Structural, Physical and Functional Characterization of Triferic (Ferric Pyrophosphate Citrate, FPC) A Novel Iron Compound for Pharmaceutical Applications

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INTRODUCTION

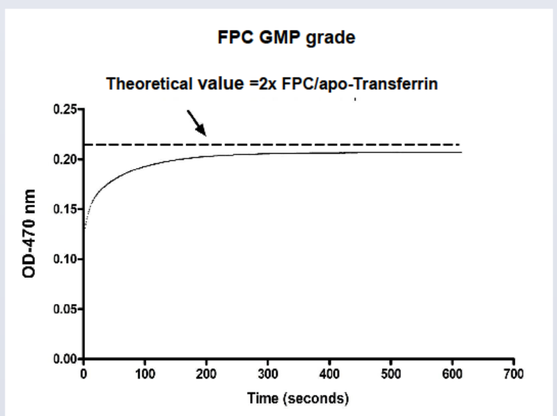
- Triferic (FPC) is a novel iron salt that is not an iron-carbohydrate complex.
- FPC replaces the 5-7 mg of iron loss that occurs with each hemodialysis procedure.
- Rockwell Medical synthesized an iron(III) compound with pyrophosphate and citrate ligands (FPC) suitable for parenteral use. FPC has improved physicochemical properties viz., solubility, solution stability and low phosphate content.
- FPC does not require processing and storage by the reticuloendothelial system to provide active iron for tissue metabolism.
- Ferric iron (Fe^{3+}) is the active form of iron required by tissues including the erythrocytes. Ferric iron is bound by transferrin to transport iron to tissues and to prevent non-transferrin-bound iron (NTBI) generation of reactive oxygen species via the Haber-Weiss reaction.
- FPC is added to the hemodialysate via the bicarbonate concentrate and diffuses across the dialyzer membrane.
- In order to further characterize FPC, investigations into the solid and solution phases were conducted.

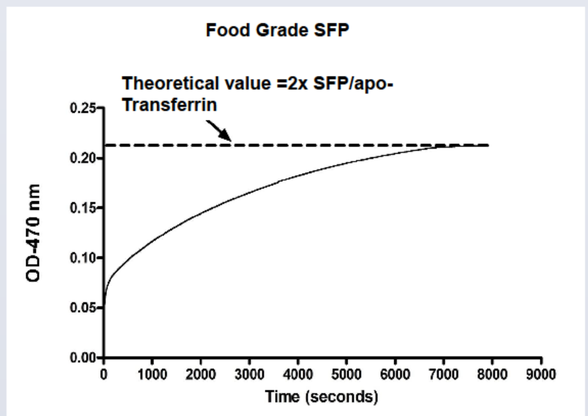
Objectives

- Demonstrate the rapid donation of FPC iron to apo-transferrin *in vitro*.
- Establish the solid and solution phase characteristics of FPC using X-ray absorption spectroscopy (XAS) to determine the co-ordination environment of iron and its neighbor atoms.
- Propose a possible solution phase structure for FPC in water.
- Establish the components of FPC bound to human transferrin.
- Demonstrate the lack of non-transferrin-bound iron (NTBI) *in vivo*.

In Vitro Donation of FPC Iron to Human Apo-transferrin

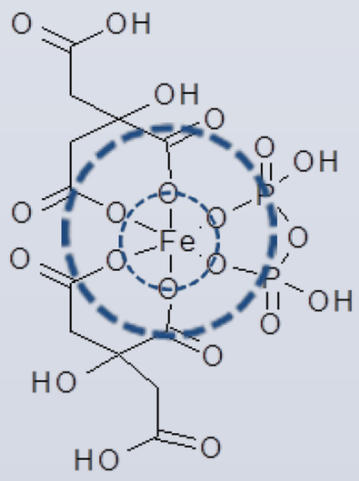
- Binding of iron from FPC to human apo-transferrin was monitored by following the change in absorbance at 470 nM, the maximum absorbance of ferric (Fe^{3+}) transferrin.
- Human apo-transferrin (hu-apo-Tf) at 40 μM was reacted with FPC at 80 μM Fe at pH 7.4 in Tris-bicarbonate buffer at 37 °C. Ferric nitriloacetic acid (Fe-NTA) and ferric citrate were used as controls. Food grade soluble ferric pyrophosphate (Paul Lohman Inc) was also used for a comparison.
- Under identical experimental conditions, 75% saturation of hu-apo-Tf occurred at <10 sec, 15 sec and >9X10^4 sec for Fe-NTA, FPC and Fe citrate respectively.
- Food grade FPC was an inefficient donor of iron to hu-apo-Tf (75% saturation >15 minutes).
- These results indicate that FPC can donate iron directly to hu-apo-Tf and the reaction proceeds with fast binding kinetics.

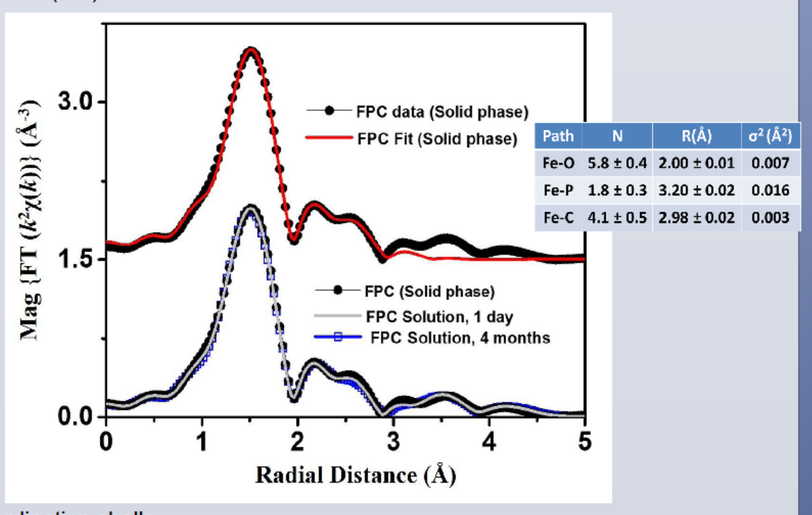




PROPOSED COORDINATION STRUCTURE OF FPC BASED ON EXAFS DATA

- X-ray powder diffraction (XRPD) and X-ray absorption spectroscopy (XAS), X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) were performed at Illinois Institute of Technology and Argonne National Laboratory by the author (BM).





Path	N	R(Å)	σ²(Å²)
Fe-O	5.8 ± 0.4	2.00 ± 0.01	0.007
Fe-P	1.8 ± 0.3	3.20 ± 0.02	0.016
Fe-C	4.1 ± 0.5	2.98 ± 0.02	0.003

- Dotted lines represent 1st and 2nd coordination shells.
- Shell by shell fitting was done using iFEFFIT software package.
- Linear combination fitting of Fe XANES data suggest that the primary coordination sphere has contributions from pyrophosphate and citrate.
- EXAFS analysis demonstrates that Fe^{+3} is complexed with O as the nearest neighbor (2.02 Å), P (3.22 Å) and C (2.98 Å) as the next-nearest neighbors

CONCLUSIONS FROM EXAFS WORK

- **FPC is a purely Fe^{3+} based compound.**
- Fe^{3+} in FPC is complexed with one pyrophosphate and two citrate molecules, such that two Fe-O-P bonds are formed and four Fe-O-C bonds are formed.
- Complexation of Fe^{3+} with two citrate molecules makes it more soluble and stable.
- The coordination environment of Fe^{3+} in FPC remains unchanged in solution phase.
- **FPC is stable in aqueous solution.**

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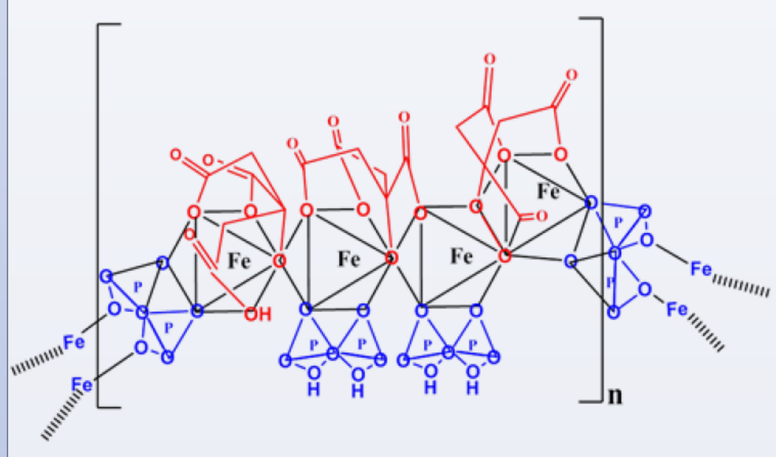
PROPOSED SOLUTION STRUCTURE OF FPC

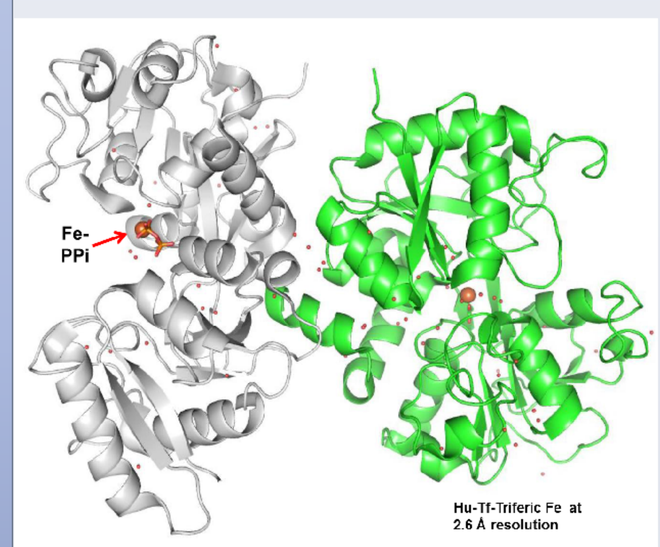


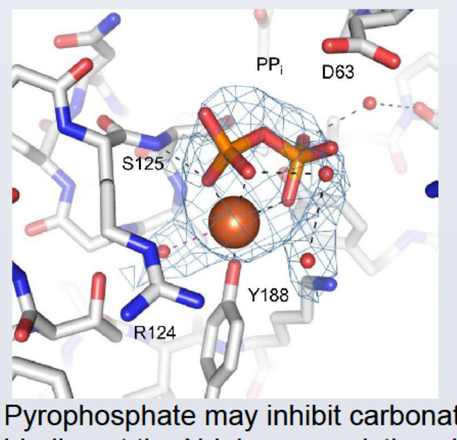
- FPC, an iron replacement product, is a mixed-ligand iron complex in which iron(III) is bound to pyrophosphate and citrate.
- The molecular formula is $Fe_4(C_6H_4O_7)_3(H_2P_2O_7)_2(P_2O_7)$.
- FPC has a relative molecular weight of approximately 1313 daltons.

TRANSFERRIN CO-CRYSTALLIZATION WITH FPC

Crystallization conditions:

- Sparse matrix screening in 96-well format with 0.4 μL of protein with 0.4 μL of precipitant equilibrated against 80 μL of reservoir at 16 °C.
- Human apo-transferrin was crystallized and structure confirmed to be iron free.
- FPC co-crystallization was performed under similar conditions.
- Crystals examined using synchrotron radiation.

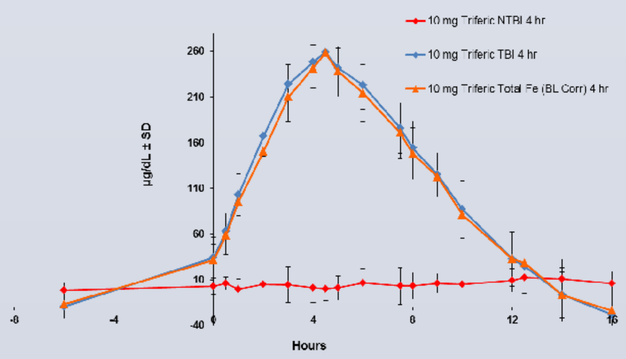




- Pyrophosphate may inhibit carbonate binding at the N-lobe synergistic anionic site in hu-Tf under study conditions
- C-lobe of hu-Tf is fully folded with carbonate in the anionic site.

- Crystal structure demonstrates a partially open di-ferric hu-Tf structure.
- FPC can donate iron to both lobes of hu-Tf:
  - The C lobe binds Fe^{+3} with carbonate as the synergistic anion
  - The N lobe binds Fe^{+3} with PP_i as a synergistic anion
- Similar to the partially open di-ferric structure with sulfate as the synergistic anion (Yang et al. Scientific Reports 2012).
- Pyrophosphate may play an important role in the donation of iron directly to hu-Tf in-vivo.

FPC ADMINISTRATION DOES NOT GENERATE NTBI



- 10 mg FPC administered IV over 4 hours to healthy volunteers.
- TSAT = ~100%.
- Total Fe and TBI measured on same sample
- No NTBI demonstrated.

CONCLUSIONS

- **Triferic, a soluble, complex iron salt, is the first parenteral pharmaceutical iron product that does not contain a carbohydrate shell and has high solubility and stability in aqueous solutions.**
- **Triferic exhibits a distinct and unique molecular structure in solid phase that remains stable and intact in solution.**
- **Triferic rapidly donates iron directly to transferrin.**
- **Co-crystallization with its natural ligand, hu-apo-Tf, reveals that Triferic donates iron to both lobes of Tf, and pyrophosphate is an integral part of the molecular iron complex.**
- **Triferic does not result in NTBI formation even at 100% TSAT.**
- **Triferic is the first drug approved by the FDA for delivery via the dialysate in CKD-HD patients.**
- **Triferic administered via dialysate, in clinical studies, reliably delivers iron, maintains hemoglobin and reduces the ESA and iron requirements in CKD-HD patients. The safety profile for Triferic is similar to placebo treated patients in clinical trials.**

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