ELECTROCHEMICAL STUDIES ON THE REMOVAL OF CADMIUM BY METHYLATED XANTHINE BASES FROM AQUEOUS SOLUTION

Md. Abdul Jabbar[*] and Md. Riad Hossain Sabuj

*Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh*

Abstract

The removal of cadmium from aqueous solution using methylated xanthine bases, such as, caffeine and theophylline was investigated. Electrochemical techniques, such as, cyclic and differential pulse voltammetry were employed for such investigation. It was found that both caffeine and theophylline interact with cadmium. It was also observed that peak current decreases for cadmium due to gradual addition of caffeine or theophylline suggesting removal of cadmium from its aqueous solution. The empirical formula of the complexes revealed from the Job's plot suggests that 1 : 2 complexes are formed for both cadmium-caffeine and cadmium-theophylline systems in aqueous KCl solution.

Key words: Removal of cadmium, cadmium-ligand complexes, electrochemical detection

Introduction

Three important methylated xanthines that occur naturally are caffeine, theobromine and theophylline. Caffeine and theophylline are methylated xanthine bases, naturally found in tea. They are also found in cocoa beans (Apgar *et al.* 1998). Caffeine and theophylline both are most widely used behavioural active substances in the world (Esimone *et al.* 2008, Fredholm *et al.* 1999, Hashimoto *et al.* 2004). They are well-known as hydrotropic agents and have the ability to solubilize a wide variety of therapeutic drugs. Caffeine and theophylline both contain two aromatic rings that affect the solubility of the aromatic anti-malarial agent (Evstigneev *et al.* 2006, Jain *et al.* 1996, Lim *et al.* 2000). Several studies on methylated xanthines show that the methyl group at the N7 site blocks the interaction of metal ions with N7 atom. The interactions of calcium and magnesium ions with caffeine and theophylline have been investigated in aqueous solution at physiological pH. Recent study (Kabir 2011) based on MOPAC semi-empirical calculation shows that the interaction site of both caffeine and theophylline is at O13 position (Scheme 1).

---

*Corresponding author, E-mail: jabbar@univdhaka.edu;
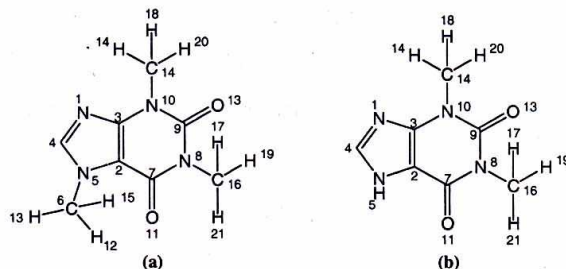
Scheme 1. Structures of (a) caffeine and (b) theophylline with atom numbering.

Cadmium (Cd) is highly toxic and responsible for poisoning through food. It is obtained as a by-product of zinc refining and used industrially in plating of steel, pigments, plastics, alloys. It is also used in nickel-cadmium batteries and in nuclear and electronic engineering (Kazantzis *et al.* 1963, Adams *et al.* 1969, Friberg 1984). It can also enter into the environment through the corrosion of galvanized pipes. The biologic half-life of cadmium is long (more than 30 years), which prolonged low level exposure leads to excessive accumulation in certain tissues, especially the kidney. Long-term exposure may lead to heart and kidney diseases, high blood pressure and even cancer (Sugira *et al.* 1979). When excessive amount of Cd(II) is ingested, it replaces Zn(II) at key enzymatic sites, causing metabolic disorder. At concentrations grater than 200 ppb, it is toxic to fishes in aquatic system. In potable waters, the normal level of Cd(II) is from 0.4 to 60 ppb. World Health Organization sets the tolerance level of Cd in drinking water at 27 nM (Niragu and Pacyna 1989).

Binding of Cd with ligands is a versatile process for the remediation of Cd from *in vivo* and *in vitro*. For example, the remediation of Cd was successfully carried out by binding it with bio-surfactants and naturally occurring acids (Deepika and Kannabiran 2010). Recent research shows that ethylenediamine-tetraacetic acid (EDTA) and glutathione (GSH) and combination of these two chelating agents facilitated efficient elimination of cadmium by the formation of corresponding Cd-complexes (Sheabar *et al.* 1989). The influence of an antioxidant agent, such as, *N*-acetyl cysteine (NAC) or mannitol on the Cd chelating ability of monoisoamyl 2,3-dimercaptosuccinate (MiADMS) was investigated *in vivo* (Tandon *et al.* 2003). *In vivo*, Cd(II) can bind to nucleic acids, or to proteins. Several investigators have demonstrated that Cd(II) is not found associated with low molecular weight compounds like free amino acids, *etc.* (Veronica *et al.* 2012). The efficiency of chelating agents is essential for increasing renal excretion of Cd(II) during acute Cd(II) intoxications. A recent report, shows that *N,N,N*-type ligands are selective for Cd(II) ions (Hirayama *et al.* 1997), which suggests that

other *N*-based ligand systems, such as amine based ligands can also be used as selective heavy metal removers.

For this reason, we choose methylated xanthine bases, such as, caffeine and theophylline as chelating agents and aim to find evidence that Cd(II) can be associated with these low molecular weight ligands as more than one ligated site is available. This will obviously help to reduce the concentration of Cd(II) from *in vivo* from liver and kidney by daily chelation therapy with caffeine and/or theophylline through renal excretion or also reduce cadmium from *in vitro* through the formation of metal-ligand complexes. Furthermore, due to our continuous interests on the study of metal-ligand interactions (Laiju *et al.* 2011, Jabbar *et al.* 2010, 2011), the present paper will give us the idea of such interactions between cadmium with caffeine and theophylline to develop therapy for Cd(II) contamination in liver or kidney as well as to remove Cd(II) from the environment to develop the green technology.

Experimental

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were employed to investigate cadmium-methylated xantine bases interactions in the present study. DPV was employed to find out the removal of cadmium from its aqueous solution by methylated xanthine bases, such as, caffeine and theophylline. Cyclic voltammetry was employed to study the nature of interaction of cadmium with caffeine and theophylline. This ultimately gives an idea of the redox behaviour of cadmium in the presence of caffeine and theophylline. Finally, Job's plots obtained from DPV data gives information about the empirical formula of the corresponding cadmium-ligand complexes. Details of the instrumentation and experimental set up are given below.

Apparatus

All the voltammetric experiments (CV and DPV) were carried out by using an Electrochemical Analyzer (Advanced Analytics, USA, Model HQ-2040) connected to a three-electrode micro cell consisting of a platinum working electrode, Ag/AgCl reference electrode and a Pt-wire counter electrode. The cell is completely shielded from any perturbing noises by a Faraday cage (C-3 cell stand, Bioanalytica USA. The Faraday Cage is a BAS C-3 cell stand connected to a N_2 gas cylinder to ensure diffusion controlled experiments. A continuous flow of N_2 was confirmed prior to any experiment for inert atmosphere.

Chemicals

Caffeine and theophylline were procured from BDH, England and was used without further purification. Cadmium chloride was purchased from Merck and was also used in the experiment without further purification. All the solutions were prepared from deionized water obtained from a milli-Q-water plant *PURIC*, Model-S. The experimental solutions were degassed with 99.99% commercially available N_2 gas from BOL, Dhaka, Bangladesh and all the runs were taken under N_2 atmosphere.

Results and Discussion

Evidence for the removal of cadmium by methylated xanthine bases

Differential pulse voltammetry (DPV) was performed for 1.3 mM Cd(II) in aqueous 0.1 M KCl solution. It was found that a sharp peak at −0.78 V *vs.* Ag/AgCl was observed in the DPV for the reduction of Cd(II) in its aqueous solution (Figure 1 A). Then a gradual addition of caffeine in the aqueous solution of Cd(II) was carried out to see the change in the diffusion current in DPV. It was observed that the diffusion current gradually decreases as the concentration of caffeine increases (Figure 1 A). In the DPV experiment, the diffusion current is directly proportional to the concentration of corresponding species. Therefore, observation in Figure 1(A) is strong evidence that the gradual addition of the ligand caffeine decreases the concentration of cadmium in aqueous solution. The similar beaviour has been observed for theophylline, which is shown in Figure 1(B).
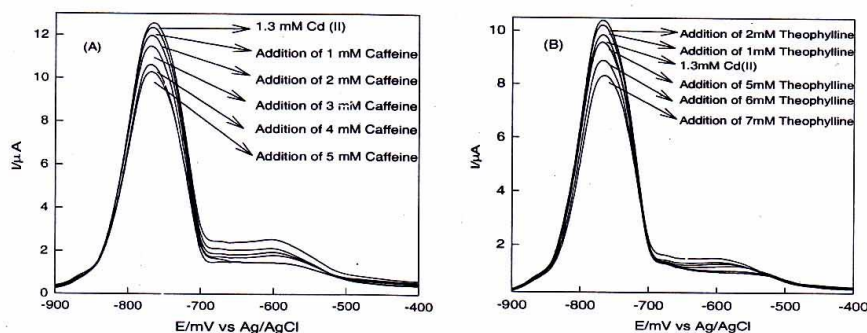

Figure 1. Recorded DPVs of 1.3 mM Cd^{2+} in 0.1 M aqueous KCl solution for the gradual addition of (A) caffeine and (B) theophylline.

DPV data for cadmium in presence of both caffeine and theophylline shifts the peak potential towards more cathodic position. This suggests that the interaction of cadmium

with both caffeine and theophylline is very strong and the reduction of Cd(II) to cadmium metal is difficult in the presence of the above ligands.

Evidence for the interaction of cadmium with caffeine and theophylline

Cyclic voltammogram of cadmium shows a multiple-step charge transfer at a platinum-disc electrode (Figure 2) in 0.1 M aqueous KCl solution. This multiple-step redox criteria even continue in the presence of caffeine (Figure 2A) and theophylline (Figure 2B). Peaks at − 0.8 V vs. Ag/AgCl has been attributed due to the reduction of Cd(II) ion in aqueous solution. The other two peaks may be arises due to the formation of meta-stable complexes in the aqueous media with water molecules. In the presence of caffeine (Figure 2A) and theophylline (Figure 2B), the redox peak height for cadmium is drastically reduced. The peak potential of cadmium has been shifted towards more cathodic region for the addition of both caffeine and theophylline. This effect has been clearly shown in Figure 2(A) and Figure 2(B). Reduction of peak heights of cadmium in the presence of caffeine and theophylline clearly indicates that these two ligands utilized cadmium to form the complexes and therefore reduce the concentration of cadmium from its aqueous solution.
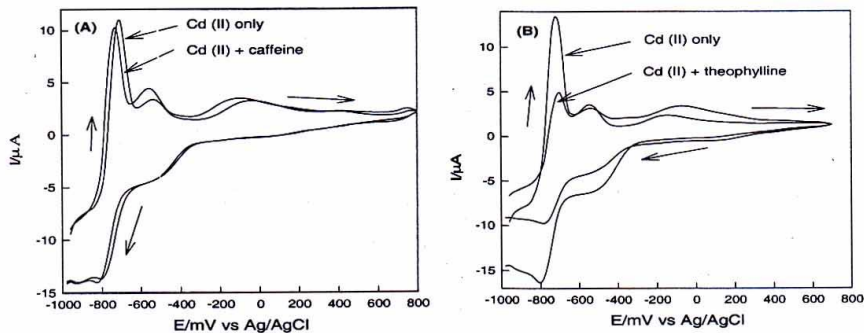

Figure 2. Comparative CVs of (A) Cd(II) in the presence and absence of caffeine and (B) Cd(II) in the presence and absence of theophylline in 0.1 M KCl solution at a platinum disc electrode. Scan rate 50 mV/s. [Cd(II)], 1.2 × 10⁻³ M, [caffeine] and [theophylline], 2.4 × 10⁻³ M.

CVs of cadmium, caffeine, theophylline, Cd-caffeine and Cd-theophylline systems were performed separately at different scan rates as shown in Figure 3. The diffusion co-efficient, D has been evaluated from the current-square root relationship from Figure 3, and by using the equation.
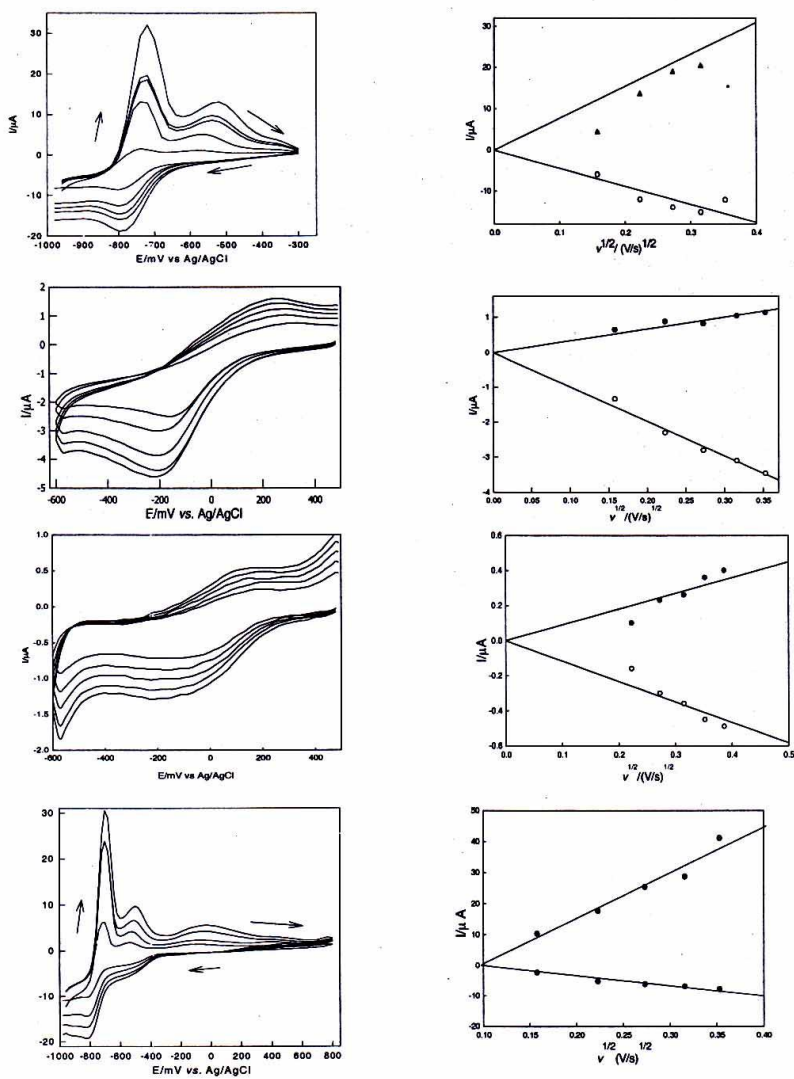
Figure 3. From up to down, CVs and current-square root of scan rate relationship Cd(II),
caffeine, theophylline and cadmium-caffeine system, respectively in 0.1M aqueous
KCl solution with a platinum-disc electrode at different scan rates. For all of the
cases, scan rates (0.025, 0.050, 0.075, 0.100 and 0.125 V/sec).

$$I_{pc} = 2.69 \times 10^5 \times n \times (\alpha n_a)^{1/2} A D^{1/2} C v^{1/2} \qquad (1)$$

To calculate D, we have considered the peak at −0.8 V in the CV. Taking the geometric area of the platinum disc electrode, A = 0.025 cm^2; concentration of cadmium for all of the cases is 1.2×10^{-3} M and the concentration of caffeine and theophylline is taken twice that of the metal ion; n = 2, $(\alpha n_a)^{1/2}$ = (obtained from the intercept from equation 2) for a quasi-reversible system, D for the different systems has been calculated

The diffusion co-efficient, D for different systems calculated by using equation (1) and Figure 3 (current-square root of scan rate relationship), are given in Table 1. From the CVs of different systems, it is clear that the redox activity of the cadmium-caffeine and cadmium-theophylline (not shown in the figure) is more prominent than that of cadmium or the ligands. The cathodic shift of the cadmium peak in the presence of caffeine or theophylline suggests that the cadmium-caffeine or cadmium-theophylline complex should be more labile and stable. The heterogeneous charge transfer rate constant, k_f for different systems has been calculated by using the theory described by Nicholson and Shain (Nicholson and Shain 1964) described in a review by Brown and Sandifer (1986). Accordingly, we calculate the k_f-value by using the following equation.

$$I_p = 0.227 nFACk_f \exp\left[\frac{\alpha n_a F}{RT}(E_p - E_0) \right] \qquad (2)$$

A plot of I_p vs. C gave a straight line with a slope $0.227nFAk_f$. The value of k_f was calculated from the slope. The intercept of the graph gave the value of αn_a. The value of αn_a can be put into equation (1) and the diffusion co-efficient, D for different species can be evaluated.

Table 1. Comparative values of diffusion co-efficient and charge transfer rate constant value data of 1.0×10^{-3} M cadmium, caffeine, theophylline, cadmium-caffeine and cadmiumd-theophylline complexes in aqueous 0.1M KCl solution with a platinum-disc electrode at scan rate 50 mV/sec.

Solution	Diffusion co-efficient value, D $\times 10^6$ (cm^2/s)	Charge transfer rate constant, $k_f \times 10^3$ (cm/s)
Cadmium, Cd(II)	20.53	2.15
Caffeine	0.049	0.018
Theophylline	0.061	0.025
Cd (II) + caffeine	9.06	0.18
Cd (II) + theophylline	5.39	0.28

The results for the D-values and k_f-values of different systems are given in Table 1. It was observed that the diffusion co-efficient as well as the heterogeneous charge transfer rate constant is higher for cadmium. As it is expected the D and k_f values for caffeine and theophylline are low and affect the diffusion and electrode kinetics for cadmium in the presence of caffeine or theophylline. Therefore, the D and k_f values for cadmium in the presence of caffeine and theophylline are lower than that of cadmium alone. This suggests that cadmium forms complexes with caffeine or theophylline. As the value of the diffusion co-efficient of the complex is lower than that of cadmium alone, it is therefore expected that introduction of caffeine or theophylline will reduce the rate of diffusion in the soil. This study also prevails that the reduction of diffusion rate of cadmium is more effective for the ligand theophylline than caffeine in aqueous electrolytic solution.

Speciation of cadmium-caffeine and cadmium-theophylline complexes

Differential pulse voltammetry (DPV) has been employed to establish the empirical formula of the complex ion in solution. For this purpose DPV of 1.5 mM solution of cadmium was carried out at a platinum-disc electrode in 0.1 M KCl solution. Then a gradual addition of the ligand caffeine was carried out in the same solution. It was observed that the peak current decreases due to gradual addition of caffeine in cadmium solution. When the addition of caffeine reached to about 3.0 mM, a sharp decrease of the peak current was observed and decreases more as the addition of the ligand caffeine in the solution. Peak current obtained from DPV data was plotted against the addition of caffeine solution (Figure 4). From this figure, it is easily seen that cadmium bonded with two molecules of caffeine. Similar result was observed for theophylline system (Figure 4).
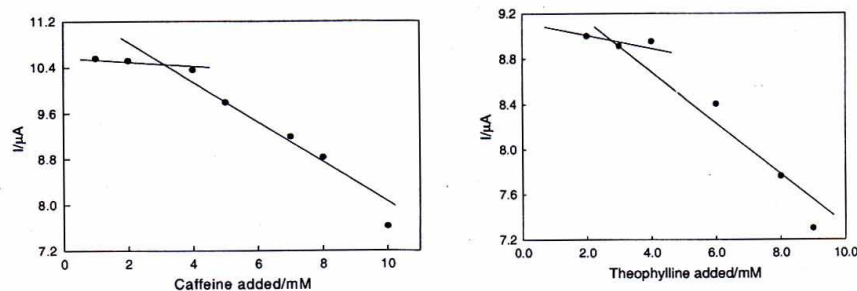

Figure 4. Peak current vs. concentration of caffeine and theophylline for the determination of the empirical formula of cadmium-caffeine and cadmium-theophylline complex.

The possibility of such bond formation can be shown if cadmium can form bridge complexes with caffeine or theophylline. It is notable and mentioned in the introduction section that the active center for caffeine and theophylline is at O13 position; the optimum energy required for the formation of cadmium-caffeine and cadmium-theophylline complexes can be possible if it forms bridge complexes according to Scheme 2.

Caffeine Cadmium-caffeine complex

Theophylline Cadmium-theophylline complex
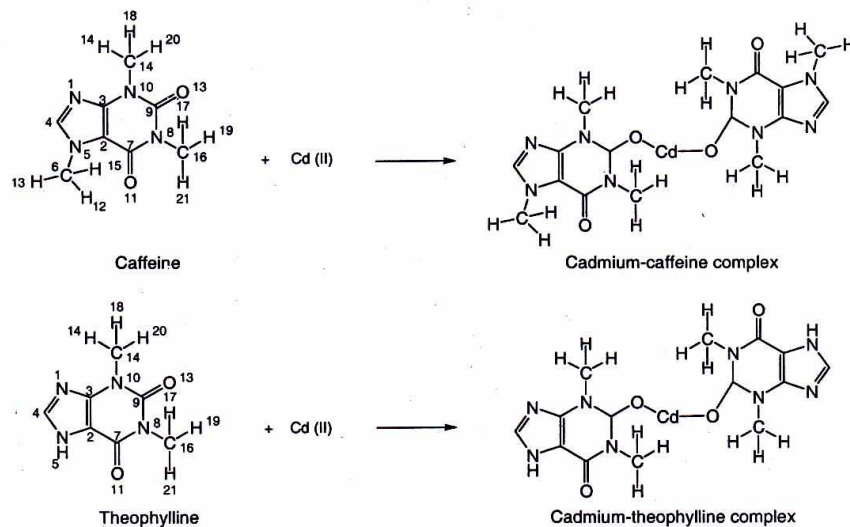
Scheme 2. Proposed structures of Cd-caffeine and Cd-theophylline complexes.

Conclusion

The removal of cadmium from its aqueous solution by methylated xanthine bases, such as, caffeine and theophylline has been successfully demonstrated in this paper. It was found that theophylline can suppress the diffusion rate of cadmium by forming a complex more prominently than caffeine in the electrolytic aqueous solution. Differential pulse voltammetric data suggests that cadmium-caffeine and cadmium-theophylline both form 1 : 2 bridge complexes in their aqueous solution. The shifting of peak potential towards more cathodic position compared to cadmium ion in the cyclic voltammetric data suggests the stability of the cadmium-caffeine and cadmium-theophylline complexes even more than cadmium ion. This also suggests that reduction of the corresponding complexes is difficult.

References

Adams, R. G., J. F. Harrison and P. Scott. 1969. The development of cadmium-induced *proteinuria*, impaired renal function and in alakaline battery workers. *Q. J. Med.* **38**: 425-443.

Apgar, L., J. T. Joan, and M. Stanly. 1998. *Caffeine*, CRC Press, Florida, USA. pp. 171.

Brown, R. E. and J. R. Sandifer. 1986. Physical Methods of Chemistry, Electrochemical Methods. Rossiter, B.W. and J. F. Hamilton. (eds), John Wiley and Sons. Inc., New York, USA. pp. 300-305.

Deepika, L. and K. Kannabiran. 2010. Biosurfactant and Heavy Metal Resistance Activity of *Streptomyces* spp. Isolated from Saltpan Soil. Brit. *J. Pharm. and Toxicol.* **1**(1): 33-39.

Esimone, C. O., F. B. C. Okoye, C. S. Nworu and C. O. Agubata. 2008. In vitro interaction between caffeine and some penicillin antibiotics. *Tropical Journal Pharmaceutical Research* **7**: 969-974.

Evstigneev, M. P., V. P. Evstigneev, S. Hernandez, A. A. Davies and B. David. 2006. Effect of a mixture of caffeine and nicotinamide on the solubility of vitamin (B2) in aqueous solution. *Eur. J. Pharm. Sci.* **28**: 59-66.

Fredholm, B. B., K. Battig, J. Holmen, A. Nehlig and E. E. Zvartan. 1999. Action of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev.* **51**: 83-133.

Friberg, L. 1984. Cadmium and the kidney. *Environ. Health Perspect* **54**: 1-11.

Hashimoto, T., Z. He., W. Y. Ma, P. C. Schmid., A. M. Bode., C. S. Yang and Z. Dong. 2004. Induction of apoptosis by caffeine. *Cancer Res.* **64**: 3344- 3349.

Hirayama, N., N. Ichitani, K. Kubono, Y. Mastuoka, H. Kokusena and T. Honjo. Extraction behavior of divalent first row transition metal ions with *N,N'*-bis(2-hydroxyphenylmethyl)-*N,N'*-bis(2-pyridylmethyl)-1,2-ethanediamine and its derivatives 1997. *Talanta* 44: 2019–2025.

Jabbar, M. A., S. Salahuddin and R. J. Mannan. 2010. Electrochemical investigation on the redox behaviour of thiamine and possible interaction with cadmium in aqueous media. *J. Bangladesh Chem. Soc.* **23**(1&2): 56-66.

Jabbar, M. A., K. Kaium, S. Salahuddin, R. A. Chowdhury and M. Q. Ehsan. 2011. Voltammetric and spectrophotometric evidences for the complexation between ascorbic acid and iron in aqueous media and its applications. *Bangladesh J. Sci. Res.* **24**: 1-14.

Kabir. M. H. 2011. Voltammetric and spectrophotometric assessment for the complexation between Caffeine and Iron in aqueous solution. Unpublished M.S. Thesis. Department of Chemistry, University of Dhaka.

Jain, N. K., A. K. Singhai and S. Jain. 1996. Hydrotropic solubilization of ketoprofen. *Pharmazie.* **51**: 236-239.

Kazantzis, G., F. V. Flynn, J. S. Spowage, D. G. Trott. 1963. Renal tubular malfunction and pulmonary emphysema in cadmium pigment workers. *Q. J. Med.* **32**: 165-192. .

Laiju, M., H. M. N. Akhtar, M. A. Mamun, M. A. Jabbar and M. Q. Ehsan. 2010. Cyclic voltammetric study on the effect of the introduction of secondary ligands on the redox behaviour of the copper-saccharin complex. *J. Natn. Sci. Foundation Sri Lanka.* **38**(2): 91-99.

Lim, L-Y. and M.-L. Go. 2000. Caffeine and nicotinamide enhances the aqueous solubility of the antimalarial agent halofantrine. *Eur. J. Pharm. Sci.* **10**: 17-28.

Nicholson, R. S. and I. Shain. 1964. Single scan and cyclic methods applied to reversible, irreversible, and kinetic systems. *Anal. Chem.* **36**: 706-723.

Niragu, J. O. and J. M. Pacyna. 1988. Quantitative assessment of worldwide contamination of air, water and soil by trace metals, *Nature,* **333**: 134-139.

Sheabar, F. Z., S. Yannal and U. Taitelman. 1989. Extracorporeal Complexation Haemodialysis for the Treatment of Cadmium Poisoning. *In Vivo* Mobilization and Removal. Pharm. & Toxicol. **65**(1): 13–16.

Sugira, Y., K. Ishizu and K0. Miyoshi. 1979. Studies of *metallobleomycins* by electronic spectroscopy, electron spin resonance spectroscopy and potentiometric titration. *J. Antibiol.* **32**(5): 453.

Tandon, S. K., S. Singh, S. Prasad, K. Khandekar, V. K. Dwivedi, M. Chatterjee and N. Mathur. 2003. Reversal of cadmium induced oxidative stress by chelating agent. *Toxicol Lett.* **145**(3): 211-316.

Veronica, O., F. Nareetsile and H. M. Kwaambwa. 2012 (*in press*). A study of the removal of heavy metals from aqueous solutions by *Moringa oleifera* seeds and amine-based ligand 1,4-bis[N,N- bis(2-picoyl)amino]butane. *Anal. Chim. Acta.*