Highly sensitive determination of trace copper in food by adsorptive stripping voltammetry in the presence of 1,10-phenanthroline

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ABSTRACT

A highly sensitive, rapid, simple and selective adsorptive stripping assay for the determination of trace copper(II) is proposed. The methodology is based on the adsorptive accumulation of copper(II)–1,10-phenanthroline complexes onto a glassy carbon electrode, followed by oxidation of the adsorbed species by voltammetric scanning using square-wave voltammetry. The influences of experimental variables on the sensitivity of the proposed method, such as the effects of pH, ligand concentration, accumulation time, accumulation potential and interferences, were investigated. Under optimal conditions, the proposed method showed linearity from 0.1 ng mL^{-1} to 50 ng mL^{-1}. The 3 S/N detection limits were 0.0185 ng mL^{-1}, and the relative standard deviations ($n=10$) were 0.09–4.71% for intra-day and 0.05–7.14% for inter-day analyses, respectively. The application of the proposed method to the direct analysis of food samples yielded results that agreed with those obtained from including inductively coupled plasma–optical emission spectrometry (ICP–OES) assays according to a paired t-test. The results are a step toward the development of an alternative and reliable analytical method for food research, which requires the direct determination of copper.

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1. Introduction

The presence of trace metals, particularly heavy metals, in parental nutrition assumes a specific relevance because some of them are potentially dangerous or even toxic. Therefore, control of the finished product's metal content is recommended. Copper is an essential element that plays an important role in many body functions, such as hemoglobin synthesis, connective tissue development, normal function of the central nervous system, and oxidative phosphorylation [1]. However, excessive intake of copper would lead to accumulation of the metal in liver cells and hemolytic crisis, jaundice, and neurological disturbances [2]. Wilson's disease (hepatolenticular degeneration) is a well-known disease caused by excessive copper [3]. However, cases of copper deficiency in patients on unsupplemented hyperalimentation are well documented [4].

Water and food are the potential sources through which copper enters human bodies; thus, the determination of copper in water and food samples could afford some important information. In addition, a high copper content can promote rancidity and off-flavors in foods and beverages. Therefore, the quantitative analysis of copper at trace or even ultra-trace concentration levels in food is required.

Several analytical methods are available for the determination of copper at low concentrations, including inductively coupled plasma–optical emission spectrometry (ICP–OES) [5], electrothermal atomic absorption spectrometry (ETAAS) [6,7], X-ray fluorescence (XRF) [8], flame atomic absorption spectrometry (FAAS) [9,10], inductively coupled plasma–mass spectrometry (ICP–MS) [11,12], and capillary electrophoresis (CE) [13]. Even though ICP–OES and AAS are the most used techniques in the determination of traces of copper, the low copper concentration level in parental solutions is not compatible with the detection limits of these techniques. To attain accurate, reliable and sensitive results, preconcentration is required before the samples are analyzed directly using the analytical technique when the concentrations of the elements in the sample are excessively low. This requirement complicates the analytical method and leads to long analysis times and high costs. Spectrophotometry is one method that is effective for the determination of copper [14]. It involves less-expensive instrumentation compared to the previously mentioned techniques. However, the drawback of this technique is that it is highly sensitive only when appropriate chromogenic reagents are available.

To improve on these weak points, electrochemical analysis with various electrodes was investigated as a potential novel analysis system. Anodic stripping voltammetry (ASV) is an established method for trace metal–ion analysis in contaminated samples such as water and food because of the sensitive, selective, rapid and portable nature of these techniques [15]. In the context of copper determination, adsorptive stripping voltammetry at a hanging-mercury-drop electrode [16–26] has been reported to provide enhanced sensitivity and specificity for quantitative analysis. The detection limits observed during the previous work are typically low enough for the detection of copper in real samples; however, most of the investigators used a mercury electrode. Thus, because of the toxicity, stability, and volatility issues that arise from the use of mercury, a need exists for the development of alternative electrodes that possess the same attractive properties as mercury. For example, adsorptive stripping voltammetry of copper has been reported using carbon-paste electrodes [27–30] and glassy carbon electrodes [15,31]. However, the determination of trace or ultra-trace levels of metal ions in real samples is a challenging task.

Electroanalysis using 1,10-phenanthroline at a glassy carbon electrode via voltammetry has been shown to indirectly quantify copper [31]. However, an important problem remains with respect to realizing a direct analysis method, and the protocol has not yet been fully explored in the analysis of real samples. In this work, we aim to develop a straightforward, rapid, simple, sensitive, and specific methodology for the trace detection of copper in food samples. The present procedure is shown to be viable for the trace detection of copper ions in food samples and does not require any sample pre-concentration during the measurement step to enhance the sensitivity. We also show that no sample pre-treatment is required for the application of the complex, which provides a sensitive and selective protocol for the analysis of copper in real complex samples.

To the best of our knowledge, this is the first report on the use of a glassy carbon electrode for the analysis of copper ions in the presence of 1,10-phenanthroline in "real world" contaminated samples.

2. Experimental

2.1. Instrumentation

Square-wave voltammetry (SWV) experiments were performed using a model PGSTAT 101 Autolab Electrochemical System controlled with the NOVA software package (Kanaalweg 29-G 3526 KM Utrecht, The Netherlands). All experiments were performed using a conventional three-electrode system with a glassy carbon (GC) electrode with a diameter of 3.0 mm as the working electrode, a platinum wire as the auxiliary electrode, and a standard Ag/AgCl (3 M KCl) reference electrode.

2.2. Reagents and solutions

Sodium chloride (NaCl) was purchased from Merck. Copper(II) sulfate ($CuSO_4$) and hydrochloric acid were purchased from BDH. 1,10-Phenanthroline was purchased from Fluka (Buchs, Switzerland). All reagents were of analytical grade. The copper(II) stock solution (100 µg mL^{-1}) was prepared by dissolving the appropriate weight of $CuSO_4$ in 0.1 mol L^{-1} NaCl at pH 4.5 (1.0 mol L^{-1} HCl was used to adjust the pH). A 0.1 mol L^{-1} stock solution of 1,10-phenanthroline was prepared by dissolving an appropriate amount of 1,10-phenanthroline in 0.1 mol L^{-1} HCl. Water with a resistivity of 18 MΩ cm^{-1}, which obtained from a Millipore Milli-

Q purification system (Millipore, Benford, MA, USA), was used throughout the experiments.

2.3. Electrochemical procedure

The GC electrode was polished with 1.0 µm and 0.3 µm alumina. After the electrode was rinsed with double-distilled water, it was sonicated in water and in absolute ethanol for 1 min each. The standard/sample (10 mL) was pipetted into an electrochemical cell. Then, 10 µL of the 1,10-phenanthroline stock solution was added, which corresponded to a final concentration of 10^{-4} mol L^{-1}. The potential was set to −0.5 V, whereas the accumulation time was set to 120 s. The square-wave voltammetric parameters were a step potential of 20 mV, a pulse amplitude of 50 mV, and a frequency of 5 Hz. The voltammograms were recorded by applying a positive-going potential from −0.2 V to +0.4 V. The oxidation peak for copper(II)–1,10-phenanthroline complex occurred at +0.05 V, and its current was used for the measurement of the copper(II) concentration. All data were obtained at room temperature.

2.4. Calibration curve, limit of detection and precision

Calibration was performed under optimized conditions with standard solutions with concentrations that ranged from 0 ng mL^{-1} to 100 ng mL^{-1} with respect to copper(II). For each standard solution, three replicate measurements were performed. The calibration data were evaluated by linear regression analysis using the Excel software package. The limit of detection and limit of quantification were determined via the 3 S/N and 10 S/N methods, which reflect the concentrations of copper(II) that produced signals that exceeded three times and ten times the blank signal, respectively. The precision of the peak heights was estimated by performing 10 replicate measurements of standard solutions that contained 0.5, 5.0 and 30.0 ng mL^{-1} of copper(II).

2.5. Sample preparation

The juices samples, which included orange juice, vegetable juice, tea and honey, were purchased from a local supermarket. The juices were filtered using Whatman No. 1 filter paper. One milliliter of each solution was transferred to a volumetric flask and diluted to 10 mL with supporting electrolyte before analysis via the electrochemical procedure. The sample solutions obtained had concentrations within the range of the calibration graph. ICP–OES assays [32] were employed for purposes of comparison.

2.6. Data analysis

Standards and samples were analyzed, and the currents were integrated. Standard curves were obtained by plotting the net current as a function of the analyte concentration and fitting the data to a linear equation. To compare the two measurement systems, which are supposed to be equivalent, the results were tested using the paired t-test.

3. Results and discussion

3.1. Adsorptive characteristics of copper(II)–1,10-phenanthroline complexes

Preliminary experiments were performed to identify the general features that characterize the preconcentration of the metal ion–1,10-phenanthroline complexes on the electrode–solution interface. Fig. 1 displays the stripping square-wave voltammograms of