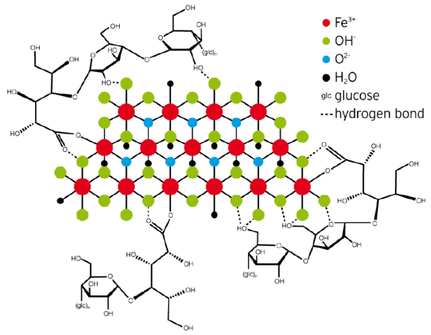
# Bioanalytical Approaches to Measure Iron Speciation in the Plasma of Patients Treated with Iron-Nanoparticle Drug Products

Heather M. Neu, Aaron D. Smith, Angela Wilks, James E. Polli, Maureen A. Kane, Tricia Y. Ting, and Sarah L. J. Michel
University of Maryland, Baltimore, MD 21201

## Background

Patients with Chronic Kidney Disease (CKD) experience severe anemia and must be treated with IV iron drugs. IV iron drugs are colloidal nanoparticles, and there is concern regarding the bioequivalence of generic and brand formulations.[1] In the US, there is a single iron product, Ferrlecit (sodium ferric gluconate) for which a generic formulation is approved (generic sodium ferric gluconate). We are performing a comparative study of the physiochemical properties of the two drugs along with a bioequivalence clinical trial on healthy patients. For the clinical trial, we are developing novel bioanalytical assays using inductively coupled plasma mass spectrometry (ICP-MS) to measure iron speciation.

## Sodium Ferric Gluconate (Ferrlecit and Generic)



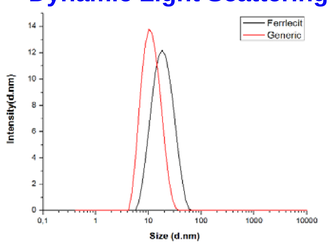




**Intravenous Iron:**
iron (III)-oxyhydroxide form stabilized by a carbohydrate complex which leads to nano-sized colloidal structures.

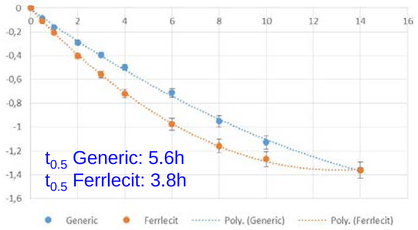
## Characterizing Ferrlecit and Generic

### Dynamic Light Scattering



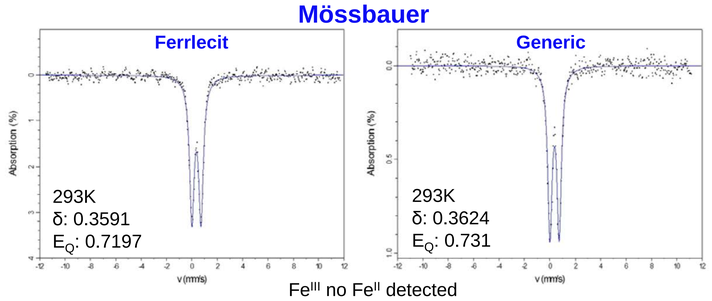

No differences in zeta potential
Generic particles are smaller

### Acid Stability




Generic is more stable against acid

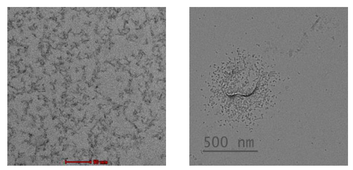
### Mössbauer




FeIII no FeII detected
Broad linewidth -> amorphous structure

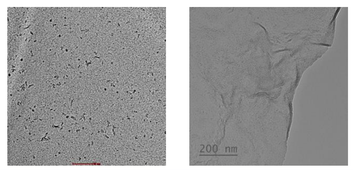
### Transmission Electron Microscopy

**Ferrlecit:**



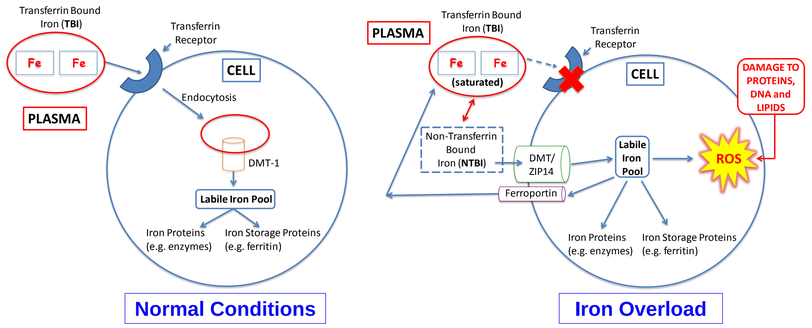

- Particles form clusters
- Amorphous structure
- Average geometrical diameter: 3.93 ± 1.08nm

**Generic:**

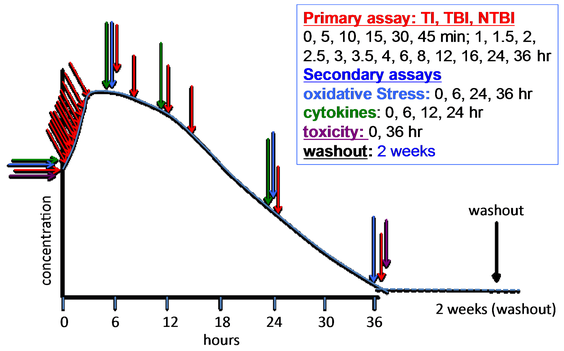



- Particles are more widely spread
- Amorphous structure
- Average geometrical diameter: 3.48 ± 0.93nm

## Iron Acquisition Pathway and Iron Overload




## Clinical Trial: Bioequivalence Study



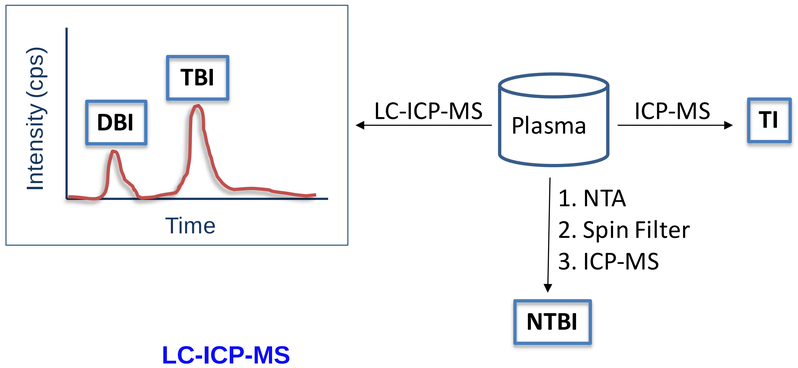

**Primary assay: TI, TBI, NTBI**
0, 5, 10, 15, 30, 45 min; 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 24, 36 hr
**Secondary assays**
**oxidative Stress:** 0, 6, 24, 36 hr
**cytokines:** 0, 6, 12, 24 hr
**toxicity:** 0, 36 hr
**washout:** 2 weeks

52 healthy subjects will be given either the generic or brand drug and the iron speciation will be measured. This will then be repeated with the other drug (crossover).

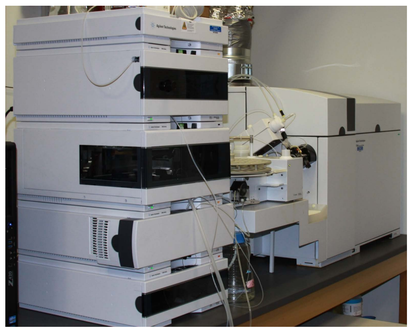
## Measuring Iron Species in Plasma

TI = TBI + NTBI
NTBI = DBI + FI + LI

**TI** = Total Iron in Plasma
**TBI** = Transferrin Bound Iron
**NTBI** = Non-transferrin Bound Iron
**DBI** = Drug Bound Iron
**FI** = Ferritin Bound Iron
**LI** = Labile Iron (Fe-Albumin, Fe-Citrate)




### LC-ICP-MS

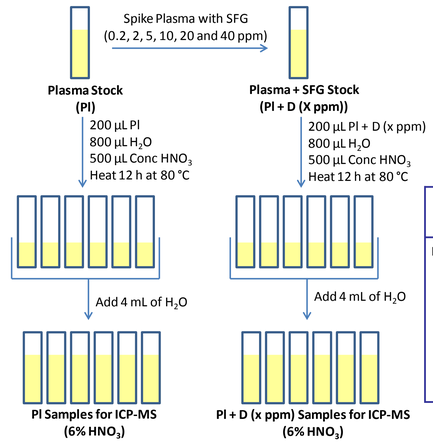


Agilent 7700 ICP-MS

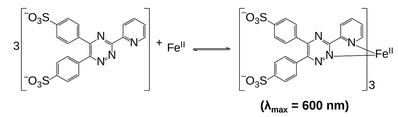
Agilent 1260 Infinity LC

BioSEC-5, 5 µm, 300 Å, size exclusion column with dimensions of 4.6 mm x 150 mm

## Ferrozine Assay vs ICP-MS for TI Measurement

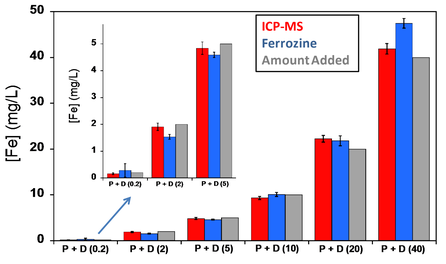



### Control: ferrozine assay

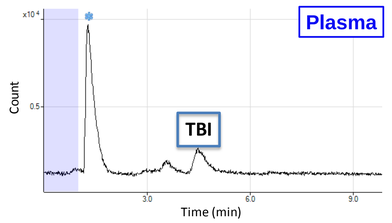


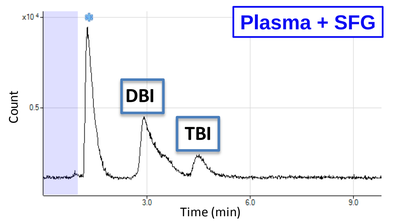

| Sample | Percent Recovered ICP-MS | Percent Recovered Ferrozine |
|---|---|---|
| Plasma spiked with 0.2 ppm SFG | 89% ± 4% | 144% ± 127% |
| Plasma spiked with 2 ppm SFG | 96% ± 7% | 77% ± 5% |
| Plasma spiked with 5 ppm SFG | 97% ± 5% | 92% ± 2% |
| Plasma spiked with 10 ppm SFG | 93% ± 3% | 101% ± 4% |
| Plasma spiked with 20 ppm SFG | 111% ± 4% | 109% ± 5% |
| Plasma spiked with 40 ppm SFG | 105% ± 3% | 119% ± 3% |

- Total iron method has been validated.
- ICP-MS and Ferrozine Assay TI measurements in good agreement
- ICP-MS readouts are more accurate than Ferrozine assay at lower concentrations




## TBI via LC-ICP-MS




Transferrin MW: around 80 kDa
SFG MW: around 300 kDa

**We can separate TBI from DBI**

## Conclusions

A comparison of the physiochemical properties of Ferrlecit and generic sodium ferric gluconate has revealed that the particle size of the generic drug is smaller than the brand. In addition, the generic sodium ferric gluconate is more stable to acid. The brand forms clusters of particles where the generic does not, suggesting that the two drugs may have different iron release properties in vivo. We have developed a novel assay to measure total iron in plasma and are developing assays to measure transferrin and non-transferrin bound iron that will use ICP-MS as the metric. These assays will be then utilized in the planned clinical trial.

## Reference

1) *European Medicines Agency Committee for Medicinal Products for Human Use* (2011) Reflection paper on non-clinical studies for generic nanoparticle iron medicinal product applications.
2) Winter WE, Bazydlo LA, & Harris NS (2014) *Lab. Med.* 45(2):92-102.
3) Pai AB, *et al.* (2007) *Pharmacotherapy* 27(3):343-350
4) Manley SA, Byrns S, Lyon AW, Brown P, & Gailer J (2009) *J. Biol. Inorg. Chem.* 14(1):61-74.

## Acknowledgements

FDA grant (UO1FD005266). We thank Peter Langguth and Julia Schnorr, Dept. of Biopharmaceutics and Pharmaceutical Technology, Johannes Gutenberg University Mainz, Germany for the physciochemial characterization of Sodium Ferric Gluconate.

University of Maryland School of Pharmacy

FDA