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The versatility of salicylaldehyde thiosemicarbazone in the determination of copper in blood using adsorptive stripping voltammetry

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Abstract

The adsorptive cathodic stripping voltammetry technique (AdCSV) is used to determine copper(II) using salicylaldehyde thiosemicarbazone (N, S- donor) as a complexing agent on hanging mercury drop electrode at pH 9.3. Variable factors affecting the response, i.e. the concentration of ligand, pH, adsorption potential and adsorption time are assessed and optimized. The adsorbed complex of copper(II) and salicylaldehyde thiosemicarbazone gives a well defined cathodic stripping peak current at −0.35 V, which has been used for the determination of copper in the concentration range of 7.85×10^{-9} to 8.00×10^{-6} M with accumulation time of 360 s at −0.1 V versus Ag/AgCl. This technique has been applied for the determination of copper in various digested samples of whole blood at trace levels.

Keywords: Adsorptive stripping voltammetry; Salicylaldehyde thiosemicarbazone; Copper; Blood samples

1. Introduction

Copper is a metal of prime environmental concern. The technique of voltammetry is suitable for determination of trace elements in environmental and biological samples, because it is highly sensitive, simple and only a very simple pretreatment of the sample is required [1].

The application of voltammetric techniques in the determination of trace copper has been reviewed [2]. Of these methods, anodic stripping voltammetry on a hanging mercury drop electrode (HMDE) has gained wide acceptance for copper determination [3]. The disadvantages of this method are the formation inter-metallic compounds with co-existing metal ions at the electrode, which can cause serious error [4], and the presence of ligand such as chloride in the sample solution, which disturbs the stripping wave [5]. To solve the disadvantages of anodic stripping voltammetry for copper determination, an organic ligand was used to complex with copper(II) with adsorptive property rather than electrolytic accumulation on the surface of the electrode.

The method of adsorptive accumulation is useful to concentrate an analyte selectively for voltammetric analysis or to concentrate the ions of metals, which have low solubility in mercury. It is well known that copper forms inter-metallic compounds with other metals at electrode in anodic stripping voltammetry. These compounds interfere considerably in copper determination. So the determination of copper by measuring its reduction current after accumulation without its electrolysis is the better method. A number of studies on the use of AdSV for the determination of copper have been reported and compared [6–9]. These methods present advantages and disadvantages in relation to the sensitivity, selectivity and resolution of the adsorptive stripping peak current.

Salicylaldehyde thiosemicarbazone (Hstsc) has been used as an ionophore for the detection of mercury using PVC based ion selective electrode technique [10]. In view of excellent properties of Hstsc towards Cu(II) [11], it was

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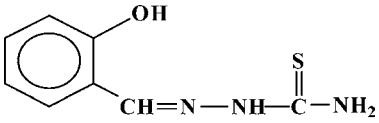
desired to extend the analytical applications of this reagent using AdCSV. In this paper, the adsorptive cathodic stripping voltammetry of the copper complex with salicylaldehyde thiosemicarbazone is investigated.



Salicylaldehyde thiosemicarbazone

2. Experimental

2.1. Apparatus and reagents

A polarograph, Metrohm, (μ-Autolab, Type-II) equipped with static mercury dropping electrode was used. The three electrode system consists of a working hanging mercury drop electrode (HMDE), platinum auxiliary electrode and Ag/AgCl saturated with KCl as reference electrode, was used to measure the cathodic current. pH measurements were made on pH meter (Elico, LI-120).

All the reagents used were of Analytical Reagent grade. A stock solution of 1×10^{-2} M copper was prepared by dissolving 99.99% pure metal (Aldrich) in 5% nitric acid. A 1.0×10^{-3} M stock solution of salicylaldehyde thiosemicarbazone (synthesis given below) was prepared in tetra-hydrofuran. The supporting electrolyte was 0.1 M ammonia/ammonium chloride buffer (pH 9.3). Working solutions were prepared by diluting the stock solutions with de-ionized double distilled water.

2.2. Synthesis of salicylaldehyde thiosemicarbaozone

Thiosemicarbazide (0.746 g) was dissolved in 75 mL deionized double distilled water by heating. It was then added to salicylaldehyde (1 g) and the reaction mixture was refluxed for 3–4 h. Crystals of salicylaldehyde thiosemicarbazone were obtained on cooling which were separated by filtration and were dried by vacuum pump. Crystals so obtained were re-crystallized from ethanol. A yield of 80% was obtained.

2.3. Blood sample preparation

Approximately 2 mL blood samples were taken from 40 children with special care, by vein puncture using disposable syringes and needles, and were placed into heparinized pretreated clean polypropylene tubes. 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. Only reagents with low background impurities were used.

2.4. Procedure

Twenty millilitres of 0.1 M ammonia/ammonium chloride buffer of pH 9.3, was pipetted into the cell along with 1.6×10^{-8} M Hstsc. Nitrogen gas was purged for 4 min. A new mercury drop was made to form and deposition was conducted for 6 min at −0.1 V, while stirring the solution. At the end of the deposition, the stirrer was switched off and 10 s elapsed to allow the solution to become quiescent. Voltammograms were then recorded by scanning the potential in the negative direction up to −0.8 V versus Ag/AgCl electrode by differential pulse stripping voltammetry with a scan rate of 5 mV/s, pulse amplitude of 50 mV/s, pulse repetition time of 1 s. The peak so obtained was labeled as $I_{p.b}$. After the background (blank) voltammogram has been obtained, aliquots of Cu(II) solution was added into the cell while maintaining the inert atmosphere inside the cell with nitrogen gas. Scanning of the potential was again performed by same method. The current amplitude was labeled $I_{p.s}$. Net peak current ($\Delta I_p = I_{p.s} - I_{p.b}$) was plotted against Cu(II) concentration to get calibration graphs.

3. Results and discussion

Figs. 1 and 2 show adsorptive stripping voltammograms of salicylaldehyde thiosemicarbazone and its complex with copper(II), respectively. These voltammograms were obtained in the presence of 0.1 M buffer of ammonia/ammonium chloride (pH 9.3), accumulation time of 6 min and deposition potential of −0.1 V versus Ag/AgCl. The reduction peak of the ligand with concentration 1.6×10^{-8} M appeared at −0.62 V. Two potential reduction sites in the ligand are C=N and C=S and these are associated with π^* molecular orbital. The π^* molecular orbital of C=S group are at low energy vis-a-vis π^* molecular orbital of C=N group. It is likely that this group (C=S) gets reduced at the said potential. This peak at −0.62 V was confirmed by the fact that there was a proportional increase in peak current/area with increase in both the concentration of reagent and increase in accumulation time. A new peak at −0.35 V appeared on adding copper(II), (in concentration range, 1.0×10^{-8} to 1.0×10^{-9} M) which was due

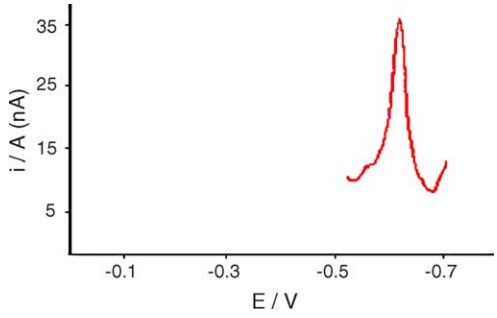


Fig. 1. Voltammogram for 1.6×10^{-8} M Hstsc after deposition of 6 min at −0.1 V in 0.1 M ammonia/ammonium chloride buffer (pH 9.3).

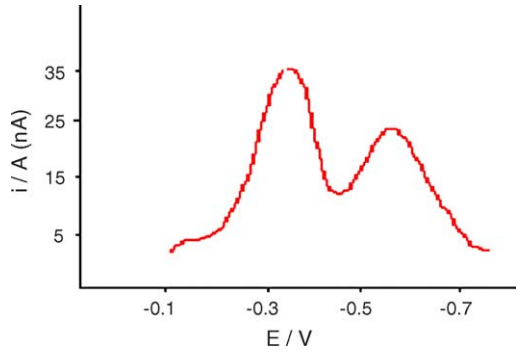


Fig. 2. Voltammogram for Cu–$(stsc)_2$ complex after accumulation for 6 min at −0.1 V in 0.1 M ammonia/ammonium chloride buffer (pH 9.3) containing 1.6×10^{-8} M Hstsc + 7.85×10^{-9} M Cu(II). Pulse amplitude 50 mV, pulse repetition time 1 s, scan rate 5 mV/s.

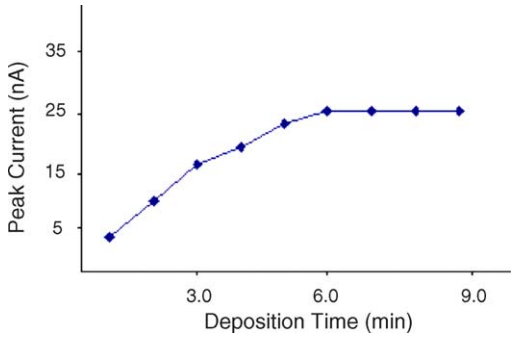


Fig. 3. Effect of deposition time. Accumulation at −0.1 V in 0.1 M ammonia/ammonium chloride buffer (pH 9.3) containing 1.6×10^{-8} M Hstsc + 7.85×10^{-9} M Cu(II). Pulse amplitude 50 mV, pulse repetition time 1 s, scan rate 5 mV/s.

to the formation of Cu–$(stsc)_2$ complex (Fig. 2). The change in concentration of ligand (explained later) showed that the complex formed had a stoichiometry of 1:2 (copper:ligand). The influences of potential scan rate on the current and on the potential of the adsorbed Cu(II)–ligand system showed a shift of the peak potential with the scan rate. This phenomenon indicates some irreversibility of the redox process, which is also characterized by stronger separation of cathodic and anodic peaks. The adsorption of the reactants at the surface of electrode usually lead to such type of characteristics [12,13], indicating an effective interfacial accumulation of the Cu(II)–ligand complex at the electrode surface.

The process of electrolysis was used to obtain some information about the reactants and products. The working electrode was mercury pool. Electrolysis was performed at a constant potential of −0.5 V. After the reduction peak current of Cu–ligand complex had dropped down to zero; a defined amount of Cu(II) was added to the cell. By using AdCSV, the peak corresponding to the reduction of Cu(II)–ligand was obtained again. The same electrolysis experiment was performed and after completion of electrolysis, a defined amount of ligand was added to the solution. In this case, no peak current was obtained in adsorptive stripping voltammetry. The above studies indicate that the reactant was copper(II) in the copper–ligand complex.

An increase in deposition time resulted in increased peak current, showing evidence for the adsorptive nature of the complex. In addition, small amounts of surfactant such as Triton X-100 and sodium dodecyl sulphate, strongly suppressed the peak current. These two phenomenons indicate that the complex was strongly adsorbed on the mercury electrode surface.

The effect of various parameters like, deposition time, concentration of ligand, deposition potential, pH, etc., were studied and optimized for the determination of copper and are discussed below.

3.1. Effect of deposition time

Deposition time from 0 to 10 min was studied and best results were obtained at 6 min, where the maximum peak current was observed. There was increase in the peak current at the initial stage up to 6 min and was constant for time longer than 6 min, because of adsorptive equilibrium between the electrode surface and the solution. The relationship of deposition time with peak current is shown in Fig. 3.

3.2. Effect of ligand concentration

The effect of concentration of Hstsc on the peak current (Fig. 4) was studied at pH 9.3 for the range 5.0×10^{-9} to 5.0×10^{-7} M. An increase in peak current up to 1.6×10^{-8} M and then leveled off, was observed. At the break point (1.6×10^{-8} M) the Hstsc concentration is just twice that of Cu(II). This suggested that the adsorbed species is the 1:2 copper:Hstsc complex. This was confirmed by changing the concentration of Cu(II) ion, with constant concentration of Hstsc.

3.3. Effect of deposition potential

Fig. 5 shows the relationship between deposition potential and peak current. It was observed that reproducible peak current was obtained at −0.1 V; however, at potential more positive than −0.1 V, the oxidation of mercury and consequent increase in the base current interfered in the

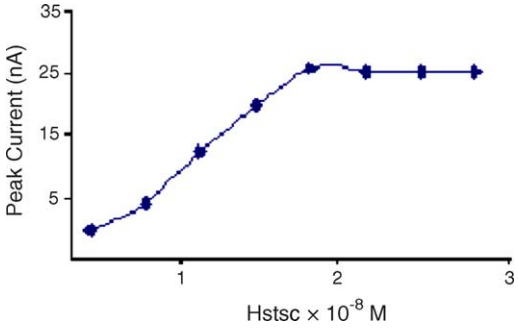


Fig. 4. Effect of ligand concentration. Accumulation for 6 min at −0.1 V in 0.1 M ammonia/ammonium chloride buffer containing 7.85×10^{-9} M Cu(II).

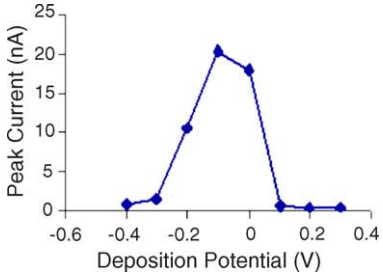


Fig. 5. Effect of deposition potential on the peak current, conditions same as in Fig. 3.

measurement of reduction current of the complex of copper. On the other hand, at more negative potential than −0.3 V the peak current decreased sharply because the reduction of complex had already taken place at −0.1 V. So deposition potential of −0.1 V was used in all subsequent analysis.

3.4. Effect of pH

Fig. 6 shows the relationship between pH and the peak current. Stability of the complex largely depends on the pH of the system. A system may become unstable with a small variation in the pH. So far the optimization of a stable complex of Cu–$(stsc)_2$ was concerned, a large variation in pH values, from 2 to 11 was studied. Initially, there was an increase in peak current with rise in pH up to 9.3 and a sharp fall in current value then onwards. This was due to the increasing complex formation of copper(II) with the ligand at the electrode surface with increasing pH. At pH more than 9.3, the precipitation of copper as $Cu(OH)_2$ occurred resulting in sharp decrease in peak current. The effect of concentration of ammonia/ammonium chloride buffer between 0.1 and 1 M was also studied and no effect as such was observed. However, all the experimentation was performed at 0.1 M concentration of buffer.

3.5. Interferences

The interference study of different ions like lead, cadmium, zinc, magnesium, calcium, manganese, barium, iron, sodium, potassium, chloride, phosphate and sulphate was made. It was observed that lead, cadmium and zinc ions

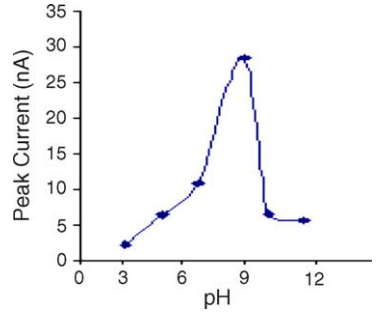


Fig. 6. Effect of pH on the peak current, conditions same as in Fig. 3.

Table 1
Determination of copper in synthetic samples

Serial number	Amount of copper added (1×10^{-9} M)	Amount of copper recovered (1×10^{-9} M)	Percentage recovery
1	8.0	7.9	98.7
2	10.0	9.6	96.0
3	15.0	14.5	96.6
4	20.0	18.7	93.5
5	25.0	24.2	96.8

interfere with copper determination when concentration was 5 mg/L, but do not interfere below 5 mg/L. 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.

3.6. Accuracy, precision and detection limits

A linear response over the concentration range of 7.85×10^{-9} to 8.00×10^{-6} M Cu(II) was observed under optimum conditions, with correlation coefficient of 0.998. The detection limit for copper was found to be 7.85×10^{-9} M. The validity of the method was further ascertained by cross method check, spike recovery (Table 1) and replicate analysis. For 10 successive determinations of 8×10^{-8}, 8×10^{-7} and 8×10^{-6} M Cu(II), relative standard deviation of 4.7, 3.9, 2.7%, respectively, were obtained.

3.7. Application to blood samples

The developed method has been applied to the blood samples for copper determination and the values have been compared with AAS (Table 2). As the amount of copper found in blood samples was quite high so dilution of the blood samples was done accordingly. Reagent blanks were taken along with each set of sample and the metal concentration observed in these blank samples were negligible because 1 mL of the digested sample was diluted to 100–200 times, while carrying out the analysis. It is evident from the table that this technique has provided better sensitivity and accuracy and can be used for on-site monitoring of environmental and biological samples.

Table 2
Determination of copper in blood samples

Site number	Location	Number of blood samples in each set.	Cu estimation by AAS[a] (1×10^4 nM)	Cu estimation by AdCSV[a] 1×10^4 nM
1	Set 1	10	0.94 ± 0.07	1.05 ± 0.008
2	Set 2	10	1.11 ± 0.06	1.18 ± 0.005
3	Set 3	10	1.68 ± 0.08	1.65 ± 0.007
4	Set 4	10	1.57 ± 0.06	1.71 ± 0.008

[a] Average value.

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