

# Voltammetric Studies of Cadmium Ion at the Mercury Electrode in the Presence of Glutathione.

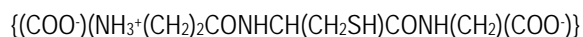
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## ABSTRACT

Voltammetric behaviors of cadmium (II) ion in the presence of a ligand with sulphur bearing group, such as glutathione:



were studied using mainly Osteryoung square wave voltammetry (OSWV) and cyclic voltammetry (CV) at a mercury electrode. The coordination chemistry of reduced glutathione (GSH) is of great importance as it acts as excellent model system for the binding of metal ions. In this study, it was observed that an addition of glutathione as ligand to solution containing Cd(II) with sulphate as supporting electrolyte caused an increase in the reduction current of Cd(II) by several factors and also with a slight cathodic shift in the reduction peak potential of Cd(II). Further assessment of the chemical and physical conditions that may favor optimum current enhancement was done by studying the effect of varying pH, supporting electrolyte concentration of ligand and metal ion, interfering ions and scan rate. The presence of 1-3 mM glutathione, a weakly complexing supporting electrolyte at pH 4-5 caused a 3.5 fold increase in the reduction current of Cd(II) and a slight negative shift in peak position. The current enhancement observed in different electrolytic media varied in the following order: sulphate > nitrate > perchlorate. The presence of other metal ion such as Cr(III) or Co(III) appear to cause further increase in the reduction current of the Cd(II)-glutathione peak while the presence of Ni(II) was found to suppress the enhanced peak current. An anion-induced adsorption mechanism can be used to account for the observed current enhancement of Cd(II) ion in the presence of glutathione.

(Keywords: voltammetric behaviors, Cd(II), GSH, glutathione, OSWV)

## INTRODUCTION

Interest in the use of mercury electrodes as a working electrode for the analysis of metal ion and sulphur bearing groups of ligands in voltammetric/polarographic experiments have been due to the strong affinity of sulphur bearing groups for mercury electrode. This is due to the large value of the formation constant of Hg-S bond ( $K_f = 10^{14}$ ) [1]. Hence some classical work on the use of ligands containing sulphur atoms as potential anchoring groups to the adsorption of metal complexes at a mercury electrode surface have been reported much earlier by Anson [2-4].

Glutathione, GSH (( $\gamma$ -Glu-cys-Gly), an amino acid, is an essential constituent of living cells. It is characterized by the formation of protonated and polynuclear complexes as it possesses a facile electron donating capacity linked to -SH group. It plays an important role in physiological functions as an antioxidant, antitoxin and enzyme cofactor. GSH is also responsible for the elimination of toxic heavy metals from the organisms, the significance of the study of heavy metal ions interaction with GSH is therefore apparent and is reflected in the works reported by Banica et al. [5] Crux et al. [6-8]. The former studied the catalytic cathodic stripping voltammetric behavior of oxidized glutathione, GSSG at a HMDE in the presence of Ni(II) ion while the latter employed the combination method of MCR and differential pulse polarography (DPP) and cyclic voltammetry in the study of complexation of Cd(II), Pb(II), and Zn(II) with GSH.

These divalent metal ions were thought to bind strongly to the sulphur atoms of cysteine and less strongly to the oxygen of the glycine carboxylate, hence lead to the formation of the proposed 1:1 and 1:2 M(II) : GSH (where M= Cd(II), Pb(II) and Zn(II)). Earlier report relied heavily on pulse technique such as differential pulse polarography and rarely using other technique modes such as

the one reported here. Thus far, current enhancement behavior of divalent metal ion Cd(II) in the presence of glutathione at a mercury electrode was rarely reported. It is therefore our intention to report the work done on Cd(II) – glutathione system using fast cyclic voltammetry, sweep square wave, chronocoulometry with specific emphasis on current enhancement observed for the Cd(II) in the presence of GSH. This report also serves as an extension of our previous work done on Cd(II) –cysteine system [9].

## EXPERIMENTAL

All chemicals used were of reagent grade purity and were used as received. 1000mg/ml Cd(II) was prepared as a stock solution from cadmium nitrate salt. A stock solution of glutathione was usually prepared fresh in doubly distilled water and used within a few hours after preparation. 0.03M sodium acetate buffer was used with adjustment to pH 4 and pH 6 by either an addition of acetic acid or nitric acid or NaOH. 0.04M triethanolamine was used for pH 7 while 0.04 M NH<sub>4</sub>OH/NH<sub>4</sub>Cl buffer was used for pH 8-10 un-buffered solution of pH<4 was used with the addition of nitric acid.

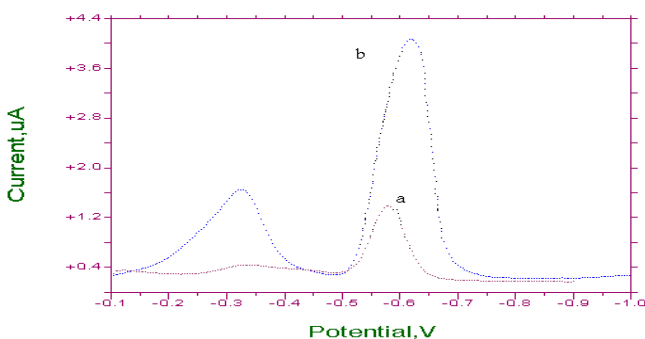
## APPARATUS

An electrochemical workstation (Model BAS100A) was used to perform, square wave voltammetry (OSWV), cyclic voltammetry (CV), chronocoulometry (CC). It was used together with CGME (Model PAR303), Controlled Growth Mercury Electrode (CGME, BAS). An electrode area of 0.034 cm<sup>2</sup> was employed in CGME. All potential were measured against the Ag/AgCl (3M NaCl) reference electrode. A platinum wire was used as a counter electrode. The experiments were carried out at 25 ± 1 °C.

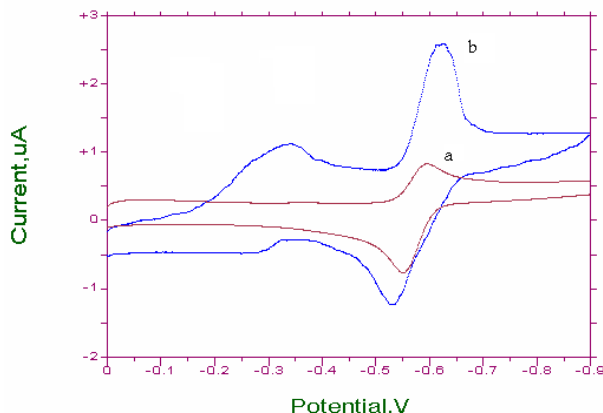
## RESULTS

The addition of glutathione to Cd(II) ion solution is found to cause a pronounced enhancement on the reduction current of Cd(II) ion and a negative shift of its peak potential by a few mV during square wave voltammetry (Figure 1(i) and cyclic voltammetry, Figure 1(ii-iii), the reduction peak appearing at -0.32V and -0.63V (vs Ag/AgCl) are due to the reduction of Hg-(Glu)<sub>x</sub> and Cd-(Glu)<sub>x</sub> respectively. As the enhancement in its reduction

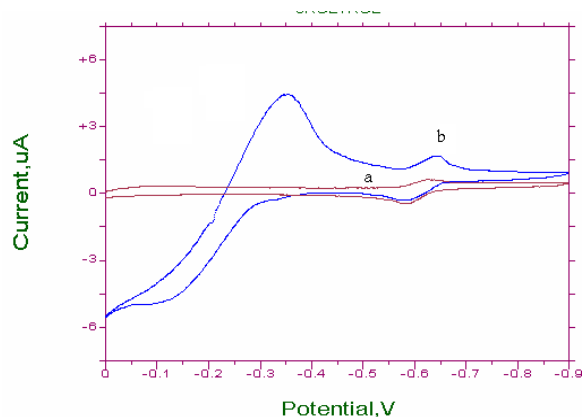
current was considerable in square wave voltammetry (Figure 1 (i)), it was used in subsequent studies.



**Figure 1(i):** Square Wave Voltammetry: 20 µg/ml Cd(II) in 0.1M Na<sub>2</sub>SO<sub>4</sub>, 3 mM acetate buffer, pH 4.7 in CGME, (a) Without Glutathione (b) With 3 mM Glutathione.



**Figure 1(ii):** Cyclic Voltammogram: 20 µg/ml Cd(II) in 0.1M Na<sub>2</sub>SO<sub>4</sub>, 3 mM Acetate Buffer, pH 4.7 in CGME, (a) Without Glutathione (b) With 3 mM Glutathione.



**Figure 1 (iii):** Cyclic Voltammogram: 20 µg/ml Cd(II) in 0.1M KNO<sub>3</sub>, 3 mM Acetate Buffer pH 4.7 in CGME, (a) Without Glutathione (b) With 3 mM Glutathione.

**Table 1:** Effect of Supporting Electrolyte on Ip and Ep of 5.62 µg/ml Cd(II) with and without 3.0mM Glutathione during Square Wave Voltammetry using CGME as a Working Electrode.

Supporting electrode	Ip (mA)		Ep (mV)		Enhancement
	No: Glu	Glu	No: Glu	Glu	
KCl	1.66	2.6	-612	-636	1.6
Na <sub>2</sub> SO <sub>4</sub>	1.46	4.9	-580	-620	3.5
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.51	3.5	-580	-616	2.3
KNO <sub>3</sub>	1.39	2.7	-564	-668	1.9
KClO <sub>4</sub>	1.32	2.69	-624	-628	2.0
KBr	1.71	1.82	-580	-590	1.1

### Effect of supporting electrolyte

Table 1 represents the effect of supporting electrolyte on the electrode reduction process of Cd(II) in the absence and presence of glutathione. Sodium sulphate appears to provide the largest current enhancement (3.5 fold) while bromide the least (1.1 fold) for Cd(II)–glutathione system. In general, the current enhancement observed in different media varied in the following order: sulphate > nitrate > perchlorate > chloride > bromide.

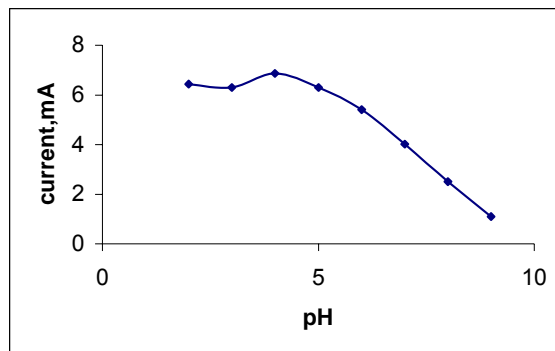
### Effect of pH

Figure 2 shows the effect of varying pH on the peak current of Cd(II)-glutathione. The optimum pH range for current enhancement appears to be between 2 to 5 with maximum enhancement at pH 4.7. Current decrease rapidly from pH 5 onward until pH 9. Hence, it is of no advantage to work under strongly alkaline condition, under which precipitation of Cd(OH)<sub>2</sub> would take place.

### Effect of varying [L]

Table 2 shows the result obtained from varying the glutathione concentration at constant Cd(II) ion concentration during square wave voltammetry. Peak current for Cd-Glu complex increases rapidly with increasing concentration of

glutathione of up to 3mM and decreases gradually from 3 mM onwards. The optimum range of ligand concentration for sensitivity enhancement is between 1 to 3 mM.



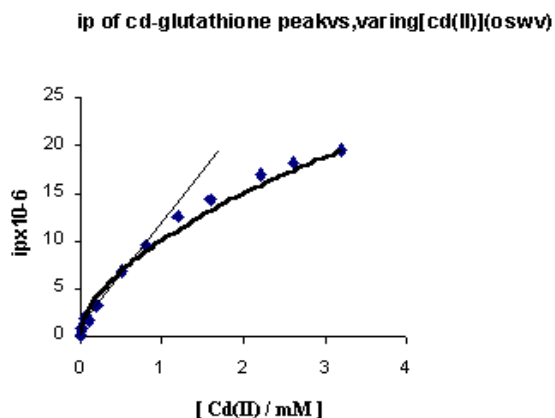
**Figure 2:** The Square Wave Voltammetry of Cd(II) Ion at 3.0 mM Glutathione, 0.1 M Na<sub>2</sub>SO<sub>4</sub>, at Different pH Ranging from 2 to 9.

**Table 2:** Effect of Varying Glutathione Concentration on Cd(II)-Glutathione System During Square Wave Voltammetry 0.1 mM Cd (II) in 1.0 M Na<sub>2</sub>SO<sub>4</sub> with 3 mM Acetate Buffer pH4.7.

Glutathione Concentration/mM	SWV i <sub>p</sub> (µA)
0.01	0.7
1.00	0.9
3.00	1.9
5.00	0.9
10.00	0.8

### Effect of varying [Cd<sup>2+</sup>]

Linearity exists below 1mM of Cd(II) ion concentration in the Cd(II) –glutathione system with correlation coefficient, R= 0.992. Above this concentration, the plot of ip vs Cd(II) ion concentration has a smaller gradient. The concentration isotherm obtained assumes characteristic of an adsorption isotherm. The linearity portion of the curve has a sensitivity response of ca. 10 µA/mM.



**Figure 3:** Calibration Curve of Varying Cd(II) Ion at 3.0 mM Glutathione, 0.1 M Na<sub>2</sub>SO<sub>4</sub>, 3 mM Acetate Buffer, pH 4.7 using Square Wave Voltammetry: 4mV Step Potential and CGME as a Working Electrode.

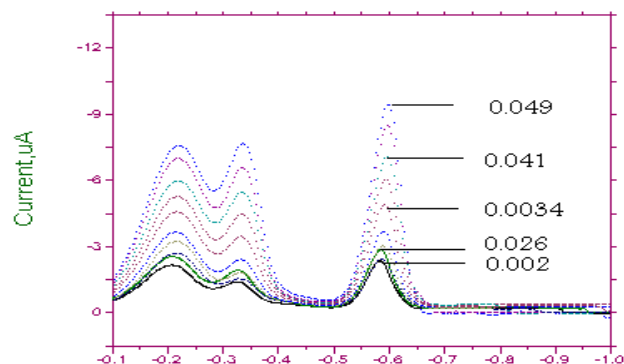
#### Effect of varying electrode area

Figure 4 (i) summarizes the effect of the surface area of electrode (HMDE) on the reduction of Cd-glutathione metal complex species. The plot of surface area vs peak current, *i<sub>p</sub>* is shown in Figure 4 (ii). As the area of the electrode increases, peak current also increases fairly proportionally as expected. Although larger area provides higher current sensitivity, an average area of 0.034cm<sup>2</sup> (equivalent to sec drop time of the CGME) was used.

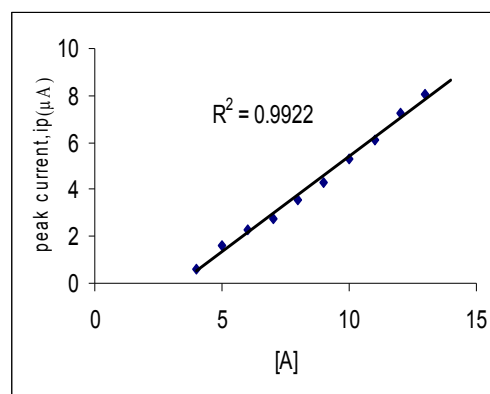
#### Effect of metal ion interference

An investigation is carried out to determine if the presence of metal ion such as Ni(II), Co(III), Cr(III), Cu(II), and Zn(II) ions could interfere in the enhancement of the reduction current of Cd(II)-glutathione complex species during square wave voltammetry. The results in Table 3, show that the presence of Co, Zn and Cr appears to enhance the reduction current of Cd-glutathione further while Ni(II), Cu(II) suppress the reduction current of Cd(II)-glutathione. The reason for the discrepancy was not obvious. Further work is required. It is interesting to note that Cd(II) ion in the presence of glutathione and Cr(III), has the greatest enhanced value, of 28.9% (Table 3). The comparison between presence and absence of glutathione was shown in Figures 5 (i) and 5 (ii) respectively, in which [Cd(II)] is held constant while [Cr(III)] is varied. Figure 5 (ii) showed that

the enhanced reduction peak of Cd(II)-Glu (*E<sub>p</sub>* = -0.7V) was further increased by the presence of increasing amount of Cr(III) ion (*E<sub>p</sub>* = -1.1V).



**Figure 4 (i):** Effect of Varying Size of Mercury Electrode, (cm<sup>2</sup>) at 1mM Cd(II), 0.1M Na<sub>2</sub>SO<sub>4</sub>, 3 mM Glutathione during Square Wave Voltammetry using CGME as a Working Electrode.



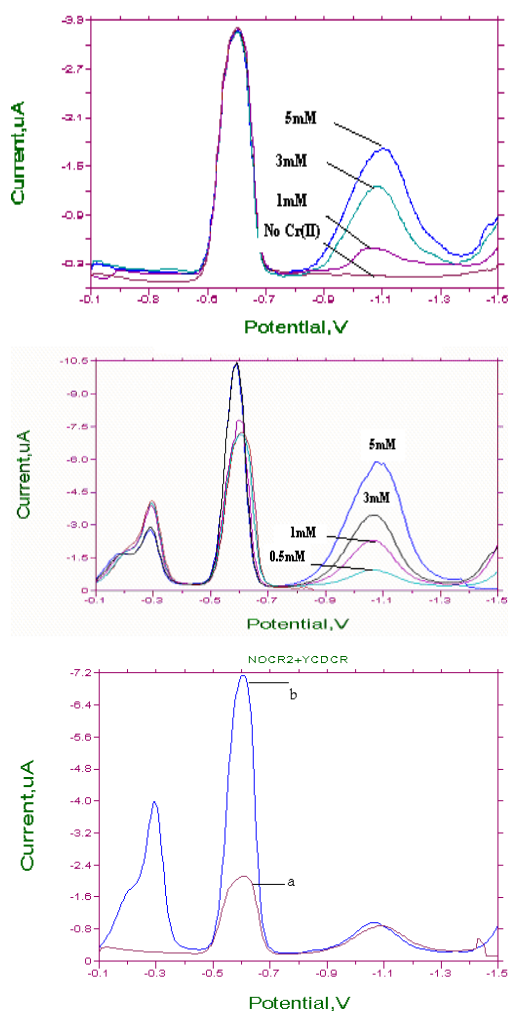
**Figure 4 (ii):** A Plot of Peak Current of Cd(II)-Glu Versus Surface Area of Condition Shown in Figure 4 (i).

However, the presence of up to 5 mM Cr(III) dose not cause any interference in Cd(II) reduction process in the absence of glutathione (Figure 5 (i)). Also, the presence of 3 mM glutathione does not change the magnitude of the reduction current of Cr(III) ion although a slight shift in peak potential is observed (Figure 5 (iii)). It is therefore evident that Cr(III) ion appears to exert a synergistic or catalytic effect on the reduction process of the adsorbed Cd(II)-Glu complex species at the Hg electrode during SWV.

**Table 3:** Effect of Metal Ion on  $i_p$  and  $E_p$  of 1mM Cd(II), 0.1M Na<sub>2</sub>SO<sub>4</sub>, 3 mM Glutathione during Square Wave Voltammetry using CGME as a Working Electrode.

Metal ion	1:10 mM (Cd: metal ion)				Percentage of current increment %
	$i_p/\mu\text{A}$ (a)	$i_p/\mu\text{A}$ (b)	$E_p/v$	$E_p/v$	
Co	7.5	9.9	-604	-592	24.1
Zn	7.7	8.2	-612	-596	6.09
Cr	7.1	10.1	-604	-588	28.9
Ni	8.4	7.5	-600	-569	-12.4
Cu	8.2	6.6	-604	-696	-23.7

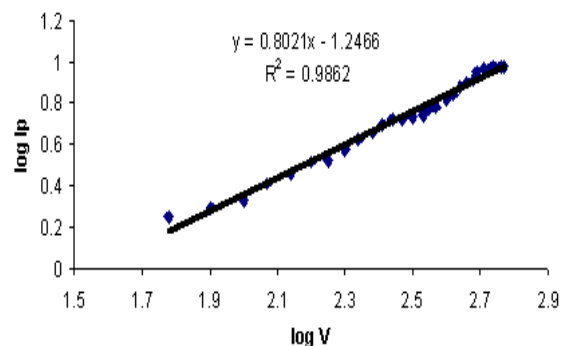
a) Absence of metal ion    b) Presence of metal ion (1:10[Cd] / [M<sup>+</sup>])



**Figure 5:** Typical OSWV Voltammograms for the Reduction of Cd(II) Ion, Cr(III) Ion in 1.0 M Na<sub>2</sub>SO<sub>4</sub>, 3 mM acetate buffer, pH 4.7: [(i), (iii a)] Without Glutathione; [(ii), (iii b)] with 3 mM Glutathione at CGME.

### Effect of Varying Scan Rate

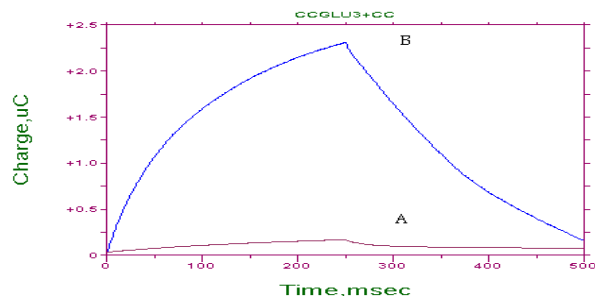
The effects of varying scan rate on the reduction current of Cd-Glu complex species are also studied. The slope of the log plot ( $\log i_p$  vs  $\log v$ ) has a value of 0.8 which is quite close to 1 for strong adsorption indicating that the Cd(II)-glutathione complex species is not very strongly adsorbed on to the Hg electrode surface.



**Figure 6:** Effect of Varying Scan Rate of Cd(II) Ion in the Presence of 3 mM Glutathione on Peak Current Obtained with Glutathione in 1.0 M Na<sub>2</sub>SO<sub>4</sub>, 3mM Acetate Buffer pH 4.7 using Square Wave Voltammetry: 4 mV Steps, with Varying Frequency, an CGME as a Working Electrode.

### Chronocoulometry

Figure 7 showed the effect of the addition of glutathione on the chronocoulomogram of Cd(II) ion in sodium sulphate electrolyte. Considerable surface charge enhancement can be observed and is evident of an adsorption process [10].

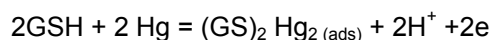


**Figure 7:** Chronocoulomogram for (a) 20  $\mu\text{M}$  Cd(II) and (b) with 0.3 mM Glutathione at pH 4.7 in 0.5 M  $\text{Na}_2\text{SO}_4$ .

## DISCUSSION

Evidence gathered from the concentration isotherm obtained from fast scan square wave voltammetry and the chronocoulometric scan rate studies of Cd(II)–glutathione system using CGME as a working electrode all point to the adsorption of the reactants of Cd(II)–glutathione complex species at the surface of mercury electrode as a controlling process. This adsorption process is deemed responsible for the current enhancement of the peak current of Cd(II) ion in the presence of excess amount of glutathione and under weakly acidic condition.

That the enhancement effect, which is pH dependent, was greatly felt only under weakly acidic condition strongly indicates that the presence of glutathione anion (RS) is a precondition for the specific adsorption of Cd(II) – glutathione complex species to take place at Hg electrode surface. It is a well know fact that a sulphur bearing group has strong affinity for mercury as sulphur form strong bond with mercury ( $\text{p}K_f(\text{Hg-S}) = 14.4$ ). The peak which appears at ca.-0.3 V (Figure 1) was associated with the reduction of glutathione (GSH) to mercury (I) glutathione by reaction with the electrode material [7]:

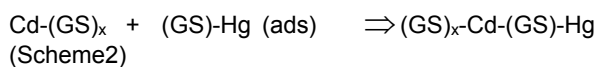
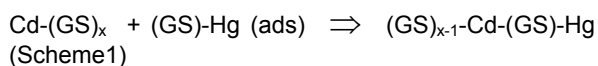


In general any species that competes with glutathione anion for the surface of the electrode would inevitably interfere in the adsorption of metal glutathione complex species hence leading to a decrease in the enhancement effect. This explains why supporting electrolyte such as perchlorate and nitrate which are weakly complexing produce higher current enhancement,

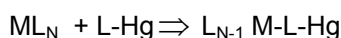
as compared to chloride. The latter which exhibits complexing ability at concentration of 0.1 M or higher was capable of competing with glutathione anion for electrode surface (Table 1).

The above findings prove that the adsorption process which causes the enhancement effect of Cd(II) peak current in the presence of glutathione was probably due to the anion–induced adsorption, in which a specifically adsorbed substance (glutathione) is bonded to the mercury electrode surface via its strong mercury mercaptide bonding and thus promotes specific adsorption of the Cd(II)–glutathione on the mercury electrode .

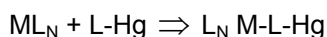
It is possible that the following reaction schemes occurred:



Scheme 1 is in accordance to the mechanism suggested by O'Dom and Murray [11] who assumed that the interaction between one particular complex species present in the solution,  $\text{M}_{\text{L}_\text{N}}$  with an anion L already adsorbed on the mercury surface, is such that it produces an adsorbed species with the same chemical composition as shown below:



The Scheme 2 is in accordance to the mechanism proposed by Anson and Barclay [12] which differs from the above in that interaction with anions already on the mercury occurs according to the following reaction:



The interaction between Cd(II) and GSH involved the formation of 1:1 and 1:2 (Cd(I):GSH) complexes have been reported by Diaz-Cruz et al. [6-8].

## CONCLUSIONS

Various evidence obtained in these studies have pointed towards the enhancement of the reduction peak current of Cd(II) observed in the presence of excess amount of glutathione at a

mercury electrode during fast sweep square wave voltammetry and cyclic voltammetry as being due to adsorption controlled process. Anion induced adsorption models proposed earlier by O'Dom and Murray [11] or Anson and Barclay [12] appear suitable to explain the observed behavior. The optimum current enhancement of Cd(II) can be observed under condition where 1 – 3 mM glutathione is used in weakly complexing electrolyte media such as sulphate or potassium nitrate adjusted to pH 4.7 using acetate buffer.

## ACKNOWLEDGEMENT

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