Advanced Characterization of Injectafer®: Carboxymaltose Structure, Iron Core Structure, and Interaction of Carboxymaltose with Iron Core

T1430-07-46

Md Abdullah Shamim[1], Tianyun Zhang[1], Nicholas Huls[1], Eric J. Munson[1], Dahui Liu[2], Kang Chen[3], Haiou Qu[3], Li Zhang[4], Wenlei Jiang[4], Yan Wang[4], and Deyi Zhang[4]

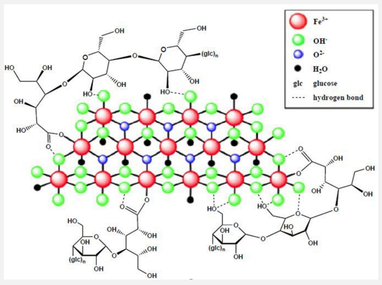
[1]Department of Industrial and Molecular Pharmaceutics, Purdue University, West Lafayette, IN
[2]Office of Pharmaceutical Quality Assessment II, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD
[3]Office of Pharmaceutical Quality Research, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD
[4]Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD

CONTACT INFORMATION: munsone@purdue.edu

aaps PharmSci 360

PURPOSE

Intravenous (IV) iron complexes are widely used for the treatment of iron deficiency anemia. The carbohydrate ligands interact with the polynuclear iron-oxyhydroxide/oxide core and thus stabilize the iron core, enabling the controlled release of iron [1]. Different types of iron colloids have different molecular weights of carbohydrate ligands. **Injectafer® (ferric carboxymaltose, FCM)** is a high molecular weight IV iron complex that allows for the administration of a maximum of 1000 mg iron in a single IV dose in as little as 15 minutes [2]. However, the carboxymaltose (CM) ligand, iron core, and the interaction of carbohydrate ligand with the iron core in Injectafer® are not fully characterized.



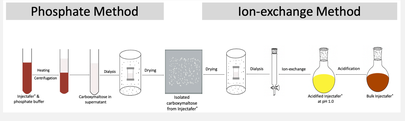
OBJECTIVES

Characterize Injectafer® to understand

I. The structure of carboxymaltose.
II. The structure of the iron core.
III. The interaction of carboxymaltose with the iron core.

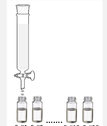
METHODS

I. Isolation of carboxymaltose from Injectafer®

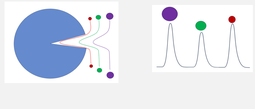


II. Molecular weight distribution of carboxymaltose in Injectafer®

a. Preparative-scale size exclusion chromatography



b. Separation is based on the hydrodynamic size of carboxymaltose



RESULTS

1. Structure of carboxymaltose

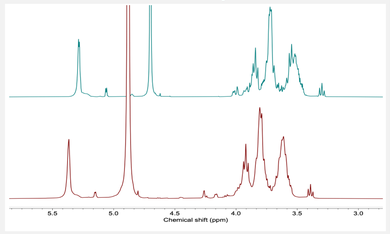


Figure 1: ^{1}H NMR spectra of isolated carboxymaltose from Injectafer® using ion exchange (top) and phosphate (bottom) methods.

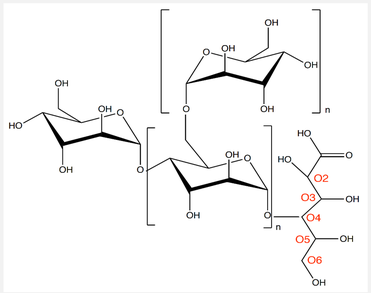


Figure 2: Representative structure of carboxymaltose from Injectafer®.

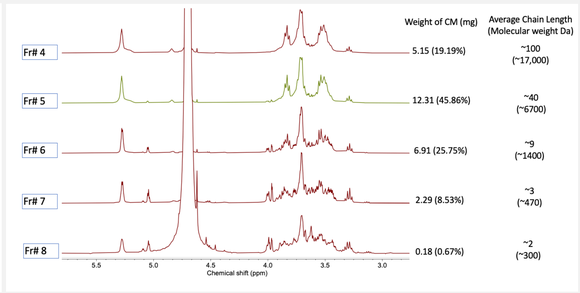


Figure 3: ^{1}H NMR spectra of preparative-scale size exclusion chromatography fractions of isolated carboxymaltose from Injectafer®.

Based on the elemental analysis (data not shown) and ^{1}H NMR spectra (**Figure 1**), the ion exchange method yields ~ 95 mg of carboxymaltose from 1 mL of Injectafer®. The isolated carboxymaltose contains different molecular weight species with an average chain length of approximately 13 as shown in **Figure 3**.

2. Iron core structure

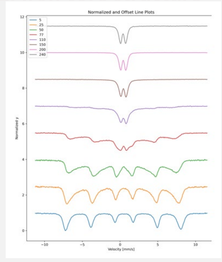


Figure 4: Mössbauer spectra of the iron core in Injectafer® ranging from 240 to 5 K (top to bottom).

Mössbauer spectra of the iron core exhibit a doublet at higher blocking temperatures (from 240 to 110 K). However, the hyperfine sextet is observed at lower blocking temperatures from 77 to 5 K.

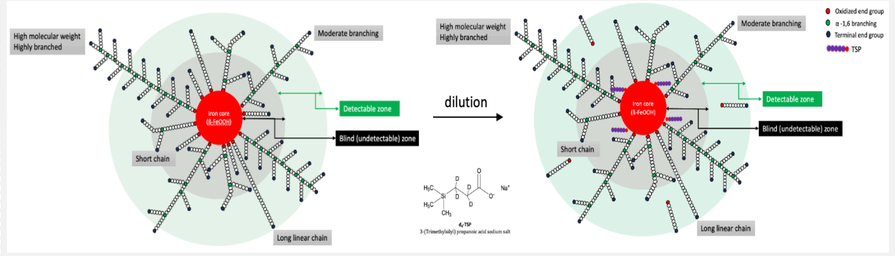


Figure 6: A simplified model of how different molecular weights of carboxymaltose interact with the iron core in Injectafer®. The highly branched long carboxymaltose ligand may wrap around the iron core instead of sticking out as drawn on the left. The low molecular weight carboxymaltose dissociates from the iron core upon dilution of Injectafer® as shown on the right. Small molecules such as 3-(trimethylsilyl)propanoic acid (TSP, pink dots) have higher binding affinity than the low molecular weight carboxymaltose and can competitively bind to the iron core.

3. Interaction of carboxymaltose with the iron core

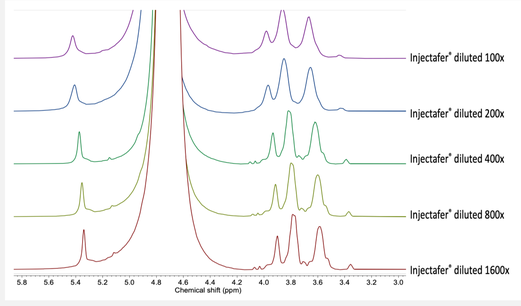


Figure 5: ^{1}H NMR spectra of diluted Injectafer® in D_2O.

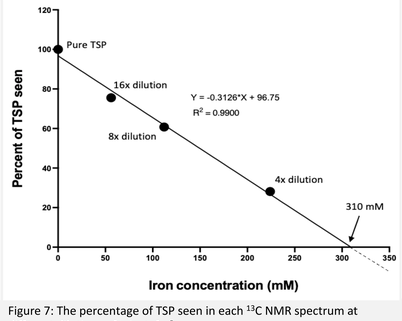


Figure 7: The percentage of TSP seen in each ^{13}C NMR spectrum at different dilutions of Injectafer®.

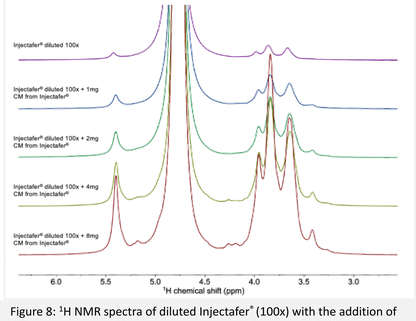


Figure 8: ^{1}H NMR spectra of diluted Injectafer® (100x) with the addition of different amounts of isolated carboxymaltose from Injectafer®.

The low molecular weight carboxymaltose starts to dissociate from the iron core at 400x dilution of Injectafer® as indicated by the presence of O2/O3 protons at ~ 4.1 ppm in **Figure 5**. The low molecular weight carboxymaltose dissociates faster (weaker interaction) than the high molecular weight carboxymaltose, as shown in **Figure 6**. Approximately 85% of the surface area of the iron core is saturated by the carboxymaltose as calculated in **Figure 7**. Low molecular weight compounds such as TSP (MW: 172 g/mol) appear to have a higher binding affinity to the iron core compared to the low molecular weight carboxymaltose. Approx. 1 mg of added isolated carboxymaltose from Injectafer® can completely bind with the iron core. Adding additional carboxymaltose will be observed as the presence of O3/O2 and terminal peaks (~ 3.45 ppm) as shown in **Figure 8**.

CONCLUSIONS

1. Structure of carboxymaltose

- To separate carboxymaltose from the iron core in Injectafer®, phosphate and ion-exchange methods were studied. Based on the ^{1}H NMR spectrum, the phosphate method yields only 63% of carboxymaltose as compared to the elemental analysis of dried Injectafer® (data not shown). The lower yield is due to several drawbacks of this method, such as heating the complex mixture at a higher temperature, which may cause degradation of the carboxymaltose chain.
- However, the ion exchange method yields 100% of carboxymaltose, although a trace amount of iron (~ 0.15%) is still present in the isolated carboxymaltose, which does not create any challenges in further analysis.
- Approximately 65% of carboxymaltose has higher branching with an average chain length of ~ 40-100. The remaining carboxymaltose consists of either long or short chains, with an average chain length of 2-9. Based on the ^{1}H NMR spectra, the average chain length of carboxymaltose is ~ 13 (weight average molecular weight ~ 2153 Da).

2. Iron core structure

- Mössbauer spectra suggest an akaganeite-like structure and a lower crystallinity of the iron core in Injectafer®.

3. Interaction of carboxymaltose with the iron core

- Overall, ~ 85% of the surface area of the iron core is saturated with carboxymaltose of various chain lengths.
- Different molecular weights of carboxymaltose have different degrees of interaction with the iron core. The low molecular weight carboxymaltose dissociates faster than the high molecular weight carboxymaltose. It suggests that the low molecular weight carboxymaltose weakly interacts with the iron core compared to the high molecular weight carboxymaltose. In addition, the interaction of carboxymaltose with the iron core is an equilibrium process and extreme dilutions are required to dissociate the weakly-bound carboxymaltose ligands from the iron core.
- Low molecular weight compounds, such as TSP, exhibit higher binding affinity – it can bind to the free space of the iron core and the free space left by the low molecular weight carboxymaltose once it is dissociated upon dilution. However, due to steric hindrance, the high molecular weight carboxymaltose, with a higher degree of branching, exhibits lower binding affinity to the iron core compared to the low molecular weight compounds (TSP) and maltodextrin, which have no carboxy group (data not shown).

REFERENCES

[1] Zou, Peng, *et al.* "Physicochemical characterization of iron carbohydrate colloid drug products." The AAPS journal 19 (2017): 1359-1376.
[2] FDA. Injectafer. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/203565s014lbl.pdf

FUNDING / GRANTS

- This research was funded through a contract from the U.S. Food and Drug Administration (FDA) (75F40121C00189). This poster reflects the view of the authors and should not be construed to represent the FDA's views or policies.

PURDUE COLLEGE OF PHARMACY

FDA