ml volumetric flask. The pH of mixture was adjusted at 2 and diluted with distilled water until 50 mL.

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