The Application of Differential Pulse Cathodic Stripping Voltammetry in the Determination of Trace Copper in Whole Blood

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Abstract

Electrochemical stripping analysis is a very important and widely used tool for the trace determination of various elements and compounds. The method is based on accumulation of Cu (II) ions onto platinum electrode, followed by the reduction of the species by differential pulse cathodic stripping voltammetry. The effect of various parameters such as pH of supporting electrolyte, accumulation of potential and time, scan rate and speed of rotating electrode on the selectivity and sensitivity were studied. The best conditions were found to be pH = 6.5 potassium acetate buffer, the accumulation potential of 0 mV (vs. Ag/AgCl), the accumulation time of 50 s, rotational speed of the working electrode 1500 rpm and the scan rate of 25 mV s⁻¹. The peak current is proportional to the concentration of copper over the range 0.53-107 ng/mL with a detection limit of 0.159 ng/mL and an accumulation time of 50 s. The developed method was validated by the analysis of reference materials certified by total blood.

Keywords: Copper; Cathodic Stripping Voltammetry; Whole Blood; Platinum Electrode

1. Introduction

Copper is an essential trace element for humans and animals. In the human organism, copper exists in two forms, the first and second oxidation form which exists the most in the human organism (Jack and Svetlana, 2009). The ability of copper to easily attach and accept electrons explains its importance in oxidative reduction processes and in disposing and removing free radicals from the organism (Lobo et al., 2010). Although scientists identified copper compounds to treat diseases in 400 B.C (Borkow et al., 2005), researchers still discover new information regarding the
biochemistry, physiology, toxicology, many clinical, laboratory and other indicators of the impact of copper in the organism (Angelova et al., 2011). Since then, evidence has accumulated that supports a requirement for dietary copper in humans for optimal function of all biological systems (Broderius and Prohaska., 2009). Copper is an element with biological significance to the cardiovascular disease patient (Kim et al., 2010). Investigation of copper functions in human bodies requires accurate, affordable, informative, low-detection-limit methods for determination of trace copper in biological samples (Prakash et al., 2008). Although a considerable number of methods for copper investigation exist, research continues in search of more sophisticated analytical approaches. Among a variety of new analytical tools under development, stripping voltammetry has been recognized as one of the most sensitive methods for trace analysis of heavy metals. Compared to its spectroscopic competitors such as atomic absorption and atomic emission spectroscopy, stripping voltammetry has great potential for on-site environmental monitoring due to its favorable portability, suitability for automation, short analysis time, low power consumption, and inexpensive equipment (Yantasee et al., 2008). These methods occupy a particular place because its applications can be extremely varied, as well in the field of the mineral analysis as in that of the organic analysis (Péricio et al., 1993). Its principle is usually based on the accumulation of metals per electrochemical reduction on electrode of platinum, followed by their qualitative and quantitative analysis. Platinum electrodes have been among the mostly used solid metallic electrodes for electroanalytical determinations (Radulescu and Danet, 2008). Among the electrochemical methods most recent, the stripping voltammetry is a precise, significant and selective method electrochemical (Brown et al., 2009). Many electroanalytical stripping procedures have been proposed for the individual determination of nanomolar concentration of copper (Quentel and Madec., 2010). In this work we will study the influence of the principal experimental parameters on the current of stripping, namely the pH of supporting electrolyte, the potential and the time of accumulation, the scan rate and the electrode rotating speed. The objective is the determination of the optimal operating conditions for each one as of these parameters in order to determine the concentration of the copper trace dissolved in whole blood.

2. Experimental

2.1. Chemicals
All chemicals used were of analytical-reagent grade or the highest purity available. Aqueous solutions were prepared by dissolving a certain amount of chemicals into high-purity deionized water (Milli-Q water system). Acids used for the analysis were the nitric acid (69.5%, Fluka) and the perchloric acid (70-72%, Merck). Stock solution of Cu(II) (1000 ppm, atomic adsorption standard, Aldrich) was prepared in deionized water. Acetate buffer solution was prepared using acetic acid and potassium hydroxide.

2.2. Instrumentation
Ten milliliters of the supporting electrolyte solution was pipetted into the voltammetric cell. The working electrode was a rotating platinum electrode. The reference electrode was an Ag/AgCl, 1 M KCl electrode. The counter electrode was a platinum wire. Measurements performed with a rotating disk electrode of platinum produced a systematic increase of the cathodic stripping peak at
subsequent runs. The platinum electrode work was polished over polishing cloths containing the
dispersions of alumina.

2.3. Blood sample preparation
Blood samples were taken from 7 healthy human with special care, by vein puncture using
disposable syringes and needles. The samples (1 mL) were then digested with nitric acid and
perchloric acid (3:1). Digested samples were made up to 5 mL using 0.25% nitric acid. Special care
was taken to avoid all contaminations (Attar et al., 2012). Only reagents with low background
impurities were used.

3. Results and discussion

Preliminary experiments were performed to select the working electrode. Many electrode materials
such as gold, platinum, silver, mercury or their alloys have been investigated for stripping
voltammetry (Fischer et al., 2006; Yantasee et al., 2008; Pan et al., 2009). Fig. 1 illustrates the eff
ect of electrode on the cathodic stripping signals of copper on four different electrodes. Whereas
rotating disk electrode platinum showed a high intensity of peak current from the other electrodes
(Au, graphite and glassy carbon). For this purpose we have

![Fig 1](image)

**Fig 1.** Differential-pulse cathodic stripping voltamograms arising from the addition of 45 ng/ml of
copper(II), accumulation time is 50 s, accumulation potential is 0 mV, pH=6.5,
electrode rotating speed 1500 rpm and scan rate is 25 mV s⁻¹.

3.1. Effect of pH
Different electrolytes were tested as supporting electrolytes (Potassium and sodium acetate buffer,
potassium hydroxide, acetic acid, perchloric acid, nitric acid and hydrochloric acid). Among these,
potassium acetate buffer (pH =6.5) gave the best response. Fig. 2 shows the relationship between
pH and the peak current. Stability of the complex largely depends on the pH of the system. The
influence of pH on the cathodic stripping voltammetry of copper peaks current was studied in the
pH range of 2.5-7.5 for solution containing 45 ng/mL. Initially, there was an increase in peak
current with rise in pH up to 6.5 and falls after this value. Therefore, the pH = 6.5 was selected as the optimum experimental condition.

![Fig 2](image-url)

**Fig 2.** Effect of pH on the peak current of 45 ng/mL copper. Experimental conditions same as in Fig. 1.

3.2. **Effect of the potential of accumulation**

Accumulation potential is an important parameter for stripping techniques that influences the sensitivity of the determination. The influence of the deposition potential on the stripping signals was studied in the potential range from 200 to -300 mV (Fig.3). The peak current was found to increase with decreasing deposition potential up to 0 mV. This value of deposition potential 0 mV was chosen.

![Fig 3](image-url)

**Fig 3.** Effect of accumulation potential on the peak current of 45 ng/mL copper (II). Experimental conditions same as in Fig. 1.

3.3. **Effect of accumulation time**

The accumulation time is always important factor in stripping voltammetric analysis because of its prevailing influence on sensitivity and detection limit of the method (Attar et al., 2013). The influence of the copper deposition time onto the platinum electrode surface was studied within 10-
60 s range. A linear dependence between deposition time and registered signal was found in the range 10-50 s, with increasing preconcentration time and mass-transfer until the saturation surface concentration is reached (Delahaye and Trachtenberg, 1957). The obtained data are presented in Fig. 4. Therefore, accumulation potential of 50 s was selected as an optimum accumulation time for further experiments.

![Accumulation Times vs Current](image)

**Fig 4.** Effect of accumulation time on the peak current of 45 ng/mL copper(II).
Experimental conditions same as in Fig. 1.

### 3.4. Effect of the scan rate

The observed stripping voltammetric signal can be further maximized by adjusting the way the applied potential was scanned. The relationship between the measured peak intensity and scan rate was found to be directly proportional over 10-50 mV s\(^{-1}\) scan rate. However, when scan rates faster than 25 mV s\(^{-1}\) were employed, the peak current decreased slightly. The influence of scan rate on the observed voltammetric signal is illustrated in Fig. 5, which indicates that scan rate value of 25 mV s\(^{-1}\) would be adequate optimum for succeeding investigations.

![Scan rate vs Current](image)

**Fig 5.** Effect of scan rate on the peak current of 45 ng/mL copper(II).
Experimental conditions same as in Fig. 1.
3.5. Effect of electrode rotating speed

Effect of electrode rotation speed is one of the important parameters that affect the accumulation efficiency. The use of a rotating-disk electrode for the preconcentration step greatly enhances the mass-transfer efficiency during preconcentration and ensures more reproducible mass-transfer conditions than stirring of the solution (Morfobos et al., 2004). In present work, the samples were agitated at various electrode rotation speed (0, 500, 1000, 1500, and 2000 rpm). The result showed that the current intensity efficiency was first improved with increased speed rotating before 1500 rpm and then decreased after that value (Fig. 6). Hence, a rotation speed of 1500 rpm was chosen for further studies.

![Fig 6. Effect of electrode rotation speed on the peak current of 45 ng/mL copper(II). Experimental conditions same as in Fig. 1.](image)

3.6. Effect on interferences

Possible interference by other metals with the cathodic stripping voltammetry of copper was investigated by the addition of the interfering ion to a solution containing 45 ng/ml of copper(II) using the optimized conditions. A study of potential interferences in the determination of copper was performed. The tolerance limit was defined as the amount of foreign ions causing a change less than 5% in the recovery of Cu(II).

3.7. Calibration data and application to blood samples

Table 1 illustrates the percentage recovery, standard deviation, accuracy and precision. It is observed that the precision for two levels is 3.77 and 5.91%. This is strong evidence that this method is precise and reproducible. Furthermore, the percentage recovery of the copper determination was tested by measurement of a standard reference material, two *levels seronorm* were used as the standard whole blood certified reference materials with the recommended concentration of copper are ranging between 95.13 to 106.07% which is an indication that this method is accurate for copper is ranging between 96.05 to 105.42 %. The linear range for copper was evaluated at 50 s deposition time. The peak current increased linearly with copper concentration over the range 0.53-107 ng/mL, with correlation coefficient of 0.998. A detection limit of 0.159 ng/ml was estimated from 10 replicate determination of blank solution under optimum conditions.
Table 1 Accuracy, precision and recovery of the method against a standard reference material

<table>
<thead>
<tr>
<th>Material</th>
<th>Mean ± SD (ng/ml)</th>
<th>Acceptable range (ng/ml)</th>
<th>Accuracy</th>
<th>Precision</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>1564±5.4</td>
<td>586.28±5.61</td>
<td>531-597</td>
<td>96.05</td>
<td>3.77</td>
</tr>
<tr>
<td>Level 3</td>
<td>1740±151</td>
<td>1834.31±159.41</td>
<td>1438-2042</td>
<td>105.42</td>
<td>5.91</td>
</tr>
</tbody>
</table>

The mean and standard deviation of seven healthy human in the western Algerian population were 890,658 ± 270,513 ng/ml. The range for all samples was 396,516 - 1271,485 ng/ml.

4. Conclusion

The determination of trace levels of copper via cathodic stripping voltammetry at a platinum rotating electrode has been evaluated for use in whole blood analyses. This method offers a practical potential for trace determination of copper, with high selectivity and sensitivity, simplicity and speed relative to other methods.

Reference


