

Polarographic Catalytic Hydrogen Wave Technique for the Determination of Copper(II) in Leafy Vegetables and Biological Samples[†]

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An economical, novel, eco-friendly and robust method for the quantification of copper(II) in various leafy vegetables, milk and blood samples has been developed using direct current catalytic hydrogen wave (DC-CHW) technique involving the formation of ammonium salts of piperidine/morpholine dithiocarbamates metal complex. Ammonium piperidine/morpholine dithiocarbamates complexed with copper(II) in the presence of NH₄Cl-NH₄OH medium at pH 6.5±1 produces CHW's at -0.35±1 V vs SCE. Consequently, various optimal parameters such as preparation medium, effect of pH, ligand concentration, metal ion concentration and indifferent ions effect on peak height were optimized to enhance the sensitivity and selectivity. The novel aspect in the work address the interaction of ammonium piperidine/morpholine dithiocarbamates with copper(II) were confirmed with cyclic voltammetry (CV) and supported by computational calculations using Density Functional Theory (DFT) methods. Furthermore, the student "t"-test and variance ratio "f"-tests indicated no significant difference between the present method and the differential pulse polarographic (DPP) method.

Keywords: Direct Current Catalytic hydrogen wave (DC-CHW) technique; Copper(II); Dithiocarbamates (Ammonium Piperidine dithiocarbamates [Amm Pip-DTC] and Ammonium Morpholine dithiocarbamates [Amm Mor-DTC]); D.C Polarography(DC); Differential Pulse Polarography (DPP); Leafy vegetables and biological samples.

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1. INTRODUCTION

Copper an essential element in all living organisms are widely used for metabolic processes and are considered as a prototype for the emergence of biologically important functional systems [1]. As a malleable metal, copper is extensively used for domestic and industrial purposes. Since high levels of copper can be detrimental to organisms, it is very useful in the control of unwanted organisms. Thus, the metal plays many roles in humans, microorganisms, plants, animals belonging to Molluscan's, Arthropodian phylum's and especially invertebrates in the development of a respiratory protein (haemocyanin) [2]. Copper occurs naturally in most vegetables, meat and grains. The study of copper in food items is of great concern, since it plays a definitive role in the intrinsic mechanisms regulating vital biological processes [3-5]. Copper toxicity is a much-overlooked cause of many important health conditions including fatigue, premenstrual syndrome, anorexia, depression, anxiety, migraine headaches, allergies (food and environmental allergies). During congenital deficiency, the copper metal accumulates in the liver, discrete areas of the brain, the cornea of the eye and other tissues causing Wilson's disease [6]. Wide varieties of clinical disorders have been associated with a dietary deficiency of copper which respond to copper therapy. They include anemia, depressed growth, neo-natal ataxia, impaired reproductive performance, heart failure and gastro-intestinal disturbances [7].

Apart from biological utility of copper, it has several applications in industrial sectors [8] including in the electrical industry and fine wires, commutator bars and high conductivity tubes. It is also used in pipe making, roof sheeting, bronze paint and insecticides. However, it is also a pollutant in the environment resulting from the industrial discharge in the form of particulate or soluble copper waste from electroplating, chemical and textile industries. As a pollutant, copper is of particular concern, because of the high degree of toxicity to aquatic organisms. In view of this determination of copper is indispensable.

Several methods for the detection of copper metal are available in literature. For instance, solvent extraction into n-butanol or cyclohexanone in the presence of oxime for determination of micro amounts of copper with polarography was reported by Rao and Rao [9,10]. Similarly, Vatamin et al [11] reported polarographic certification analysis of standard samples of copper-based alloys. Copper(II) in presence of HCl-thioglycolic acid, 1,10-phenanthroline exhibited an adsorptive catalytic wave which was applied for trace amounts of copper in waste and natural waters with a detection limit $0.03 \mu\text{g mL}^{-1}$ [12]. Thiosemicarbazones of benzaldehyde, salicylaldehyde, biacetyl, benzyl, acetyl acetone, acetonyl acetone in DMF were employed for the determination of copper by polarography [13]. Polarographic adsorptive complex wave method was established for the determination of micro amounts of copper in human hair samples [14]. Biernat and Syzmaszek [15] reported the polarographic waves of copper(II) in the presence of sulphuric acid and thiourea applied for the analysis of copper(II) in various environmental samples.

Reversible two electron reduction waves were obtained at pH 1.2 - 4.2 and 6.1 -11.9 for copper(II)-2-amino butanoate solution system with detection of 0.6 ppm for the determination of copper(II) in various environmental samples [16]. Lei et al [17] developed a method for the detection of copper(II) in aluminum alloys using sodium acetate buffer and benzoin oxime. Polarographic

method was employed for the analysis copper in Dutch rennet cheeses by dry ashing of a sample in presence of chloride-ammonia background electrolyte and anhydrous sodium sulphite [18]. An indirect polarographic technique for the determination of copper(II) was established based on the highly stable complex formation of Cu(II)-Salicyladehyde thiosemicarbazone by Palaniappan and Revathi [19]. Antipyrine was used as an electro-analytical reagent for the analysis of trace quantities of copper(II) in the presence of sodium perchlorate medium using catalytic currents coupled to polarography [20]. Voltammetric investigation was done for the quantification of copper(II) with xanthates as a chelating ligands at DME using dc polarography [21].

Thioglycollic acid-1,10 phenanthroline system exhibits an adsorptive catalytic waves with copper(II), which was applied for the quantification of copper(II) in waste and natural waters [22]. An indirect kinetic method [23] was established for the determination of trace copper in human hair and finger nails based on catalytic function to the reaction between ethyl orange and ascorbic acid. The polarographic peak is observed at -0.30 V vs SCE. A post-column method was developed for the determination of toxic metals as metal-1-(2-pyridylazo)-2-naphthol complex using ion-exchange chromatography [24]. On-line preconcentration technique with nanometer-sized alumina packed micro column was employed for analysis of trace metals with ICP-AES in various environmental samples [25]. Gallic acid immobilized Amberlite XAD-16 was synthesized for the development of column and batch method to analyze Cr(III), Mn(II), Fe(II), Co(II), Ni(II) and Cu(II) in waste water samples with FAAS [26]. Pre-capillary complexation methodology was adopted for the analysis of alkali, alkaline and transition metals in presence of glycolic acid & α -hydroxyisobutyric acid with capillary electrophoresis [27]. Voltammetric studies were carried out with nanocrystalline diamond thin film electrode to analyze Ag(I), Cu(II), Pb(II), Cd(II) and Zn(II) in different environmental samples [28]. Alumina hollow fiber was developed for the determination of copper(II), manganese(II) and nickel(II) in water systems with inductively coupled plasma-optical emission spectroscopy [29]. Multivariate calibration techniques were developed for the simultaneous determination of copper and iron with 1-(2-Pyridylazo)-2-naphthol in AOT Micellar Solution by Ghasem et al [30]. Chemometric methods were also adopted for solving theoretical and experimental problems in chemistry to analyze the metal ion estimation in complex mixture from spectral data [31, 32].

Literature survey revealed applicability of dithiocarbamates has not been used so far, as electro-analytical complexing reagents for the determination of copper(II) in various leafy vegetables, milk and blood samples. Accordingly, it is desirable to develop a more facile, novel, sensitive, selective, rapid and economical method for the quantification of copper(II) by synthesizing ammonium piperidine/morpholine dithiocarbamates (Amm Pip/Mor-DTC) that gives catalytic hydrogen currents with the metal [copper(II)] at dropping mercury electrode. The present eco-friendly method does not require elaborate cleanup procedure and extraction of copper(II)-Amm Pip/Mor-DTC complexes into the organic solvents, and thus the usage of hazardous and environmentally unfriendly chemicals such as chloroform and carbon tetrachloride [33, 34] are prohibited. This paper describes the polarographic determination of copper(II) using Amm Pip/Mor-DTC in ammonium chloride-ammonium hydroxide medium and the results were supported by CV and computational calculations. It was found that it gives a pronounced direct current-catalytic hydrogen wave (DC-CHW) with peak potential at -

0.35±1 V vs SCE and diffusion half wave current at ($E_{1/2}$) at -0.51 V vs SCE in $\text{NH}_4\text{Cl-NH}_4\text{OH}$ medium.

2. EXPERIMENTAL

2.1. Apparatus

The current-voltage curves were recorded using a D.C. Polarographic analyzer, model *CL-357* coupled with model LR-101 strip chart recorder manufactured by Elico Private Ltd (Hyderabad, India). Effects of mercury height on polarographic currents were studied using D.C. recording polarograph model *CL-25* of Elico Pvt Ltd, Hyderabad. The current-voltage curves were recorded using polarographic analyzer, model *CL-362* coupled with optional printer manufactured by Elico Private Limited (Hyderabad, India). Cyclic voltammetric studies were performed with a 797 VA Computrace (Metrohm, Herisau, Switzerland). All pH measurements were made using pH meter, model *LI-120* (Elico Pvt. Limited, India) with combined electrode of pH range 0-13.

2.2. Reagents and Chemicals

All the experiments were performed at 25 °C using freshly prepared solutions. Double distilled mercury and deionized water were used. The dissolved oxygen in the solutions was removed by passing nitrogen (99.8% purity) gas for 10 -15 minutes. Standard metal ion solution was prepared by taking accurately 3.928 g L^{-1} of CuSO_4 to get 1 $\mu\text{g mL}^{-1}$ and adding 3 mL of concentrated HNO_3 corresponding to the anions of the salts to suppress the hydrolysis. Ammonium Chloride (1 M) (S.D Fine Chemicals, India) was prepared by weighing 53.49 g of ammonium chloride (Anala R) and dissolving in 1000 mL of deionized water.

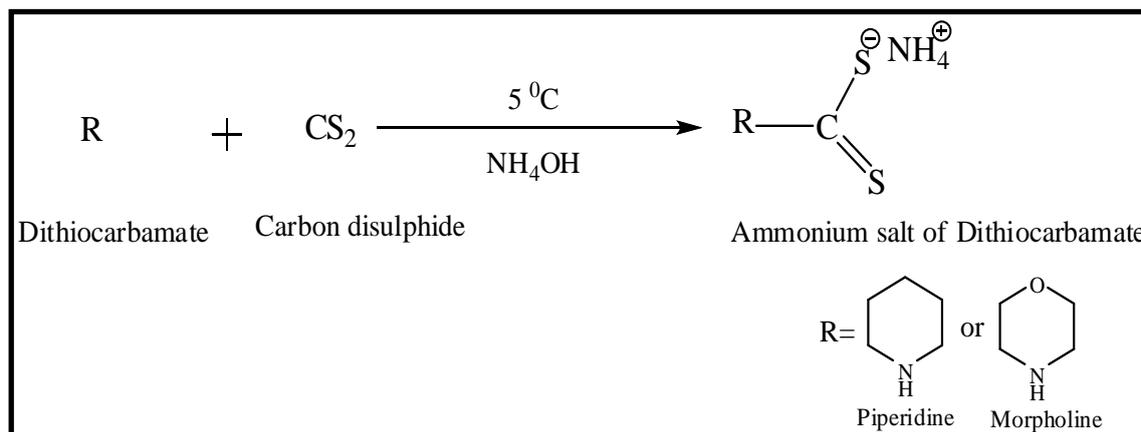
For pH adjustments, the ammonium hydroxide (5%) and HCl (1%) (S.D Fine Chemicals, India) solutions were prepared. Potassium iodate (S.D Fine Chemicals, India) and sodium sulphite (S.D Fine Chemicals, India) were also prepared in deionizer water using AnalaR samples. Triton X-100 was prepared in W/V basis. 0.200 g of Triton X-100 was weighed and dissolved in 100 mL doubly distilled deionized water in a standard flask. Gelatin (Difco laboratories, USA) was prepared by weighing accurately 0.125 g and dissolving in 25 mL of deionized water.

2.3. Method

2.3.1. Synthesis of ammonium salts of piperidine/morpholine dithiocarbamates

Carbon disulphide (80 g) was slowly added to a solution of piperidine/ morpholine (85 g) of each in 25 mL of distilled water at 5 °C with constant stirring, followed by addition of ammonium hydroxide (20%) for neutralization. The resultant reaction mixture was warmed at room temperature and washed repeatedly 2-3 times with purified acetone. The product was purified by recrystallization

in acetone [35] which has melting points of 196-199 °C (Amm Pip-DTC) and 182-185 °C (Amm Mor-DTC) at 740 mm pressure as shown in Scheme 1.



Scheme 1. Reaction route for the preparation of ammonium salt of piperidine/morpholine dithiocarbamates

2.3.2. Computational calculations for copper(II)-dithiocarbamates complexes

In order to better understand the possible coordination of synthesized dithiocarbamates with the copper(II) metal (see Figure 1), Density Functional Theory (DFT) calculations were performed in the presence/absence of ammonium ions.

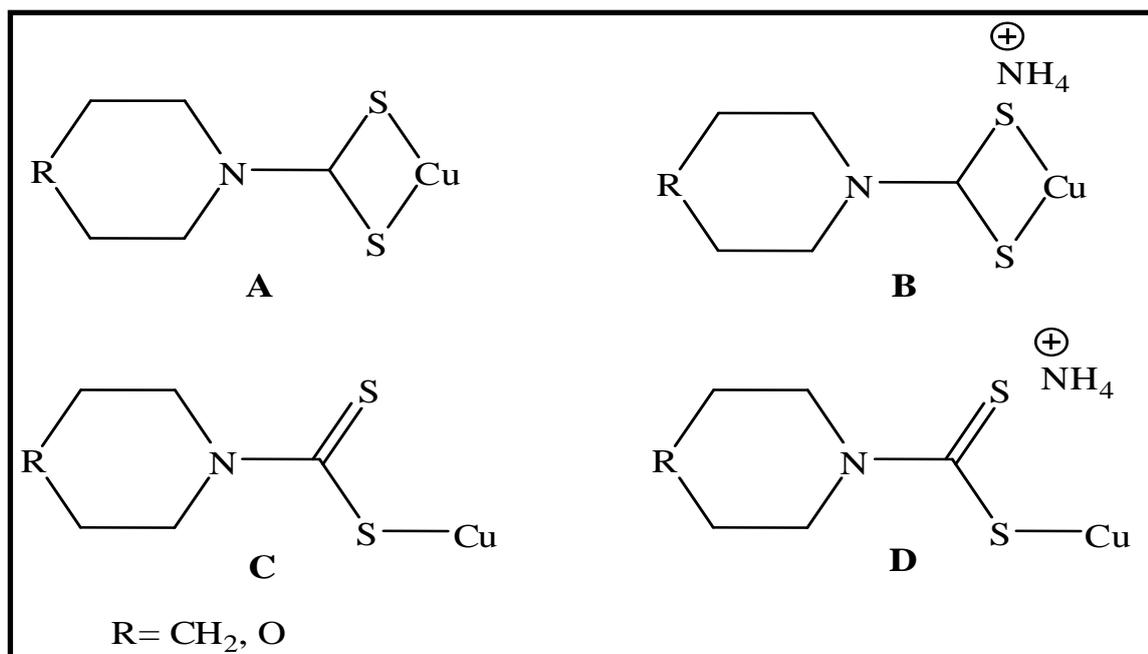


Figure 1. Possible coordination for the both single and double coordination of sulfur atoms with copper(II) in the presence/absence of ammonium ions of ammonium salt of piperidine/morpholine dithiocarbamates

All starting structures (A-D, Figure 1) were fully optimized at the B3LYP level using the 6-31G*/lanl2dz mixed basis sets. All calculations were performed using the Gaussian 03 computer program [36].

2.3.3 Procedure for determination of Copper(II) by DC-CHW technique

A measured volume of the $\text{NH}_4\text{Cl-NH}_4\text{OH}$ buffer and ligand (Amm Pip-DTC and Amm Mor-DTC) solutions at optimum pH were made up to 100 mL in beaker with distilled water and then adequate amount transferred into the polarographic cell followed by addition of the required sample or standard copper(II) solution. The dissolved oxygen was expelled by bubbling pure nitrogen through the analyte solution for 15 min. Polarogram of the solution were recorded using D.C. Polarography at -0.35 and -0.36 V vs SCE in ammonium chloride-ammonium hydroxide medium for Amm Pip-DTC and Amm Mor-DTC respectively. Dithiocarbamates (Amm Pip-DTC and Amm Mor-DTC) or simple metal ions in the medium do not give any current signal at the potential mentioned.

2.3.4 Preparation of samples

To test validity of the proposed method, different leafy vegetables and biological samples were collected, processed for the quantification of copper(II) after complexation with Amm Mor-DTC in presence of $\text{NH}_4\text{Cl-NH}_4\text{OH}$ medium by evolution of catalytic hydrogen currents using dc polarography and differential pulse polarography in real samples.

2.3.4.1. DC-CHW technique for analysis of copper(II) in vegetables

3.5 g of leafy vegetables were collected from local sources and digested by dry ash method. The mass was made up to 100 mL with deionized water and determined as per aforesaid procedure in

2.3.3. The obtained data is presented in Tables 3a-b.

2.3.4.2. DC-CHW technique for analysis of copper(II) in milk samples

A 100 mL sample of milk was added drop wise into a crucible and heated to 450 -500 °C for 1 hr to remove moisture and evaporate it without frothing. The obtained dark ash was dissolved in 3 mL of concentrated HNO_3 and evaporated, and again dissolved in the 3 mL of dilute HNO_3 . The sample was filtered and filtrate was made up to 25 mL in a volumetric flask with deionized water. Required volume of the sample was used for the analysis of copper(II) using aforesaid procedure in 2.3.3. The results obtained are show in Tables 3c-d.

2.3.4.3. DC-CHW technique for analysis of copper(II) in blood samples

Blood samples obtained from local sources were digested by mixing 30 ml of sample, 10 ml of deionised water, 18 ml concentrated HNO_3 and 5 mL of 30% H_2O_2 in a 100 ml beaker. The mixture was heated while stirring until the volume decreased to half than filtered. The filtrate was made up to a 250 ml volumetric flask with deionized water for the analysis of copper(II) using the aforesaid procedure in 2.3.3. The results obtained are tabulated in Tables 3c-d

3. RESULTS AND DISCUSSION

3.1. Optimization studies of experimental parameters

Various optimal conditions developed for the quantification of copper(II) with catalytic hydrogen current technique are reported below.

3.1.1. pH effect

The effect of pH ranging from 5 – 10 on solution containing 1.0 ppm of copper(II) in 0.3 M ammonium chloride for Amm Pip-DTC/Amm Mor-DTC as complexing agents were studied, by adjusting with ammonium hydroxide. With increasing pH, the height of the catalytic wave increased after attaining a maximum peak current of pH 6.6 for Amm Pip-DTC and pH 6.4 for Amm Mor-DTC, the wave height decreased with further increase in pH. The maximum wave heights of the polarograms were selected as optimum pH which was maintained in all other studies. The results are presented in Figure 2.

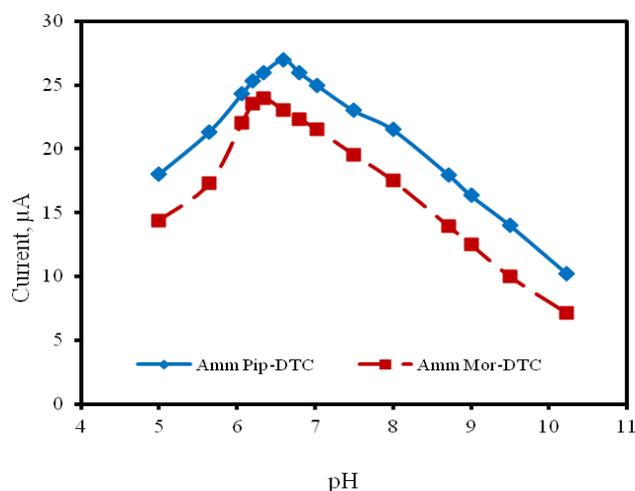


Figure 2. Effect of pH on copper(II)-Amm Pip-DTC[conditions:- pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0 mM, copper(II)= 0.05 ppm] and copper(II)-Amm Mor-DTC[conditions:- pH=6.4, supporting electrolyte= 0.3 M, Amm Mor-DTC= 3.0 mM, copper(II)= 0.05 ppm] complex systems.

3.1.2. Effect of supporting electrolyte concentration

The effect of ammonium chloride concentration (between 0.1 to 0.8 M) on the nature of current-voltage curves at dropping mercury electrode (DME) for Amm Pip-DTC and Amm Mor-DTC complexes has been studied, keeping the copper(II) ion concentration at 0.05 ppm. The polarograms were well defined in NH_4Cl of 0.3 M for both dithiocarbamates and the peak height was decreased beyond this concentration as shown in Figure 3. Therefore, the optimum concentration (0.3 M) was maintained for further studies.

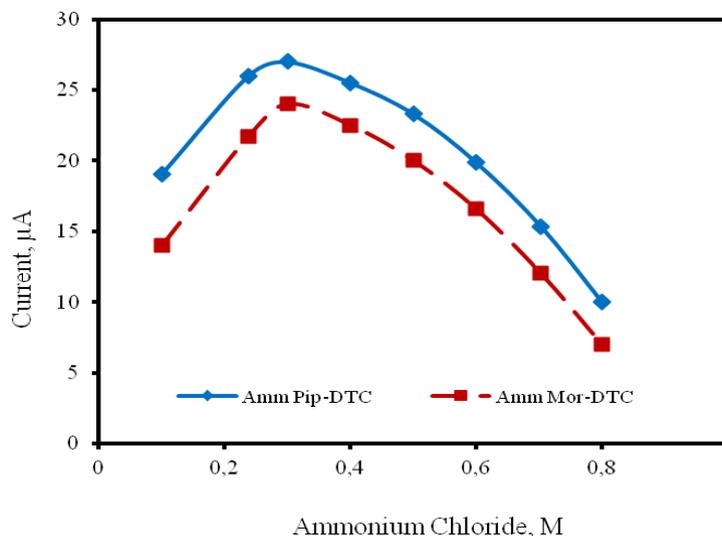


Figure 3. Effect of supporting electrolyte concentration on copper(II)-Amm Pip-DTC [conditions: - pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0 mM, copper(II)= 0.05 ppm] and copper(II)-Amm Mor-DTC [conditions: - pH=6.4, supporting electrolyte= 0.3 M, Amm Mor-DTC= 3.0 mM, copper(II)= 0.05 ppm] complex systems.

3.1.3. Effect of dithiocarbamates concentration

The solution of copper(II) ion, and ammonium chloride containing 0.05 ppm and 0.3 M respectively were kept constant and while the dithiocarbamate was varied from 0.5 to 6.0 mM. The pH of the solution was maintained at 6.6 and 6.4 for Amm Pip-DTC and Amm Mor-DTC respectively. The peak current does not vary linearly with concentration of ligand (Amm Pip-DTC/Mor-DTC) which was a typical characteristic nature of catalytic wave (Figure 4a).

From the results it was observed that the wave height increased linearly with dithiocarbamate concentration up to 2.0 mM for Amm Pip-DTC and 3.0 mM for Amm Mor-DTC. With further increase in Amm Mor-DTC concentration the wave height is independent of concentration and shows that the complex was stable. Therefore, the concentration of the ligands where the maximum wave height obtained is fixed for quantitative studies.

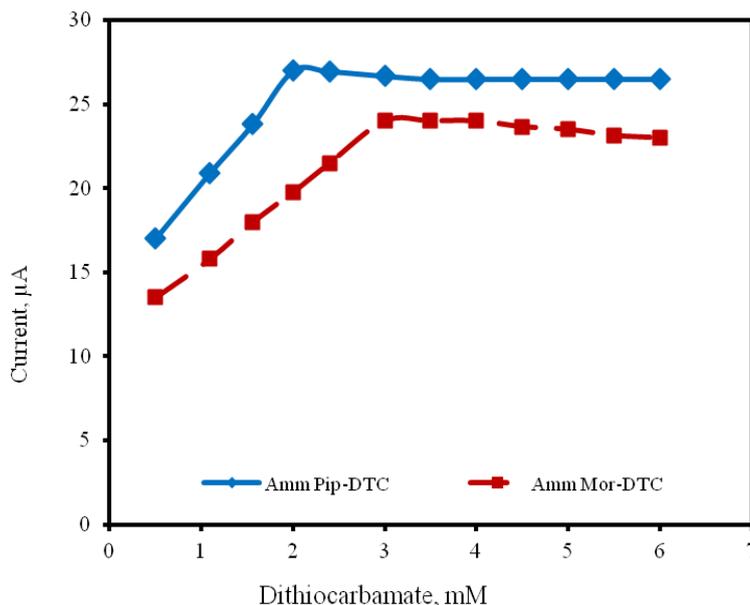


Figure 4(a). Effect of ligand concentration on copper(II)-Amm Pip-DTC [conditions:-pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0 mM, copper(II)=0.05 ppm] and copper(II)-Amm Mor-DTC[conditions:- pH=6.4, supporting electrolyte= 0.3 M, Amm Mor-DTC= 3.0 mM, copper(II)= 0.05 ppm] complex systems.

The peak potential of the catalytic wave shifted towards more negative potentials on increasing the dithiocarbamates concentration, up to the maximum concentration reported above and remained constant beyond these concentrations. Plot of $\{[dithiocarbamates]/i_p\}$ Vs $\{[dithiocarbamates]\}$ was a straight line (Figure 4b) and confirms that adsorption phenomenon was involved in the electrode reaction process.

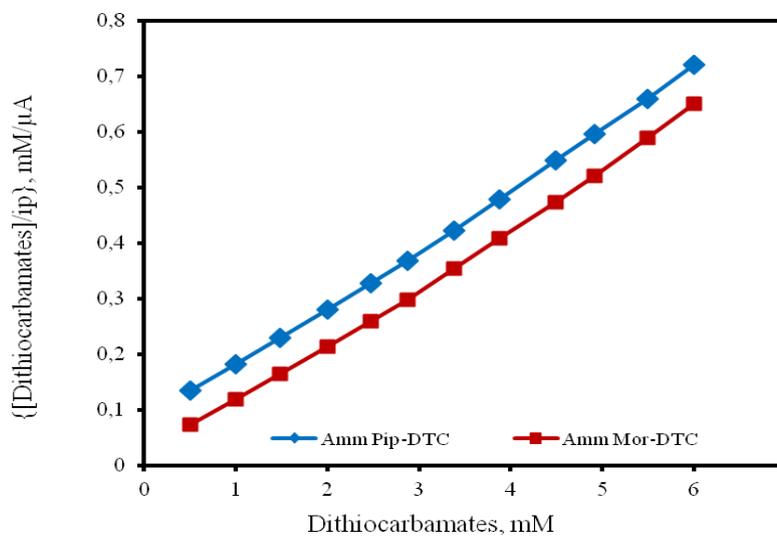


Figure 4(b). Langmuir adsorption isotherm plot of copper(II)- Amm Pip-DTC conditions:- pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0 mM, copper(II)= 0.05 ppm] and copper(II)-Amm Mor-DTC[conditions:- pH=6.4, supporting electrolyte= 0.3M, Amm Mor-DTC= 3.0 mM, copper(II)= 0.05 ppm] complex systems.

3.1.4. Effect of mercury pressure

The effect of the height of the mercury column on the polarograms of copper (II) [0.05 ppm] in quantitative experimental conditions were noted and found that the catalytic current as well as i_c/\sqrt{h} decreases with the increase in height of the mercury column indicating the catalytic nature of the current. The experimental observations are recorded in Table 1.

Table 1. Effect of mercury pressure on copper (II)-dithiocarbamate complex systems

| S.No | Height of the mercury column, cm | Amm Pip-DTC | | Amm Mor-DTC | |
|------|----------------------------------|------------------------|----------------|------------------------|----------------|
| | | Current, μA | i_c/\sqrt{h} | Current, μA | i_c/\sqrt{h} |
| 1 | 17 | 26.00 | 4.528 | 23.00 | 3.198 |
| 2 | 21 | 27.00 | 6.240 | 24.00 | 5.487 |
| 3 | 26 | 26.50 | 4.145 | 23.50 | 3.224 |
| 4 | 31 | 25.25 | 3.043 | 22.25 | 2.551 |
| 5 | 36 | 24.00 | 2.169 | 21.00 | 1.984 |

Conditions for copper(II)-Amm Pip-DTC system:-pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0 mM, copper(II)= 0.05 ppm]. Conditions for copper(II)-Amm Mor-DTC[conditions:- pH= 6.4, supporting electrolyte= 0.3 M, Amm Mor-DTC= 3.0 mM, copper(II)= 0.05 ppm].

3.1.5. Effect of maximum suppressor

The effect of surface active substances, gelatin in the range 0.005 to 0.01% and Triton X-100, 0.002 to 0.004 % on the catalytic wave height of copper (II)-Dithiocarbamate systems was investigated.

Table 2. Effect of maximum suppressors on copper(II)-dithiocarbamate complex systems

| Sample No | Maximum suppressor, % | Current, μA | |
|-----------------|-----------------------|------------------------|-------------|
| | | Amm Pip-DTC | Amm Mor-DTC |
| A. Gelatin | | | |
| 1 | 0.000 | 26.00 | 24.00 |
| 2 | 0.005 | 14.00 | 15.00 |
| 3 | 0.010 | 13.00 | 15.00 |
| B. Triton X-100 | | | |
| 1 | 0.000 | 26.00 | 24.00 |
| 2 | 0.005 | 24.50 | 23.75 |
| 3 | 0.010 | 24.00 | 23.00 |

The catalytic wave decreases sharply up to 0.005% gelatin concentration and with further increase in concentration of the surface active material, the wave height was decreased by only about 2%. The peak potential shifted towards positive potentials and the catalytic peak became round shaped. The suppression of the catalytic wave was found to be within 0.002 to 0.005 % Triton X-100 and was small compared to gelatin. The peak potential shifted towards positive potentials in this case also. The observations are presented in Table 2.

Conditions for copper(II)-Amm Pip-DTC system:-pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0 mM, copper(II)= 0.05 ppm]. Conditions for copper(II)-Amm Mor-DTC[conditions:- pH=6.4, supporting electrolyte= 0.3 M, Amm Mor-DTC= 3.0 mM, copper(II)= 0.05 ppm].

3.1.6. Effect of temperature

The current-voltage curves of copper(II)-dithiocarbamate systems were recorded at various temperatures i.e. 15 to 45 °C. It was found that with increase in temperature the wave height increased and temperature coefficient value decreased gradually. The current, i_c became completely temperature independent above 30 °C.

3.1.7. Effect of copper(II) ion concentration on peak current

At fixed concentration of dithiocarbamates, (2.0 mM Amm Pip-DTC & 3.0 mM Amm Mor-DTC) and 0.3 M ammonium chloride adjusting the pH to their optimum values, the metal ion concentration was varied between 0.05 to 6.0 ppm and the proportionality of the peak current was studied. The results obtained are shown in Figure 5.

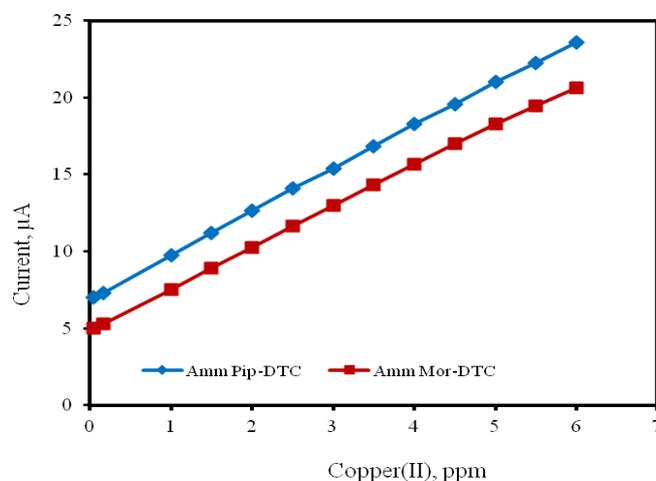


Figure 5. Effect of concentration of copper(II) on peak current of copper(II)-Amm Pip-DTC[conditions:- pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0mM] and copper(II)-Amm Mor-DTC[conditions:- pH=6.4, supporting electrolyte= 0.3 M, Amm Mor-DTC= 3.0 mM] complex systems.

The peak current increased linearly with copper concentration in the range 0.05 to 6.0 ppm (Figure 4b). The method suggests that the quantification of trace and ultra-trace levels of copper(II) was possible in unknown samples. No changes were observed in the shape of the wave throughout the copper(II) concentration range.

3.1.8. Effect of indifferent cations

The effect of neutral salts and replacement of monovalent cations with divalent cations leads to the changes both in the height and the potential of the catalytic wave. The concentration of NH_4Cl in the solution was kept constant and several amounts of different chlorides were added to the polarographed solutions of 0.05 ppm copper(II) in the fixed concentrations of dithiocarbamates at corresponding pH. Three alkali chlorides, potassium, sodium and lithium and divalent cation, calcium were used. From the Figures 6a-b, it was clear that with increase in concentration of chlorides the wave height decreased gradually for calcium chloride when compared to other chlorides.

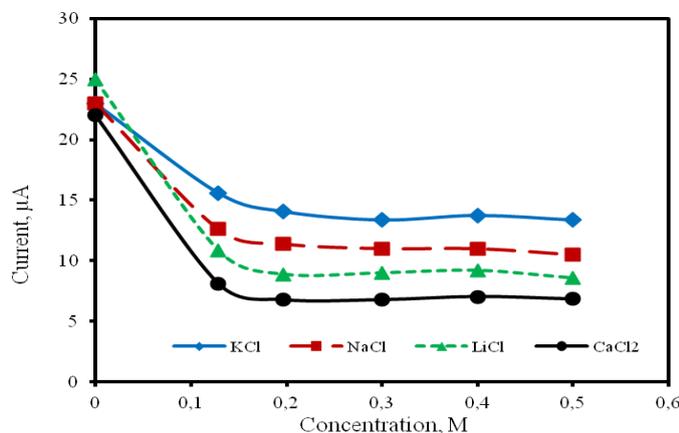


Figure 6(a). Effect of indifferent ions on copper(II)-Amm Pip-DTC complex system [conditions:- pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0 mM, copper(II)=0.05 ppm]

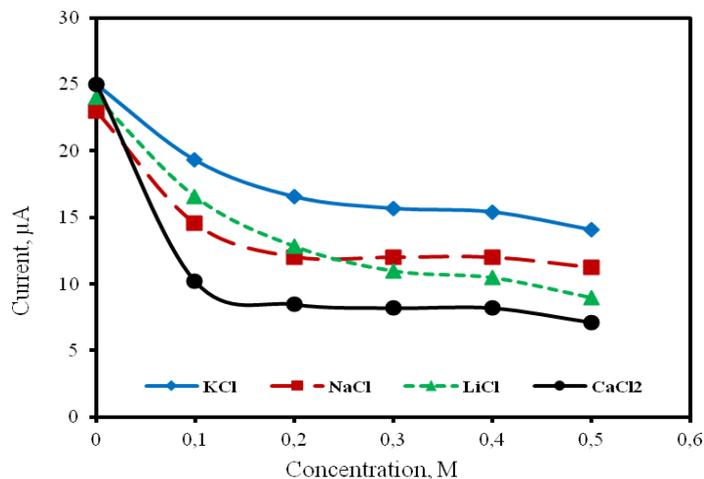


Figure 6(b). Effect of indifferent ions on copper(II)-Amm Mor-DTC complex system [conditions:- pH=6.6, supporting electrolyte= 0.3 M, Amm Pip-DTC= 2.0 mM, copper(II)=0.05 ppm]

3.2. Cyclic voltammetric studies of dithiocarbamates-copper(II) complexes

The cyclic voltammetric studies have been utilized to elucidate and confirm the possible mechanism between ammonium salts of piperidine/morpholine dithiocarbamates and copper(II) in addition to computational calculations (density functional theory method). Figures 7a-b shows cyclic voltammograms of Amm Pip-DTC and Amm Mor-DTC in a 0.3 M $\text{NH}_4\text{Cl-NH}_4\text{OH}$ electrolyte solution of pH 6.6 and 6.4 respectively.

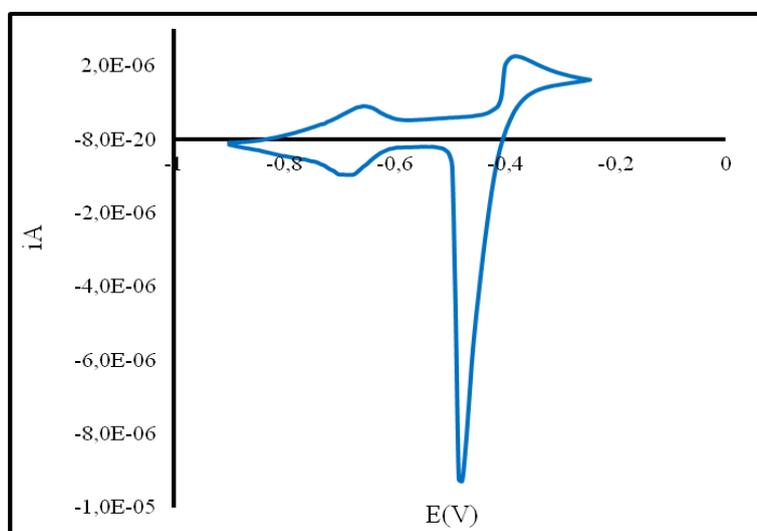


Figure 7(a). Cyclic voltammograms for 2.0 mM Amm Pip-DTC in 0.3 M $\text{NH}_4\text{OH-NH}_4\text{OH}$ at pH 6.6

On the hand Figure 7c-d shows cyclic voltammograms of Amm Pip-DTC and Mor-DTC in presence of 0.001 ppm copper(II) standard and 0.3 M $\text{NH}_4\text{Cl-NH}_4\text{OH}$ electrolyte solution of pH 6.6 and 6.4 respectively.

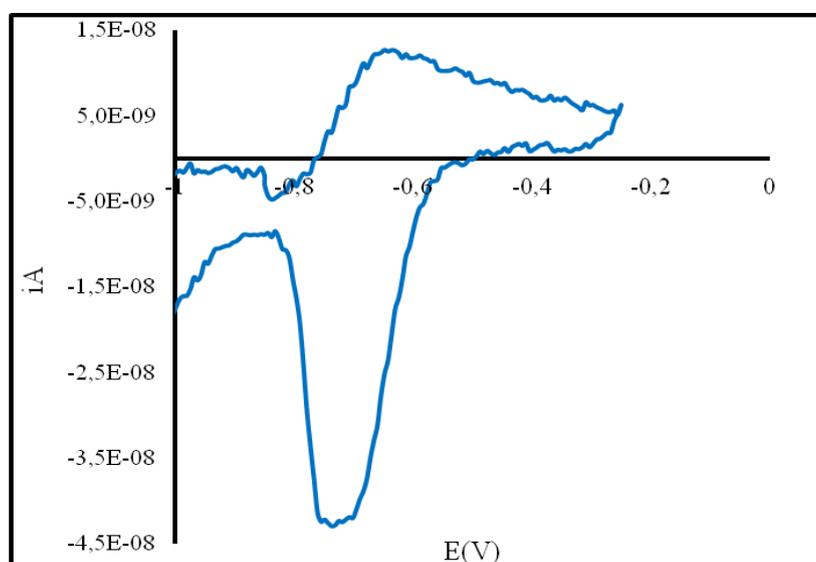


Figure 7(b). Cyclic voltammograms for 3.0 mM Amm Mor-DTC in 0.3 M $\text{NH}_4\text{OH-NH}_4\text{OH}$ at pH 6.4

On comparing Figures 7a and c, two reversible peaks were observed in at $E_{1/2}$ of -0.48 and -0.68 respectively while Figure 7c shows only one reversible peak at $E_{1/2} = -0.64$. The shift in potential and disappearing of the first peak confirms that there are interactions between Amm Pip-DTC and copper(II). Similarly in Figure 7b, the $E_{1/2}$ at -0.68 is shifted to -0.59 in Figure 7d also confirming the Amm Mor-DTC and copper(II) interactions. Interestingly both ligands showed similar behavior with $E_{1/2} = -0.64$ and -0.59 for Amm Pip-DTC and Amm Mor-DTC respectively. However Amm Mor-DTC showed a more pronounced cathodic peak, this could be due to the presence of “O” in the Morpholine ring. A similar behavior was also observed in computational calculations.

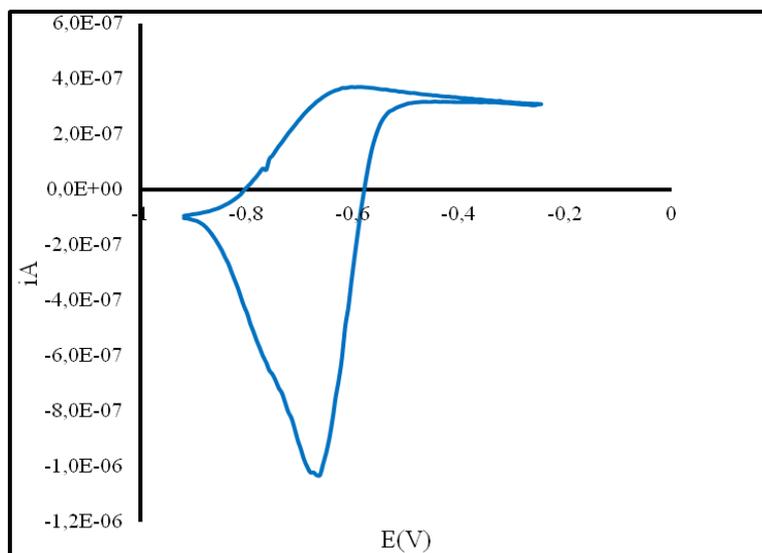


Figure 7(c). Cyclic voltammograms for 2.0 mM Amm Pip-DTC + 0.05 ppm copper(II) in 0.3 M $\text{NH}_4\text{OH-NH}_4\text{OH}$ at pH 6.6

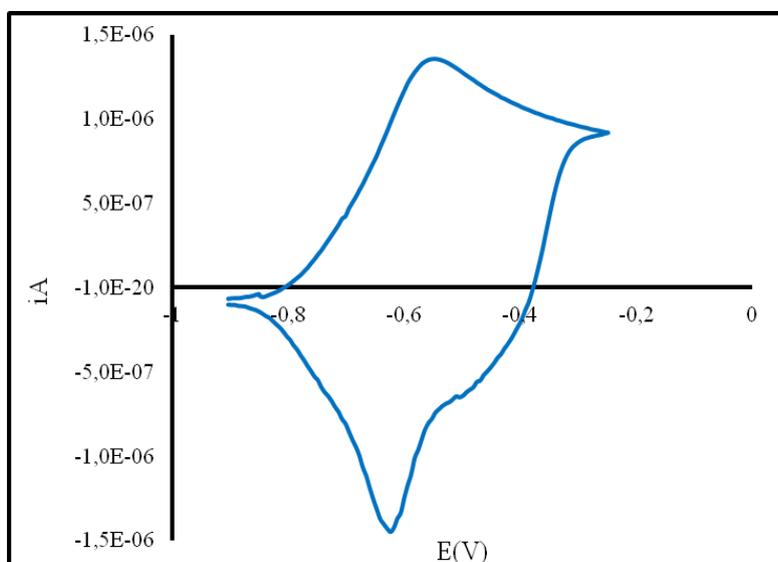


Figure 7(d). Cyclic voltammograms for 3.0 mM Amm Mor-DTC + 0.05 ppm copper(II) in 0.3 M $\text{NH}_4\text{OH-NH}_4\text{OH}$ at pH 6.4

3.3 Computational studies of dithiocarbamates-copper(II) complexes

The optimized geometries pictorially represented in Figures 8-9 were verified by second derivative frequency calculations for their true ground state minima. Clearly, in the absence of ammonium ion (NH_4^+), both structures A and C (Figure 1), irrespective of the starting coordination/amines, were optimized to similar geometries with copper metal centrally coordinated by both sulfur atoms of the dithiocarbamates (Figure 8a-b). The optimized energies of complexes of morpholine (Figure 8a) and piperidine (Figure 8b) with copper were found to be -541.6596833 Hartree and -505.7721889 Hartree, respectively.

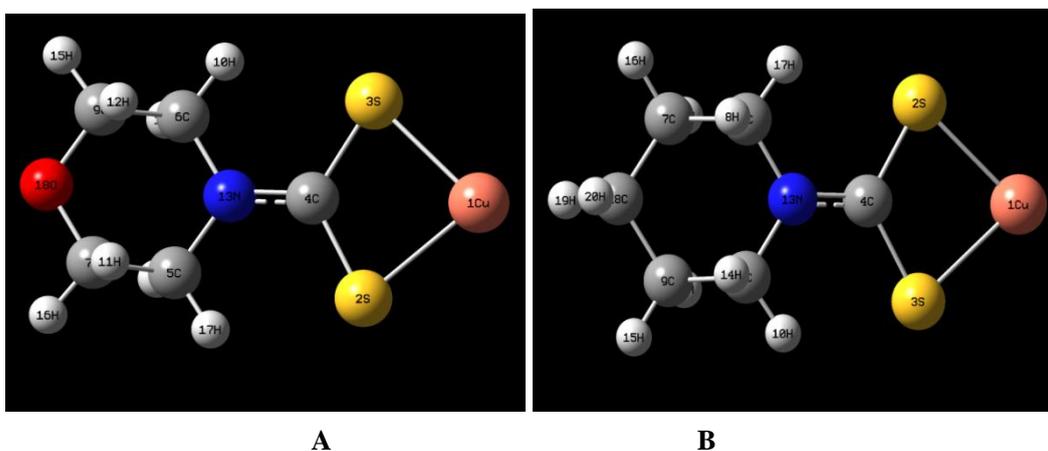


Figure 8. Optimized complexes of copper metal with morpholine dithiocarbamate (a) and piperidine dithiocarbamate (b) in the absence of ammonium ion

The bond distances between the copper and sulfur atoms were found to be 2.38 Å in both dithiocarbamates (Figures 8a-b). The presence of NH_4^+ ion in the complexes B and D (Figure 1), shifted the coordination *via* single sulfur atom in both amine dithiocarbamates, with bond distance 2.41 Å between the bonded sulfur and copper atom in their optimized structures, as depicted in Figures 9 a-b with energies -598.7700982 Hartree and -562.8998656 Hartree respectively.

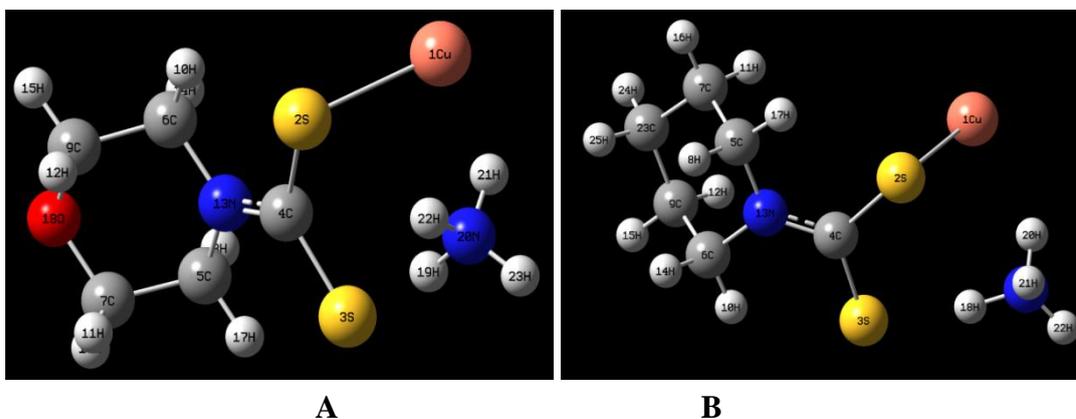


Figure 9. Optimized complexes of copper metal with morpholine dithiocarbamate (a) and piperidine dithiocarbamate (b) in the presence of ammonium (NH_4^+) ion

Hence, it is believed that the morpholine/piperidine dithiocarbamates coordinate preferably *via* single sulfur atom with the copper metal probably due to decreased electron density on other sulfur atom in the vicinity of NH_4^+ ion. In the absence of NH_4^+ ion, both sulfur atoms are equally available for coordination due to uniform distribution of electron density.

3.3. Proposed method evaluation of DC-CHW technique

The proposed DC-CHW technique was critically evaluated with regard to reproducibility, accuracy and detection limits for analysis of copper (II) in leafy vegetables, milk and blood samples.

To test reproducibility of the proposed method, six replicate analysis of each sample were run. A % R.S.D in the range of 0.050 to 2.62 was obtained as shown in Tables 3a-d. The accuracy of the proposed method was evaluated by comparing the results with those obtained by the other methods as reported in the literature [24-29] and also with the DPP method. The analytical data presented in Tables 3a-d shows that the present method was more facile, sensitive, selective and sensitive than the reported methods in the literature [24-29].

Table 3(a). Analytical data for the quantification of copper(II) with Amm Pip-DTC in leafy vegetable samples

| Sample ^a Scientific/ Local name | DC-CHW method | | DPP method | | <i>t</i> -test* | <i>f</i> -test** |
|--|----------------|------------------------------|----------------|------------------------------|-----------------|------------------|
| | Amm Pip-DTC | | Amm Pip-DTC | | | |
| | Cu(II), ppm | Recovery(%)±RSD ^b | Cu(II), ppm | Recovery(%)±RSD ^b | | |
| <i>Hibiscus cannabinus/</i> <i>gongura</i> | 1.134 | 98.20±2.20 | 1.137 | 98.50±2.45 | 1.59 | 0.37 |
| <i>Celosia argentea/</i> <i>gurugaku</i> | 1.182 | 98.50±2.18 | 1.182 | 98.50±2.18 | 1.25 | 0.20 |
| <i>Spinacia oleracea/</i> <i>palaku</i> | 1.109 | 99.20±1.90 | 1.115 | 99.50±1.65 | 1.17 | 0.63 |
| <i>Amaranthus graecizans/</i> <i>Sirraku</i> | 1.055 | 99.45±1.45 | 1.059 | 99.85±1.65 | 1.42 | 0.41 |
| <i>Alternanthera sessilis/</i> <i>ponagantaku</i> | 1.124 | 97.80±2.49 | 1.124 | 97.80±2.49 | 1.38 | 0.33 |

Conditions:- NH_4Cl : 0.3M, Amm Pip-DTC: 2.0, mM, pH: 6.6,

^a5 mL of the concentrated sample is used,

^bRelative Standard Deviation (n=6),*1% level of significance,**5% level of significance.

All samples were spiked with 1.0 ppm copper(II) standard

The developed method was compared with the DPP method in terms of Student's "*t*"-test and Variance ratio "*f*"-test as shown in Tables 3a-d. The analytical data summarized in Tables 3a-d

suggest that the percentage of copper(II) recovery from vegetables and biological samples ranges from 80.00 to 100.00 % with R.S.D(%)= 2.62, suggesting a good agreement between the two methods.

Table 3(b). Analytical data for the quantification of copper(II) with Amm Mor-DTC in leafy vegetable samples

| Sample ^a Scientific/ Local name | DC-CHW method | | DPP method | | t-test* | f-test** |
|--|---------------|------------------------------|-------------|------------------------------|---------|----------|
| | Amm Mor-DTC | | Amm Mor-DTC | | | |
| | Cu(II), ppm | Recovery(%)±RSD ^b | Cu(II), ppm | Recovery(%)±RSD ^b | | |
| <i>Hibiscus cannabinus/</i> gongura | 1.128 | 97.80±2.15 | 1.131 | 98.10±2.28 | 1.34 | 0.43 |
| <i>Celosia argentea/</i> gurugaku | 1.000 | 98.00±2.26 | 1.008 | 98.80±2.30 | 1.10 | 0.17 |
| <i>Spinacia oleracea/</i> palaku | 1.082 | 99.20±2.52 | 1.088 | 99.80±2.61 | 1.06 | 0.90 |
| <i>Amaranthus graecizans/</i> Sirraku | 1.145 | 99.20±1.90 | 1.149 | 99.20±2.14 | 1.29 | 0.25 |
| <i>Alternanthera sessilis/</i> ponagantaku | 1.188 | 97.55±2.62 | 1.188 | 98.00±2.20 | 1.37 | 0.41 |

Conditions:- NH₄Cl: 0.3 M, Amm Mor-DTC: 3.0 mM, pH: 6.4,

^a5 mL of the concentrated sample is used,

^bRelative Standard Deviation (n=6), *1% level of significance, **5% level of significance,

All samples were spiked with 1.0 ppm copper(II) standard

Table 3(c). Quantification of copper(II) in biological samples with Amm Pip-DTC

| Samples | DC-CHW method | | | DPP method | | t-test* | f-test** |
|----------------------|-------------------|-------------|------------------------------|-------------|------------------------------|---------|----------|
| | Amm Pip-DTC | | | Amm Pip-DTC | | | |
| | Cu(II) Added, ppm | Cu(II), ppm | Recovery(%)±RSD ^b | Cu(II), ppm | Recovery(%)±RSD ^b | | |
| Milk samples | | | | | | | |
| 1 | 0.2 | 0.265 | 93.00±0.22 | 0.272 | 95.10±0.11 | 1.20 | 0.14 |
| 2 | 0.4 | 0.470 | 94.50±0.18 | 0.450 | 92.50±0.14 | 1.09 | 0.09 |
| 3 | 0.6 | 0.685 | 100.00±0.30 | 0.680 | 100.00±0.36 | 1.55 | 0.25 |
| 4 | 0.8 | 0.850 | 98.80±0.15 | 0.910 | 99.80±0.29 | 1.23 | 0.60 |
| 5 | 1.0 | 1.220 | 99.20±0.10 | 1.210 | 99.10±0.10 | 1.30 | 0.72 |
| 6 | 1.2 | 1.250 | 98.50±0.45 | 1.320 | 99.30±0.34 | 1.44 | 0.34 |
| Blood samples | | | | | | | |
| 1 | 0.2 | 0.250 | 85.00±0.14 | 0.263 | 92.00±0.20 | 1.50 | 0.26 |
| 2 | 0.4 | 0.430 | 90.00±0.25 | 0.439 | 95.50±0.15 | 1.28 | 0.15 |
| 3 | 0.6 | 0.650 | 97.20±0.10 | 0.705 | 98.10±0.38 | 1.10 | 0.62 |
| 4 | 0.8 | 0.825 | 99.00±0.30 | 0.833 | 99.75±0.05 | 1.79 | 0.39 |
| 5 | 1.0 | 1.220 | 92.50±0.15 | 1.230 | 95.00±0.10 | 1.32 | 0.20 |
| 6 | 1.2 | 1.300 | 95.10±0.44 | 1.411 | 98.16±0.24 | 1.65 | 0.48 |

Conditions:- NH₄Cl: 0.3 M, Amm Pip-DTC: 2.0 mM, pH : 6.6,

^a5 mL of the concentrated sample is used,

^bRelative Standard Deviation(n=6), *1% level of significance, **5% level of significance

Moreover, Amm Pip-DTC showed much better recoveries in all samples tested (results shown in Tables 3a and c).

Table 3(d). Quantification of copper(II) in biological samples with Amm Mor-DT

| Samples | DC-CHW method | | | DPP method | | t-test* | f-test** |
|----------------------|-------------------|-------------|------------------------------|-------------|------------------------------|---------|----------|
| | Amm Mor-DTC | | | Amm Mor-DTC | | | |
| | Cu(II) Added, ppm | Cu(II), ppm | Recovery(%)±RSD ^b | Cu(II), ppm | Recovery(%)±RSD ^b | | |
| Milk samples | | | | | | | |
| 1 | 0.2 | 0.250 | 90.00±0.20 | 0.270 | 95.00±0.17 | 1.18 | 0.12 |
| 2 | 0.4 | 0.460 | 92.50±0.14 | 0.450 | 92.50±0.15 | 1.00 | 0.06 |
| 3 | 0.6 | 0.680 | 100.00±0.26 | 0.680 | 98.33±0.25 | 1.52 | 0.37 |
| 4 | 0.8 | 0.890 | 98.75±0.09 | 0.910 | 100.00±0.25 | 1.83 | 0.52 |
| 5 | 1.0 | 1.190 | 99.00±0.05 | 1.210 | 99.00±0.12 | 1.98 | 0.60 |
| 6 | 1.2 | 1.290 | 98.33±0.25 | 1.320 | 99.16±0.08 | 1.49 | 0.29 |
| Blood samples | | | | | | | |
| 1 | 0.2 | 0.220 | 80.00±0.02 | 0.250 | 90.00±0.10 | 1.26 | 0.20 |
| 2 | 0.4 | 0.390 | 85.00±0.07 | 0.420 | 90.00±0.10 | 1.15 | 0.11 |
| 3 | 0.6 | 0.680 | 96.66±0.20 | 0.690 | 95.00±0.16 | 1.91 | 0.57 |
| 4 | 0.8 | 0.930 | 98.75±0.28 | 0.970 | 98.75±0.09 | 1.54 | 0.36 |
| 5 | 1.0 | 1.100 | 90.00±0.11 | 1.120 | 88.00±0.05 | 1.30 | 0.24 |
| 6 | 1.2 | 1.350 | 98.33±0.23 | 1.390 | 99.16±0.08 | 1.63 | 0.42 |

Conditions:- NH₄Cl: 0.3 M, Amm Mor-DTC: 3.0 mM, pH : 6.4,

^a5 mL of the concentrated sample is used,

^bRelative Standard Deviation(n=6), *1% level of significance, **5% level of significance

3.4. Comparison of direct current-catalytic hydrogen wave (DC-CHW) technique with differential pulse polarography and reported methods in literature

In catalytic hydrogen wave technique, it was desirable to do comparison between the results obtained with dc polarography and differential pulse polarography. Standard addition method was used in this investigation for the quantification of copper(II) in leafy vegetables and biological samples. The polarograms obtained from dc polarography were shown in Figure 10a-b and the results obtained by this method were further supported by differential pulse polarography (polarograms not shown).

Figure 11 a-b clearly indicated the good correlation between both recovery values for the quantification of copper(II) in vegetables and biological samples. This method was found to be sensitive, selective, specific, reliable and rapid and may be successfully applied for the analysis of copper(II) using dithiocarbamates as complexing agents in various samples of environmental importance as compared to the other techniques reported in the literature (see Table 4). This improvement observed in DC-CHW is due to Copper(II)-Dithiocarbamate [Cu⁺²-DTC]_{ads} complex which undergoes reduction from +2 to zero valent state complex [Cu⁰-DTC]_{ads} at mercury electrode in the adsorbed state. The zero valent state metal complex was basic in nature, which undergoes protonation accepting proton from the solution [Cu⁰-DTCH⁺]_{ads} in the adsorbed state and further undergo again reduction to liberate hydrogen.

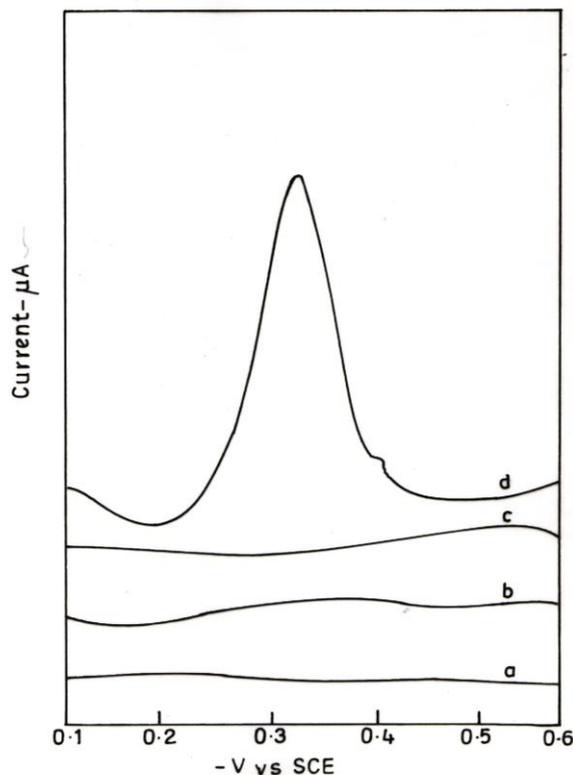


Figure 10(a). Polarographic curve of copper(II) in $\text{NH}_4\text{Cl-NH}_4\text{OH}$ medium in the presence of Amm Pip-DTC[conditions:- (a) 0.3 M NH_4Cl , pH ~6.6, (b) a + 2.0 mM Amm Pip-DTC, (c) a + 1.0 ppm copper(II), (d) b + 1.0 ppm copper(II)]

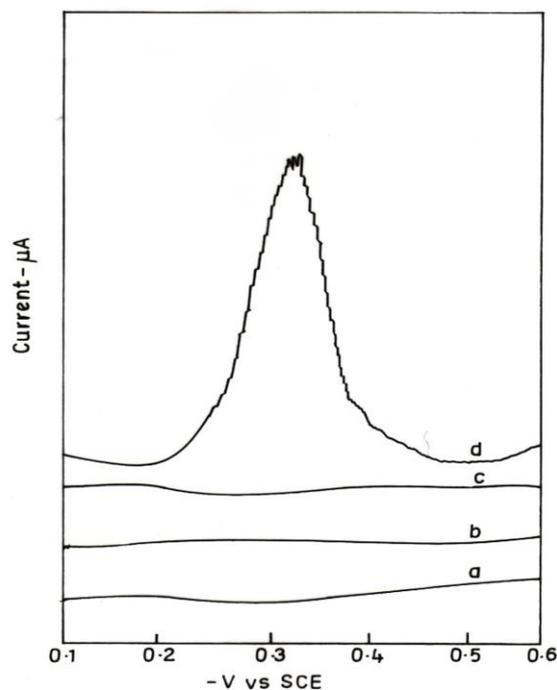


Figure 10(b). Polarographic curve of copper(II) in $\text{NH}_4\text{Cl-NH}_4\text{OH}$ medium in the presence of Amm Mor-DTC[conditions:- (a) 0.3 M NH_4Cl , pH ~6.4, (b) a + 3.0 mM Amm Mor-DTC, (c) a + 1.0 ppm copper(II), (d) b + 1.0 ppm copper(II)]

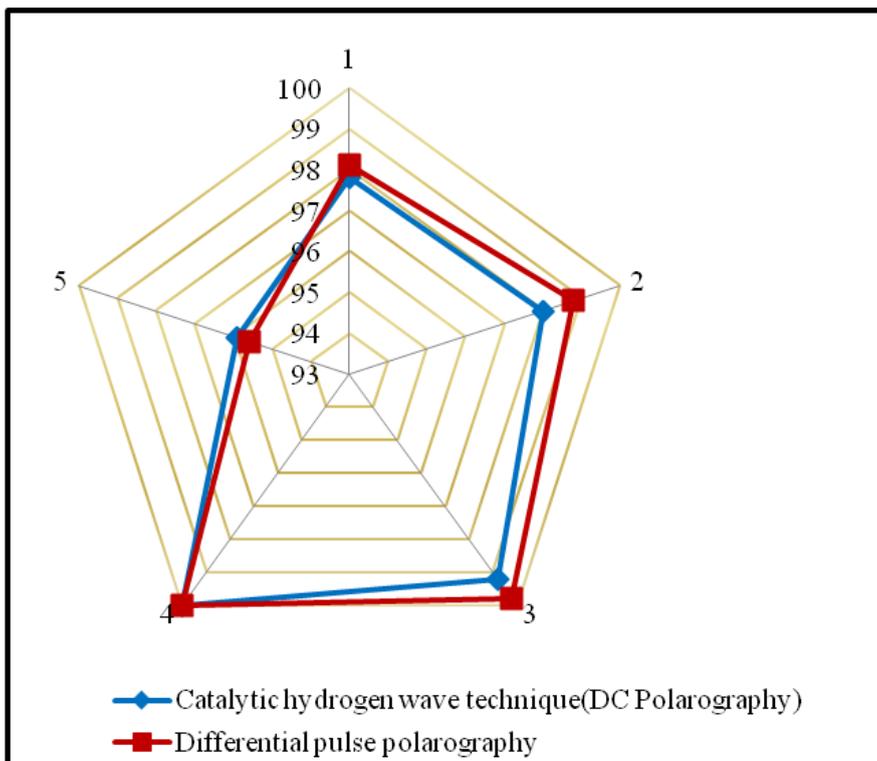


Figure 11(a). Radial plots for correlation of recoveries between direct current-catalytic hydrogen wave technique(DC-CHW) and Differential pulse polarography for quantification of copper(II) in vegetables

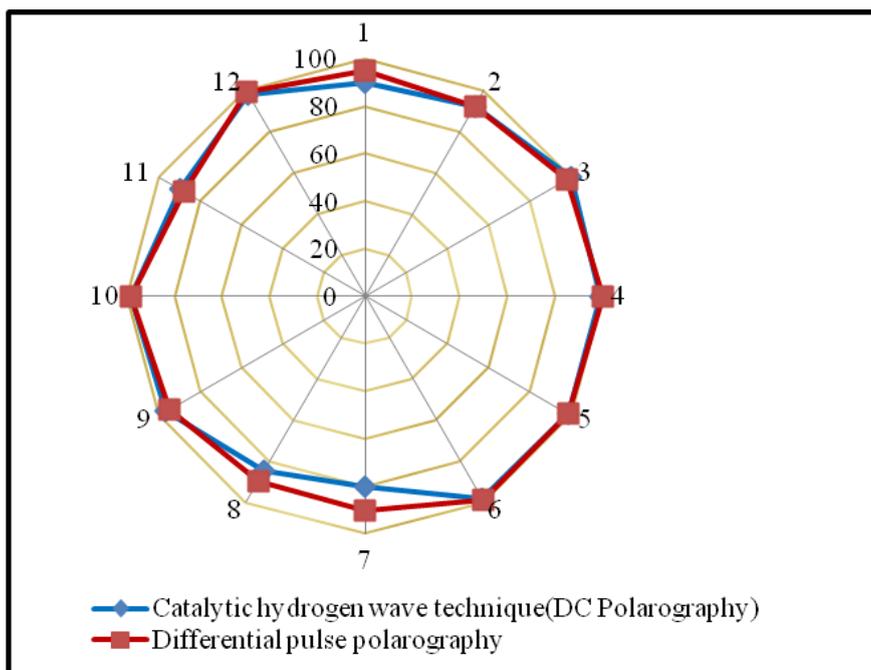


Figure 11(b). Radial plots for correlation of recoveries between direct current-catalytic hydrogen wave technique(DC-CHW) and Differential pulse polarography for biological(milk & blood) samples

Table 4. Comparison analytical data of present method with reported methods for the determination of copper(II) in environmental samples

| Metal ions analyzed | Reagent | Method/Technique | Detection Limits | Remarks | Ref |
|---|----------------------------------|--------------------------|------------------------|--|----------------|
| Cu(II) & Ni(II) | Xanthates | Polarographic/ DC-CHW | 1.0 ppm | Less sensitive and unstable | [20] |
| Cu(II) | Xanthates | Polarographic/ DC-CHW | 0.1 ppm | Economical & eco-friendly ligands, but low detection limits | [21] |
| Cu(II), Cd(II), Co(II) & Mn(II) | Tris(hydroxymethyl)methylamine | Polarographic/ DC-SCC | 5.0 ppm | Employed elaborate procedure, low detection limits and complex was unstable | [37] |
| Cu(II), Cd(II), Ni(II), Zn(II) & Mn(II) | Tris (hydroxymethyl) methylamine | Polarographic/ DC-SCC | 6.0 mg L ⁻¹ | Poor detection limit and complex was unstable | [38] |
| Cu(II), Co(II) & Ni(II) | Hexamethylenetetramine | Polarographic/ DC-SCC | 1.0 ppm | Poor detection limit and less sensitive | [39] |
| Cu(II) | Dithiocarbamates | Polarographic/ DC-CHW | 0.001 ppm | Economical & eco-friendly ligand, good detection limits, inexpensive instrumentation, facile methodology | Present method |

DC-CHW= Direct current-catalytic hydrogen wave, DC-SCC= Direct current-simple catalytic current

4. CONCLUSION

The present method for the quantification of copper(II) in leafy vegetables, milk and blood samples by direct current-catalytic hydrogen wave (DC-CHW) technique coupled with DC polarography and differential pulse polarography was facile, sensitive, and selective for the analysis of copper(II) in leafy vegetables, milk and blood samples. New synthesized dithiocarbamates viz., ammonium salt of piperidine/morpholine dithiocarbamates was fairly soluble in water and can be synthesized in classical and ordinary laboratories. Methodology developed in this study was economical and environmental friendly without any usage of solvent for extraction and can be extended for the routine analysis of toxic metal ions in large commercial and research laboratories. The obtained cyclic voltammetric results confirmed the formation of dithiocarbamates and its complexes with copper(II). DFT calculations supports the possible coordination site of piperidine/morpholine dithiocarbamates for copper(II) in the presence/absence of ammonium ion.

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