BLOOD PROCESSING FOR IRON CHELATION USING MICROREACTORS

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PROBLEM

Genetic blood disorders like thalassemia and hemochromatosis require regular blood transfusions leading to iron accumulation in the body. Because the body cannot naturally remove excess iron, patients with iron overload can experience severe tissue and organ damage.

OPPORTUNITY

Current treatments administer chelating agents to a patient's bloodstream to bind iron. The resulting molecules are inert and can be removed by the liver. However, this treatment is expensive and can lead to adverse side-effects. Our opportunity is to design a device with chelators immobilized in microchannels to remove iron externally.

OBJECTIVES

- Devise an effective immobilization mechanism to attach an iron chelator to a polymer surface for application to a microreactor device.
- Compare the ability of the chelator in free solution to that of the chelator immobilized on a surface in extracting iron from serum.

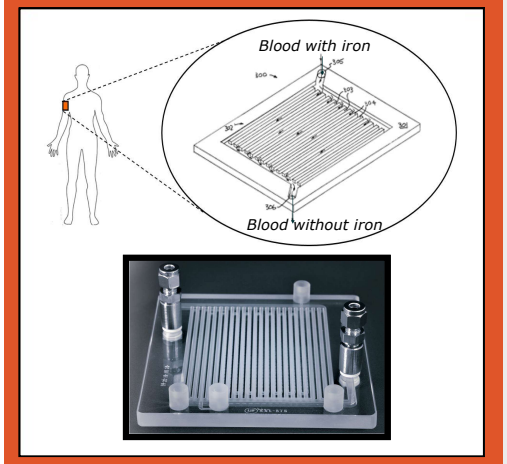


***Figure 1**: Diagram displaying the relative size of the envisioned extracorporeal microreactor and a realistic envision of the microreactor design. Bottom image taken from Amar Equipments Pvt. Ltd.*

CHELATOR PROPERTIES

Name: Deferoxamine (DFO)

Chemical Formula: $C_{25}H_{48}N_3O_4$

Color in solution: Clear

Binding Ratio: One DFO molecule binds one iron

Name (bound with iron): Ferrioxamine (FO)

Chemical Formula: $C_{25}H_{48}N_3O_4Fe$

Color in solution: Orange/brown

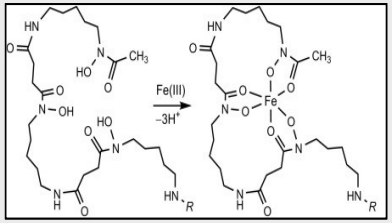
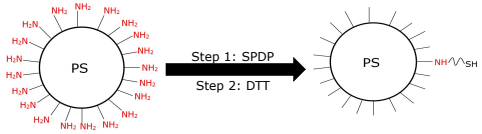


***Figure 2**: Deferoxamine (left) binds with one ferric ion to form ferrioxamine (right). The R-group represents a hydrogen atom for DFO in solution and represents the immobilization surface for the DFO immobilized.*

***Figure 3**: Test tubes showing the color of DFO (left), iron (middle), and FO (right) in solution.*

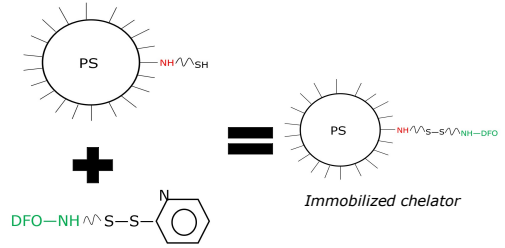
IMMOBILIZATION METHODS

Aminated microspheres are modified using the SPDP short-chain crosslinker and then reduced to produce a sulfhydryl end.



Deferoxamine is modified with the SPDP short-chain crosslinker.

These products are then combined and react to form polystyrene microspheres with immobilized DFO.



MATERIALS & EQUIPMENT

- Aminated 1 µm polystyrene microbeads to model the anticipated microreactor material
- Succinimidyl 3-(2-pyridyldithio)propionate (SPDP) short-chain crosslinker for amine-to-sulfhydryl conjugation
- Dithiothreitol (DTT) reducing agent for disulfide bonds
- VICTOR 3V Multilabel Plate Reader Spectrometer for quantitation analysis
- Centrifuge for separation of microbeads from supernatant

EXPERIMENTAL RESULTS

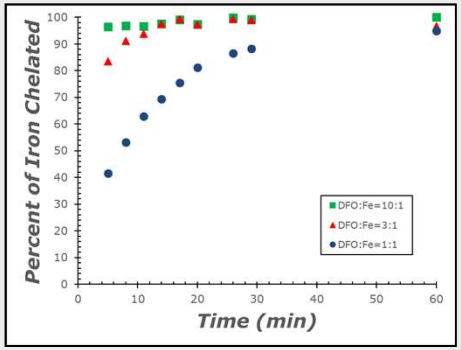


***Figure 4**: Kinetics of ferric iron chelation over 60 minutes. Higher DFO concentrations increased the chelation rate.*

EXPERIMENTAL RESULTS

- Deferoxamine is an effective chelator of iron in free solution, even if it is present in a minimum stoichiometric ratio (1 to 1).
- Sodium hydroxide is an effective regeneration agent for DFO. Most of the iron is released (~97%) when sodium hydroxide is added to a solution containing ferrioxamine.[1]
- Preliminary experiments and observations indicated that deferoxamine was successfully immobilized onto the surface of the microbeads. This immobilized DFO retains functionality as a chelator and still binds to iron (see below).

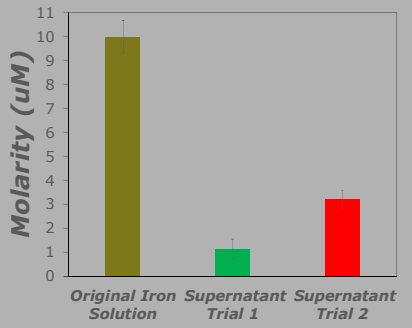


***Figure 5**: After combining a 10 µM iron solution with immobilized-DFO microbeads, the molarity of free iron dropped, indicating 90% iron removal in the first trial and 70% iron removal in the second trial. The amount of immobilized DFO present in each trial was uncertain, but results indicate that immobilization of DFO is occurring.*

FUTURE WORK

- Evaluate the chelating performance of immobilized DFO vs. DFO in solution.
- Evaluate the immobilized DFO performance with serum.
- Design and create a physical model of the microreactor.
- Simulate microreactor performance using MATLAB and COMSOL.

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REFERENCES

[1] – Saito, Hiroshi. *Method of Determination of Iron in the Form of Ferrioxamine in Urine.* Clinical Chemistry, Vol. 1, No.1, 1971
[2] – Touma, Jad. *Treatment of Iron Overload Patients via Extracorporeal Chelation in Microfluidic Devices: Research Report.* Oregon State University. Fall 2017

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