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SPECTROPHOTOMETRIC AND CYCLIC VOLTAMMETRIC STUDY OF INTERACTION OF Fe(III) WITH VITAMIN B_3 AND VITAMIN B_6

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ABSTRACTS

The interaction of Fe(III) with niacin and pyridoxine was investigated by UV-Vis. spectrophotometry. The metal to ligand ratio for Fe(III)- niacin system was found to be approximately 1 : 3 and for Fe(III)-pyridoxine system it was 1 : 1. The redox properties of uncoordinated Fe(III)/Fe(II) system is reversible at platinum electrode and in presence of the ligands the system was found to be quasi-reversible in nature. It was also found that in every case the system was diffusion controlled.

Key words: Cyclic voltammetry, Redox reaction, Vitamin B_3, Continuous variation method, Slope ratio method

INTRODUCTION

Vitamin is an organic compound required as a nutrient in tiny amounts by an organism. Vitamins help the animal organism to perform some specific physiological functions and regulation of metabolic processes, which are vital to life (Liberman and Bruning 1990, Chatterjee 1972, Claus and Tayler 1965, Finer, 1973). Vitamins are known as accessory dietary factors and are only necessary in very small amount. Pyridoxal, pyridoxamine and pyridoxine are different forms of vitamin B_6. All three compounds are efficiently converted to the biologically active form of vitamin B_6, pyridoxal phosphate. Pyridoxal phosphate functions as a coenzyme for the amino group transformation (Voet and Voet 1995, Garrett and Grishamn 1995). Niacin (nicotinic acid and nicotinamide) is another water soluble B-vitamin. In the blood, brain, kidney and liver it is converted to the coenzyme of nicotinamide. Tryptophan is an amino acid, which is a provitamin of niacin. Nicotinic acid is an essential component of mammalian diet.

Iron is of fundamental importance to the growth, development and well-being of almost all living organisms. Multiple biological systems have evolved for the uptake, utilization, storage, and homeostasis of iron in microbes, plants and mammals. Both iron deficiency and iron overload are found extensively in humans; the intimate links between iron and oxidative stress are associated with a wide range of pathologies. Iron overloads and deficiencies are important factors in the health of humans and are therefore a key target in drug development. Iron is essential for all living organism, although in excess

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amount it is dangerous via catalyzing the formation of reactive oxygen species (Crichton 2009).

In a previous paper, the authors reported the cyclic voltammetric studies of Fe(III)-vitamin B_6 complex in HEPES buffer at carbon paste electrode (Shaikh *et al*. 2006). Solid product of Fe-vit B_6 complex was isolated and then CV studies were carried out by dissolving it in HEPES buffer. The redox process was irreversible. Here, in this communication the authors report the cyclic voltammetric study of interaction between Fe(III) and two water soluble vitamins, pyridoxine (vitamin B_6) and nicotinic acid (vitamin B_3) at platinum electrode in the solution medium.

MATERIALS AND METHODS

This study was carried out using an Epsilon Electroanalyser developed by Bioanalytical System Inc., U.S.A. The purpose of the potentiostat is to impose a cyclic linear potential sweep on the working electrode and to output the resulting current potential curve. This sweep is described in general by its initial (E_i), switching (E_s) and final (E_f) potentials and scan rate (V/s).

The experiments were done in a pyrex glass micro cell with Teflon cap. A platinum electrode was used as working electrode, which was cleaned by using alumina powder on polishing cloth. Ag/AgCl electrode and Pt wire were used as reference and counter electrodes, respectively.

An AGE (Velp Scientifica) magnetic stirrer with a Teflon coated magnetic bar and a pH meter (Orion 2 Star made by Thermo Electron Corporation) was employed for stirring and measuring the pH value of the solutions, respectively. Preparation of the solutions was done using volumetric flask and pipettes made of Pyrex glass. The spectroscopic study was done by UV-1800 (Shimadzu Corporation, Japan) using a pair of quartz cell.

Chemicals used in the experiments were: Ferric nitrate (BDH), pyridoxine hydrochloride and niacin (Nicotinic acid) (ESKYAF, Bangladesh), potassium chloride and sodium perchlorate monohydrate (MERCK, Germany). The buffers were prepared using sodium acetate (MERCK, Germany) and acetic acid (Sigma-Aldrich Laborchemikaline, GmbH). For cleaning and all other purposes deionized water was used and 99.997% nitrogen (BOC, Bangladesh) was used for purging purpose.

RESULTS AND DISCUSSIONS

The interaction between Fe(III) and two B vitamins, pyridoxine hydrochloride and niacin were studied using cyclic voltammetry. The composition of the complexes in solution was determined spectrophotometrically (Hill and MacCarthy 1986).

The ratio at which Fe(III) forms complexes with pyridoxine was determined using continuous variation method (Job's method) and slope ratio method.

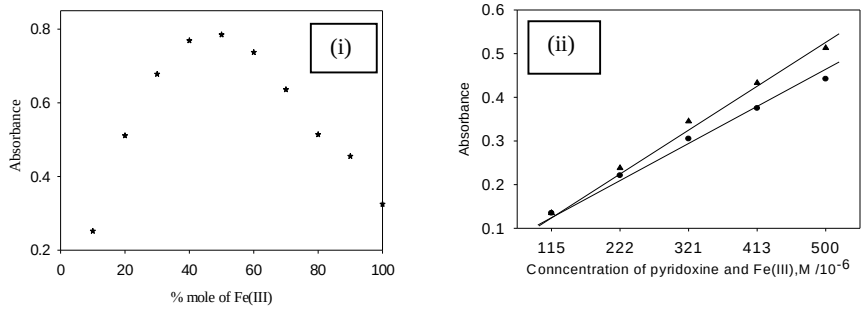

Fig. 1. (i) Variation of absorbance with the % moles of Fe(III). (ii) Variation of absorbance with concentration of Fe(III) and pyridoxine.

Fig. 1(i) exhibits that, the absorbance of the Fe-pyridoxine solution increases gradually with the increase of the % mole of Fe(III) until maximum absorbance reached at approximately 50 - 50 mole ratio and then gradually decreases with the increase of Fe(III) concentration, which indicates that the metal to ligand ratio in the complex is approximately 1 : 1. The absorbance vs concentration plots for the metal and ligand solution are plotted in Fig. 1(ii) and the calculated slope ratio is 1 : 1. So it can be concluded that Fe(III) forms complex with pyridoxine in 1 : 1 ratio in the aqueous medium. The metal to ligand ratio in Fe(III)-niacin complex was measured using same method and found to be 1 : 3.

First of all the redox behavior of Fe(III) was studied and then redox behavior of Fe(III) in presence of pyridoxine and niacin was studied using cyclic voltammetry at platinum electrode. The cyclic voltammogram of 3 mM $Fe(NO_3)_3$ in 0.1. M KCl was taken in the potential range (800 - 300 mV), at scan rate 200 mVs^{-1} (Fig. 2i).

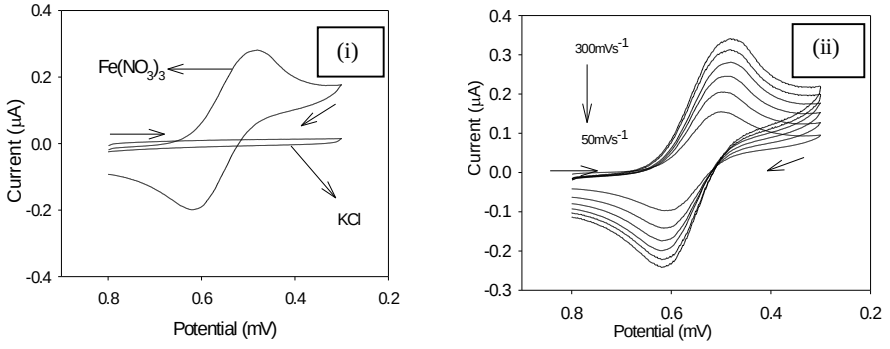

Fig. 2. (i) CV of 3 mM $Fe(NO_3)_3$ in 0.1 M KCl and the supporting electrolyte 0.1 M KCl at 200 mVs^{-1}. (ii) CV of 3 mM $Fe(NO_3)_3$ in 0.1 M KCl at 300, 250, 200, 150, 100 and 50 mVs^{-1}.

From the Figure it is seen that there is one cathodic peak at potential 480.3 mV and one anodic peak at potential 619.7 mV. The reactions involve for corresponding cathodic and anodic peaks are,

cathodic reaction $Fe(III) + e \rightarrow Fe(II)$ and
anodic reaction $Fe(II) - e \rightarrow Fe(III)$

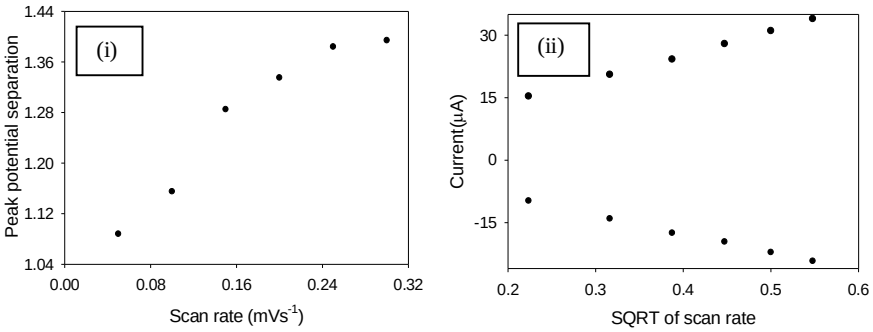

Fig. 3. (i) Variation of peak potential separation against scan rate. (ii) Variation of peak current against SQRT of scan rate.

The cyclic voltammograms of $Fe(NO_3)_3$ solution at different scan rates in the same potential range are shown in Fig. 2(ii). The current-potential data, peak potential separation, peak current ratio of the voltammograms at different scan rates have been studied carefully.

From the Fig. 2(ii) it is seen that for the cathodic peaks, the peak potentials are gradually decreased and for the anodic peaks, the peak potentials are gradually increased as the scan rate increased. But the increasing and decreasing rate of potential is very small. This behavior can be described by slower charge propagation, probably due to difference in solvation and or permeability (Shaikh *et al.* 2006, Akhtar *et al.* 2008).

It is also observed that the peak current increases with scan rate. In a slow voltage scan the diffusion layer grows much further from the electrode in comparison to a fast scan. Consequently, the flux to the electrode surface is considerably smaller at slow scan rates than it is at faster rates. As the current is proportional to the flux towards the electrode the magnitude of the current becomes lower at slow scan rates and higher at high scan rates. The general conclusion is that the redox system is diffusion controlled (Bard and Faulkner 1980).

Current passing through either a Galvanic or an electrolytic cell requires a driving force (potential) to overcome the resistance of the ions to move towards the anode or the cathode. This force follows Ohm's law and is equal to the product of current in amperes and the resistance of the cell in Ohms. The force is generally referred to as the Ohmic

potential or the IR drop. From Fig. 3(i) it is clear that with increasing scan rate, the peak potential separation increases because the cathodic peak shifts towards negative potential and that of anodic towards positive potential. Here the cause is the effect of IR drop (Shaikh *et al.* 2006).

Fig. 3(ii) illustrates that with increasing SQRT of scan rate ($\nu^{1/2}$), the peak currents increases, giving the conclusion that the process is adsorptive controlled (Bard and Faulkner 1980, Hasan *et al*. 2012). The peak current ratio is found to be very near to unity, which implies the system to be reversible.

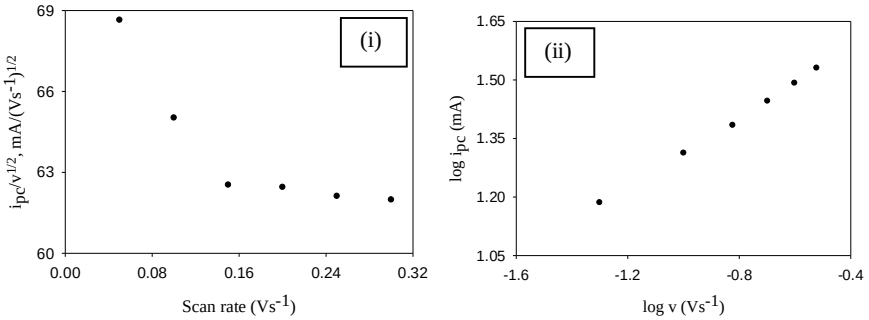

Fig. 4. (i) Variation of peak current function against scan rate. (ii) Variation of log (current) against log (scan rate).

Fig. 4(i) shows lowering of peak current function with increase of scan rate. The Fig. 4(ii) demonstrates linear increment of log (current) vs log (scan rate) plot. The slope is less than unity, which again indicates that the process is diffusion controlled (Devid and Grosser 1993, Nicolson and Shain 1964).

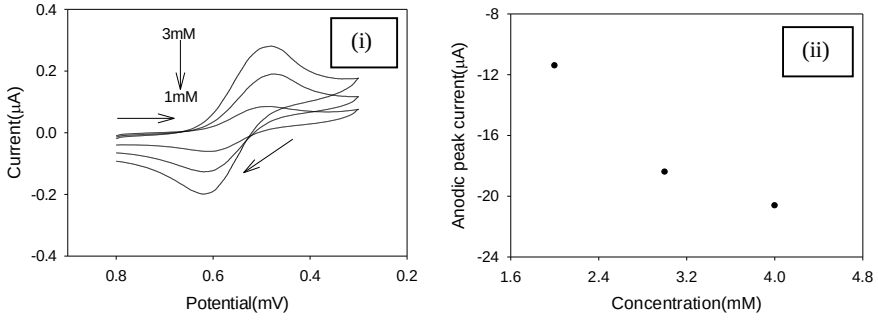

Fig. 5. (i) Cyclic voltammograms of 3, 2 and 1 mM $Fe(NO_3)_3$. (ii) Variation of peak current with concentration.

The cyclic voltammograms of $Fe(NO_3)_3$ of various concentrations (1, 2 and 3 mM) in 0.1 M KCl at 250 mVs^{-1} scan rate are shown in Fig. 5(i) and the variation of anodic peak current with concentration of iron is shown in Fig. 5(ii). With the increase in

concentration there is a gradual linear increase in peak current, which may be due to the presence of a large amount of electroactive species at higher concentration (Mascus *et al*. 1996). The peak current increases with concentration also give the idea that the system may be diffusion controlled.

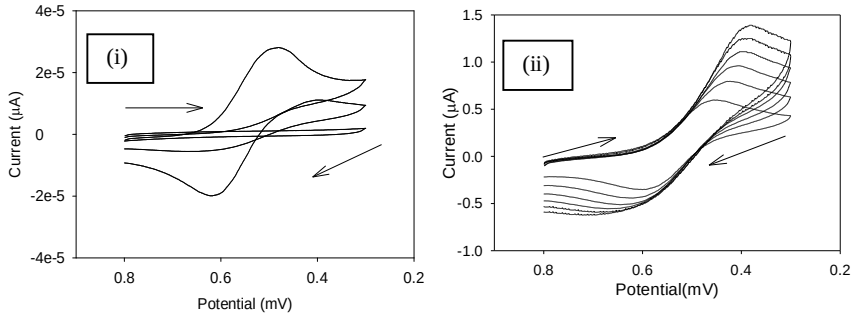

Fig. 6. (i) CVs of 3 mM $Fe(NO_3)_3$, 3 mM $Fe(NO_3)_3$ in presence of 3 mM niacin and 3 mM niacin in 0.1 M KCl at 200 mVs^{-1}. (ii) CVs of 3 mM $Fe(NO_3)_3$ in presence of 3 mM niacin in 0.1 M KCl at 300, 250, 200, 150, 100 and 50 mVs^{-1}.

The cyclic voltammograms of 3 mM $Fe(NO_3)_3$, 3 mM niacin and equimolar mixture of $Fe(NO_3)_3$ and niacin were taken in the same potential range and at the same scan rate. The CVs are shown in Fig. 6(i). In the CV of metal-ligand mixture, there is a cathodic peak at potential 398.3 and one anodic peak at 617.1 mV. The voltammograms demonstrate that both the cathodic and anodic peak position of Fe(III)-Fe(II) system in presence of niacin moves towards right. Moreover, the intensity of the peaks has been modified. This behavior confirms the interaction between Fe(III) and niacin, and formation of Fe(III)-niacin complex. The reactions involved on the electrodes may be written as,

cathodic reaction : Fe(III)-niacin + e $\rightarrow$ Fe(II)-niacin and

anodic reaction : Fe(II)-niacin – e $\rightarrow$ Fe(III)-niacin

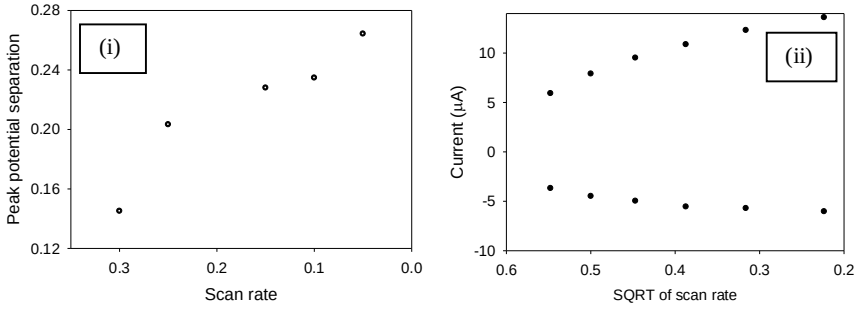

Fig. 7. (i) Variation of peak potential separation against scan rate. (ii) Variation of peak current with SQRT of scan rate.

A series of cyclic voltammogram of a 1 : 1 mixture of $Fe(NO_3)_3$ and niacin in 0.1 M KCl at different scan rates are shown in Fig 6(ii). The parameters obtain from the figure are listed in Table 1.

Table 1. Current-potential data, peak potential separation, peak current ratio of the voltammograms of $Fe(NO_3)_3$ in presence of niacin in 0.1 M KCl.

$V(s^{-1})$	$V^{1/2}$	E_{pa} Volt(+)	E_{pc} Volt(+)	i_{pa} μA(-)	i_{pc} μA(+)	$\Delta E=E_{pc}\sim$ Epa (Volt)	i_{pa}/i_{pc}
0.300	0.5477	0.643.5	0.3792	6.03	13.60	0.2643	0.443
0.250	0.5000	0.6244	0.3897	5.71	12.31	0.2397	0.463
0.200	0.4472	0.6263	0.3983	5.55	10.87	0.2280	0.513
0.150	0.3873	0.6268	0.4040	5.50	9.64	0.2168	0.570
0.100	0.3162	0.6273	0.4240	4.48	7.75	0.2033	0.578
0.050	0.2236	0.6006	0.4555	3.69	5.92	0.1451	0.623

V = Scan rate; $V^{1/2}$ = SQRT of scan rate; Epa = Anodic peak potential; Epc = Cathodic peak potential; ipa = Anodic peak current; ipc = Cathodic peak current; ΔE = Peak potential separation;

The parameters indicate that for cathodic peaks, the peak potentials are gradually increased and for anodic peaks, the peak potentials are gradually decreased as scan rate increased. But in both case the change is very minute. The data in Table 1 also express that the peak current increases with scan rate. This can be rationalized by considering the size of the diffusion layer and the time taken to record the scan. So, the redox system (electron transfer process) in Fe(III)-niacin complex is also diffusion controlled (Shaikh *et al*. 2006, Akhtar *et al.* 2008, Bard and Faulkner 1980).

The plot 7(i) shows that with the increase of scan rate, the peak potential separation increases because the cathodic peak shifts towards negative potential and that of anodic towards positive potential. The Randles-Sevcik plot, Fig. 7(ii) expresses that with increasing $v^{1/2}$, the peak currents increases, which indicate that the process is diffusion controlled as well as adsorptive controlled. Considering all observations and the peak current ratio it can be said that the charge transfer process in Fe(III)-niacin complex is quasireversible.

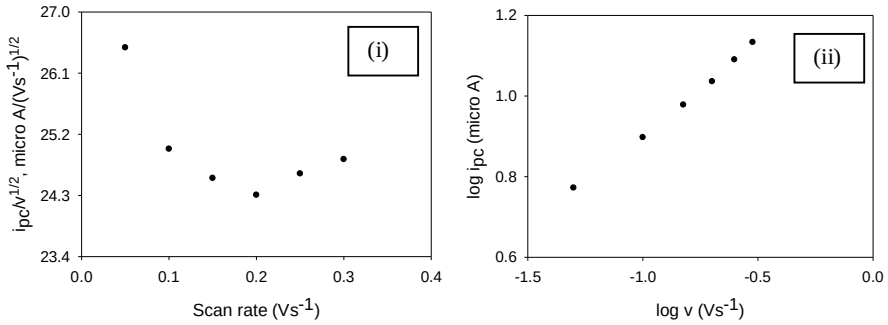

Fig. 8. (i) Variation of peak current function against scan rate. (ii) Variation log (current) against log (scan rate).

Fig. 8(i) demonstrates variation of peak current function with increase of scan rate. The Fig. 8 (ii) shows linear increment of log (current) vs log (scan rate) plot. The slope is less than unity, which again indicates that the process is diffusion controlled (Devid and Grosser 1993, Nicolson and Shain 1964).

The cyclic voltammograms of 3 mM $Fe(NO_3)_3$, 3 mM pyridoxine and equimolar mixture of $Fe(NO_3)_3$ and pyridoxine was taken in the same potential range and at the same scan rate. The CVs are shown in Fig. 9(i). In the CV of metal- ligand interaction, there are two cathodic peaks at potentials 388.7 and 510.3 and one anodic peak at 594.8 mV. The voltammograms demonstrate that both peak position and peak current of the cathodic and anodic peak of Fe(III)-Fe(II) system have been modified in presence of pyridoxine. This behavior confirms the interaction between Fe(III) and pyridoxine.

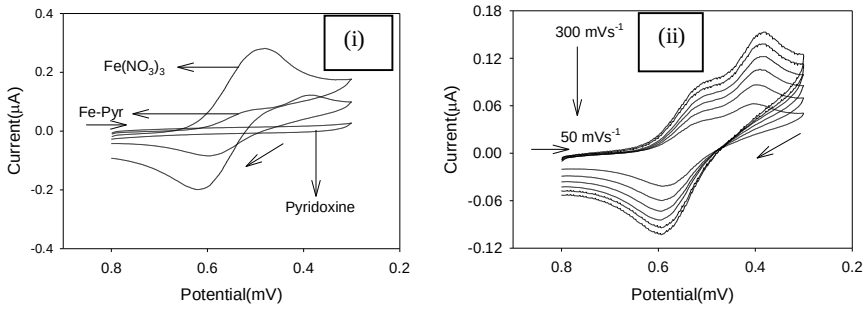

Fig. 9. (i) CVs of metal solution (3 mM $Fe(NO_3)_3$), ligand solution (Pyridoxin) and metal-ligand mixture at 200 mVs^{-1}. (ii) CVs of metal-ligand mixture at 300, 250, 200, 150, 100 and 50 mVs^{-1} scan rate.

The pair of peaks at 388.7 and 594.8 mV may be due to Fe(III)-Fe(II) system of the iron-pyridoxine complex. The hump like peak at 510.3 mV may be due to the ligand part of the complex.

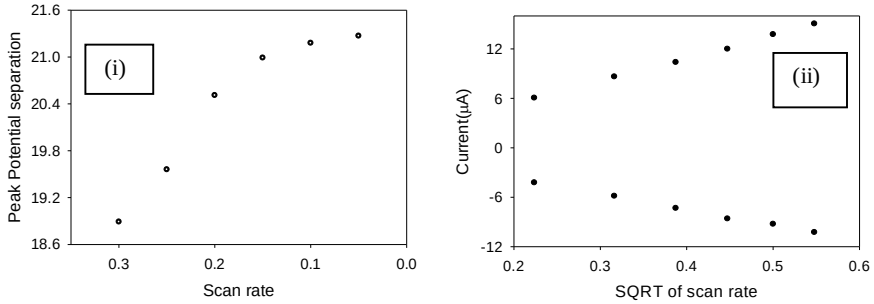

Fig. 10. (i) Variation of peak potential separation against scanrate. (ii) Variation of peak current with SQRT of scan rate.

To investigate the scan rate effect, a series of cyclic voltammograms of 3 mM Fe(III) in presence of 3 mM pyridoxine in 0.1 M KCl at different scan rates were taken which are overlaid and shown in Fig. 9(ii). The parameters obtain from the figure are listed in Table 2.

Table 2. Current-potential data, peak potential separation, peak current ratio of the voltammograms of $Fe(NO_3)_3$ in presence of pyridoxine in 0.1 M KCl.

$V(s^{-1})$	$V^{1/2}$	E_{pa} Volt (+)	E_{pc} Volt (+)	i_{pa} μA (-)	i_{pc} μA (+)	$\Delta E=E_{pc} \sim E_{pa}$ Volt	i_{pa}/i_{pc}
0.300	0.5477	0.5948	0.3821	10.25	15.05	0.2127	0.681
0.250	0.5000	0.5948	0.3830	9.25	13.76	0.2118	0.672
0.200	0.4472	0.5948	0.3849	8.60	11.99	0.2099	0.717
0.150	0.3873	0.5948	0.3897	7.32	10.38	0.2051	0.705
0.100	0.3162	0.5939	0.3983	5.85	8.03	0.1956	0.729
0.050	0.2236	0.5920	0.4031	4.22	5.52	0.1889	0.808

V = Scan rate; $V^{1/2}$ = SQRT of scan rate; Epa = Anodic peak potential; Epc = Cathodic peak potential; ipa = Anodic peak current; ipc = Cathodic peak current; ΔE = Peak potential separation.

Table 2 reveals that for cathodic peaks, the peak potentials gradually increased and for anodic peaks, the peak potentials are more or less same as scan rate increased. But in both case the change is very minute. This behavior can be described by slower charge propagation, probably due to difference in solvation and permeability.

The data in Table 2 also shows that the pattern of peak current, peak potential etc are almost same as that of metal niacin complex. The increment of peak current with $v^{½}$, the increment of peak position separation with scan rate is due to IR drop. The fact is that for positive (cathodic) current, the actual working electrode potential is less negative than the applied (measured) potential, while for negative (anodic) current, the shift is in the positive direction. Considering the peak current ratio, peak separation, and other factors discussed above it can be said that the redox system in iron-pyridoxine complex is quasireversible and the charge transfer process is diffusion controlled and adsorptive controlled.

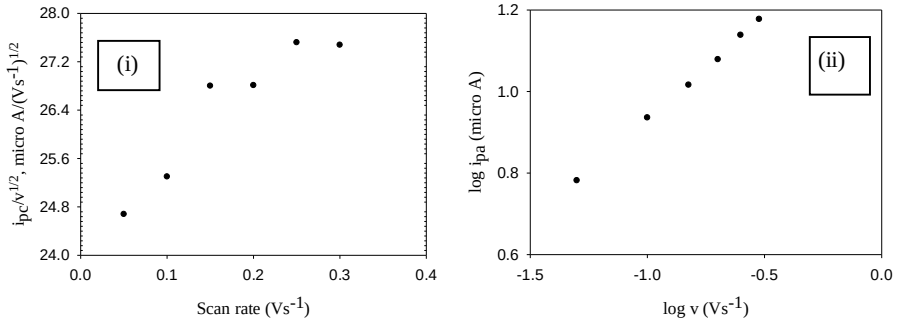

Fig. 11. (i) Variation of peak current function against scan rate. (ii) Variation of log (current) against log (scan rate).

Fig. 11(i) shows variation of peak current function with increase of scan rate and Fig 11 (ii) shows linear increment of log (current) vs log (scan rate) plot. The slope is less than unity, which again indicates that the process is diffusion controlled (Devid and Grosser 1993, Nicolson and Shain 1964).

To investigate the effect of concentration on the interaction of Fe(III) and pyridoxine, cyclic voltammograms were taken where the concentration of pyridoxine was kept fixed and was let to interact with $Fe(NO_3)_3$ of various concentration. The following figure shows the voltammograms.

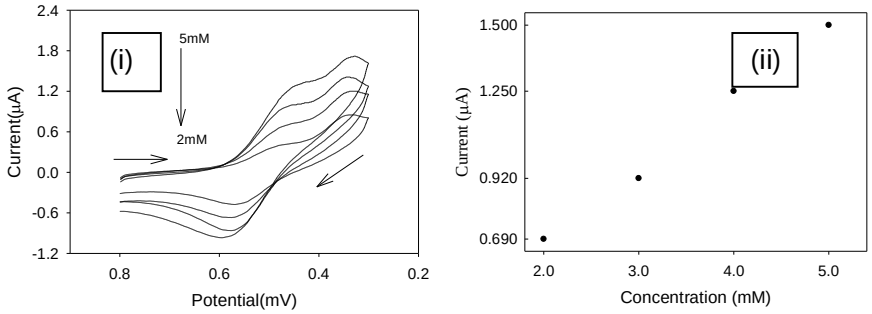

Fig. 12. (i) Voltammogram of mixture of 3 mM pyridoxine and 5, 4, 3 and 2 mM $Fe(NO_3)_3$. (ii) Current vs concentration graph.

The voltammograms indicate a regular pattern of interaction between Fe(III) and pyridoxine in which the cathodic peak gradually shift towards right and the anodic peak moves to left as the concentration of Fe(III) is increased. The peak current increases with concentration also give the idea that the system may be diffusion controlled (Mascus *et al*. 1996).

CONCLUSION

The interaction of Fe(III) with pyridoxine and Niacin in solution was confirmed by UV-Vis spectrophotometry. The ligand to metal ratio for the complexes was calculated by using two independent methods (continuous variation and slope ratio method). The ligand to metal ratio for Fe(III)-pyridoxine system was found to be approximately 1 : 1 by both the methods. Similarly the metal to ligand ratio for Fe(III) with niacin was found to be 1 : 3.

The redox properties of Fe(III)/Fe(II) system has been studied in absence and presence of the ligands pyridoxine and niacin in KCl. Uncoordinated Fe(III)/Fe(II) system is reversible and in presence of the ligands the system was found to be quasi-reversible in nature. It was also found that in every case the system is diffusion controlled. Pyridoxine was found to interact more strongly than niacin.

REFERENCES

Akhtar, H. M. N, A. A. Shaikh and M. Q. Ehsan. 2008. Cyclic voltammetric study of the redox behavior of Fe(II)/Fe(III) systems forming during the oxidation of Fe(II) complexes with saccharin and with saccharin and 1,10-phenanthroline. *Russian J. Electrochem*. **44**(12): 1403-1408.

Bard A. J. and L. R. Faulkner. 1980. *Electrochemical Methods, Fundamentals and Applications*. NY, USA: John Wiley and Sons.

Chatterjee, C. C. 1972. *Human Physiology*. Vol. 1. Calcutta, India: Central Agency.

Claus, E. and V. E. Tayler. 1965 *Pharmacognosy*. London, UK: Kimpton.

Crichton, R. 2009. *Iron Metabolism: From Molecular Mechanisms to Clinical Consequences*. NY, USA: Wiley.

Devid, K. and J. Grosser 1993. *Cyclic Voltammetry, Simulation and Analysis of Reaction Mechanism*. NY, USA: Wiley-VCM.

Finer, I. L. 1973. *Organic Chemistry*. New Delhi, India: Dorling Kindersley Pvt. Ltd.

Garrett, R. H. and C. M. Grishamn. 1995. *Biochemistry*. NY, USA: Saunders College Publishing.

Hasan, M. M., M. E. Hossain, M. A. Mamun and M. Q. Ehsan. 2012. Study of redox behavior of Cd(II) and interaction of Cd(II) with proline in the aqueous medium using cyclic voltammetry. *Journal of Saudi Chemical Society*, **16**: 145-151.

Hill, Z. D. and P. Mac Carthy. 1986. Novel approach to Job's method: An undergraduate experiment. *J. Chem. Edu*. **63** (3): 162.

Liberman, S. and N. Bruning. 1990. *The real Vitamin and Mineral Book*. NY, USA: Avery Publishing Group.

Mascus, M., F. Pariente, Q. Wu, A. Toffanin, P. J. Shapleigh and H. D. Abruna. 1996. Electrocatalytic reduction of nitric oxide at electrodes modified with electropolymerized films and their application in cellular NO determination. *Anal. Chem*. **68:** 3128-3134.

Nicolson, R.S. and I. Shain. 1964. Theory of Stationary Electrode Polarography; Single Scan and Cyclic Methods Applied to Reversible, Irreversible, and Kinetic Systems. *Anal. Chem*. **36** (4): 706.

Shaikh, A. A., M. Begum, A. H. Khan and M. Q. Ehsan. 2006. Cyclic voltammetric studies of the redox behavior of iron(III)-vitamin B6 complex at carbon paste electrode. *Russian J. Electrochem*. **42** (6): 620-625.

Voet, D. and J. G. Voet. 1995. *Biochemistry*. NY, USA: Wiley and Sons.

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