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Journal Name:	International Research Journal of Pure and Applied Chemistry
Manuscript Number:	2014_IRJPAC_10977
Title of the Manuscript:	Investigations of ferrous thiamine complex by cyclic voltammetry
Type of the Article	Original Research Article

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Extensive revision of grammar, typing, spelling and style; this is necessary, for the scientific content to be better highlighted. Numerous examples along the Manuscript, of words phrases needing correction/reformulation. Detailed examples are given at the end of this file. For the Section Results and Discussions: -the plot of Ipa and Ipc values versus thiamine HCl concentration, at different scan rates: here, the same values could be also differently exploited: I would suggest to check also the linear dependence on the square root of the scan rate, to investigate <u>if</u> the process is diffusion – controlled and observes Randles-Sevcik equation. I propose detailing even shortly, how the equilibrium constants were calculated from the plot of the current intensities (Ipa, Ipc) and the concentrations of thiamine and ferrous ion, at different scan rates (it is known that the peak current is related to concentration, but also to the square root of the scan rate). Please, reformulate more clearly and use parantheses: „...,which was calculated from by the Ipa value at each scan rate, but Ipc equilibrium constant values were the same only up to 300 mV/s (Table 1).”	Fig 7 is corrected again in which concentration of thiamine is plotted against Ipa and Ipc. Randles Sevcik equation is used which shows the reversible reaction and diffusion control process. Randles Sevcik equation is applied on that complex formation reaction for determination the diffusion coefficient and reversibility of the reaction. $I_p = -2.69 \times 10^5 n^{3/2} C D^{1/2} v^{1/2} \quad \text{-----}$ - Eq 1 The graph is plotted between $v^{1/2}$ and Ipa or Ipc, straight line was obtained with 0.992 coefficient of regression (Fig. 5). It shows that the reaction is reversible and is occurring under diffusion control system. The average diffusion coefficient values were found between 400 to 500 for both Ipa and Ipc current by using Randles Sevcik equation (Eq-1). Mention the procedure for determination of equilibrium constant . The equilibrium constant values are



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		calculated by using the following formulas $2M + A \leftrightarrow M_2A$ $K_{eq} = [M_2A]/[M]^2 [A]$ $i_d = a[M_2A]$ $[M] = [M]_{eq} - 2 [M_2A]_{eq}$ $[A] = [A]_{eq} - [M_2A]_{eq}$ where 'i' is diffusion current, $[M_2A]$ is concentration of complex form between metal M and ligand L, 'a' is voltametric constant (containing upon various factors such as diffusion coefficient, area of electrode, number of electron involved in reaction, nature, distance between electrodes etc.). The graph is plotted between $v^{1/2}$ and I_{pa} or I_{pc}, straight line was obtained with 0.992 coefficient of regression (Fig. 5). The values of equilibrium constants are increasing with variable concentration of metal ion upto 300 mV/s given in Table 1.
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Minor REVISION comments	For the discussion given for Fig. 5, see lines 126-127, please correct the text in order to be consistent with the graph: The graph ~~was plotted between the lpc values of complexes versus concentrations of thiamine HCl~~ was not showed the straight line did not show a linear dependence, due to the involvement of thiamine in equilibrium with HCl, therefore the vitamin becomes not available for complexation at very low concentration. I also suggest updating the section References, with several more recent examples. To this aim, several novel aspects regarding the theme approached could be introduced in the section Introduction. For instance regarding thiamine as a ligand for ferrous ion, its electroactivity etc.	Line 231-234: The graph plotted between lpc values of complexes versus the concentrations of thiamine HCl. The pot is not showing a linear dependence, due to involvement of the thiamine in equilibrium with HCl, this is also showing that the thiamine is unavailable for complexation at very low concentration. Fig. 7. The role of bivalent metals in enzymatic action of thiamine is determined by the bonding mode of the metals to thiamine (Stamatis, et al., 2007). Early studies show metal ion binds to thiamine or its derivatives through N1 of pyrimidine ring or pyrophosphate group and form ionic salt such as $[Th]^{2+}[MX_4]^{2-}$, $[Th]^{2+}([MX_3])_2$, or $(Th^+)_2[MX_4]^{2-}$. M could be Zn^{2+}, Co^{2+}, Ni^{2+}, X could be Cl^-, Br^- and Th is for thiamine (Stamatis, et al., 2007; Malandrinos, et al., 2000). The presence of positive charge on thiazolium ring and protonation of N1 of pyrimidine ring formed a double charged species. Some partial positive charge migrated toward the sulfur position of the thiazolium ring. This delocalized charge was balanced by bonding of nitrogen, sulfur and oxygen with metals ions.
Optional/General comments	The Introduction Section presents the importance of B1 vitamin (thiamine) as coenzyme; as well, its behaviour in acidic / alkaline media is described, including the electrochemical one. The section Experimental presents the reagents and necessary	



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	instrumentation. The Section Results and Discussions presents -the plot of I_{pa} and I_{pc} values vs thiamine HCl concentration at different scan rates, and the linear dependence noticed; -the voltammetric behaviour of different working standard solution of Fe^{2+} having constant concentration of thiamine HCl at 100 mV/s. I_{pc} values sharply increased, and formed a dip for a molar ratio of thiamine HCl to ferrous ion between two to three, and then remain constant. This sharp increase showed two or three ferrous ions may be involved in complex formation. - the dependence between I_{pa} of thiamine HCl complexes solution in acid media versus different concentration of ferrous ion, proved that all complexes showed approximately the same cathodic and anodic potential, but difference was observed in their current intensity values. The I_{pa} and I_{pc} values of thiamine HCl were lower than the I_{pa} or I_{pc} values of the ferrous thiamine complexes. The dependence between the I_{pc} values of complexes and the concentrations of thiamine HCl was not linear due to the involvement of thiamine in equilibrium with HCl, so it was concluded that thiamine was not available for complexation at very low concentration. - the plot of I_{pa} and I_{pc} values versus variable concentration of thiamine and ferrous ion at different scan rates aimed at calculating the equilibrium constant values of the complexes. A 1:2 thiamine to ferrous ion molar ratio was established as the most appropriate one. -the stability of the formed complex at different pH values has been investigated and potassium chloride pH 6 was found the most appropriate. -the mechanistic study of the electron transfer reaction during complexation shows aspects consistent with previously published studies.	
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	The Conclusion Section underlines the main findings of the present research. Nevertheless, phrase reformulations are strongly recommended, as explained at the end of this file. I recommend extensive revision of grammar, typing, spelling, style, for the scientific content to be better highlighted. Numerous examples are along the Manuscript, of words phrases needing correction/reformulation. Some suggestions are marked in red, as below. Lines 9-11: Abstract “The results showed different behaviour of thiamine (4.3mM) in the presence of ferrous ion (4.3mM), to which attributed with decreases of cathodic and increases of anodic currents were attributed. The mole ratio between thiamine to and ferrous ion...” Lines 10-11: The mole ratio between thiamine and ferrous ion... Line 27, please correct spelling: “thiazolium ring”, not “thiozolium”. Lines 45-46: “...,the involvement of thiamine in complexation with ferrous ion in acidic medium was investigated. The mechanism of such (this type of)	The different mole ratio of ferrous thiamine complexes is assessed. Acidified KCl (1M) solution is used as supporting electrolyte. Mole ratio of complex is established 1:2 (thamine: Ferrous) 100 mV/s scan rate is chosen as optimum. Line 46, thiazolium 104, versus Line 64-65: In present study, the involvement of thiamine in complexation with ferrous ion in acidic KCl medium is
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	reaction was also studied. Page 64-65: “The solutions containing complexes were scanned at 50, 100, 200, 300, 400, 500 mV/s.....” Line 69: “the oxidized species was reduced back during the reverse...” Line 75, please correct “versus”, not “verses”. Same for line 107. Line 78: On further increase in scan rate didn't make sense a proper relationship could not be established between I_{pa} and I_{pc} on one hand and the scan rate, on the other. Line 89: Ferrous ion did not show peaks within the selected potential window.... Lines 89-91: The overlay of anodic peaks of complexes formed between thiamine HCl and ferrous ion at 100 mV/s, showed that the complex formation was completed for a 1:2 ratio, thiamine to ferrous ion. Lines 93-94: While the increased current is for other mole ratio composition showed the presence of free or uncomplexed thiamine. Lines 95-96: Even if complexes were forming formed at higher molar ratio, but they were extremely unstable,	investigated. The mechanism of this type of reaction is also studied. Line 89-90 The solutions containing complexes are run at 50, 100, 200, 300, 400 and 500mV/s. Line 85-86 The oxidized species is noticed to be reduced back during the reverse scan from 0 V to initial potential -0.8 V. Line 106-107: On further increase in scan rate than 300mv/s, there is no proper relationship is established. Line 129-132: The overlaid plot of cathodic peaks of complexes formed between thiamine HCl and ferrous ion at 100m V/s, is confirming that the complex formation is optimized at 1:2 thiamine to ferrous ion ratio, which is noticed with decrease in current. Line 133-134: For other mole ratio compositions, increase in current is due to the presence of free or un-complexed thiamine. Line 134-136: Even if complexes were formed at higher molar ratio, they were extremely unstable, and therefore thiamine is released.
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	and therefore thiamine is released, at more than 1:2 molar ratio (between?, here please specify!), that results in an increase of the current intensity value (Fig. 3.). Lines 126-127: The graph was plotted between the <i>lpc values of complexes versus concentrations of thiamine HCl</i> was not showed the straight line did not show a linear dependence, due to the involvement of thiamine in equilibrium with HCl, therefore the vitamin becomes not available for complexation at very low concentration. Line 180: "The complex formation was related to the release of hydrogen from the thiamine molecule". Lines 185-188: The ferrous ion-thiamine complex was synthesized in aqueous methanolic solution, in which the molar ratio between the ferrous ion and thiamine was found to be 3:1 at pH 6, determined with the help of different techniques, while for/in the acidified (?) 1 M KCl solution (pH 6) the 2:1 molar ratio ferrous ion to thiamin was confirmed. Lines 184-185: Once the N1 was protonated, N4, N3 and sulphur of thiazolium ring can be formed bonds with ferrous atoms (ions?) in such a way that they join the two molecules of thiamine by bridge. Lines 192-193, please reformulate more clearly: <i>But they didn't gives sensible results accept potassium</i>	Line 231-235: The graph plotted between <i>lpc values of complexes versus the concentrations of thiamine HCl</i>. The plot is not showing a linear dependence, due to involvement of the thiamine in equilibrium with HCl, this is also showing that the thiamine is unavailable for complexation at very low concentration. Fig. 7. Line 239-297: The variable concentrations of ferrous complex solutions are also prepared in different buffer solution, such as sodium acetate (pH 4), potassium hydrogen phthalate (pH 5 & 7) and sodium tetraborate (pH 8) and scanned at 100mV/s, but they are giving meaningless results except for potassium chloride at pH 6. Line 322-326: In case of AFe_2 complex, thiamine may oxidize and converts into $A^+ Fe_2$ which
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	chloride of pH 6. Fig. 6 Suggestion: <i>But they did not give sensible results, hence potassium chloride pH 6 was accepted....and here the phrase should be completed. Fig. 6</i> Lines 241-242: In case of AFe²⁺ complex, it was oxidized in the same way to AFe²⁺ and reduced into AFe²⁺ at fast scan rate and may consecutely be converted into AFe⁺ and Fe. Lines 254-256: The electrochemical study of the mole ratio between ferrous ion and thiamine in the complex was carried out by cyclic voltammetry using a Pt working electrode vs Ag/AgCl reference electrode. Lines 256-260: The acidified 1M KCl (pH 6) was the suitable electrolyte for complex as compared to the different buffers solutions, such as sodium acetate (pH 4), potassium hydrogen phthalate (pH 5 & 7) and sodium tetraborate (pH 8). A 1: 2 mole ratio was exist determined between thiamine and ferrous ion in acidified 1 M KCl (pH 6) solution, which was confirmed to the noticed decrease in the I_{pa} and I_{pc} values at the appropriate scan rate 100 mV/s.	further reduces at fast scan rate. At slow scan rate A⁺ Fe₂ may converted into A⁺Fe and that reduces in the presence of Fe atom and converts into AFe₂ complex or may be converted into A⁺ and Fe atom. A⁺ reduces into A and reacted with two moles of Fe atoms and formed AFe₂ complex. Line 242-243 Electrochemical study for ferrous and thiamine complex are carried out by cyclic voltammetry using Pt electrode vs Ag/AgCl reference electrode. Line 344-349: The acidified 1M KCl (pH 6) is found to be a suitable electrolyte for the complex formation as compared to different buffer solutions such as sodium acetate (pH 4), potassium hydrogen phthalate (pH 5 & 7) and sodium tetraborate (pH 8). Mole ratio of 1:2 is established between thiamine to ferrous ion in acidified 1 M KCl (pH 6) solution. Scan rate of 100mV/s is selected. The complexation reaction is found reversible and average diffusion coefficient value is observed between 400 - 500 for both I_{pa} and I_{pc} current.
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