



Brief Report

How Reproducible Is Feraheme[®] (Ferumoxytol Injection)? Comparison of Size, Zeta Potential, and Complement Activation of Different Batches over 15 Years

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Abstract: Ferumoxytol injection, also known as Feraheme[®], is an approved IV injectable iron supplement and an experimental MRI contrast agent. Initially, it was approved as an IV bolus agent, but its use was later limited to a slow infusion drip due to high levels of infusion reactions. We collected various batches of ferumoxytol with expiration dates ranging from 2010 to 2025 and compared their size and zeta potential. Since nanoparticle surface properties can affect infusion reactions, we conducted a dot blot immunoassay to measure complement C3 opsonization with ferumoxytol preparations. We observed differences in nanoparticle size and zeta potential between batches and a 2.5-fold variation in complement activation. Interestingly, older batches from 2010 showed more uniform size distribution and lower complement activation than some of the newer batches. This finding may be valuable to the nanomedicine community and regulatory authorities.

Keywords: ferumoxytol; Feraheme; iron oxide nanoparticle; size; batch; zeta potential; complement; variability



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1. Introduction

It is widely recognized but rarely acknowledged that nanoparticles synthesized in academic laboratories are incompletely characterized and lack demonstration of synthetic reproducibility [1]. Academic reports are further confounded by incomplete descriptions of tests used for nanoparticle acceptance, quality control specifications, and failure/acceptance ratios of syntheses. Hopefully, this situation does not apply to pharmaceutically approved nanoparticles where substantial reports of synthesis, quality control methods, animal toxicology, and human testing are required for regulatory approval. Unfortunately, all this information is unavailable to the general scientific community. Furthermore, problems associated with the scale-up of syntheses, as well as the transfer of synthetic procedures from “in-house” to contract manufacturing organizations (CMOs), may fail to recognize small changes in nanoparticle physicochemical properties even though all quality control specifications are met [2]. Ferumoxytol (Feraheme[®]) is a clinically approved ultrasmall superparamagnetic iron oxide nanoparticle (USPION) coated with reduced carboxymethyl dextran T-10 for iron deficiency [3]. Ferumoxytol was initially approved as a bolus agent and was administered as a bolus injection in earlier clinical studies [4,5]. While ferumoxytol was proven to be safe in the initial clinical studies, subsequent studies revealed the emergence of infusion reactions in patients. The US Food and Drug Administration (FDA) put a black box warning on the label, necessitating premedication and slow infusion [6]. While the reason for this has not been thoroughly investigated, ferumoxytol went through a series of production and manufacturer changes associated with scale-up, which could explain the physiological deviations seen from the earlier in-house preparations. In this short report, we ask how reproducible ferumoxytol preparations are when sampled over fifteen years.

We address this question by comparing various batches of ferumoxytol injections using size measurements, zeta potential, and deposition of the C3 complement component in human serum. These measurements show a drift to larger size, increased zeta potential, and variable C3 complement activation over 15 years.

2. Materials and Methods

All ferumoxytol samples were obtained from Advanced Magnetix or the Anschutz Medical Center Infusion Pharmacy and stored in sterile vials at 4 °C. The expiration dates of the various batches studied spanned from 2010 to 2025 (Table 1). For size determination, ferumoxytol was diluted to an iron concentration of 1 mg Fe/mL in deionized water. While mixing, 10 µL of standard phosphate buffered saline was added to 3 mL of water (25 °C), followed by 10 µL of diluted ferumoxytol (this resulted in a pH of 6.9). The solution was maintained for three minutes at 25 °C and then introduced to a Malvern ZetaSizer Nano. The signal was acquired for 5 min. For zeta potential measurements, ferumoxytol was diluted to an iron concentration of 1 mg Fe/mL in deionized water. While mixing, 10 µL of 0.15 M sodium chloride was added to 1 mL of water (25 °C), followed by 10 µL of diluted ferumoxytol. The solution was maintained for three minutes at 25 °C and then introduced to a Malvern ZetaSizer Nano. Data were acquired as intensity-weighted diameter in duplicates. C3 complement measurements were determined in human serum from a healthy male volunteer using a validated dot blot immunoassay [7] at a final concentration of 0.25 mg/mL in 75% serum. Goat IgG fraction against human C3 (Cat#55033) that was previously validated to recognize activated C3 isoforms was from MP Biomedicals (Solon, OH, USA), and secondary antibody donkey anti-goat-IRDye800CW was from Li-COR Biosciences (Lincoln, NE, USA).

Table 1. Batches of ferumoxytol and size measurements. The measurements were performed at the same time on all batches (repeated in duplicates).

	Expiration Date	Lot	Z-Average nm	Poly Dispersity Index	Peak 1 Mean Intensity	Peak 2 Mean Intensity	Pk 1 Area Intensity	Pk 2 Area Intensity
1	02/2010	06021502	30	0.19	32	0	100	0
1	02/2010	06021502	29	0.15	33	0	100	0
2	02/2014	10021802	33	0.23	38	4362	98	3
2	02/2014	10021802	31	0.20	33	4320	97	4
3	09/2023	LT7285	38	0.24	42	4510	96	4
3	09/2023	LT7285	41	0.17	38	5451	99	1
4	06/2024	AL8360D	43	0.28	51	4136	95	5
4	06/2024	AL8360D	46	0.25	63	3888	98	2
5	11/2024	AM4444B	52	0.32	60	4718	95	5
5	11/2024	AM4444B	51	0.31	60	4716	96	4
6	04/2025	AN1173C	70	0.18	38	0	100	0
6	04/2025	AN1173C	77	0.17	37	0	100	0
7	08/2025	AM8228B	45	0.18	33	0	100	0
7	08/2025	AM8228B	52	0.14	36	0	100	0
8	11/2025	AP4322B	37	0.24	37	5224	97	3
8	11/2025	AP4322B	36	0.24	42	3980	96	4

3. Results

The results are presented in Table 1 and Figure 1. Table 1 includes all sizing information and shows consistent increases in all parameters measured as a function of expiration date starting in 2010 and ending in 2025. Zeta potential values stayed between -30 mV and -40 mV for all batches (Figure 1A), whereas C3 deposition was highly variable (Figure 1B). The size (Z-average) showed a direct but not significant correlation with complement activation (Figure 1C).

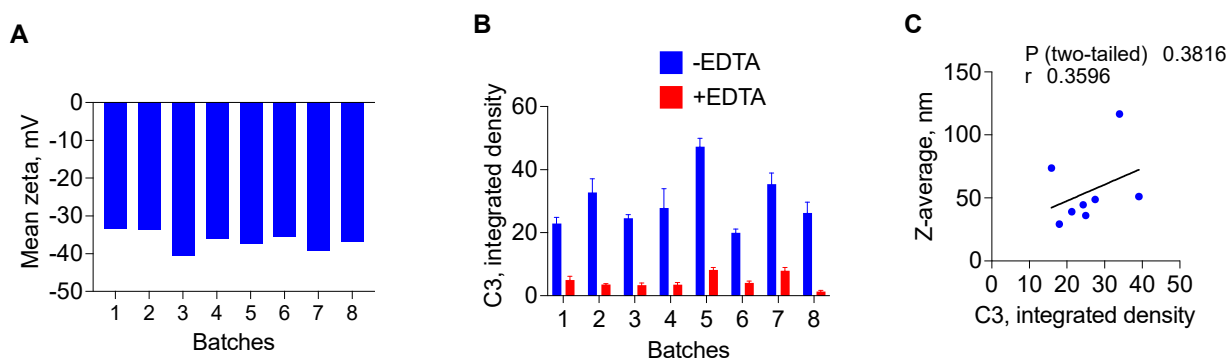


Figure 1. (A) Zeta potential, (B) complement C3 deposition. Blue bars, human serum; red bars, serum plus 10 mM EDTA; (C) correlation of complement with size (Z-average) across batches. The measurements were taken at the same time on all batches. The data are an average of between two (size) and three (zeta potential and complement) technical replicates.

4. Discussion

While we could not find any specific FDA document dealing with iron oxide nanoparticles, the guidance related to liposomes and nanomaterials [8,9] appears to indicate that the agency expects the manufacturer to monitor the size changes on the nanoparticle. Our data suggest substantial changes in the size of the product, with older batches anecdotally having a smaller size, as described in earlier publications [10]. It is important to note that anomalies may arise in the synthesis of nanoparticles that are too subtle to detect both before and after particles are approved for sale. These alterations in the properties of nanoparticles may or may not lead to the degradation of the safety and efficacy of the nanoparticle.

Due to the proprietary nature of ferumoxytol synthesis, we relied on the information for the synthesis of ferumoxytol found in US patent 7553479 [11]. Ferumoxytol is prepared from ferric and ferrous chlorides, carboxymethyl-reduced dextran, water, and ammonium hydroxide. From these simple ingredients, we note several points in the acquisition of starting materials and synthesis of ferumoxytol that may affect issues related to synthetic reproducibility. These include lot-to-lot uniformity of dextran both in molecular weight and branching, uniformity in the synthesis of carboxymethyl-reduced dextran, the method of dissolving and mixing all components prior to adding ammonium hydroxide, and the addition of ammonium hydroxide. Numerous examples of “successful” syntheses and “failed” syntheses are presented within the patent to support the claims. Unfortunately, no single example within the patent is identified as a pharmaceutical product.

An example of a subtly small change to dextran that results in a large and unpredicted change in the synthesis of iron oxide nanoparticles is illustrated by the reduction of the terminal glucose residue of dextran. This reduction affects only one of sixty glucose residues in a 10,000 Dalton dextran but results in a 10-fold decrease in the amount of dextran used for particle synthesis compared with the amount of native dextran used in an otherwise identical synthesis. Furthermore, particles prepared with reduced dextran yield a stable particle of identical size pre- and post-autoclaving. Particles prepared with native dextran are not stable when autoclaved [12].

In a second example, we note that phase 3 clinical studies administered ferumoxytol as an undiluted IV push injection. Quoting from the publication, “Finally, the ability to administer ferumoxytol in dosages up to 510 mg as a rapid intravenous injection could facilitate iron deficiency anemia management in the physician’s office” [13]. This observation suggests that ferumoxytol represented a major step forward in the safe administration of an iron oxide nanoparticle. The addition of a black box warning on the label [6] could be a result of subtly small changes caused by scale-up and use of a contract manufacturer. We further suggest these changes were undetected by the standard quality control procedures for approval of ferumoxytol production lots. Another possibility is batch-to-batch variation, but there was not enough statistical power to confirm this, and we had no access to batches manufactured close to each other.

We believe that ferumoxytol remains a safe and effective drug for treating anemia. However, we urge those responsible for releasing ferumoxytol to stay vigilant about the quality and uniformity of both ferumoxytol production and changes that may occur in the bottle before the expiration date.

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