Effect of Chelator Size & Charge on Iron(II) Release from Ferritin Nano cage

A

Dissertation

Submitted in Partial fulfilment for The Degree of Master of Science in Chemistry under

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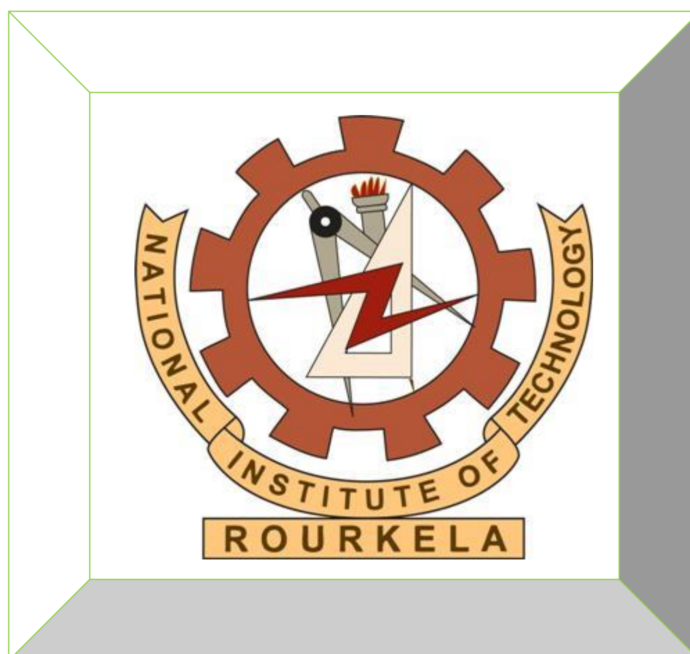

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CERTIFICATE

This is to certify that the dissertation entitled "**Effect of chelator size and charge on Iron(II) release from Ferritin nanocage** " being submitted by **Mr. Santanu Mondal** to the Department of Chemistry, National Institute of Technology, Rourkela 769008, for the award of the degree of Master Of Science in Chemistry, is a record of bonafide research carried out by him under my supervision and guidance. The dissertation report has reached the standard fulfilling the requirements of the regulations relating to the nature of the degree.

I further certify that to the best of my knowledge Mr. Mondal bears a good moral character.

Place: NIT-Rourkela

Date: **Dr. Rabindra K. Behera**

Effect of Chelator Size and Charge on IRON(II) Release from Ferritin Nano Cage

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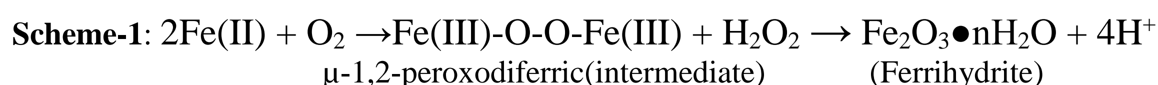
Introduction

Iron is a crucial metal for virtually all living organisms, but Iron(II) which has toxic effect. Ferritin is a class of iron packing proteins dispersed in mammals, plants and bacteria. Iron-free (apoferritin) molecules are hollow sphere and molecular weight is 450,000 Da. This protein class plays a central role in storage and detoxification of excess iron in a living cell in controlled fashion. Ferritin is present mainly in the cytoplasm of spleen, liver and bone marrow in mammals. Although the prime sequences of ferritins are vary, their 3D structures of 24 structurally equivalent subunits accumulated into a cage-like oligomer and related by 4-, 3-, and 2-fold symmetry axes (1, 3) are highly preserved. Each subunit has four helices (*Fig-1*(A-D)), each of which is made of ~30 amino acid residues. In general, the outer shell diameters of the spherical ferritin made of 24 subunits are~12 nm, where the inner cavity diameters are ~8 nm. Up to 4500 iron atoms are to be placed in the inner cavity of ferritin (1, 3). To date, three major metal binding sites have been recognized in ferritin. These display some common aspects connected to the iron storage mechanism: (i) they exist around the 3-fold symmetry channel, (ii) create a ferroxidase centre in the four-helix bundle, and (iii) create a nucleation site facing the inner cavity of ferritin (4). It has been proposed that iron atoms pass through the hydrophilic channels that cross the ferritin shell around the 3-fold symmetry axes of ferritin (5). This funnel-like channel is surrounded by hydrophilic residues, like aspartic acid and glutamic acid, which have been confirmed to serve as ligands for metal ions, such as ferrous, calcium, or zinc bivalent ions (6). With respect to the ferroxidase centre and nucleation centre, two distinct subunits, heavy (H) and light (L) chains, are gotten in mammalian ferritin (7, 8, 9). The H chain possesses the ferroxidase site (10), which is located inside the 4-helix bundle of each subunit and is accountable for oxidation of ferrous iron atoms to produce the μ-oxo-bridged Fe(III) species and that is precursor of iron and its formation is shown in scheme-1. On the other hand, the L-type ferritin lacks the ferroxidase centre, but holds the nucleation centre. The nucleation centre is composed by few glutamate residues facing the inner cavity of the ferritin shell, and eases ferrihydrite nucleation (11, 12, 13). In mammals, the H and L chains form a heteropolymer and cooperatively share the role of iron storage *in vivo* (14).



Scheme-1: $2Fe(II) + O_2 \rightarrow Fe(III)\text{-}O\text{-}O\text{-}Fe(III) + H_2O_2 \rightarrow Fe_2O_3 \bullet nH_2O + 4H^+$

μ-1,2-peroxodiferric(intermediate) (Ferrihydrite)

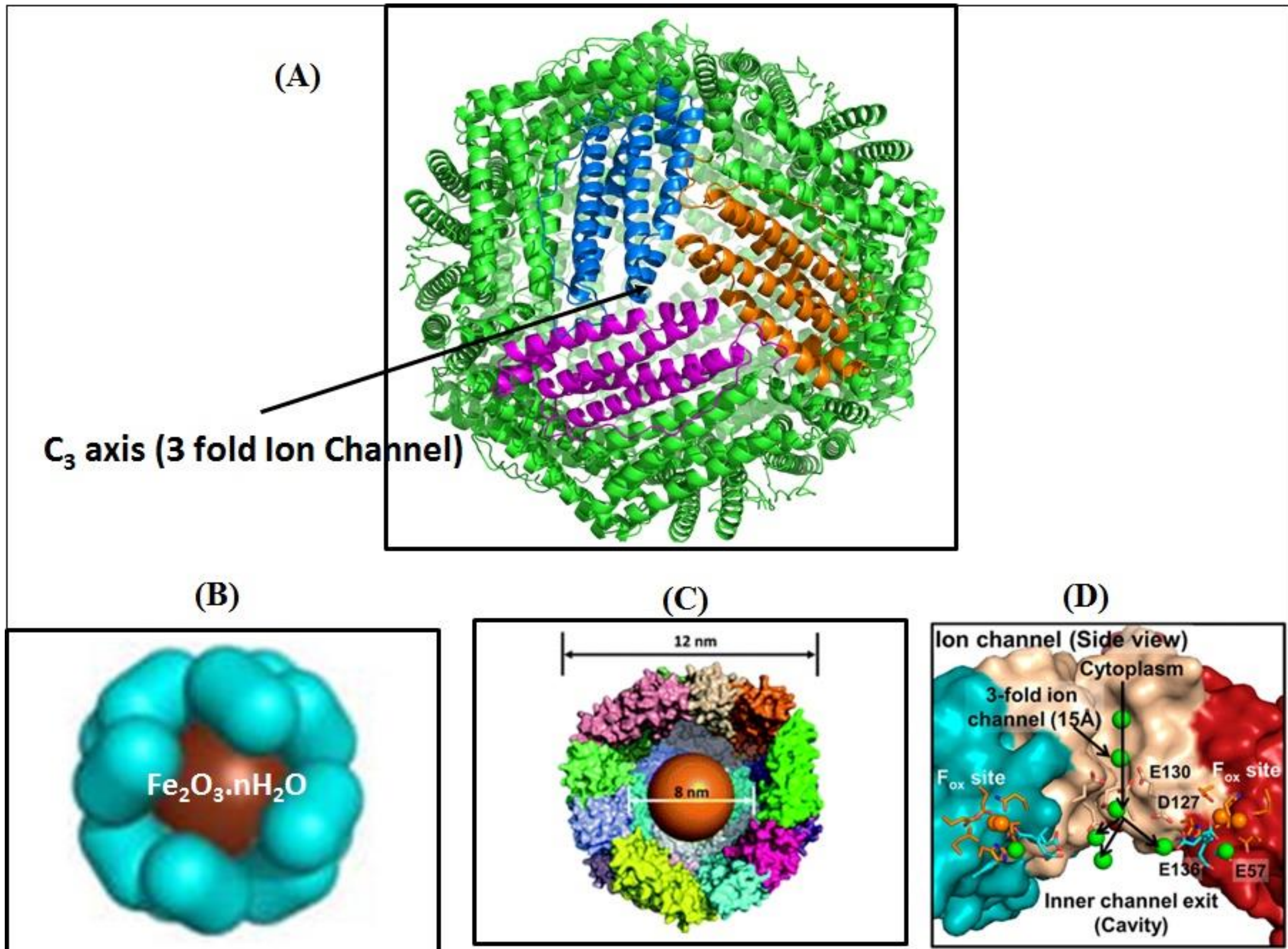


***Fig-1*: Ferritin nano cage made of 24 subunits with C_3-axis(A); Mineralized ferritin containing ferrihydrite($Fe_2O_3 \bullet nH_2O$)(B);Outer shell diameter of ferritin is 12nm and inner cavity diameter is 8nm(C); 3-fold channel of ferritin made by Glutamate(E) and aspartate(D) amino acids (D)**

The mechanism of iron mobilization from ferritin remains poorly established. The ferritin iron core is a comparatively stable entity with a very low iron dissociation rate in the lack of reducing agents. Several pathways of iron release from ferritin have been suggested. For many years the iron (Fe) chelator, desferrioxamine (DFO), has been used for the treatment of β-thalassemia and other diseases of Fe overload (15, 16). For occasion, mobilization of Iron(III) cations from ferritin can occur through reduction by flavinmononucleotide(17), ascorbate(18), glutathione(19), sodium dithionite(20), polyphenols(21), superoxide(22) and other agents. Alternatively, Iron(III) cations can be

mobilised from ferritin by hydroxamates (23) catechols (24) and other synthetic chelators (25) with or without transitional reduction to iron(II) cations (26).It is thought to be the charged and size of chelators may assist the mobilization of ferritin iron from ferritin channel. The size of 3-fold channels of ferritin is fixed. Here Bipyridine, disodium salt of 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (known as ferrozine: FZ) and phenanthroline has been used as Fe(II) potent chelator for mobilization ferritin-Fe from ferritin nano cage and structure of these chelators are shown in *Fig-2*. Bipyridine and phenantholine are hydrophobic in nature while ferrozine shows hydrophilic and hydrophobic behaviour. All these three cheletors form complex with Fe(II) in 3:1 ratio.

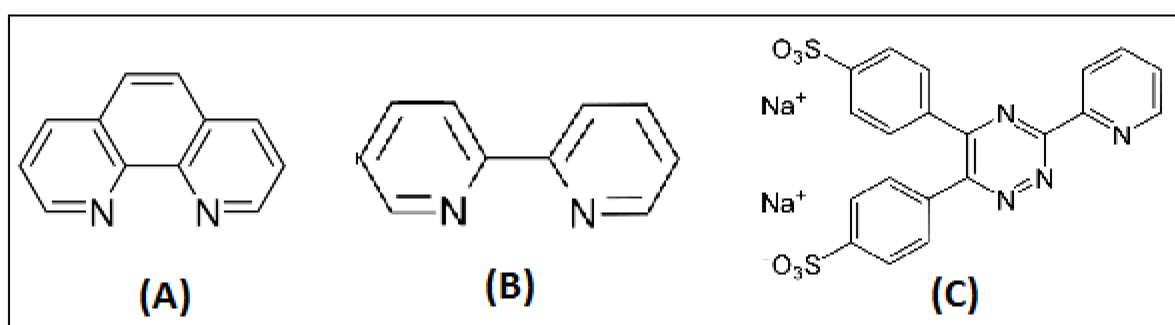
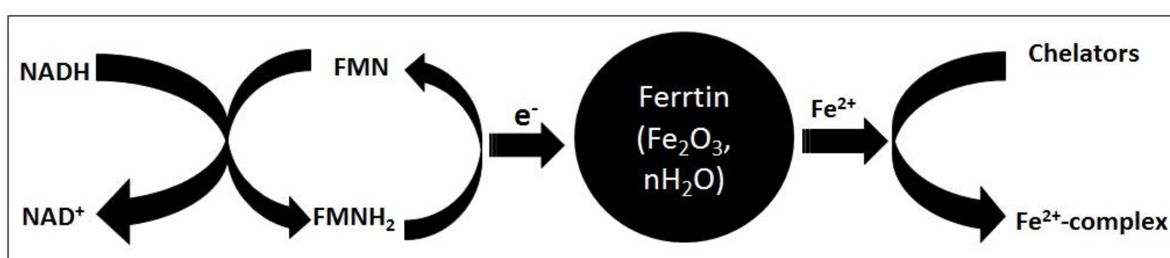


Fig-2: **Chelators structure:1,10-Phenantholine(Phen)(A); 2,2-Bipyridine(BiPy)(B); Ferrozine(Fz)(C)**

The present study to assay "The effect of chelators size and charge on Ferritin-Fe(II) release from ferritin nanocage". In our study NADH and FMN used as reducing agent. Here FMN acts as electron mediator inside the ferritin protein. $NADH/NAD^+$ system produced electrons, that electrons go inside the ferritin by help of FMN and take part in reduction of ferritin-Fe(III) to Fe(II), and released from ferritin core. Finally external chelators form complex with released iron and that complex absorbed UV-Vis light. The total overview of iron release from ferritin is shown in scheme-2.



Scheme-2: $NADH/NAD^+$ system produced e^- that electrons go inside the ferritin by help of FMN and release Fe(II).Finally external chelators form complex with released iron and that complex absorbed UV-Vis light.

1. *Methods and Materials*

We have synthesised Frog-M ferritin in E.coli. Without any purification by standard reported method WT Frog-M ferritin was loaded with 480 iron atoms in presence of oxygen. FMN and NADH were used as bio-reducing agent couple in iron release kinetics of ferritin protein, which help the conversion of Fe(III) to Fe(II) inside ferritin protein. Mentioned three ligands (Fz, Bipy and Phen) were used as Iron(II) chelator. All experiments were performed in 100mM MOPS-NaCl buffer, pH 7.0 at 25°C in CARY-300 UV-Vis Spectrophotometer. In pairing quartz cuvette of 1ml, calculated concentration of mineralised ferritin protein, FMN, NADH, ligands and MOPS buffer were mixed , allowed the reaction for 30 min. and taken UV-Vis spectrum in respect to the rate of reductive and non-enzymatic mobilization of iron release from mineralised ferritin. Three different kind of experiments were performed in this study by using three different Fe(II) potent chelators .

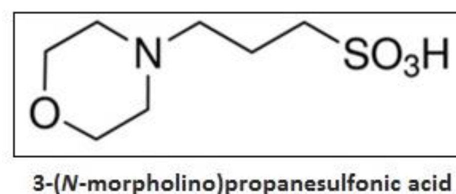
Required chemical reagents

Commercial available Bipyridine, Phenantholine, Ferrozine, FMN, NADH and MOPS buffer were used. Overexpressed FROG-M ferritin in E.coli used in this study.

1.1 Preparation of reagents:-

- *Preparation of 100mM MOPS and NaCl buffer, pH 7.0 :*

MOPS is a sulphuric acid derivative of organic molecule, its full name is 3-(*N*-morpholino)propanesulfonic acid and structure is shown in right side. For preparation of 100ml 100mM MOPS, 2.09g of MOPS was dissolved in 100ml MQ water and stirred for few minutes. After that few drops of concentrated HCl was added to MOPS solution to maintain the pH of MOPS solution at 7.0.The pH measurement was performed in pH-meter. Finally 0.58g NaCl (100 mM) was mixed with 100ml 100mM MOPS solution.



3-(*N*-morpholino)propanesulfonic acid

- *Mineralization of WT Frog-M ferritin by Fe(II) ion:*

The concentration of commercial WT Frog-M ferritin was measured byBradford protein assay. The stock concentration of protein was 38.95µM. For loading 480 iron atoms per cage of 1mL 2.08µM WT Frog-M ferritin, 53.4µL of 38.95µM WT ferritin, 20µL of 50mM $FeSO_4$ and 946.6µL of 100mM MOPS buffer was added in 2mL of eppendrof and kept in room temperature for 2hrs, after that kept in refrigerator for overnight.

- *Preparation of Fe(II) potent Chelators solution:*

Ferrozine , 2,2'-bipyridine and 1,10-phenantholine are easily soluble in water. Solubility of phenantoline in water is 2.6mg/mL, that's why preparation of high concentrated of phen-MQ water solution is difficult. The stock concentration of ligands were 25mM, 1.68mM and 10mM for 2,2'-bipyridine, 9,10-phenantholine and Ferrozine respectively. Separately measure the maximum absorption and molar absorptivity of chelators and Fe(II)-chelators complex.

- *Preparation of FMN and NADH solution*:

The solution of FMN and NADH are sunlight sensitive and easily degrade, thereby the concentration of these solution are not constant for long time. About 1-3mg of both FMN and NADH was dissolved in 1mL MQ water in 2mL separated eppendrof. After that from known λ_{max} and molar extinction coefficient, the concentration of these two solution were estimated by UV-Vis Spectrophotometer.

1.2 Kinetic study-A: *Mineralized Ferritin-Iron (0.1mM) ,FMN (1mM),NADH (1mM) and chelators (0.9mM) at pH 7.0,25^{0}C*

The Iron release kinetic studies of mineralised ferritin were performed in CARY-300 UV-Vis spectrophotometer .Mineralized Ferritin-Iron (0.1mM), FMN(1mM), NADH(1mM) ligands (BiPy,Phen and Fz) (0.9mM) and Mops-NaCl buffer (100 mM) were mixed in a 1mL quartz cuvette open to ambient air at pH 7.0 and 25^{0}C. Each reactions were allowed for 30 min inside the 1mL cuvette in UV-Vis Spectrophotometer. As three ligands-Fe(II) complex absorbed UV-Vis light at different wave length, therefore three different set of iron

release kinetics study of mineralized ferritin were performed in this study. UV-Vis light was monitored at 562nm, 511nm, and 522nm, for Ferritin-Fe(II)-Fz complex, Ferritin-Fe(II)-Phen complex and Ferritin-Fe(II)-Bipy complex respectively. The iron release time course profile is showed in *Fig-6.*

1.3 **Kinetic study-B*: Mineralized Ferritin-Iron (0.1mM) ,FMN (2.5mM),NADH (2.5mM) and chelators(0.9mM) at pH 7.0, 25^{0}C:***

In this plan of kinetic study of iron release from mineralised ferritin-iron, all reagents were mixed in same concentration and condition which is stated in previous experiment only change in concentration of FMN and NADH. Both FMN and NADH were mixed 2.5mM instead of 1mM with other reagents in 1ml quartz cuvette at pH 7.0 and 25^{0}C.In this case same time period was allowed for reaction of each ligands. The UV-Vis light was monitored at stated wavelength in previous experiment for particular Ferritin-Fe(II)-chelator complexes. The iron release time course profile is showed in *Fig-7.*

1.4 **Kinetic study-C*: Mineralized Ferritin-Iron (0.1mM) ,FMN (1mM),NADH (1mM) and chelators (1.8mM) at pH 7.0,25^{0}C:***

Here only chelators concentration was changed to 1.8mM from 0.9mM. All other reagents having same concentration were mixed with 1.8mM ligands in 1ml Quartz cuvette at pH 7.0 and 25^{0}C. To maintain pH 7.0, like previous experiments 100 mM MOPS-NaCl buffer was added to 1ml cuvette and absorption spectrum were recorded for 30 min in terms of iron release kinetic profile. Similar to previous experiment the UV-Vis light was monitored at particular wavelength for particular chelators-Ferritin-Fe(II) complexes. The iron release time course profile is showed in *Fig-8.*

2. Results and discussion:

2.1 Complexion of chelators with Fe(II)

For chelation purpose three distinct chelator Bipyridine(BiPy), Phenantholine(Phen) and Ferrozine(Fz) were used, all formed strong complex with Fe(II). The ratio of chelators and Fe(II) in complex is 3:1 . Phen and Bipy were hydrophobic in nature and Fz has both hydrophilic and hydrophobic character. Maximum absorption value of UV-Vis experiment of these three chelators-iron complex is showed in Table-1. It is thought to be 100% of Fe(II) ions formed complex with chelators without using any kind of reducing agent. The UV-Vis spectrum of Fe(II)-chelators complex using reducing agent $NH_4OH.HCl$ showed there was no change in absorption value of complex. Thus the rate of complexion is first compared to rate of oxidation of Fe(II) to Fe(III) by dissolved Oxygen. Absorption spectrum of all these three complex of Fe(II)-chelators were achieved in CARY-300 UV-Vis spectrophotometer and shown in *Fig-3*.The structure of chelators (*Fig-2*) demonstrate that all chelators are organic molecule with internal pi-bond conjugation thus chelators should absorb UV-Vis light. Therefore separately measure absorption of UV-Vis light of chelators and absorption spectrum is shown in *Fig-4.* Absorption spectrum of chelators reveal that chelators does not interfere in the absorption spectral range of complexes.

Complex	λ_{max}(nm)	ε($mM^{-1}cm^{-1}$)	Condition
Fe(II)-Fz	**562**	**25.4**	**i)pH=7.0,ii)25°C**
Fe(II)-BiPy	**522**	**8.54**	**i)pH=7.0,ii)25°C**
Fe(II)-Phen	**511**	**11.2**	**i)pH=7.0,ii)25°C**

***Table-1*:λ_{max} value of Iron(II)-chelators complex. 562nm (ε=25.4 $mM^{-1}cm^{-1}$) for Fe(II)-Fz; 522nm (ε=8.54 $mM^{-1}cm^{-1}$) for Fe(II)-BiPy; 511nm(ε=11.2$mM^{-1}cm^{-1}$) for Fe(II)-Phen.All absorption spectrum of complexes was recorded at pH 7.0 and 25^0C.**

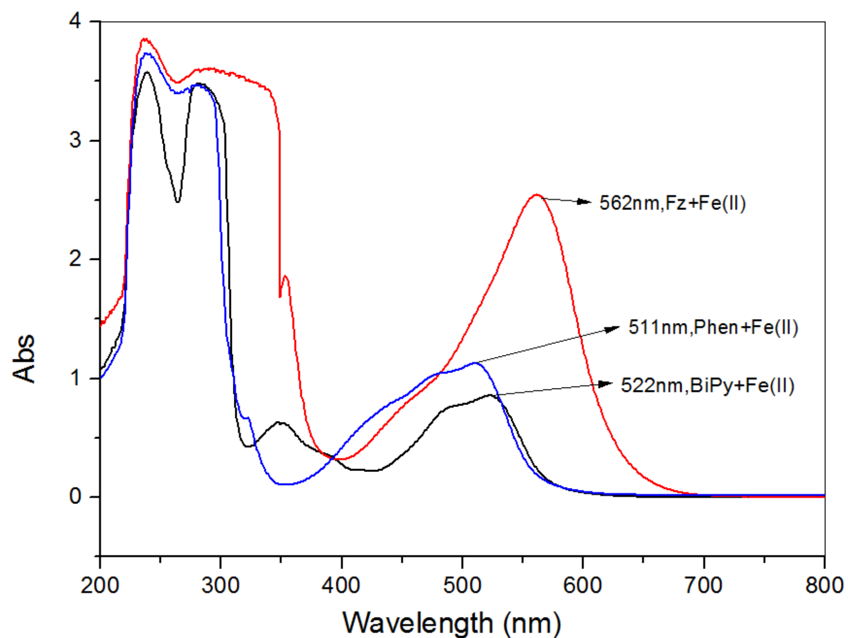


Fig-3: **Absorption spectrum of all Fe(II)-chelators complexes. BiPy-Fe(II) complex absorbed UV-Vis light at 522nm; Phen-Fe(II) complex absorbed UV-vis light at 511nm; Fz-Fe(II) complex absorbed UV-vis light at 562nm. All absorption performed in CARY-300 UV-Vis Spectrophotometer**

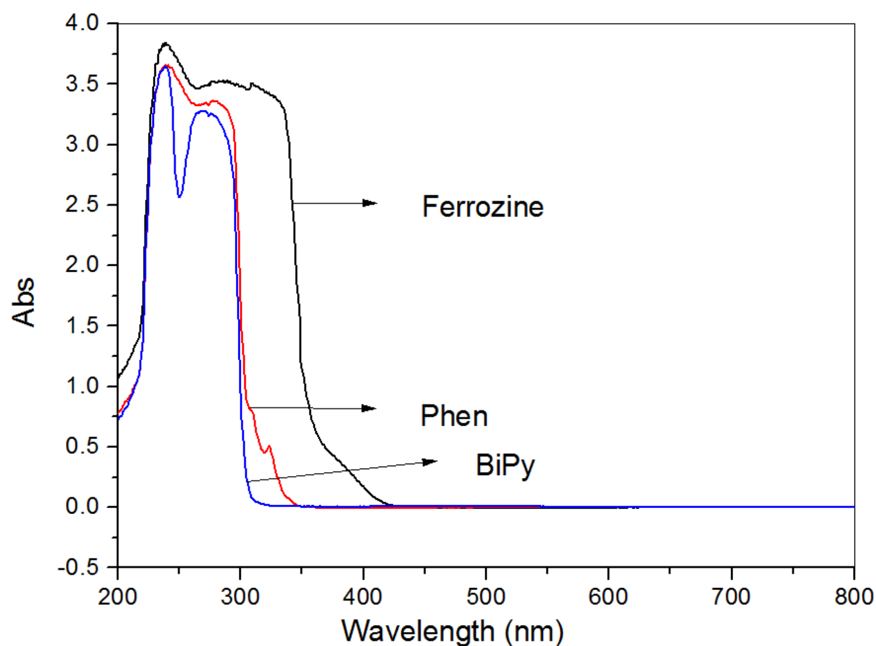


Fig-4 **: Absorption spectrum of chelators. All three chelators do not absorbed UV-Vis light in the absorption spectral of UV-Vis light of Fe(II)-chelators complex**

2.2 Absorption Spectrum of reducing agent NADH and FMN:

In entire experiment Nicotinamide adenine dinucleotide(NADH) and Flavin mononucleotide (FMN) were used as reducing agent and electron mediator inside the ferritin respectively. Nicotinamide adenine dinucleotide (NAD+) experience reversible reduction of the nicotinamide ring. The NADH go through oxidation (dehydrogenation), giving up two hydrogen atoms. NADH has water soluble cofactors that passage readily from enzyme to another. Flavoproteins are enzymes that catalyse redox reactions using either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as coenzymes. These coenzymes are derived from the vitamin riboflavin. Although the flavin coenzymes are water soluble. The reduction potential (E^0_{red}) of FMN at 25^0C is -0.30(V) and that of NADH is -0.32(V)(27). FMN showed maximum absorption at 450nm (ε=12.2$mM^{-1}cm^{-1}$) and NADH showed maximum absorption at 340nm (ε=6.22$mM^{-1}cm^{-1}$). The UV-Vis spectrum of FMN and NADH shown in *Fig-5*.

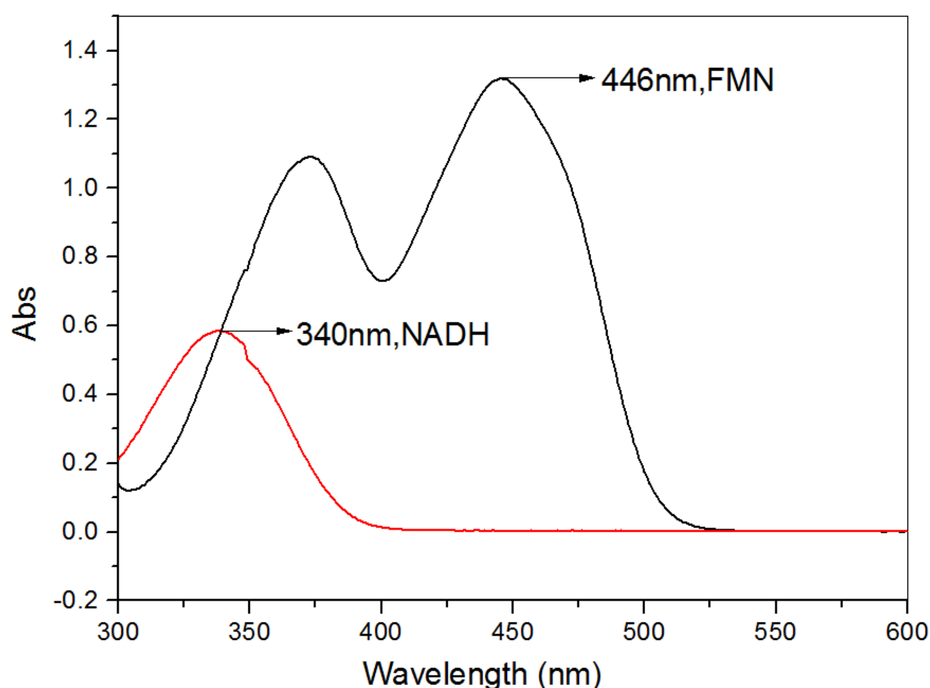


***Fig-5*: Absorption spectrum of NADH and FMN. FMN absorbed UV-Vis light at 450nm (ε=12.2$mM^{-1}cm^{-1}$) and NADH absorbed UV-Vis light at 340nm (ε=6.22$mM^{-1}cm^{-1}$).The absorption spectrum recoded on CARY-300 UV-Vis spectrophotometer**

2.3 ***Kinetic-study-A (Mineralized Ferritin-Iron(0.1mM), FMN (1mM),NADH (1mM) and chelators (0.9mM) at pH 7.0,25^{0}C)***

The iron release time course (*Fig-6*) of kinetic-study-A (Mineralized Ferritin-Iron (0.1mM), FMN (1mM), NADH (1mM) and chelators (0.9mM) at pH 7.0, 25^{0}C) is biphasic. Here 1mM of NADH and FMN and 0.9mM of chelators was used for mobilization of ferritin iron. Each chelator releases different amount of ferritin iron from ferritin nanocage. The results of kinetic-study-A is showed in *Table*-2. Within 30 minutes 29-30% ferritin iron is released from mineralized ferritin by Ferrozine chelator, 27-28% ferritin iron is released by Bipyridine and 20-21% ferritin iron released by Phenantholine. Percentage of released ferritin-iron is depend on character of chelators.

The structure of chelators (*Fig-2*) demonstrate that the size of chelators in increasing order is as follows, Bipyridine, Phenantholine and Ferrozine. Ferrozine is hydrophilic in nature with negative charged and both Phenantholine and Bipyridine are hydrophobic.

Although the size of Ferrozine is bigger compare to other two chelators, still it effectively release high percentage of ferritin iron from ferritin nanocage compare to Bipyridine and Phenantholine. Inside the Ferritin protein nanocage, there is a charge gradient from positive to negative. Ferrozine is hydrophilic molecule with negative charge. The Ferrozine is able to increase the charge gradient. Charge gradient of protein assistances the ferritin iron release from ferritin nanocage, and consequently high amount of ferritin iron released from ferritin nanocage by Ferrozine chelator.

Phenantholine and Bipyridine do not able to increase the charge gradient of protein as they are hydrophobic in nature. Only size of these two chelators play crucial role in mobilization of ferritin iron. Bipyridine is able to close approach toward 3-fold axis of ferritin compare to Phenantholine as the size of Bipyridine is small and that result in difference in percentage of iron release by chelators.

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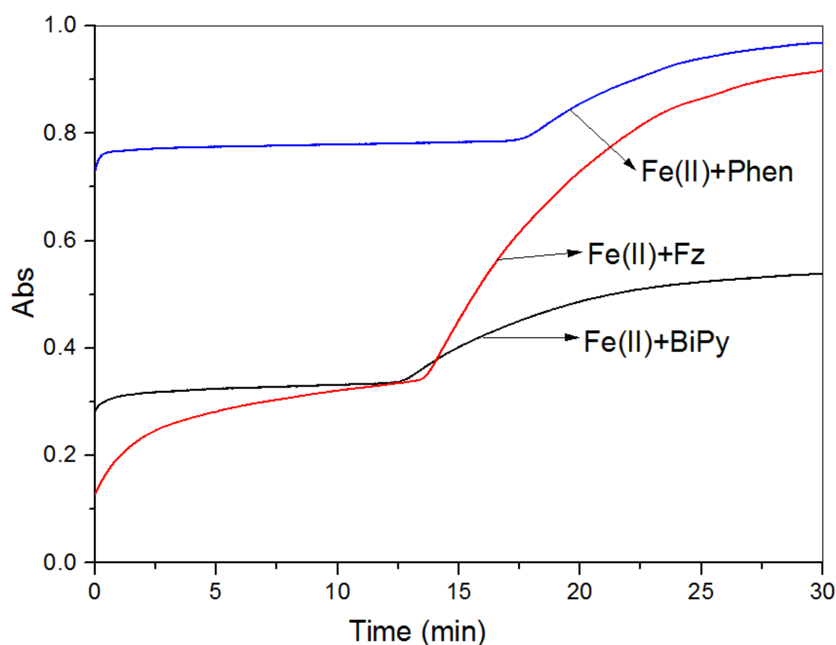

Fig-6: Time course of Kinetic study-A (Mineralized Ferritin-Iron (0.1mM), FMN (1mM), NADH (1mM) and chelators (0.9mM) at pH 7.0, 25^{0}C)

2.4 <u>Kinetic study-B*: (Mineralized Ferritin-Iron (0.1mM) ,FMN (2.5mM),NADH(2.5mM) and chelators(0.9mM))at pH 7.0, 25^{0}C:*</u>

Here the time course profile of iron release from ferritin is also biphasic (*Fig-7*).The kinetic-study-B (Mineralized Ferritin-Iron (0.1mM), FMN (2.5mM), NADH (2.5mM) and chelators (0.9mM) at pH 7.0, 25^{0}C) performed with high concentration of NADH and FMN. Here the rate of ferritin iron release is faster compare to Kinetic-study-A within same time scale. Like kinetic-study-A, initially the rate of ferritin iron release is slow. 47-48% of ferritin iron released (*Table-2*) by Ferrozine and 42-43% of ferritin iron released (*Table-2*) by Bipyridine.

The absorption spectrum of FMN (*Fig-5*) demonstrate that FMN start absorbing UV-Vis light from 500nm. The maximum absorption of Fe(II)-Phen complex is 511nm. The FMN concentration is decreased during the reaction of iron release, therefore the absorption of FMN also decreased, that leads to negative rate constant of ferritin iron release by Phenantholine. Thus Kinetic-study-B not able to perform with Phenantholine.

The concentration of NADH and FMN is high, since high percentage of ferritin iron reduced to Fe(II) and that leads to 47-48% of ferritin iron released by Ferrozine and 42-43%

of ferritin iron released by Bipyridine within same time scale. Thus concentration of FMN and NADH have significant effect on the mobilization of ferritin iron from ferritin nano cage.

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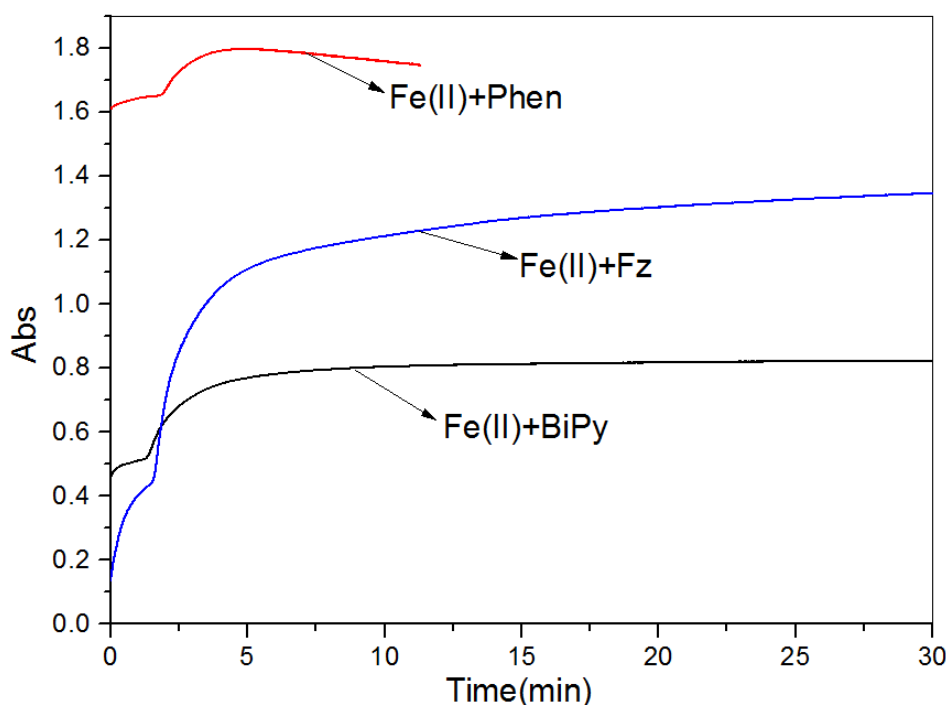


Fig-7: Time course of Kinetic study-B (Mineralized Ferritin-Iron (0.1mM), FMN (2.5mM), NADH (2.5mM) and chelators (0.9mM)) at pH 7.0, 25^{0}C)

2.5 ***Kinetic-study-C (Mineralized Ferritin-Iron(0.1mM), FMN (1mM), NADH (1mM) and chelators (1.8mM) at pH 7.0, 25^{0}C)***

Kinetic-study-C (*Fig-8*) also showed biphasic time course profile of ferritin iron release. The kinetic-study-C (Mineralized Ferritin-Iron (0.1mM), FMN (1mM), NADH (1mM) and chelators (1.8mM) at pH 7.0, 25^{0}C) is performed with high concentration of chelators. Like kinetic-study-A and kinetic study-C, here also the initial rate of ferritin iron release is slow. The kinetic-study-C is not accomplished by Phenantholine as the solubility of Phenantholine in MQ water is low. The chelators cannot enter actively inside the 3-fold axes of ferritin channel as size of chelators bigger than pore size of ferritin, thus complexion is not possible inside the ferritin nano cgae and consequently the results of kinetic-study-C

(*Table-2*) merge with that of kinetic-study-A. Therefore, the concentration of chelators do not have remarkable effect on mobilization of ferritin iron in reductive pathway.

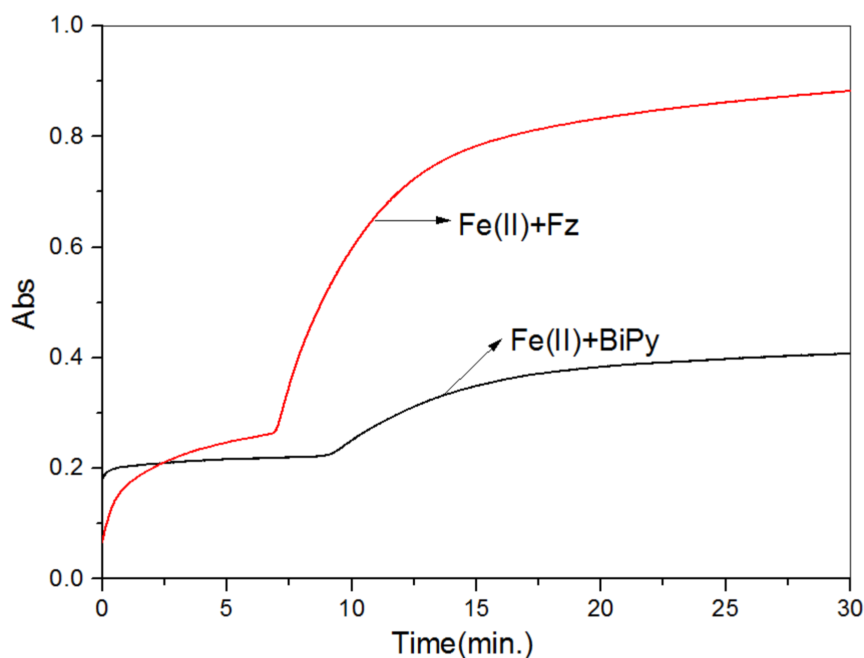


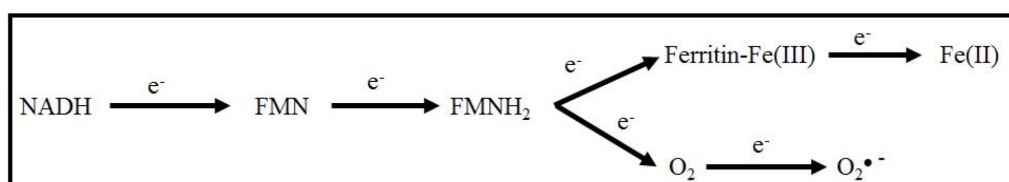
Fig-8: Time course of Kinetic study-C (Mineralized Ferritin-Iron(0.1mM, FMN (1mM), NADH (1mM) and chelators (1.8mM) at pH 7.0, 25^{0}C)

Table-2: Results of Kinetic study-A (Mineralized Ferritin-Iron (0.1mM), FMN (1mM), NADH (1mM) and chelators (0.9mM) at pH 7.0, 25^{0}C); Kinetic study-B (Mineralized Ferritin-Iron (0.1mM), FMN (2.5mM), NADH (2.5mM) and chelators (0.9mM)) at pH 7.0, 25^{0}C) and Kinetic study-C (Mineralized Ferritin-Iron(0.1mM), FMN (1mM), NADH (1mM) and chelators (1.8mM) at pH 7.0,25^{0}C) in terms of % of iron release from mineralised ferritin.

	Kinetic study-A		***Kinetic study-B***		***Kinetic study-C***	
Name of the chelators	**ΔAbs.**	***% of Fe(II) release***	**ΔAbs.**	***% of Fe(II) release***	**ΔAbs.**	***% of Fe(II) release***
Ferrozine(Fz) **($\varepsilon_{Fe\text{-}Fz}$=25.4mM^{-1}cm^{-1})**	**0.74 ± 0.03**	**29.43 ± 1.03**	**1.21 ± 0.01**	**47.74 ± 0.07**	**0.81 ± 0.01**	**31.8 ± 0.04**
Bipy **($\varepsilon_{Fe\text{-}Bipy}$=8.54mM^{-1}cm^{-1})**	**0.23 ± 0.01**	**27.22 ± 1.27**	**0.37 ± 0.01**	**43.26 ± 0.94**	**0.22 ± 0.01**	**26.3 ± 0.06**
Phen **($\varepsilon_{Fe\text{-}Phen}$=11.2mM^{-1}cm^{-1})**	**0.23 ± 0.01**	**20.17 ± 0.38**	___	___	___	___

Reduction of flavin mononucleotide (FMN) by NAD(P)H is catalysed by flavin reductases (28) which are expressed in bacterial and mammal cells. However, the enzyme is not commercially available, and the non-enzymatic reaction between NADH and FMN can be conveniently used for modelling the iron release *in vivo* (29). Despite strongly negative reduction potential, the NAD+/NADH system alone is not an efficient reducing agent of ferritin Iron(III) cations in ferritin cavity and thus FMN as electron transporter is employed to mobilize ferritin iron.

All iron release time courses are biphasic in nature. Initially the rate of iron release is slow and later rate of iron release is fast. During the reaction concentration of FMN is consumed by dissolved molecular Oxygen and ferritin Iron(30). Maximum concentration of dissolved oxygen in 1mL MQ water is about 200-250 μM (3). It is thought to be the electrons are transported to ferroxidase site through aromatic ring of amino acids. Thus reduction of ferritin-Fe(III) to ferritin-Fe(II) takes time. Therefore, there is competition between dissolved molecular oxygen and ferritin-Fe(III) to capture the electrons and the competition of electron capture is shown in scheme-3. The dissolved molecular oxygen initially capture almost all electrons with faster rate compare to ferritin-Fe(III) as they are naked and result in slow rate of ferritin iron release initially and biphasic nature of iron release courses of ferritin. All the iron release time course profile demonstrate that, initially the ferritin iron is released with slow rate as there is change in absorption and the rate of ferritin iron release become faster when almost all dissolved molecular oxygen is depleted. The time period of depletion of dissolved molecular oxygen depends on concentration of FMN and NADH. The high concentration of FMN and NADH depleted the dissolved molecular oxygen with high rate. As the kinetic-study- B (Mineralized Ferritin-Iron (0.1mM), FMN (2.5mM), NADH(2.5mM) and chelators(0.9mM) at pH 7.0, 25^0C) carried out with high concentration of FMN and NADH compare to other two kinetic study, since depletion of molecular oxygen is fast, that lead to high rate of ferritin iron release in kinetic-study-B.



Scheme-3: In reductive assay electrons are captured by dissolved molecular O_2 and Ferritin-Fe(III). By capturing electrons O_2 convert poisonous superoxide radical and Ferritin-Fe(III) convert to Fe(II) state

Conclusion:

The results revealed that the concentration of FMN and NADH have significant effect on rate of ferritin iron release from ferritin nanocage in reductive pathway. The hydrophilic and small chelators remarkably release ferritin iron from ferritin nano cage. The concentration of chelators do not enhance the rate of mobilization of ferritin iron from ferritin nano cage.

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