

INTRODUCTION

Iron is an essential metal used as a single ingredient or as a component in various pharmaceutical products, for the formation of hemoglobin and for the oxidative process of living tissues, to treat anemia (Martaindale, 1999). A large number of methods have been reported for the determination of iron in different matrices, with and without complex formation (Moffat, 1986; Charyulu *et al.*, 1987; Hernandez and Carrillo, 1988; Koscielniak and Parczewski, 1988; Talanova and Smirnova, 1995; Yi *et al.*, 1996; Fang *et al.*, 1996; Kaplan and Amadeo, 1996; Lu *et al.*, 1997; Nie *et al.*, 1999; Shapovalova *et al.*, 1999; Aizawaa *et al.*, 2000; British Pharmacopoeia, 2000; Lam *et al.*, O'Hagan, 2001). The most commonly reported method is complexation of iron with 1,10-phenanthroline monohydrate (OPT). While other reported methods are by complex formation with certain other reagents such as quinaldohydroxamic acid (Hernandez and Carrillo, 1988), 1-amino-4-hydroxy-anthraquinone (Abubakar *et al.*, 1993) salicyldoxime (Rue and Bruland, 1995), cibacron blue F3GA (Arica *et al.*, 1998). The rate of change in the concentrations of the reactants and products are used to characterize rate of different simultaneous chemical reactions, which ultimately depends on its mechanism. From a phenomenological point of view, it depends on the parameters like reagent's concentration (sometimes also of the product concentrations), temperature, and presence of a catalyst or an inhibitor. The stoichiometric coefficients for a chemical equation are determined by comparing the slopes of concentration-time plots for the reactants and products.

EXPERIMENTAL

Materials and equipments

All reagents were of analytical grade from BDH Chemical Ltd., Poole, U.K. The deionized water, used through out the experiment was freshly prepared in the lab from double distilled water. All the glasswares were of Pyrex glass, washed with chromic acid followed by a thorough washing with water and finally rinsed with double distilled water. Absorbance measurements were carried out on a double beam UV-Visible spectrophotometer Model 1201 Shimadzu, Japan.

Preparation of solutions

Potassium iodide solutions 0.01M - 0.04M were prepared by appropriately diluting 1 molar stock solution. Similarly ceric(IV) ammonium sulfate stock solution (1 M) was diluted to a concentration of 0.01M. Copper(II) sulfate stock solution (0.01M) was prepared by dissolving 65.35 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in water in a 100 ml volumetric flask and made up to the mark. Aliquots were prepared having concentration of 1×10^{-3} , 8×10^{-4} , 6×10^{-4} and 4×10^{-4} M by serial dilution.

Ferrous ammonium sulfate (0.01M) stock solution was prepared by dissolving 392.0 mg of FeSO_4 in 100 ml volumetric flask. 1,10-orthophenanthroline (0.03M) stock solution was prepared by dissolving 594.6 mg in small amount of deionized water by sonication in 100 ml volumetric flask and volume made up to the mark with the same solvent.

Metal-ligand complexation

Preparation of tris (1, 10-orthophenanthroline) iron (II) complex

Tris(1,10-orthophenanthroline)iron(II) complex (ferroin; figure 1) was prepared by adding ligand 1,10-orthophenanthroline (in slight excess to avoid mono or bis complex formation) to ferrous ammonium sulfate solution of required concentration (1×10^{-4} M, 4×10^{-5} M, 6×10^{-5} M and 8×10^{-5} M), resulting in a wine red solution of tris(1,10-orthophenanthroline)iron(II) complex.

Preparation of tris (1, 10-orthophenanthroline) iron (III) complex

Tris(1,10-orthophenanthroline)iron(II) complex was oxidized to tris (1,10-orthophenanthroline) iron(III) complex with Ce(IV) , which is blue in color. Concentration of the Ce(IV) was same as of iron(II) that is 0.01M.

Methodology

Ferrous ammonium sulfate (0.01M) was reacted with OPT (0.03M) in 1:3 ratio in cold, mixed thoroughly, which resulted in wine red tris(1,10-orthophenanthroline)iron(II) complex $[\text{Fe}(\text{OPT})_3]^{+2}$. To this complex, Ce(IV) solution was added until the formation of blue colored complex. This blue complex of iron(III)OPT was not stable as compared to iron(II) OPT. Copper sulfate solution was mixed with potassium iodide solution in 1:9 ratio, 2.6ml of this was taken in a 3 ml quartz cell and 0.4 ml of tris(1,10-orthophenanthroline)iron(III) complex solution was added to the same cuvet. The reaction was monitored by measuring the absorbance at 510 nm as a function of time, till the reaction was completed.

Effect of catalyst and reducing solution

To study the effect of varying concentration of KI and CuSO_4 on the rate of reaction, in a first set of experiments, concentration of CuSO_4 was kept constant and concentration of KI was varied from 0.01 - 0.04 M and in the second set of experiment concentration of KI was kept constant and concentration of CuSO_4 was varied similarly. In these sets of experiments 2.6 ml of reducing solution was taken in a 3 ml quartz cell already placed in a UV/visible spectrophotometer, followed by the addition of 0.4 ml of tris(1,10-orthophenanthroline)iron(III) complex with a syringe and absorbance was measured at 510 nm as a function of time.

Table 1
Variation in rate constant with respect to Fe(opt)₃

S. No	<— with respect to Fe(OPT) ₃ —>		<— with respect to KI —>	
	Fe(OPT) ₃ (Moles)	K _{observed}	KI (Moles)	K _{observed}
1	4x10 ⁻⁵	0.0065	0.01	0.0065
2	6x10 ⁻⁵	0.0065	0.02	0.0095
3	8x10 ⁻⁵	0.0065	0.03	0.010
4	1x10 ⁻⁴	0.0065	0.04	0.0108

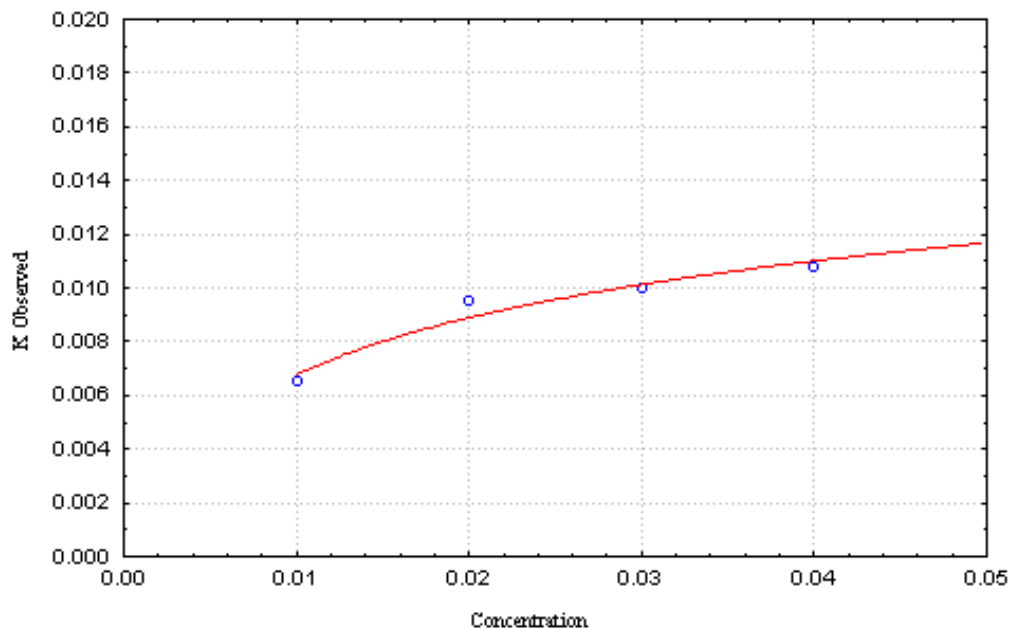


Fig. 1: Variation in rate constant with respect to concentration of KI.

Effect of concentration of Iron (III)OPT

Similar experiment as described above was carried out to study the effect of varying concentration of Iron(III)OPT on the rate of reaction. The concentration of Iron(III)OPT was kept 4x10⁻⁵, 6x10⁻⁵, 8x10⁻⁵ and 1x10⁻⁴ M.

RESULTS AND DISCUSSION

Kinetics and mechanism of 1, 10-orthophenanthroline iron complexation

Like other transition metal ions, Fe²⁺ forms many coordination compounds in which the iron ion is surrounded by various Lewis bases, called ligands. Many of these coordination compounds are colored, unlike aqueous ferrous ion itself. If the colored complex is stable and forms quantitatively, it can be used to measure of the amount of iron present. 1,10-phenanthroline or O-phenanthroline is a bidentate chelating agent, which forms a stable red complex with ferrous iron. This procedure is selective for Fe²⁺, even in the presence of other metals, for several reasons. Fe²⁺ reacts more rapidly with this ligand as compared to many other metals, the complex is exceptionally stable and the intense red color due to absorption of light at a wavelength of 510 nm is specifically due to Fe²⁺, whether other cations

or complexes may be present. Ammonium acetate buffer was used to maintain the pH for optimum complex formation while hydroxylamine hydrochloride was added as a reducing agent to intercept oxygen and prevent oxidation of ferrous iron to ferric iron. The effect of interfering species (solvents, other molecules exceptants etc.) was accounted for by running a reagent blank.

Kinetics with respect to copper sulfate

Presented kinetic studies utilize CuSO₄.5H₂O as a positive catalyst and this is confirmed by the gradual increase in the absorbance of a reacting mixture proportional to CuSO₄ concentration (figure 2). The initial rate in concentration of Fe(Opt)₃ were proportional to the concentration of the initially added copper(II) ions indicating that concentration of CuI at the steady state are also proportional to CuSO₄ added.

With respect to potassium iodide

In presented kinetic studies rate of reaction was observed to be proportional to the concentration of potassium iodide to a certain extent, after which rate became constant. The I₃⁻ concentration formed at time 30 sec in the reaction between I⁻ and Cu⁺² increased with the increase in iodide

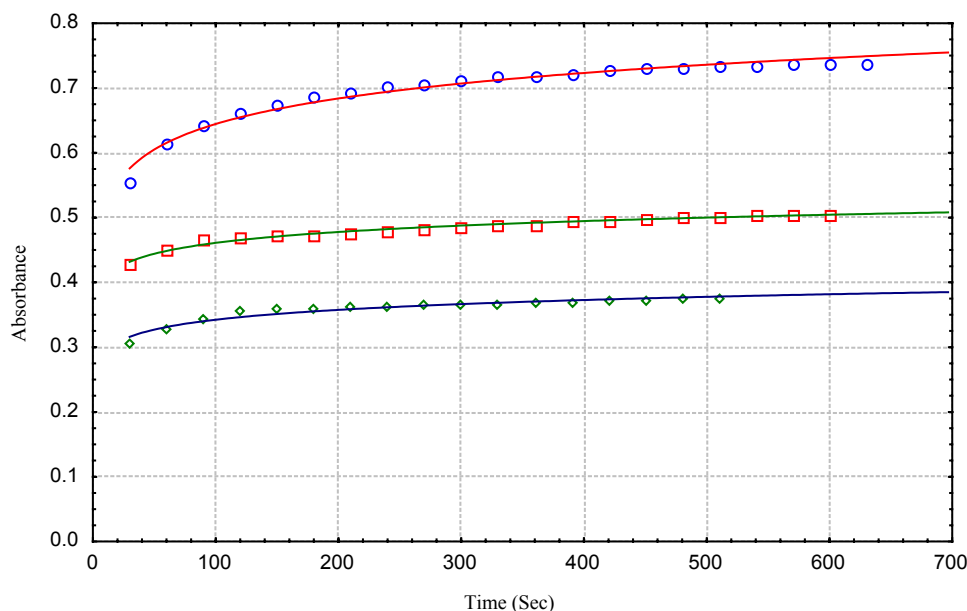


Fig. 2: Change in absorbance of reacting mixture in presence of different concentrations of copper sulfate.

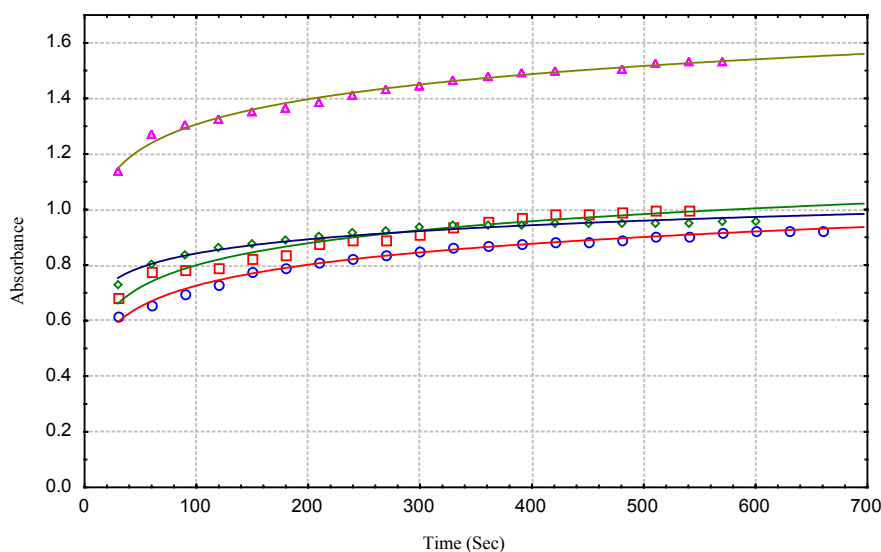


Fig. 3: Change in absorbance of reacting mixture in presence of different concentration of iron(III) OPT.

concentration from 0.01–0.04 M. Addition of $\text{Fe}(\text{OPT})_3$ in the reacting solution of I^- and Cu^{+2} at 30 seconds resulted in increased reaction rate until I^- concentration becomes 0.04M, after which became constant. There was no effect of further increase in the iodide ion concentration (figure 1, table 1). Concentration aligned trend line in figure 2 after 0.04M is due to the formation of a less inactive species, CuI^{2-} for the oxidation. Thus the reaction order with respect to Potassium Iodide is fractional-order.

With respect to $\text{Fe}(\text{III})\text{OPT}$

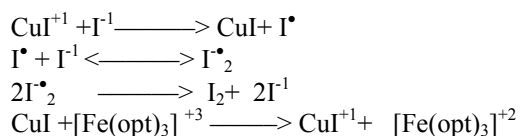
Change in $\text{Fe}(\text{III})\text{OPT}$ concentrations in the presented kinetic studies results in the increase in reaction rate (figure 3). The initial rate of reaction $= \delta[\text{I}_3^-]/\delta t$ increased with the

increasing concentration of $[\text{Fe}(\text{OPT})_3]^{+3}$. The plots of $\ln\{[\text{I}_3^-]_{t=\alpha} - [\text{I}_3^-]_{t=0}\}$ vs time shows rectilinear graph, which is conducive to the fact that this reaction is of first order with respect to $\text{Fe}(\text{OPT})_3$, being a rate determining step in a chain reaction. In the plot of concentration of $[\text{Fe}(\text{opt})_3]^{+3}$ against K -observed the constant value of K -observed is another evidence of the fact that the described reaction is first order (table 1).

Expected mechanism of reaction

All the results obtained are accounted for, by the following mechanism





Considering the rate determining step, the reaction order with respect to $\text{Fe}(\text{opt})_3$ was found to be first order.

CONCLUSION

Human body regulates quantity of iron through a protein based reservoir 'Ferritin' (figure 4), it releases stored iron in case of iron deficiency in blood while in case of overloading ferritin stores the excess iron.

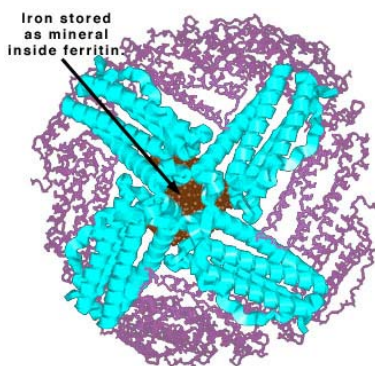


Fig. 4: Three-dimensional x-ray crystallographic representation of ferritin.

Insight II molecular-modeling system from Molecular Simulations, Inc. (Frey *et al.*, 1995; Lawson 1991 and Theil 1987).

Iron is stored in the Fe(III) oxidation state inside ferritin. It is stored in the ferritin core as Fe(III) in a crystalline solid having chemical formula $[\text{FeO}(\text{OH})]_8[\text{FeO}(\text{H}_2\text{PO}_4)]$. This mineral can be represented by ferrihydrite, $\text{FeO}(\text{OH})$ and mineral is attached to the inner wall of the sphere. Iron can only release the ferritin by first changing from the Fe(III) to the Fe(II) oxidation state. In the Fe(II) state, iron breaks away from the lattice as the Fe^{2+} ion. The positive charge of the Fe^{2+} ion attracts the electronegative oxygen atoms of water, and so water "cage" forms around the ion. (In the water cage, six water molecules surround the ion at close range). Thus, iron becomes soluble as a hydrated Fe^{2+} ion, $\text{Fe}(\text{H}_2\text{O})_6^{2+}$, and can be released from the ferritin protein via the channels in the spherical shell (shown above). Once the iron is soluble (as $\text{Fe}(\text{H}_2\text{O})_6^{2+}$) it will be released from ferritin shell through the three-fold channels of the ferritin due to polarity.

From the presented work it is clearly evident that I^- acts as reducing agent which can reduce Fe(III) to Fe(II) and oxidize itself to I_2 . This is a potential reaction to cause release of iron from ferritin as Fe(II), while simultaneously

increasing I_2 in blood, resulting in increased toxicity. Conversion of Fe(III) to Fe(II) do not require any specific pH so the feasibility of the occurrence of this reaction enhanced further more. On the other hand this reaction also provides a clue for mechanism of the CuSO_4 toxicity, as concentration of copper can favor the reduction of Fe(III) to Fe(II).

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