Electrochemical behavior of copper ion on N-methyl piperazine substituted organic compound modified Wax Impregnated Graphite Electrode

N. RAMALAKSHMI* and B. MARICHAMY

Post Graduate and Research Department of Chemistry,
Presidency Collge (Autonomous), Chennai (INDIA)
*rrama_subhar@yahoo.co.in

Abstract

Fabrication of 2, 5 bis (4-methyl piperaz-1yl-methyl) 1-4-hydroquinone modified Wax Impregnated Graphite Electrode (WIGE) was used for highly selective, sensitive accumulation and detection of copper ions in real samples. Techniques like cyclic voltammetry(CV) and electrochemical impedance spectroscopy(EIS) were used to investigate ligand modified WIGE and the ion permeation through it. The electrochemical behavior of copper ions on ligand modified WIGE was studied through scan rate effect and pH study. The results indicated that CV peak currents have a linear relationship with concentration in the range of 0.05mM to 5mM, with a correlation coefficient of 0.983. The detection limit could be estimated 5×10^{-5}M for Cu(II) ion by cyclic voltammetric method. The interference study was performed for equal molar concentrations of Cu(II), Pb(II) and Hg(II) ions on ligand modified WIGE.

Key words: electrochemical impedance spectroscopy, cyclic voltammtry, copper redox system, interference study, sensor.

Introduction

The Complexation of organic compounds with selected metal ions has a wide variety of applications in medicinal chemistry, surface chemistry, and analytical chemistry. Complexation of medicinal substances with metal ions influence the bioavailability of drugs in the body and the biological action affects the stability of medicinal compounds since a large number of metals are taken into the body system either with drugs or in the form of diet.[1] The complex formation has been suggested as one of the important mechanisms for certain drug action.[2] The metal chelating phenomena are used to reduce the toxic effect of drugs in

human physiology[3]. The study of redox behaviour of biologically and biochemically important compounds are gaining importance because such redox phenomena are close to natural processes occurring in human and other living organisms.

Electrode modification through the self-assembled process profits from chemical specificity, rapid response, high sensitivity, antifouling effect and possibility for in situ immobilization of biological recognition agents (e.g enzymes)[4-10]. Although it is possible to have a wide range of organic functionalities on the surface of the electrode with the desired properties of self assembled mono layer's tail groups. One of the limitations of this research area involves the synthesis and testing of macro cyclic ligands with selectivity and sensitivity for a target metal ion. Exploiting the molecular level control over the fabrication of a sensing interface afforded by SAM's for metal ions was first demonstrated by Rubinstein *et al.*,[11-13] where a modified gold electrode with mixed SAM's which detect a low levels of Cu^{2+} ions (10^{-7}M) with minimal interface from Fe^{2+} ions, was introduced.

Peptide modified electrodes exhibit high sensitivity to copper[14,15] which is attributed to the stable 4N co-ordinate complex. Already reported Cu^{2+} sensors are such as electro polymerized polyindole[16], carboxylated thia-crown ether[17], salenschiff base[18], SAM of mercapto compounds on gold nano particles[19], iminodiacetic acid[20] had been used. In the present work electrochemical behaviour of copper on N-methyl piperazine substituted organic compound modified wax impregnated graphite electrode is studied using electrochemical analyser. This method is applied to detect the metal ions in real samples with high selectivity by detecting the optimum range.

2. Experimental

2.1. Chemicals and solutions :

N-methyl piperazine (spectro chem.), formaldehyde solution (rankem), hydroquinone (Himedia), ethanol (CHINA), acetate buffer, potassium chloride, copper suphate (mercuric chloride(univerasal lab reagent). All chemicals were of analytical grade and were used without further purification. All solutions were prepared with triple distilled water.

2.2 Apparatus :

Cyclic Voltammetry (CV) and EIS were performed with a CHI 660C electrochemical workstation (CH Instruments, Austin, USA). The working electrode was a impregnated graphite electrode (WIGE, 3mm in diameter). An Ag/AgCl (saturated KCl) electrode and platinum wire were used as the reference electrode and counter electrode, respectively. All electrochemical experiments were carried out in a single compartment electrochemical cell at room temperature. Scanning electron microscopy (SEM) images were obtained from HR-SEM (Quanta 200F). For emission spectroscopy measurement of copper, mercury ICP-Opitcal Emission Spectrometer (Optima 5300DV) was used.

2.3 Fabrication of Wax impregnated Graphite Electrode :

Fabrication of this type electrode is

easy and low cost one. The graphite rod present in battery cell (3mm diameter) was separated out and kept in conc. HCl for 24 hours. And then the electrode was rinsed with triple distilled water and kept in ethanol for 24 hours. The porous containing graphite rod was obtained. This rod was warmed with paraffin wax and porous part of graphite rod was filled with wax. The graphite rod was cooled at room temperature. The paraffin wax has inert electron transfer and mass transfer. Thus bottom and top part of the rod was polished using emery sheet for electrical contact. This paraffin wax impregnated graphite rod was used as a working electrode.

2.4 Electrode pretreatment and activation :

Prior to each experiment, WIGE electrode was exhaustively polished with E4-400 Silicon carbide emery paper and rinsed with water. In the case where activated surface electrode was needed, the polished WIGE was immersed in 1% conc. HCl solution and the potential was cycled from -0.5 to +0.5V at 0.100V/s of scan rate until a stable voltammogram was obtained. After this, the electrode was rinsed in triple distilled water and kept in room temperature.

2.5 Synthesis and characterization of (2, 4 bis (4-methyl piperazin-1-yl-methyl)-1,4 hydroquinone) (L^1) :

The ligand was synthesized by taking mixture of formaldehyde solution (3.5ml, 0.035mol) and N-methyl piperazine (3.8ml, 0.035mol) in ethanol (15ml) and stirred for 24hours. Then p-hydroxyl phenol (2g, 0.017mol) in ethanol (10ml) solution was added and stirred for 12 hours. The resulting solution was

refluxed in oil bath at $50^{\circ}C$ for 18 hours. The excess of ethanol was evaporated and stand for 24 hours. The brown color solid was obtained. The crude sample was recrystalised from ethanol. IR spectrum (KBr disc, /cm^{-1}) 3340 (OH) br, H1 NMR (δ ppm in CDCl$_3$) ~2.52 (br,s, 16H methylene protons), ~3.68 (s,4H, benzylic protons), ~7.8 (s,2H, Ar-H).
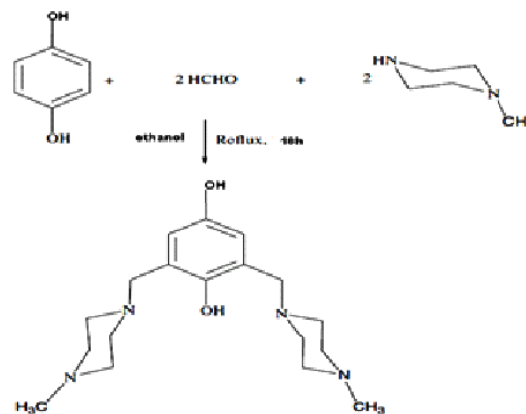

Fig. 1

2.6 Preparation of the ligand modified Wax Impregnated Graphite Electrode :

The ligand modified WIGE was prepared by electrochemical deposition of the ligand on the electrode surface which was achieved by applying potential cycling between -0.2 to 0.8V at different scan rates and different number of cycles.
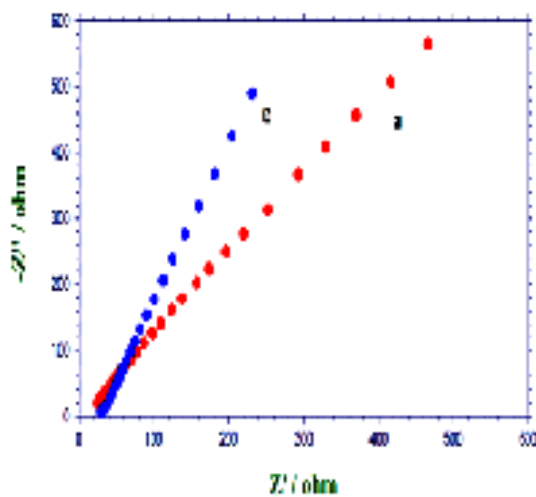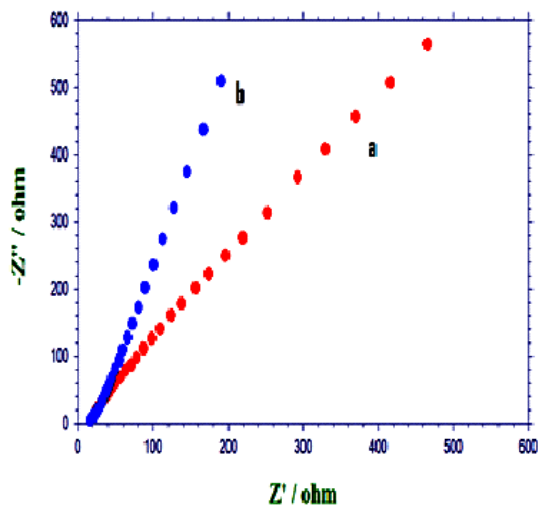
3. Results and Discussion

3.1 Characterization of ligand modified WIGE by Cyclic Voltammetry and Electrochemical Impedance Spectroscopy :

Electrochemical methods are simple and low operating cost methods for the study of the interfacial even at modified electrodes.

Among the electrochemical methods, Electrochemical Impedance Spectroscopy and cyclic voltammetry are powerful informative and non destructive method that can be used to study the interfacial events[21-23] and serve as a transducer to trace the blocking behaviors[24], electrostatic interactions by charged surfaces with charged probes[25] and diffusion behaviour/ electro catalytic effects at modified electrodes[26]. It is well known that electrochemical impedance spectroscopy (EIS) is an effective tool for studying the boundary properties of surface-modified electrodes. R_f, the semicircle diameter at higher frequencies in Nyquist plot of EIS can be used to describe the boundary properties of the electrode, because it controls the interfacial electron transfer rate of the redox probe between the solution and the electrode. Its value varies when different substances are absorbed on the electrode surface[27]. Fig (2) shows the results of ac impedance spectroscopy on bare graphite and different amount of ligand modified WIGEs in 1 x 10^{-3} M Hg^{2+} ions. As the result, the bare WIGE shows an almost straight line that is characteristic of a diffusion

limiting step of the electrochemical method. From the values of varying –Z"/ohm for the different modified electrodes proves that ligand film is successfully immobilized on the WIGE surface. This fact was checked by cyclic voltammetry experiment in 1 x 10^{-3}M Hg^{2+} ion solution in 0.5M KCl medium[28] on different amount of ligand modified WIGE by electro deposition. The anodic peak current of Hg (II) was decreased on ligand modified WIGE by electro deposition in the range of -0.2 to 0.8V at 100mV/s with 26cycles. The reason is the ligand can act as an inert electron and mass transfer blocking layer[29], and it hinders to diffuse Hg(II) toward the electrode surface, which obviously proves that ligand film is successfully immobilized on the WIGE surface. The results shows that, there is a remarkable enhancement in Hg^{2+} ion oxidation and as well as reduction peak currents (Fig. 3) when WIGE modified by electro deposition of the ligand on the electrode surface by applying potential cycling between -0.2 to 0.8V at 20mV/s with 5cycles. Based on the EIS and CV results, we can decide that ligand is successfully immobilized on the WIGE surface.
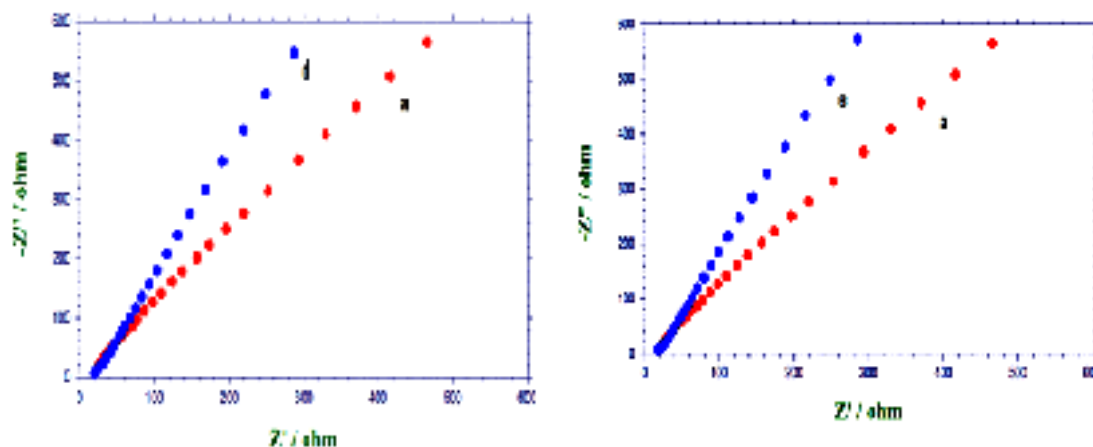
Fig. 2. Electrochemical impedence spectroscopy (EIS) of 1 x 10^{-3}M Hg(II) on clean polished bare WIGE (a) and different amount of ligand modified WIGE by electrodeposition (b) 20mV/s; 5cycles (c) 40mV/s; 5cycles, (d) 20mV/s; 10cycles, (e) 100mV/s; 26 cylcles
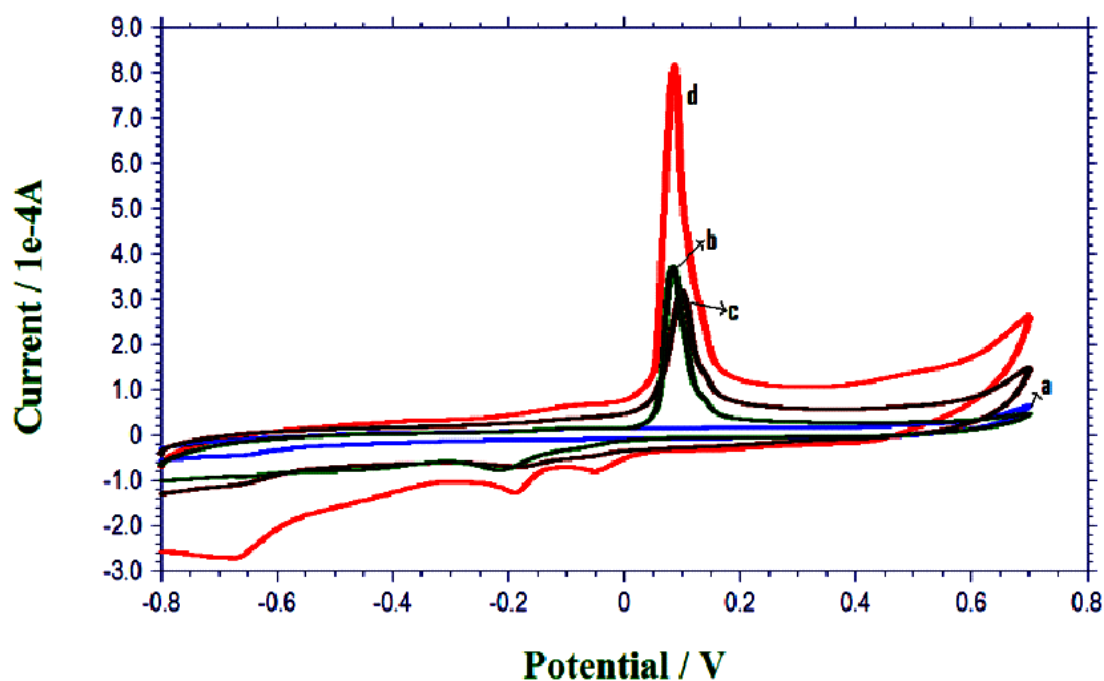

Fig. 3. CV of ligand/WIGE in 0.5M KCl solution (a). CVs of 1x 10^{-3} Hg(II) at a clean freshly polished bare WIGE (b), and different amount ligand modified WIGE by electro deposition with scan rate 100mV/s; 26 cyles (c), and 20mV/s; 5cyles(d).
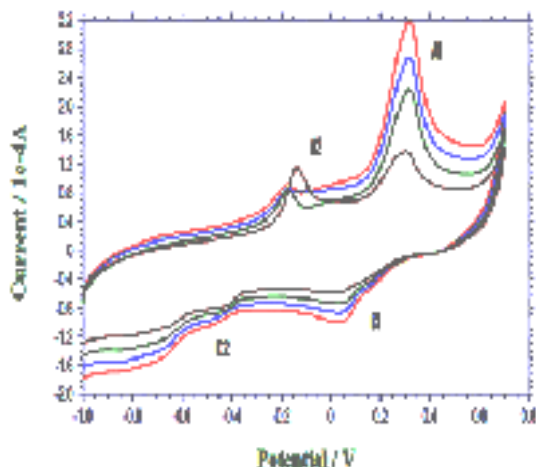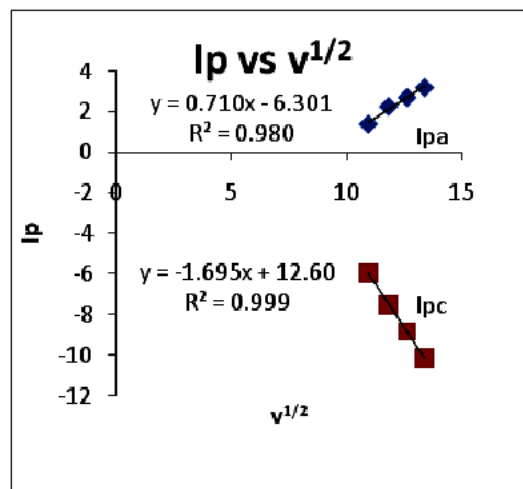
3.2 Scan rate effect

Fig. 4

Fig. 5

Fig. 4 Cyclic Voltammogram for 1×10^{-3}M Cu(II) ion on ligand modified WIGE at different scan rates 120, 140, 160 and 180mV/s. Fig 5. A plot for Ipa vs $V^{1/2}$.

Voltammograms were recorded for 1mM Cu(II) solution on ligand modified WIGEat varying scan rates (Fig. 4). The peak currents (Fig. 5) of copper ion increased linearly with the scan rate in the range of 120 to 180 mV/s. The linear regression equations are expressed as Ipa=$0.710v^{1/2}$-6.301 and Ipc=-$1.695v^{1/2}$+12.60 which suggesting that the redox reaction of Cu(II)/Cu(0) is surface adsorbed process. This is because that the surface-adsorbed process becomes the rate determining step of the electrode reaction. However, the Ip of copper ion on ligand modified WIGE was proportional to the scan rate from 120 to 180mV/s with a co-efficiencies of 0.980 and 0.999, demonstrating that the diffusion process of copper redox system is slower than the surface adsorbed process and the electrode reactions of copper ion is controlled by diffusion of copper ions from the solution to the modified electrode. Also, a shift in peak potentials is observed with the value of Ipc/$v^{1/2}$ increasing with increase in scan rates (Table 1). This implies that the chemical step does not precede electron transfer. From the voltammetric data, it has been observed that: (i) the Epc and Epa values change with increase of scan rate, (ii) the ΔEp (> 60mV) increases with increase of scan rates, and (iii) the cathodic (Ipc) and and anodic (Ipa) peak currents are not equal. This shows that the process is quasi reversible involving one electron transfer. The Ipa^2/Ipc^2 =1.473(>1) for A2/C2 redox couple and Ipa^1/ Ipc^1 =0.234 (<1) for A1/C1 redox couple *i.e.* the oxidation charges were slightly higher than the reduction charges at A1 oxidation state and controversy result is observed at the oxidation state A2 Such a result is expected for copper which undergoes facile oxidation process due to its complexation structure. The chemical steps for the redox system is explained follow.
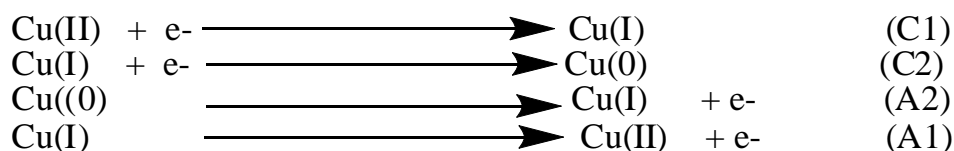
$$Cu(II) + e- \longrightarrow Cu(I) \qquad (C1)$$
$$Cu(I) + e- \longrightarrow Cu(0) \qquad (C2)$$
$$Cu(0) \longrightarrow Cu(I) + e- \qquad (A2)$$
$$Cu(I) \longrightarrow Cu(II) + e- \qquad (A1)$$

Table 1. Effect of scan rate on the behaviour of ligand/WIGE for 1mM
Cu(II) in 0.5M KCl solution

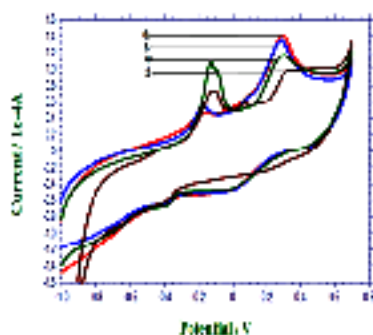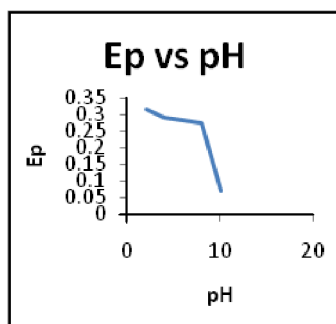| Scan rate (mV/s)	Ipa^1 $X10^4A$	Ipa^2 $X10^5A$	Ipc^1 $X10^4A$	Ipc^2 $X10^5A$	Epa^1/V	Epa^2/V	Epc^1/V	Epc^2/V	$Ipc^1/V^{1/2}$
120	1.388	11.69	-5.936	-7.936	0.303	-0.137	0.029	-0.426	-0.541
140	2.254	8.644	-7.522	-8.661	0.318	-0.173	0.036	-0.435	-0.636
160	2.690	8.814	-8.814	-9.654	0.318	-0.170	0.036	-0.461	-0.696
180	3.179	9.467	-10.14	-10.13	0.316	-0.175	0.0406	-0.442	-0.755

pH study

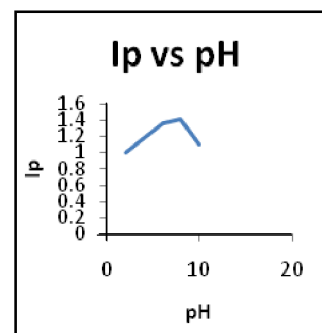Fig. 7   Fig. 8(a)   Fig. 8(b)

Fig. 7. Cyclic Voltammogram for 1mM Cu(II) on ligand modified WIGE at different pH's 2,4,6,8.
Fig. 8(a). A plot of Ep vs pH. Fig. 8(b). A plot of Ip vs pH.

The influence of the pH on the voltammetric behaviour was tested for different pH's 2.0-8 at scan rate of 140mV/s. The best results with respect to shape, sensibility and reproducibility of the peak current were obtained at pH 8.0. Cyclic voltammograms recorded for ligand modified WIGE in the presence of 1mM Cu(II) ion in 0.5M KCl solution exhibits a well-defined peak at 0.38V in the whole pH range investigated (pH 2.0-8.0). The effect of pH on peak cyclic voltammogram of ligand-Cu(II) complex are shown in Fig.7 As can be seen, the ip of the formed complex increases on going from pH

2.0 to 8.0, where the maximum value is obtained at pH 8.0. In larger pH values than 8.0 the *i*p decreases abruptly. From *E*p vs. pH (Fig. 8a), it is observed that the oxidation potential of ligand-Cu(II)complex shifted to more negative values with pH increase, indicating the presence of a chemical reaction with participation of protons[30]. From the *i*p vs.pH (Fig. 8b) relationship it is seen that different ionic species are formed, whose present different diffusion coefficients in the solution. For analytical purposes, pH 8.0 was therefore chosen as best work conditions.

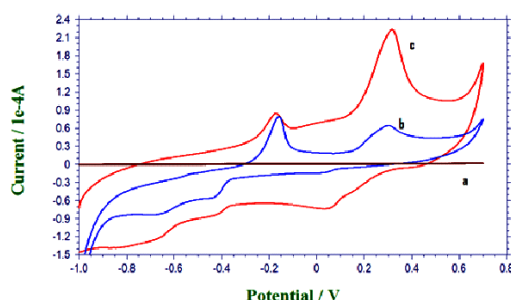**Sensing of copper ion on ligand modified WIGE**

Fig. 9

Cyclic voltammogram for a) ligand modified WIGE in 0.5M KCl solution b) 1mM Cu(II) on bare WIGE c) 1mM Cu(II) on ligand modified WIGE.

CVs for (a) 0.5M KCl solution (b) 1.0 mM Cu (II) on bare WIGE and (c) 1.0 mM Cu (II) on ligand modified WIGE at a scan rate of 140 mVs^{-1} are shown in Fig. 9. It was observed that the voltammograms are almost similar to those of pure Cu (II) ion system. The co-ordination attachment of ligand with Cu(II) forming a complex is evident by the peak separation and enhancement of peak current. The cyclic voltammograms show that both the cathodic as well as anodic peaks are enhanced as compared to the unmodified one. This happens due to increased uptake of copper ions by the chemically modified electrode through complexation. Table 2 shows the effect of modification on peak currents observed by cyclic voltammetry. The peak current enhanced for the ligand modified WIGE and also the peaks are sharper. This could be expected, as the cavity size of ligand is closer to the diameter of cupric ion (1.54 Å). As ligand is insoluble in water it did not leach out. So, the ligand modified WIGE was used for further studies and potassium chloride was used as supporting electrolyte as it is the least complexing medium. The potential value of the anodic peaks is shifted towards negative value and the cathodic peaks is shifted towards positive value giving a large peak separation, which is the characteristic of complexation[31].

Table 2. Effect of modification on the cyclic voltammetric peak currents for 1mM Cu(II) in 0.5M KCl at 140mV/s.

	Ipa^1 x 10^4	Epa^1	Ipa^2 x 10^5	Epa^2	Ipc^1 x 10^5	Epc^1	Ipc^2 x 10^5	Epc^2	Ipc^3 x 10^5	Epc^3
Bare WIGE	0.659	0.303	8.107	-0.160	-1.52	0.027	-5.714	-0.435	-8.530	-0.680
Ligand modified WIGE	2.254	0.318	8.644	-0.171	-7.52	0.041	-8.583	-0.433	-	-

Calibration Graph :

Potential / V

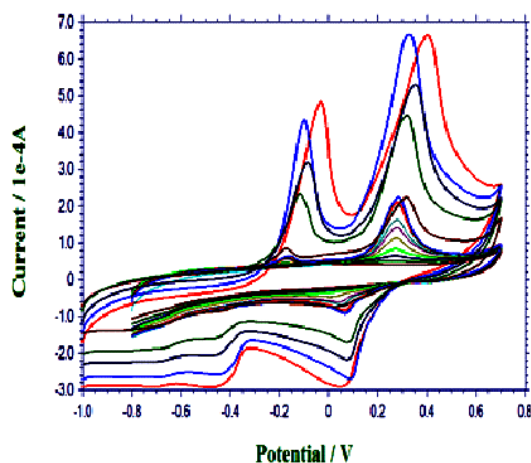Fig. 10

Fig. 11

Fig. 10. Cyclic Voltammogram for different concentrations of copper(II) in the range of 0.05mM to 5mM at 140mV/s on ligand modified WIGE. Fig. 11. A plot of Ip vs Conc.

Fig. (10 & 11) shows the cyclic voltammogram and calibration curve of Cu(II) in different concentrations (0.05 mM to 5mM) in 0.5M KCl respectively. At lower concentration one reduction peak and at higher concentration two reduction peaks were found. This is due to the presence of following reaction was present at higher concentrations. $Cu(II)+ e- \longrightarrow Cu(I)$ and $Cu(I) + e- \longrightarrow Cu(0)$. The calibration plot for Cu(II) was performed at the ligand modified WIGE Cu(II) solutions with a good linearity of anodic current versus concentration described by the linear regression equation $Y=4.484x+0.072$ $R^2=0.983$. The detection limit of the method based on ligand modified WIGE for the determination Cu(II) ion was found to be 5×10^{-5}M by cyclic voltammetry.
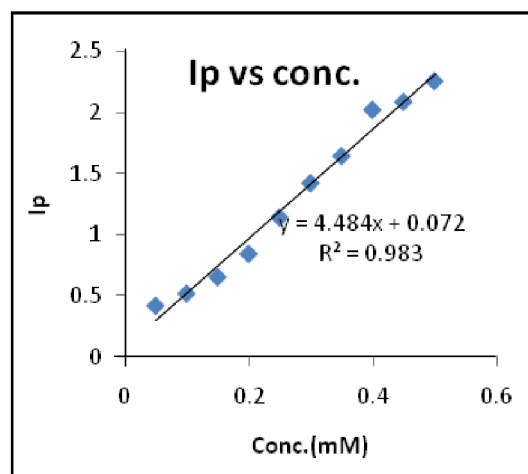
Interference study :

Fig. 12 shows the cyclic voltammograms of Pb(II), Cu(II) and Hg(II) in their mixture on ligand modified WIGE and bare WIGE. The redox peaks of the three metal ions overlapped on bare WIGE (curve a), and the peak current is much lower than that on the ligand modified WIGE (curve b). At the ligand modified WIGE, six oxidation peaks appear obviously at about -0.43V (Pb), 0.04, 0.35, 0.8 V (Cu) and 0.2, 0.6V (Hg). This result demonstrated that the anodic peaks of Cu(II), Pb(II) and Hg(II) are well separated from each other on the ligand modified WIGE. In unmodified WIGE more than six oxidation peaks were obtained. This may be due to formation of oxides of metal ions. But in ligand modified WIGE well separation of peaks and limited anodic peaks were appeared. This shows that oxides of metal ion formation were prohibited by formation of least stable complex of the metal ions with the ligand on WIGE. Hence, it is possible to simultaneously

detect Cu(II), Pb(II) and Hg(II) on the ligand modified WIGE. The presence of copper is highly interfered with the other heavy metal ions, even in nano molar level[32]. The nitrogen containing ligands are more preferable to form complex with copper as compared with mercury[33]. Under these conditions, the effect of interference was studied by addition of equal concentrations of Pb(II), Hg(II) ions. None of them were found to affect the Cu (II) peaks when present in equal concentration. The peak currents obtained for the samples were the same as that obtained for Cu(II) on ligand modified WIGE which shows that these ions do not interfere even when present together in a sample.
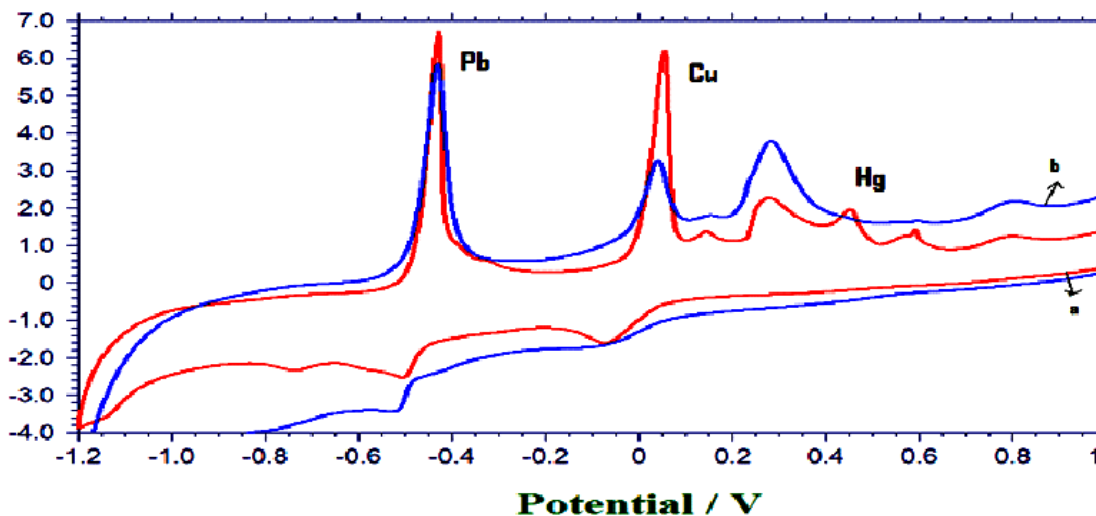

Fig. 12. Cyclic Voltammogram for 1mM Pb(II), 1mMCu(II) and 1mM Hg(II) on ligand modified WIGE in 0.5M KNO$_3$ at 140mV/s.
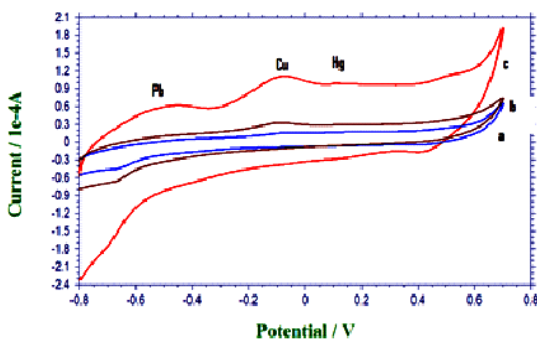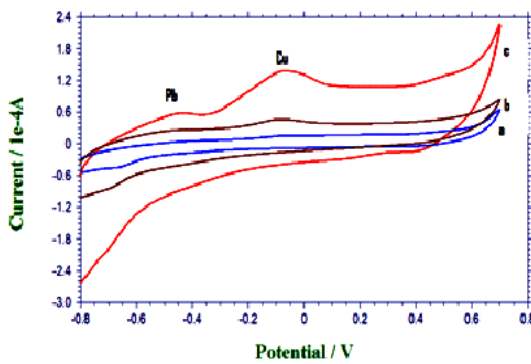
Application in real samples :

Fig. 13

Fig. 14

Fig.13 &14 Cyclic Voltammogram for metal ions in tap water and rubber industry effluent respectively.

The utilization of the proposed method in real sample analysis was investigated by direct analysis of tab water and rubber industry effluent. The tap water & rubber industry effluent are taken separately and mixed with 0.5M KCl solution and used as electrolyte solution. Fig (13, 14) shows ligand modified WIGE sensed highly copper ion even in presence of lead and mercury ions on both the real samples. The presence of lead, mercury, copper ions in real samples is confirmed from the OCP-Emission Spectroscopy results. Therefore, the ligand modified WIGE can provide effective method for detection of copper ion in real samples.

Conclusion

This study demonstrates an analysis scheme for detection of heavy metals using cyclic voltammetry technique. After proper optimization of the experimental conditions, this method was suitable for detection of metal ions in real samples. Interference study shows that, simultaneously detection of Cu(II), Pb(II) and Hg(II) ions on the ligand modified WIGE is possible. According to HSAB (Hard and Soft Acids and Bases) theory, the N-methyl piperazine is a kind of hard base, while the Cu(II) is beyond border line acid, and then it can be adsorbed onto the surface of ligand modified WIGE. Ligand modified WIGE has special selection of Cu(II) even in presence of Pb(II) and Hg(II). Symmetrical ligands has special selective and sensitive on copper ion which is proved from the results. Thus ligand modified WIGE behave as a copper ion selective electrode.

References

1. Williams, D.R. In. The metals of life: The solution chemistry of metal ions in Biological systems. Van Nastrand. Reinhold, London, New York, p. 98 (1971).

2. Goodman and Gillman In: The pharma-cological Basis of Therapeutics, 7th Edn, McMillan pub.Co.New York, p.474 (1985).

3. Quayum, M.E., Mannan, R.J and Rahman, S.G., *J. Pharm. Sci., 3,* 24-28 (2004).

4. Mandler, D. and Turyan, I., *Electroanalysis 8*, 207-213 (1996).

5. Wink, Th; Van Zuilen, Z. J., Bult, A. and Van Benmekan, W.P. *Analyst,* 122, 43R-50R (1997).

6. Mirsky V. M., *Trends Anal. Chem., 21,* 439-450 (2002).

7. Postleth Waite, T.A. Hutchison, J.E., Hathcock, K.W. and Murray, R.W. Langmuir *11,* 4109-4116 (1995).

8. Rahman, M.A., Won, M.S. and Shim, Y.B *Anal. Chem. 75,* 1123-1129, (2003).

9. Mohadesi, A. and Taker, M.A., *Talanta, 72,* 95-100 (2007).

10. Wang, F., Liu, Q., Wu, Y. and B.J. Electroanal *Chem. 630,* 49-54, (2009).

11. Rubinstein, I., Steinberg, S., Tor, Y., Shanzer, A. and Sagire, *J. Nature, 332,* 426-429, (1998).

12. Steinberg, S., Tor. Sabatani, E. and Rubinstein, I., *J. Am. Chem. Soc., 113,* 5176-5182 (1991).

13. Steinberg, S., and Rubinstein. I., Langmuir.

8, 1183-1187 (1992).

14. Yang, W.R., Chow, E., Willett, G.D., Hibbert, D.B. and Gooding, J., *J. Analyst 128,* 712-718 (2003).

15. Chow, E., Hibbert, D.B. and Gooding, J.J. *Electrochem. Commun. 7,* 101-106 (2005).

16. Pandey, P.C. Sens. Actuators B., *54,* 210-214 (1999).

17. Wakida, S.I., Sato, N& Saito. K. Sens. *Actuators (B) 130,* 187-192 (2008).

18. Ghiachi, M., Rezai, B. and Arsahdi, M. Sens. *Actuators(B), 139,* 494-500 (2009).

19. Mashadizadeh, M.H., Eshardari, Kh., Foroumadi, A. and Shafiee A, *Talanta, 76,* 497-502 (2008).

20. Lina, M., Chob, M., Choea, W. S., Sonc, Y. and Lec.,Y. Electrochim. Acta, 54, 7012-7017 (2009).

21. (a) Macdonalds, R. (1987). Impedence Spectroscopy, 1st ed., New York, Wiley.b) Janek, R.P. and Fawcett, W.R., *J. Phys. Chem. B, 101,* 8550-8558 (1997).

22. Flink, S., Boukamp, B.A., van den Berg, A., van Veggel, F.C.J.M. and Reinhoudt, D.N., *J. Am. Chem. Soc, 120,* 4652-4657 (1998).

23. Alfonta, L., Katz, E. and Willner, I., *Anal. Chem, 72,* 927-935 (2000).

24. Shervedani, R.K. and Mozaffari, S.A.

Surf. Coat. Technol., 198, 123-128 (2005).

25. Mozaffari, S.A., Chang, T. and Park, S.M. *J. Phys. Chem. C, 113,* 12434-12442, (2009).

26. (a) Shervedani, R.K. and Mozaffari, S.A. *Anal. Chem., 78,* 4957-4963, 2006. (b) Mozaffari, S.A., Chang, T. and Park, S.M., *Biosens. Bioelectron, 26,*74-79 (2010).

27. Liu, Y.J., Yin, F., Long, Y.M. *et.al. J. Colloid Interface Sci.* 258, 75, (2003).

28. A. Manivannan, M.S. Sheera, Quantification of merucy in flue gas emission using boron-doped diamond electrochemisty, (2003).

29. Fei Wang, Jian Liu, Yan-Ju Wu, Yu-Mei Gao and Xu-Feng Huang *Journal of the Chinese Chemical Society, 56,* 778-784 (2009).

30. J.A. Ocana, F.J. Barragan, M. Callejón, *Analyst 125,* 2322, (2000).

31. Bard A.J. and Faulkner, L.R. In*:* Electrochemical Methods: Fundamentals and Applications, John Wiley and Sons, p. 205 (1980).

32. Edith Chow, J. Justin Gooding *Electroanalysis, 18, (15),* 1437-1448 (2006).

33. J. Justin Gooding, D. Brynn Hibbert and *Wenrong Yang sensors, 1,* 75-90 (2001).