

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 thiozolium 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 of 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 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.

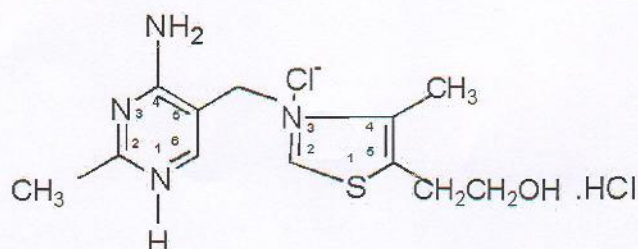


Fig. 1. Structure of thiamine hydrochloride.

2. EXPERIMENTAL

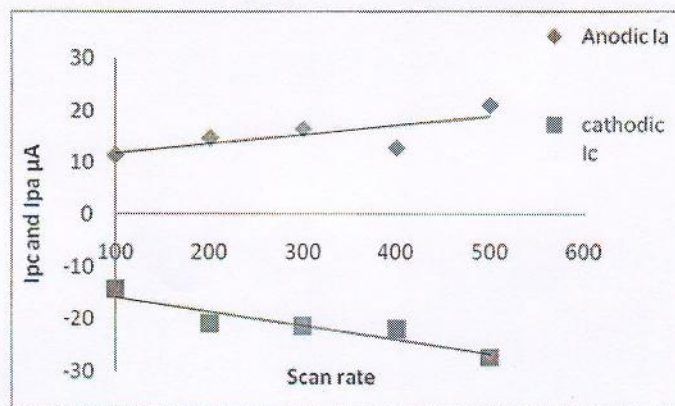
Free thiamine of analytical grade was obtained from Sigma chemical co. Ferrous ammonium sulphate hexahydrate, sodium hydroxide, oxalic acid, hydrochloric acid, potassium chloride from Aldrich AG. All solutions were prepared with double distilled deionize water. The pH was recorded on a 370 pH meter (Jenway, 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

75 cathodic peaks at approximately -505 and -615 mV/s and their plot of Ipa and Ipc verses
 76 scan rate showed straight line with correlation coefficient 0.988 regarding Ipa and -
 77 0.999 regarding Ipc up to 300 mV/s. ^{Further} On further increase in scan rate didn't make sense or
 78 proper relation with Ipa and Ipc. The peaks values for potential difference of thiamine
 79 were same as reported in literature. (Sutton, et al 2004). Magnitudes of Ipa and Ipc for
 80 complexes were increasing with ^{the} increase in scan rate Fig. 2.



88 **Fig. 2.** Plot of Ipa and Ipc values of thiamine HCl at different scan rates.

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

^{composition ratio}

^{the}

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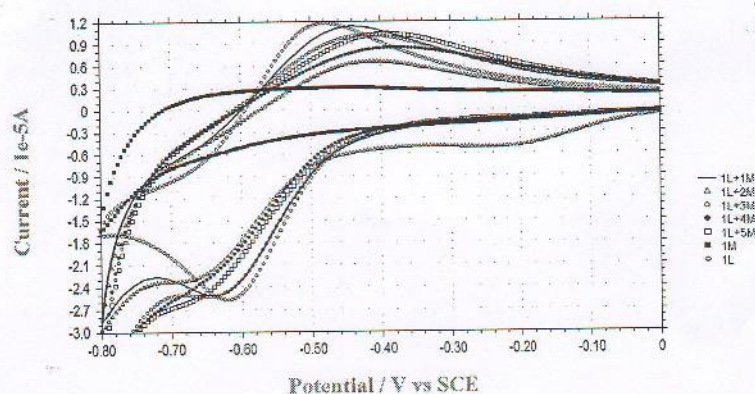


Fig. 3. Voltammogram of different working standard solution of Fe^{2+} having constant concentration of thiamine HCl at 100 mV/s.

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.

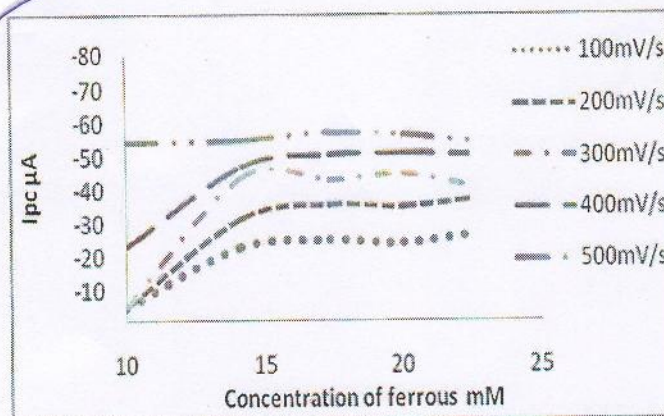
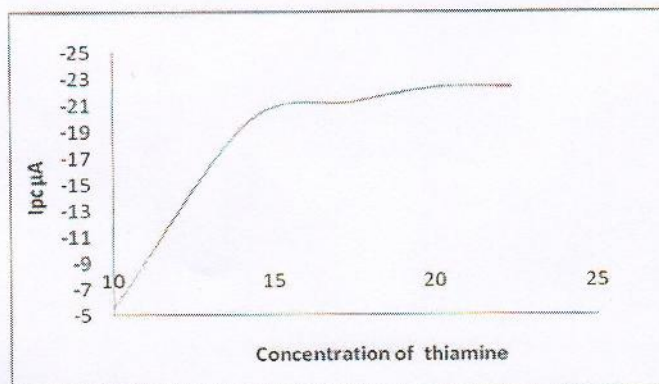


Fig. 4. Plot between I_{pa} versus variable concentration of ferrous ion than thiamine HCl complexes solution in acidify pH at 100mV/s scan rate.

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

124 of thiamine HCl ^{were} was lower than Ipa or negative Ipc values of ferrous thiamine
 125 complexes. The Ipa and negative Ipc values of complexes found to ^{were} ~~be increased~~ ^{increase} with ^{the increasing}
 126 concentration of thiamine HCl but it was not linear. The graph was plotted between the
 127 concentrations of thiamine HCl verses Ipc values of complexes was not showed the ?
 128 straight line due to the involvement of thiamine in equilibrium with HCl therefore not
 129 available for complexation at very low concentration Fig. 5.



141 **Fig. 5.** Plot between Ipc verses variable concentration of thiamine HCl than ferrous ion
 142 complexes solution in acidify pH at 100mV/s scan rate.

144 The equilibrium constant values of all complexes ^s were calculated through plot of Ipa and
 145 Ipc values verses variable concentration of thiamine and ferrous ion at different scan
 146 rates. It results that 1:2 thiamine to ferrous mole ratio was appropriate ~~one~~. All mole ratio
 147 complex solutions showed approximately same equilibrium constant. Which was
 148 calculated by Ipa at each scan rate, but Ipc equilibrium constant values were same only
 149 upto 300 mV/s Table 1.

The results showed

g which

163 Table 1. Equilibrium constant values determined from Ipa of different mole ratio
 164 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

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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.

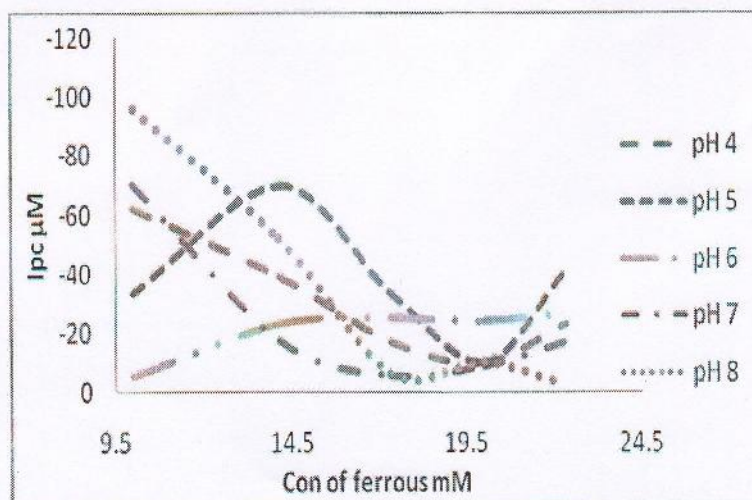
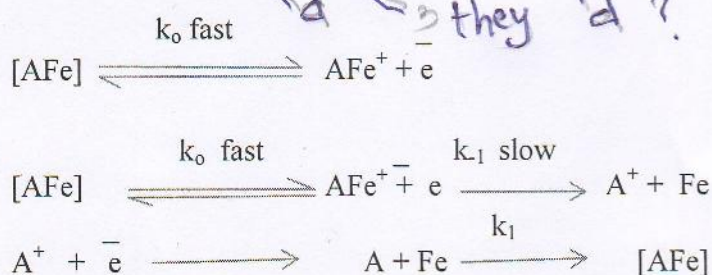


Fig. 6. Plot between I_{pc} versus variable concentration of ferrous ion than thiamine HCl complexes in acetate (pH 4), potassium hydrogen phthalate (pH 5 & 7), potassium chloride 1M (pH 6), sodium tetraborate (pH 8) buffer solutions at 100 mV/s.

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.



241 In case of AFe₂ complex ⁹ ~~in the same way~~ it was oxidized into AFe₂⁺ and reduces into
242 AFe₂ at fast scan rate and may be converted into AFe⁺ and Fe. AFe⁺ may ~~be reacted~~ with
243 Fe and form complex AFe₂ or it may be converted into A⁺ and Fe at slow scan rate.



253 4. CONCLUSION

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

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