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Determination of Ultra Trace Levels of Copper in Whole Blood by Adsorptive Stripping Voltammetry

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ABSTRACT. A selective and sensitive method for simultaneous determination of copper in blood by adsorptive differential pulse cathodic stripping voltammetry is presented. The procedure involves an adsorptive accumulation of Cu(II)-ETSC (4-ethyl-3-thiosemicarbazide) on a hanging mercury drop electrode, followed by a stripping voltammetry measurement of reduction current of adsorbed complex at about −715 mV. The optimum conditions for the analysis of copper (II) ion are : pH 10.3, concentration of 4-ethyl-3-thiosemicarbazide 3.25×10^{-6} M and an accumulation potential of −100 mV. The peak current is proportional to the concentration of copper over the range 0.003−125 ng/mL with a detection limit of 0.001 ng/mL and an accumulation time of 60 s. Moreover, with the use of the proposed method, there is a considerable improvement in the detection limit, the linear dynamic range and the deposition time, compared with the methods of adsorptive stripping voltammetry for the determination of copper. The developed method was validated by analysis of whole blood certified reference materials.

Key words: Adsorptive cathodic stripping voltammetry, Copper, 4-Ethyl-3-thiosemicarbazide, Whole blood

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.[1] 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.[2,3] Although scientists identified copper compounds to treat diseases in 400 B.C,[4] researchers still discover new information regarding the biochemistry, physiology, toxicology, many clinical, laboratory and other indicators of the impact of copper in the organism.[5] Copper is necessary in human nutrition for normal iron metabolism and the formation of red blood cells. Anemia is a clinical sign of deficiency of copper.[6–8] Investigation of copper functions in human bodies requires accurate, affordable, informative, low-detection-limit methods for determination of trace copper in biological samples.[9–12] Although a considerable number of methods for copper investigation exists, research continues in search of more sophisticated analytical approaches.

Electroanalytical techniques have undergone many important developments in recent decades. This has stemmed from a better understanding of electrode processes and improvements in instrumentation, which have allowed faster measurements to be made under better-controlled conditions, particularly those involving pulse voltammetric techniques.[13,14] The stripping voltammetry is the most sensitivity one because it has a preconcentration step on the electrode surface prior to recording the voltammogram.[15–17] The technique is based upon adsorptive accumulation of the metal ion complexed with a suitable ligand at the electrode (HMDE) and then scanning the potential of the electrode in the negative direction.[18–20] Many electroanalytical stripping procedures have been proposed for the individual determination of nanomolar concentration of copper.[21–24]

The present study attempts to describe a new adsorptive cathodic stripping procedure for simultaneous determination of trace amounts copper in whole blood by using 4-ethyl-3-thiosemicarbazide (ETSC) as a complexing agent onto the hanging mercury drop electrode.

EXPERIMENTAL

Equipment and Materials

Adsorptive cathodic stripping voltammetry (AdCSV) was carried out on an MDE 150 polarographic stand. Measurements were carried out with a hanging mercury drop electrode, in a three-electrode arrangement. The auxiliary electrode was a wire of platinum with a considerably larger surface area than that of HMDE. An Ag|AgCl (KCl 3 M) was used as reference electrode. A magnetic stirrer and stirring bar provided the convective transport during accu-

mulation. The whole procedure was automated and controlled through the programming capacity of the apparatus with Trace-Master 5 PC software. The solutions were deoxygenated with high-purity argon for 5 min prior to each experiment.

Procedure

Ten milliliters of the supporting electrolyte solution was pipetted into the voltammetric cell. The solution was purged with argon for 5 min in the first cycle and 50 s for each successive cycle. The accumulation potential −100 mV was applied for 60 s to a fresh mercury drop while the solution was stirred. The stirring was stopped for a period of 20 s and then, the potential was scanned from −100 mV toward more negative values using differential pulse (DP), (modulation time, 20 ms; modulation amplitude, −50 mV; interval time, 0.1 s; potential step, 5 mV, resulting in a scan rate of 50 mV/s). Each scan was repeated three times with a new drop for each analyzed solution and the mean of these voltammograms obtained. Copper stripping peak was registered at about −715 mV, and their current used as a measure of copper concentrations.

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. A 5×10^{-3} M solution of chelating agent 4-ethyl-3-thiosemicarbazide (Sigma-Aldrich 97%) was prepared by dissolving the appropriate amount of ETSC in deionized water. Acetate buffer solution was prepared using acetic acid and potassium hydroxide.

Blood Sample Preparation

The procedure consisted in placing 0.5 mL of whole blood in a long-necked 50 ml flask together with 2 mL HNO_3/$HClO_4$ mixture (3:1 v/v). The temperature of this mixture was slowly increased to150 ºC for 4 h in a hot plate, and then the temperature was maintained at 180 ºC until the evaporation of half of the acids. After cooling the flasks at room temperature, 2 mL from the same acid mixture was added and re-evaporated until ~ 0.5 mL was left. After cooling, the digested blood samples were made up to 5 mL using 0.25% nitric acid.[25] Special care was taken to avoid all contaminations. All the chemicals used were of suprapur quality.

RESULTS AND DISCUSSION

Preliminary experiments were performed to identify the general features which characterise the behaviour of Cu (II) and 4-ethyl-3-thiosemicarbazide systems on mercury drop electrode. *Fig.* 1 shows cathodic stripping differential pulse voltammograms of the Cu(II)-ETSC after accumulation at −100 mV for 60 s. Curve (a) shows the voltammograms of a solution containing 50 ng/mL of Cu (II) in the absence of ligand. A very small cathodic peak is found at −480 mV. In addition, curve (b) shows the differential pulse voltammogram of 4-ethyl-3-thiosemicarbazide, in the absence of Cu (II), that produced one peak current at −680 mV. The presence of copper 50 ng/ml and ligand 3.25×10^{-6} M in the same cell gives curve (c), The Cu (II)-ETSC complex has strong adsorption at the mercury electrode and produces a strong reductive peak current at −715 mV. All the above facts indicate that Cu (II) and 4-ethyl-3-thiosemicarbazide really produced a new complex, and this complex was electroactive and could be produced by a reduction peak current at −715 mV. The possible mechanisms of metal accumulation on the electrode-solution interface in adsorptive stripping voltammetry, via complex formation, were given by Paneli et al.[26] According to these authors, it seems that the presence of π-electrons in the ligand molecule favours the adsorption process. 4-ethyl-3-thiosemicarbazide has different functional groups so it can be coordinated with copper ion through donating groups, depending on the pH of the solution. For the best

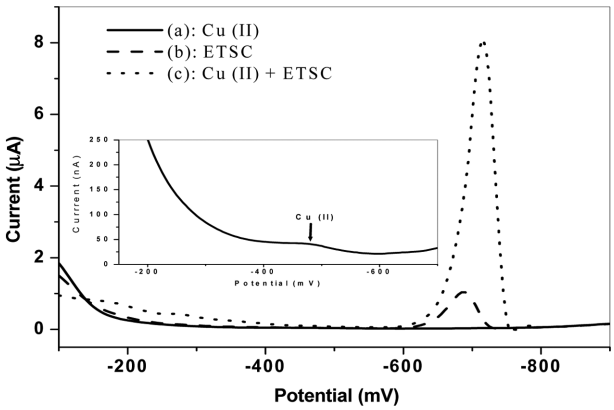

***Figure* 1.** Adsorptive stripping voltammetry for Cu-ETSC system. $C_{ETSC}=3.25\times10^{-6}$ M, $C_{Cu(II)}=50$ ng/ml, accumulation time is 60 s, accumulation potential is −100 mV, pH=10.3, Stirring rate 400 rpm and Scan rate is 50 mV/s.

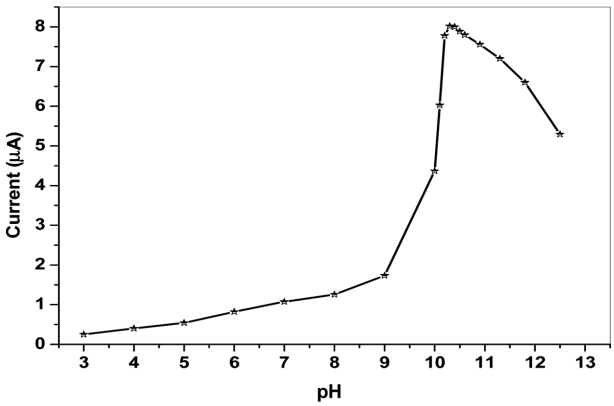

***Figure* 2.** Effect of pH on the peak current of 50 ng/mL copper. Other conditions are the same as in *Fig.* 1.

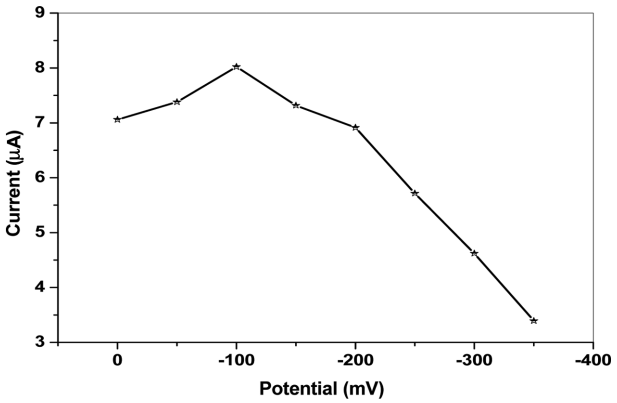

***Figure* 3.** Effect of accumulation potential. Other conditions are the same as in *Fig.* 1.

sensitivity in simultaneous determination of copper the influence of different parameters such as supporting electrolytes, ligand concentration, deposition time and potential, stirring and scan rate were investigated.

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 10.3) 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 copper peaks current was studied in the pH range of 3.0–12.5 for solution containing 50 ng/mL each of metal ion and 3.25×10^{-6} M of *ETSC*. The system may become unstable with a small variation in the pH. So far, the optimization of a stable complex of Cu-ETSC was concerned. Initially, there was an increase in peak current with rise in pH up to 10.3 and falls after this value. This is due to the increasing complex formation of copper (II) with the ligand at the electrode surface with increasing pH. At pH more than 10.3, the precipitation of copper as $Cu(OH)_2$ occurred resulting in sharp decrease in peak current.[27] Therefore, the pH 10.3 was selected as the optimum experimental condition.

Effect of Accumulation Potential

The effect of accumulation potential on the stripping peak current of the complex was examined over the potential range of 0 to −350 mV. As shown in *Fig.* 3, the peak current increased with changing potential from 0 to −100 mV. The peak current decreased due to changing potential from −100 to −350 mV due to complex reduction during adsorption step. An accumulation potential of −100 mV was used for the optimized analytical procedure.

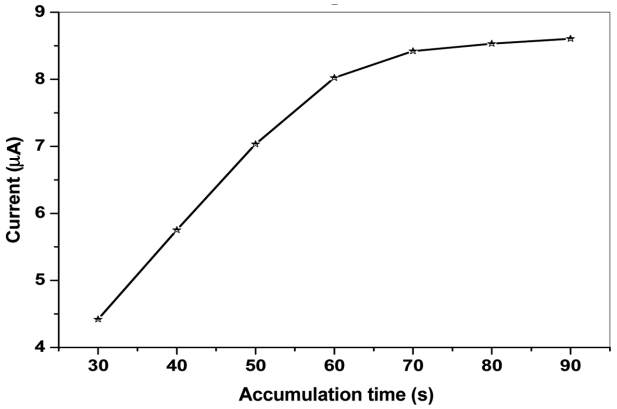

***Figure* 4.** Effect of accumulation time on the peak current. Other conditions are the same as in *Fig.* 1.

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. The effect of accumulation time on the peak current of complex (Cu-ETSC) was carried out as shown in *Fig.* 4 after increasing the accumulation time from 60 s onwards. The variation of adsorption time between 0 and 90 s at an adsorption potential of −100 mV showed that the peak current increased with the increase of accumulation time up to 60 s and then decreased. However, further increase of accumulation time does not cause the obvious enhancing of the stripping peaks current, which is probably due to the saturation loading of the electrode surface.[28] Therefore, accumulation potential of 60 s was selected as an optimum accumulation time for further experiments.

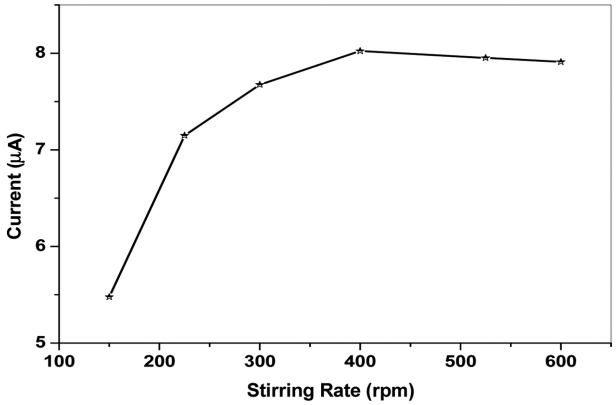

***Figure* 5.** Effect of stirring rate on the peak current of copper (II). Other conditions are the same as in *Fig.* 1.

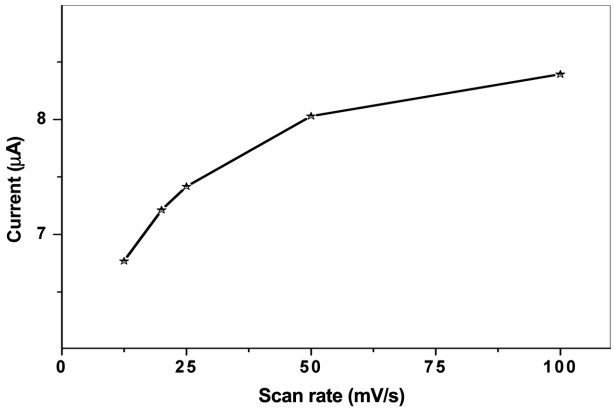

***Figure* 6.** Effect of scan rate on the peak current copper (II). Other conditions are the same as in *Fig.* 1.

Effect of Stirring of the Solution

In order to increase the adsorption rate, stirring of the solution during the accumulation of analyte seems to be effective. To prevent the perturbation of the adsorption rules; optimization of stirring speed is suggested. We observed that the accumulation amount of Copper-ligand on the hanging mercury drop electrode enhanced as stirring speed increased until reaching a maximum value at about 400 rpm and then decreased at higher stirring speed *Fig.* 5. This may be attributed to more distribution of the solution that may perturb the adsorption stripping governed in the system. Therefore, 400 rpm was suggested as optimum stirring speed for the accumulation of copper-ETSC on the mercury electrode.

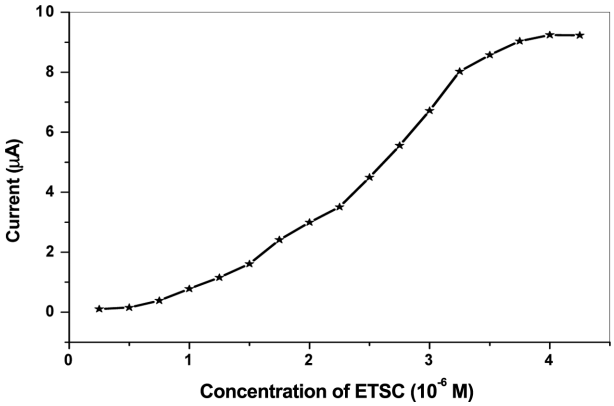

***Figure* 7.** Effect of 4-ethyl-3-thiosemicarbazide concentration on the peak current of copper (II). Other conditions are the same as in *Fig.* 1.

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 12.5–100 mV/s scan rate (from studied range 12.5–100 mV/s). However, when scan rates faster than 50 mV/s were employed, the peak current decreased slightly. The influence of scan rate on the observed voltammetric signal is illustrated in *Fig.* 6, which indicates that scan rate value of 50 mV/s would be adequate optimum for succeeding investigations.

Influence of 4-Ethyl-3-thiosemicarbazide Concentration

The influence of 4-ethyl-3-thiosemicarbazide concentration on the sensitivity of proposed method was studied for the range 2.5×10^{-7}– 4.25×10^{-6} M. The obtained results *Fig.* 7 show that with increasing the 4-ethyl-3-thiosemicarbazide concentration up to about 3.25×10^{-6} M, the cathodic stripping peaks current of Cu-ETSC increased and then were leveling off at higher concentrations. This is due to the competition of ETSC with Copper complexes for adsorption on the HMDE. So, an optimum 4-ethyl-3-thiosemicarbazide concentration of 3.25×10^{-6} M was selected for further experiments.

Table 1 shows a comparison between detection limit, linear dynamic range and accumulation time of the proposed method and those previously reported. According to the results, the limits of detection of these methods are reported to be not good.[29,30] Some others need a long deposition time,[31–34] or have a low linear dynamic range,[35,36] in a recent work has a good detection limit and low linear dynamic range.[37] But in the current paper, the deposition time is short (60 s), and detection limit is very low compared to many previously reported research works.

***Table* 1.** Some critical points in present work compared with some previous works performed by adsorptive stripping voltammetry applied for determination of copper

Ligand	LOD (ng/mL)	Dynamic range (ng/mL)	Accumulation time (s)	Reference
Morin	0.6	0.2–130	60	29.
Thymolphtalexone	0.4	0.5–100	60	30.
2,7-PADN	0.51	0.6–64.0	240	31.
Bis (acetylacetone) ethylenediimine	1.02	3.18–63.6	600	32.
Nuclear fast red	0.2	1–100	180	33.
Cyclopentanone thiosemicarabzone	0.2	0.1–99.8	150	34.
DMG and catechol (mixed)	0.03	0.03–6.35	60	35.
5,5-Dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3 thiosemicarbazone	0.49	0–35	60	36.
Thiosemicarbazide	0.007	0.01–90.0	60	37.
4-Ethyl-3-thiosemicarbazide	0.001	0.003–125	60	This work

***Table* 2.** Tolerance limit of foreign ions on copper (II) (50 ng/mL) determination by proposed procedure

Foreign ions	Tolerance limit [$W_{Foreign\ ion}/W_{Cu(II)}$]	Recovery (%)
Zn^{2+}	1000	98.9
Pb^{2+}	1000	95.6
K^{+}, Na^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}	850	98.7
Cr^{+3}	500	96.8
Cr^{+6}	500	95.1
Cd^{2+}, Mn^{2+}	500	97.8
Co^{2+}	250	95.3

Effect on Interferences

Possible interference by other metals with the adsorptive stripping voltammetry of copper was investigated by the addition of the interfering ion to a solution containing 50 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). *Table* 2 shows the results. As can be seen a very good selectivity is achieved. As most of the samples, especially, biological samples do not contain these interfering ions at such a high concentration, so the specificity of this method for such samples cannot be ignored. However, no such interference was observed with other ions as mentioned above.

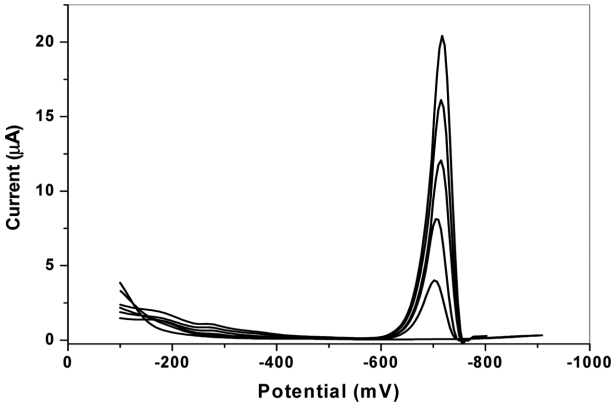

***Figure* 8.** Voltammetric response from adsorptive stripping voltammetry of 25, 50, 75, 100 and 125 ng/ml copper concentration and $C_{ETSC}=3.25\times10^{-6}$ M measured on a mercury electrode in pH=10.3 potassium acetate buffer using a 60 s accumulation time at a potential of −100 mV, Stirring rate 400 rpm and Scan rate is 50 mV/s.

Accuracy, Precision and Detection Limits

A linear response over the concentration range of 0.003 to 125 ng/mL Cu(II) was observed under optimum conditions, with correlation coefficient of 0.999 *Fig.* 8. The detection limit for copper was found to be 0.001 ng/mL. The accuracy, recovery and precision of the method used were tested in 10 replicate tests with a reference materiel (Seronorm Trace Elements Whole Blood, levels 1 and 3, Billingstad, Norway) (*Table* 3). All samples and standards were analyzed by duplicates.

***Table* 3.** Accuracy, precision and recovery of the method against a standard reference material

Material	Mean ± SD (ng/mL)		Acceptable range (ng/mL)	Accuracy (%)	Precision (%)	Recovery (%)
	Certified	Measured				
(level 1, MR4206)	564±5.4	550.191±5.268	531–597	97.55	2.05	98.67
(level 3, O512627)	1740±151	1673.392±145.219	1438–2042	96.17	2.71	96.01

The mean and standard deviation of fifteen healthy human in the western Algerian population (Tlemcen city) were 886.468±248.936 ng/mL. The range for all samples was 439.016–1271.774 ng/mL. No significant differences were observed in blood copper concentrations after applying to them the Student's t-test.

CONCLUSION

The present study demonstrates that adsorptive stripping voltammetry of copper based on accumulation of copper-4-ethyl-3-thiosemicarbazide complex can be used to determine trace amounts of copper in real samples. The detection limit of this technique is 0.001 ng/mL copper (II) at a collection period of 60 s. In conclusion, this method offers a practical potential for trace determination of copper with high selectivity, sensitivity, simplicity and speed that have not been present together in the previously reported systems.

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