

## **Original Research Article**

### **Investigations of ferrous thiamine complex by cyclic voltammetry.**

#### **ABSTRACT**

The ferrous thiamine complexes with different mole ratio were prepared in acidified KCl (1M) solution and their mole ratio was determined. Redox behaviour of complexes and mechanism of the reactions were investigated by using platinum electrode in cyclic voltammetry. The result showed different behaviour of thiamine (4.3mM) in presence of ferrous (4.3mM) which was attributed with decreases cathodic and increases anodic currents. The mole ratio between thiamine to ferrous was found to be 1:2 in acidic medium at 100 mv/s scan rate. The mechanism of the complexation reaction was tried to found by these results.

**Key words:** Thiamine, ferrous ammonium sulphate hexahydrate, Unmodified electrode, Stiochiometry, Scan rate.

#### **1. INTRODUCTION**

Vitamin B1 plays a very important role in biological compounds and used as a coenzyme. It belongs to that group of enzymes which was involved in acyl transfer reaction. Deficiency of thiamine causes the beriberi disease in human body (Dodi et al., 1996; Malandrinos, et al., 2000; Hu, et al., 1999). Thiamine and their derivatives can undergo many structural changes at different pH values. In alkaline medium thiamine HCl may be converted into thiochrome (Maier, 1957). Therefore it is stable in acidic media and fully protonated with ionizable proton at the N1 (pKa = 5) and C2 (pKa = 19) of pyrimidine and thiazolium ring respectively Fig.1 (Malandrinos, et al., 2000; Washabaugh, et al., 1988; Jordan, et al., 1982). Literature shows electrochemical behaviour of thiamine linked with ionization at C2 position of thiazolium ring and it was considered as key reactivity of thiamine (Hu, et al 1993; Breslow, 1958). Catalytic behaviour of thiamine was found to be attributed with 4 – amino pyrimidine ring when N1 protonated (Hu, et al., 1999; Jordan, et al., 2003; Schellenberger, 1982). Polarographic study of thiamine in acidic media was not favourable because mercury electrode can bind with electroactive species of thiamine and participate as a catalyst in redox reaction (Kissinger, 1996). pH and electrode compositions play a very important role in electrochemical determination of thiamine compound (Kasim, 2000). Platinum compounds have low electrode potential for hydrogen discharging as compare to mercury electrode (Band and Faulkner, 2001). In acidic and neutral medium, reduction of thiamine was attributed with multiple adsorption peaks (pre and post adsorption and hydrogen discharge) which were observed at the mercury electrode (Malandrinos, et al.,

2000; Moorthy, et al., 1981). This behaviour produces more difficulties in determination the redox properties of thiamine while platinum electrode potential window depends upon the composition of solution and pH. In the presence of Pt electrode acidic solution of thiamine ( $\text{pH} < 3.5$ ) shows two peaks which were related to two redox processes such as reduction of  $\text{H}^+$  and formation of  $(\text{Th})^{+2}$  ion. This reduction peak of hydrogen can be removed by using high pH solution (Sutton, et al., 2004).

In present study, involvement of thiamine in complexation with ferrous in acidic medium was investigated. Mechanism of such reaction was also studied.

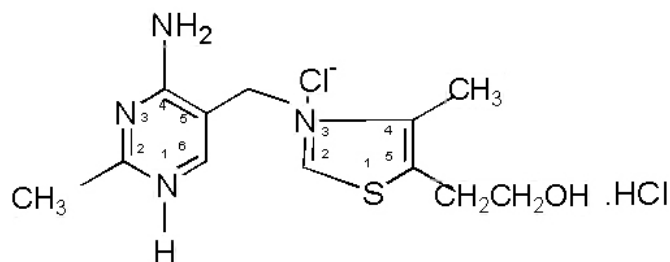


**Fig. 1.** Structure of thiamine hydrochloride.

## 2. EXPERIMENTAL

Free thiamine of analytical grade was obtained from Sigma chemical co. Ferrous ammonium sulphate hexahydrate, sodium hydroxide, oxalic acid, hydrochloric acid, potassium chloride from Aldrich AG. All solutions were prepared with double distilled deionize water. The pH was recorded on a 370 pH meter (Jenway, E.U). Cyclic voltammetry measurement was performed using Computerized Electrochemical System, Model CHI – 760D.

4.3 mM ferrous ammonium sulphate and thiamine hydrochloride solution were prepared separately by using the acidified KCl (1M having about 6 pH) solution. The two sets of working standard solutions with different mole ratio 1:1, 1:2, 1:3, 1:4, 1:5 of thiamine to ferrous ion and ferrous ion to thiamine were prepared in acidified KCl solution. The solution of complexes were scan at 50, 100, 200, 300, 400, 500 mV/s. The base line of KCl 1 M solution was recorded at Pt electrode vs Ag/AgCl reference electrode at  $25 \pm 1^\circ\text{C}$ . The horizontally straight line was observed in both sets of solution. The potential range was set from -0.8 V to 0 V and then reversed back to 0 V to -0.8 V. The oxidation wave was seen in the potential range of forward scan going from -0.8 V to 0 V. The oxidize specie was reduce back during the reverse scan from 0 V to initial potential -0.8 V.

## 3. RESULT AND DISCUSSION

The working standard solutions of complexes in which thiamine HCl concentration was fixed than ferrous ion, scanned at several scan rate. The optimum scan rate was found to be 100 mV/s for 1:2 thiamine to ferrous complex. Thiamine HCl showed anodic and

cathodic peaks at approximately -505 and -615 mV/s and their plot of  $I_{pa}$  and  $I_{pc}$  verses scan rate showed straight line with correlation coefficient 0.988 regarding  $I_{pa}$  and – 0.999 regarding  $I_{pc}$  up to 300 mV/s. On further increase in scan rate didn't make sense or proper relation with  $I_{pa}$  and  $I_{pc}$ . The peaks values for potential difference of thiamine were same as reported in literature. (Sutton, et al 2004). Magnitudes of  $I_{pa}$  and  $I_{pc}$  for complexes were increasing with increase in scan rate Fig. 2.

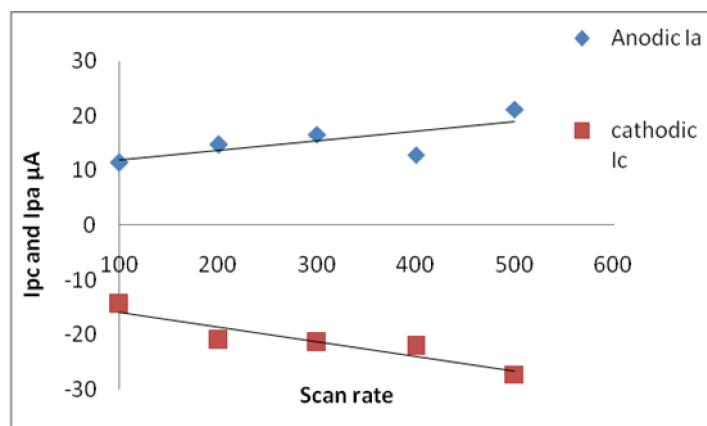


**Fig. 2.** Plot of  $I_{pa}$  and  $I_{pc}$  values of thiamine HCl at different scan rates.

Ferrous ion didn't show peaks at selected potential window. The overlay of anodic peaks at 100 mV/s of complexes, thiamine HCl and ferrous ion showed that the formation of complex was completed in 1:2 thiamine to ferrous complex which was attributed with decrease in current. This complex peak was not sharp from the upper side and showed the formation of compact type of complex. While the increased current in other mole ratio composition showed the presence of free or uncomplex thiamine. Even it higher mole ratio complexes were forming but they were extremely unstable therefore releases thiamine more than 1:2 ratio, that increase current value Fig. 3.

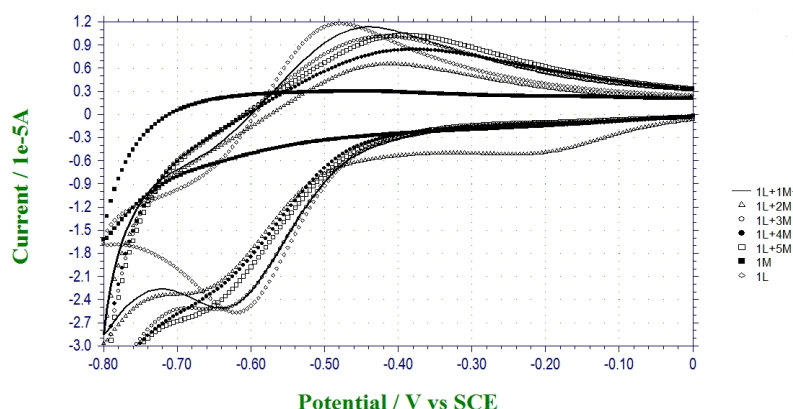


**Fig. 3.** Voltammogram of different working standard solution of  $\text{Fe}^{2+}$  having constant concentration of thiamine HCl at 100 mV/s.

The graph plotted with  $I_{pc}$  versus variable mole ratio of ferrous ion at different scan rates showed that the negative  $I_{pc}$  values sharply increased and form a dip between two to three mole ratio of thiamine HCl to ferrous ion and then remain constant Fig. 4. This sharp increase showed two or three ferrous may be involved in complex formation.

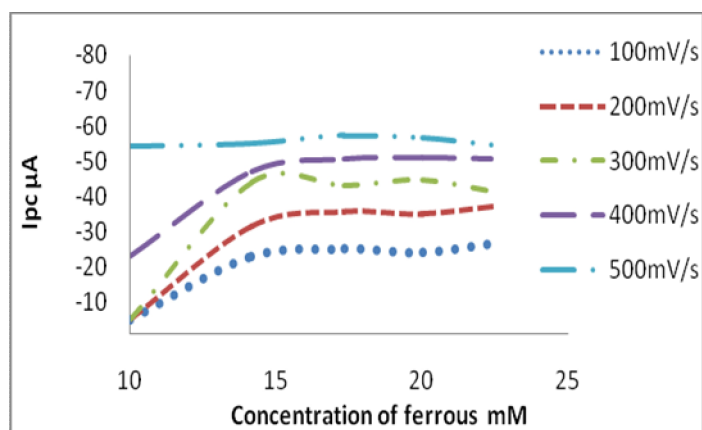


**Fig. 4.** Plot between  $I_{pa}$  versus variable concentration of ferrous ion than thiamine HCl complexes solution in acidify pH at 100mV/s scan rate.

The ferrous ion to variable thiamine mole ratio complexes solutions were scanned at 100 mV/s. All complexes showed approximately same cathodic and anodic potential as thiamine HCl but difference was observed in their current values. The  $I_{pa}$  or  $I_{pc}$  values

of thiamine HCl was lower than  $I_{pa}$  or negative  $I_{pc}$  values of ferrous thiamine complexes. The  $I_{pa}$  and negative  $I_{pc}$  values of complexes found to be increased with concentration of thiamine HCl but it was not linear. The graph was plotted between the concentrations of thiamine HCl verses  $I_{pc}$  values of complexes was not showed the straight line due to the involvement of thiamine in equilibrium with HCl therefore not available for complexation at very low concentration Fig. 5.

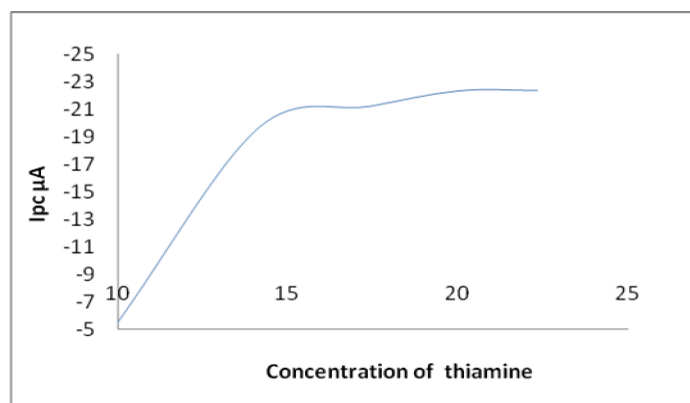


**Fig. 5.** Plot between  $I_{pc}$  verses variable concentration of thiamine HCl than ferrous ion complexes solution in acidify pH at 100mV/s scan rate.

The equilibrium constant values of all complexes were calculated through plot of  $I_{pa}$  and  $I_{pc}$  values verses variable concentration of thiamine and ferrous ion at different scan rates. It results that 1:2 thiamine to ferrous mole ratio was appropriate one. All mole ratio complex solutions showed approximately same equilibrium constant. Which was calculated by  $I_{pa}$  at each scan rate, but  $I_{pc}$  equilibrium constant values were same only upto 300 mV/s Table 1.

Table 1. Equilibrium constant values determined from  $I_{pa}$  of different mole ratio complexes at each scan rate

| Scan rate mV/s | Equilibrium Constants                         |                                              |
|----------------|-----------------------------------------------|----------------------------------------------|
|                | At constant concentration of thiamine (4.3mM) | At constant concentration of ferrous (4.3mM) |
| 100            | 0.02                                          | 0.166                                        |
| 200            | 0.015                                         | 0.269                                        |
| 300            | 0.026                                         | 0.33                                         |
| 400            | 0.0108                                        | 0.141                                        |
| 500            | 0.013                                         | 0.181                                        |

The complex formation was related to release of hydrogen from the thiamine. Literature shows the redox behaviour of thiamine was also related to the protonation of the  $-NH_2$  group at the C4 position as well as N3 of pyrimidine ring. N1 nitrogen was most basic in nature as compare to 4 amino group (Malandrinos, et al., 1998). Once the N1 was protonated N4, N3 and sulphur of thiazolium ring can be formed bond with ferrous atoms in such a way that they join the two molecules of thiamine by bridge. The ferrous thiamine complex was synthesized in aqueous methanolic solution in which mole ratio between ferrous to thiamine was found to be 3:1 at pH 6 which determined with the help of different techniques. While the acidify 1 M KCl solution (pH 6) confirmed the 2:1 mole ratio between ferrous to thiamine. It was confirmed by the above results. The variable concentrations of ferrous complex solutions were also prepared in different buffer solution such as sodium acetate (pH 4), potassium hydrogen phthalate (pH 5 & 7) and sodium tetraborate (pH 8) and scan at 100 mV/s. But they didn't gives sensible results accept potassium chloride of pH 6. Fig. 6.

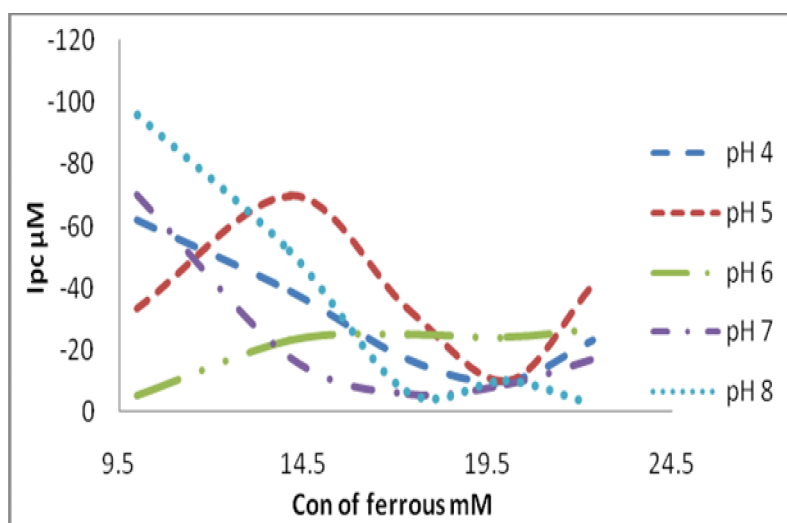
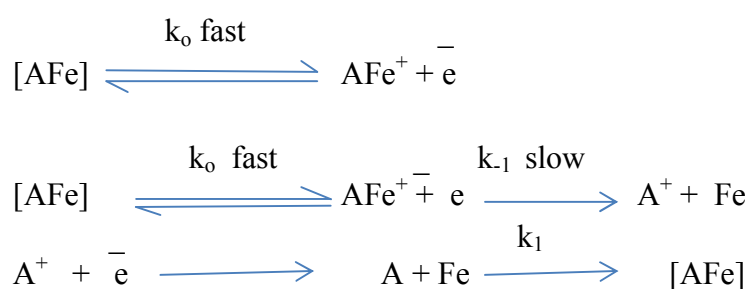
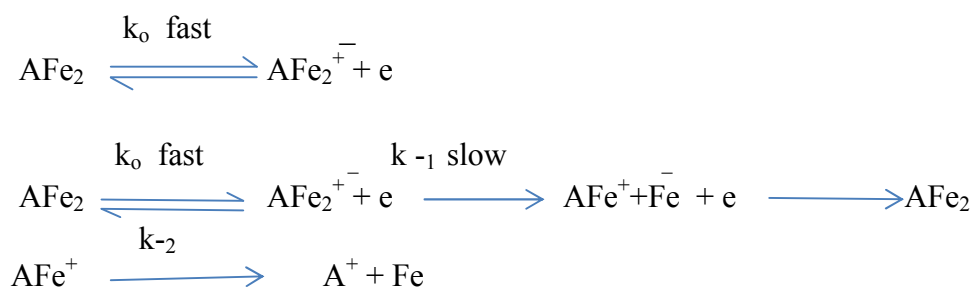


**Fig. 6.** Plot between  $I_{pc}$  verses variable concentration of ferrous ion than thiamine HCl complexes in acetate (pH 4), potassium hydrogen phthalate (pH 5 & 7), potassium chloride 1M (pH 6), sodium tetraborate (pH 8) buffer solutions at 100 mV/s.

The mechanism of complexation reaction may be derived by assumption that one electron transfer reaction may occur in one step at fast scan rate and in two steps at slow scan rate. Literature also shows the one electron transfer reduction process of thiamine was occurs in aqueous solution (Siddiquia and Pitre, 2001). In 1:1 mole ratio complex AFe (where A is ligand) was oxidized at Pt electrode and converted into  $AFe^+$  which receive electron at the fast scan rate or it may be converted into  $A^+$  and Fe at slow scan rate than  $A^+$  was reduce into A and converted into AFe complex. The charges of complexes were not considered here it will be balance by some counter ions.



In case of  $\text{AFe}_2$  complex in the same way it was oxidized into  $\text{AFe}_2^+$  and reduces into  $\text{AFe}_2$  at fast scan rate and may be converted into  $\text{AFe}^+$  and  $\text{Fe}$ .  $\text{AFe}^+$  may be reacted with  $\text{Fe}$  and form complex  $\text{AFe}_2$  or it may be converted into  $\text{A}^+$  and  $\text{Fe}$  at slow scan rate.



## 4. CONCLUSION

Electrochemically studied of the mole ratio between ferrous and thiamine in complex was carried out by cyclic voltammetry using Pt electrode vs Ag/AgCl reference electrode. The acidified 1M KCl (pH 6) was suitable electrolytes for complex as compare to the different buffers solutions such as sodium acetate (pH 4), potassium hydrogen phthalate (pH 5 & 7) and sodium tetraborate (pH 8). 1: 2 mole ratio was exist between thiamine to ferrous ion in acidified 1 M KCl (pH 6) solution which was attributed to decrease in  $I_{pa}$  and  $I_{pc}$  value at the appropriate scan rate 100 mV/s. The equilibrium constant values were calculated through  $I_{pa}$  values were approximately same and confirmed the 1:2 mole ratio between the thiamine and ferrous in the complex.

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