Waters™

Application Note

SEC- and FFF-MALS Multi-Attribute Quantification of Colloidal Iron Drug Products

Martin Kurnik, Marisa Herrera, Michelle Chen

Waters Corporation, United States

Published on October 30, 2026

Abstract

Intravenous iron administration is used to treat iron deficiency anemia in multiple medical specialties including nephrology, gastroenterology, cardiology, oncology, and gynecology. Current drug products like iron sucrose, ferric carboxymaltose, and ferumoxytol are designed to circumvent free iron toxicity by delivering the iron as nanometer-sized colloidal complexes consisting of a polynuclear Fe (III)-oxyhydroxide core stabilized by a carbohydrate shell. Complex attributes, such as total iron content, free iron and carbohydrate content, molar mass, size, shape, surface charge, stability, and immunogenicity, may influence dosing, pharmacological activity, clearance, and safety, and side effects include hypophosphatemia, transient proteinuria, cardiac and liver issues, and anaphylaxis. Consequently, robust and advanced biophysical characterization is needed in addition to basic analytical assays and clinical data for regulatory agency approval of both innovator and generic iron-based nano-colloidal products. This application note provides examples of how size-exclusion chromatography and field-flow fractionation coupled to multi-angle light scattering (SEC-MALS and FFF-MALS, respectively) can meet this characterization need and be used as orthogonal tools for high-resolution multi-attribute quantification in the development and production of safe and effective iron-carbohydrate complex drug products.

Introduction

Intravenous iron products are iron-carbohydrate complex drugs used to treat iron-deficiency anemia associated with, for example chronic kidney disease or congestive heart failure when the patient is non-compliant to oral iron therapy.[1] There are ~1.2 billion patients globally, corresponding to ~15% of the world population, and the market is expected to grow. This growth is, however, contingent on new product launches and new approvals for specific conditions and associated with multiple challenges. For example, rare but life-threatening anaphylaxis and other adverse drug reactions as well as differences in clinical efficacy and tolerability between originator and follow-on products have been reported. The molecular mechanisms behind these adverse reactions are not fully understood. Consequently, a combination of clinical safety data and biophysical characterization by multiple, orthogonal techniques are required for regulatory agency approval of both originator and generic drugs, and patient monitoring and management of hypersensitivity reactions are advised during administration.[2–4]

Approved iron carbohydrate preparations are diverse in terms of composition and properties. The iron content ranges from 12.5 mg/mL for sodium ferric gluconate to 100 mg/mL in ferric derisomaltose, with other products such as the commonly used iron sucrose, the older iron dextran, and the more recent ferumoxytol all having intermediate iron content. In addition to iron, all these products contain a carbohydrate component critical to safe administration and identity as well as the relative amounts of carbohydrate and iron also varies between them, from a 15-fold mass concentration excess in iron sucrose, to much lower carbohydrate excess in products with polymeric sugars like ferric carboxymaltose and ferumoxytol. There can also be substantial differences in the size and stoichiometry of the resulting complexes, with e.g., iron sucrose containing on average smaller particles with different shape than the particles in ferric carboxymaltose and other products made with polymeric sugars.[1] These differences between intravenous iron originator products may impact their pharmacological activity.

Size-exclusion chromatography (SEC) and field-flow fractionation (FFF) coupled to multi-angle light scattering (MALS) are excellent solutions for high-resolution quantification of colloidal iron drug products. Specifically, multiple attributes that may impact pharmacological activity may be quantified from a single SEC/FFF-MALS measurement, including complex iron content and composition, free iron and carbohydrate ligand amounts, and nanoparticle molar mass, size, and shape. SEC and FFF separate molecules and particles by size, or hydrodynamic volume. In SEC, the sample is applied to a column containing a stationary phase with controlled pore size distribution. Large particles are excluded from the stationary phase pores to greater extent than small particles and thus elute earlier. FFF, in contrast, uses an open channel without stationary phase. Two flows are applied to achieve size-based separation: a parabolic channel flow along the channel, and a perpendicular

crossflow. Small particles diffuse more effectively against the crossflow and further away from the bottom of the channel than large particles and consequently move through the channel at a higher flow velocity of the channel flow, eluting before larger particles. The crossflow exits the channel through a semi-permeable membrane at the bottom of the channel, typically made from regenerated cellulose or polyethersulfone.

Iron sucrose is currently the only colloidal iron product where guiding documentation for characterization is available.[5] The protocol relies on column calibration, which generally may be limited in applicability and accuracy since molar mass quantification is critically reliant on reproducible retention time or elution volume and column calibration assumes that the analyte and the calibration standard have identical or at least similar conformation, density or compactness, and extent of column interaction. Mismatches between calibration standard and analyte attributes may consequently yield inaccurately measured molar masses. In the specific context of iron sucrose, the molar mass standard used typically has a different chemistry than the drug product and the calibration curve uses a third order fit to peaks that are not baseline separated, which may complicate quantification and compromise the accuracy of the molar mass determination. Beyond total molar mass, additional attributes of the entire iron carbohydrate complex may also impact pharmacological activity, and a simple single-detector analysis may not be able to capture all the relevant attributes, demanding additional tools for comprehensive characterization. In contrast, SEC/FFF-MALS with differential refractive index (dRI) and UV/vis absorbance concentration detection measures the molar mass and other key attributes directly, slice-by-slice across the chromatogram/fractogram, makes no assumptions about polymer shape or density, and does not require known molar mass standards or reproducible retention times, as quantification is not reliant on a standard curve.

Experimental

The samples were transferred from sealed product vials using sterile syringes and needles before measurements. The vials were stored and handled following the manufacturers' guidelines over the course of the experiments. Samples were diluted ten-fold (iron sucrose) or 100-fold (ferric carboxymaltose, ferumoxytol) in 0.9% NaCl or phosphate-buffered saline prior to measurement.

SEC separation was performed with a Waters XBridge™ Premier Protein 250 Ångström pore size column. FFF separation was performed using a separation method optimized for colloidal iron products on an Eclipse™ FFF Instrument with a 10 kDa molecular weight cutoff regenerated cellulose membrane in a 350 µm fixed-height short channel connected to an Arc™ HPLC System pump and FTN-R sample manager. PBS was used as the

mobile phase. A DAWN™ MALS photometer with a WyattQELS™ module for online DLS, an Optilab™ differential refractometer, and a UV Detector set to 280 nm wavelength were used for online detection. The same MALS, dRI and UV Detectors can be used for both SEC and FFF, allowing the analyst to switch between SEC and FFF mode on the same station. The FFF System was controlled by VISION™ Software. Unless otherwise noted, reported uncertainties are the standard deviations from ten replicate measurements for SEC-MALS and three replicate measurements for FFF-MALS, respectively.

Results and Discussion

In the first case study, SEC-MALS was used for quantitative comparison of two lots of an iron sucrose reference listed drug (RLD) approved and commonly used to treat iron-deficiency anemia in patients with chronic kidney disease. Combined MALS, UV/vis absorbance, and dRI detection enables multi-attribute quantification from a single experiment. The results are tabulated in Table 1.

Sample	M_w main peak (kDa)	M_w / M_n	Total injected iron (µg)	*m* Fe main peak (µg)	*m* carbohydrate in inclusion limit (µg)	R_h (nm)	Conformation plot slope
SEC-MALS							
Iron sucrose RLD Lot 1	264 ± 3	1.80 ± 0.02	20	18.76 ± 0.08	297 ± 5	5.38 ± 0.01	0.48 ± 0.01
Iron sucrose RLD Lot 2	262 ± 4	1.75 ± 0.02	20	18.67 ± 0.02	277.0 ± 0.4	5.28 ± 0.01	0.44 ± 0.04
Ferric carboxymaltose RLD Lot 1	2443 ± 11	1.19 ± 0.01	10	8.91 ± 0.09	–	12.18 ± 0.05	N.D.
Ferric carboxymaltose RLD Lot 2	2508 ± 16	1.19 ± 0.01	10	8.67 ± 0.09	–	12.26 ± 0.06	N.D.
Ferumoxytol RLD	2110 ± 31	1.59 ± 0.01	6	4.90 ± 0.07	–	12.24 ± 0.07	N.D.
FFF-MALS							
Iron sucrose RLD Lot 2	283 ± 9	1.94 ± 0.09	20	19.68 ± 0.01	–	6.5 ± 0.1	0.44 ± 0.01
Ferric carboxymaltose RLD Lot 2	2802 ± 69	1.53 ± 0.06	12.5	12.20 ± 0.07	–	12.61 ± 0.06	0.35 ± 0.01

Table 1. Multi-attribute quantification of colloidal iron drug products by SEC/FFF-MALS.

The molar mass from MALS with dRI concentration detection for the large iron-carbohydrate complexes in the main peak, measured directly slice by slice across the chromatogram without the need for a molecular weight column calibration standard, is effectively identical for the two lots (264 ± 3 kDa and 262 ± 4 kDa, respectively), despite slight differences in UV elution profiles. In contrast to molar masses obtained using column calibration, these molar masses measured by MALS are insensitive to the chromatography conditions, including retention time variation due to flow rate and injection amount. The measured IUPAC dispersity, defined as the ratio of the weight-average and number-average molar mass (M_w/M_n) is also identical for the large iron-carbohydrate complexes (Figure 1, Table 1).

The same SEC-MALS experiment also quantifies the particle size, compactness, and iron and sucrose amounts (Figure 2, Table 1). Specifically, more than 90% of the iron in the sample is contained within the large complexes in the main peak (18.76 ± 0.08 µg and 18.67 ± 0.02 µg of the injected 20 µg, respectively), with only a minute amount existing as smaller species. For sucrose, which is present at a 15-fold mass excess in this iron sucrose RLD, the vast majority exists as free sucrose that elutes in the inclusion limit of the column (297 ± 5 µg and 277 ± 0.4 µg of the injected 300 µg, respectively). Only less than 10% of the total sucrose mass is contained in the large iron complexes of the main peak. Moreover, Lot 2 appears to contain slightly lower total sucrose concentration than Lot 1 (Table 1) and somewhat lower amount of the intermediately-sized iron species eluting between the main peak and the inclusion limit (Figure 1).

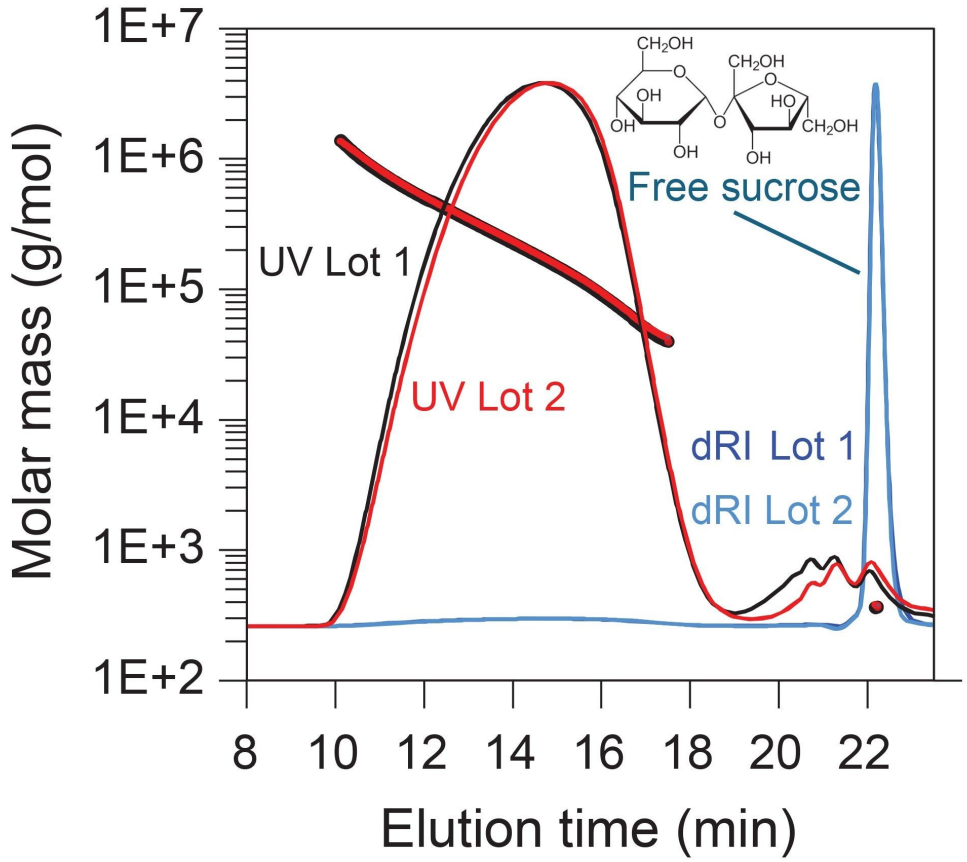

Figure 1. SEC-MALS-UV-dRI separation of two lots of the same iron sucrose product. The large iron-sucrose complexes in the two lots have effectively identical molar mass distributions.

Like for the molar mass, dispersity, and main peak iron content, the size and conformation of the main peak species are also effectively identical for the two lots of this RLD (Table 1), based on the hydrodynamic radius (Figure 2) and the complex conformation plot slope (Figure 3).

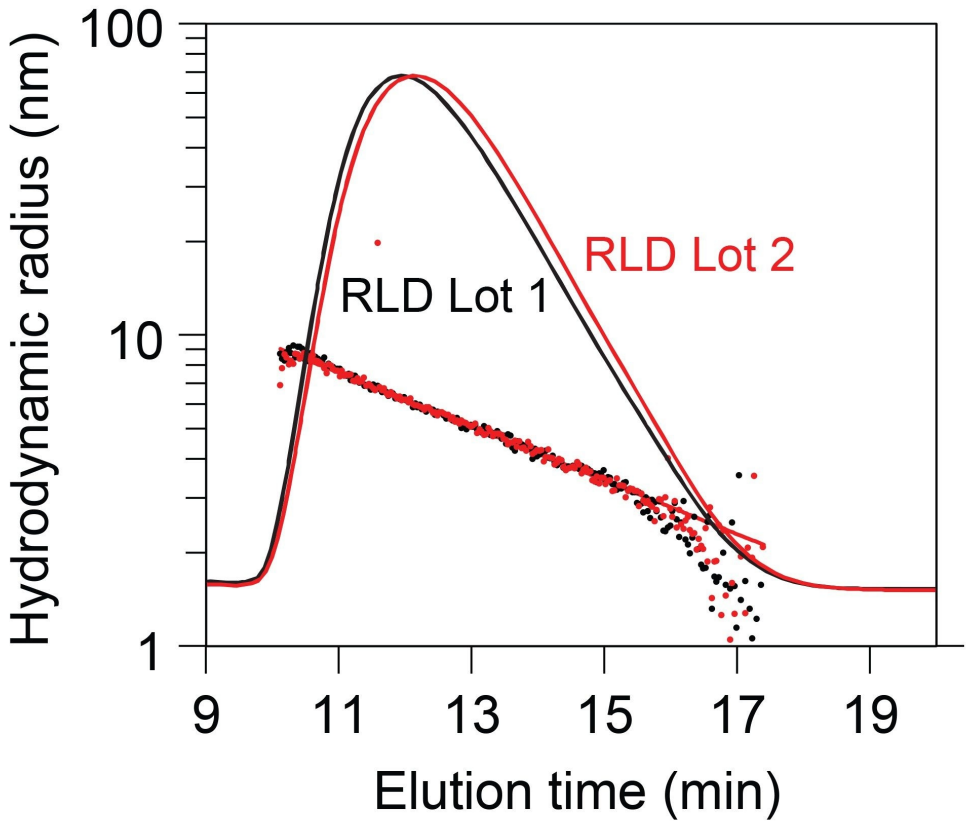

Figure 2. Hydrodynamic radius quantification of two lots of the same iron sucrose product. The size distribution of the large iron-sucrose complexes in the two lots are effectively identical.

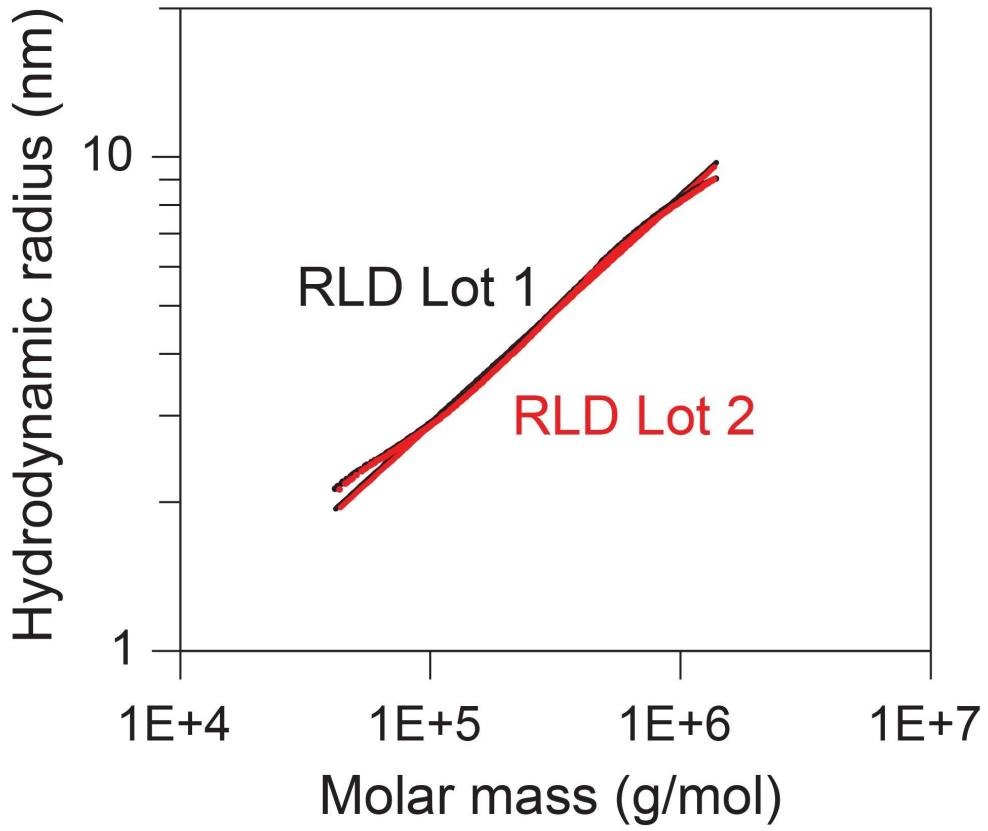

Figure 3. The conformation plot of the two iron sucrose lots confirms that the complexes in both lots have effectively identical conformation and compactness.

FFF-MALS is an excellent complement to SEC-MALS for comprehensive characterization of colloidal iron products. In the FFF experiment, species like free iron and low-molecular weight sugars like sucrose are small enough to exit through the pores of the semi-permeable membrane at the bottom of the channel and will therefore not reach the detectors downstream of the channel outlet. Only species that are large enough to not pass through the membrane will be detected. The combined FFF-MALS and SEC-MALS results thus allow for easy identification and comprehensive quantification of relative amounts of both small molecules and large molecules. The present experiment used a membrane with 10 kDa molecular weight cut-off, but cut-offs as small as 2 kDa cut off can be used to retain and detect species with lower molar masses. As seen in Figure 4, only a single peak is detected in the FFF experiment, and its attributes are consistent with those of the main peak in the SEC-MALS experiment (Table 1). This result confirms that the species in the additional peaks in the SEC-MALS

experiment are from species small enough to pass through the FFF membrane. The molar mass and size trends across the peak also corroborate that the apparent peak shoulder seen in the fractogram is not a discrete species, but rather an artifact arising from the crossflow gradient used in the FFF method that can be avoided by tweaking the FFF method crossflow. FFF also provides orthogonal quantification of the relative amounts of the different species in the sample (Table 1). The dilution control module of the Eclipse FFF instrument also adds flexibility when evaluating the concentration dependence of complex stability and size.

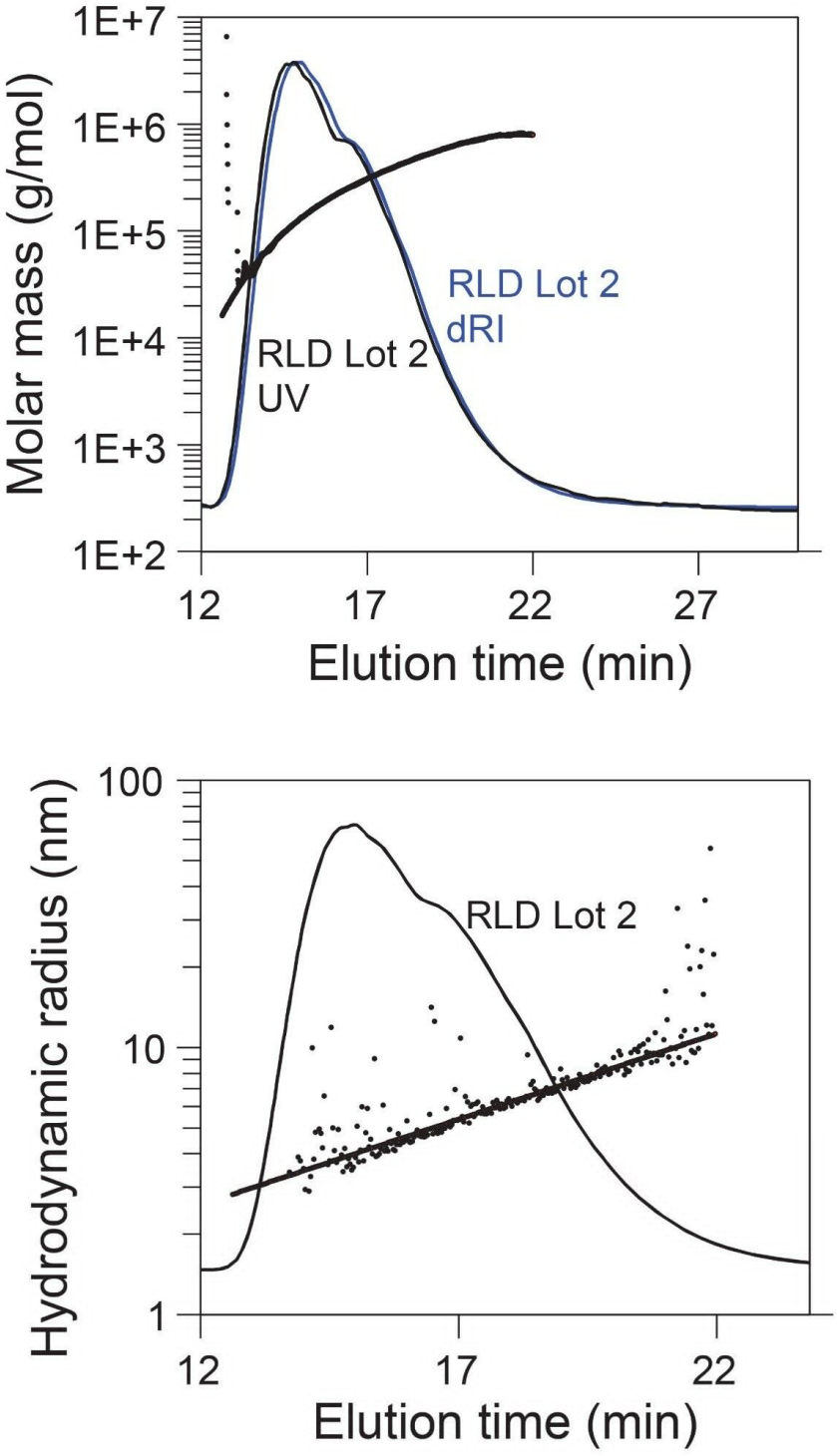

Figure 4. Iron sucrose molar mass and hydrodynamic radius measured by FFF-MALS.

Smaller species and free sucrose seen by SEC-MALS are not detected in the FFF-MALS experiment as they exit the channel through the membrane when crossflow is applied.

In the second case study, SEC-MALS was used for multi-attribute quantification of two different colloidal iron products made with polymeric sugars (Table 1). The top panel in Figure 5 shows ferumoxytol, another FDA-approved product for treatment of iron deficiency anemia in chronic kidney disease patients. In contrast to iron sucrose, ferumoxytol is made with iron oxide nanoparticles coated with carboxymethyl-dextran. Iron is only present in the main peak, and unlike iron sucrose (Figure 1), there are no trailing UV peaks from smaller iron species. The ferumoxytol particles have both higher molar mass and an average hydrodynamic radius that is about twice that of the iron sucrose particles, and the sample also has somewhat lower dispersity than iron sucrose (Table 1).

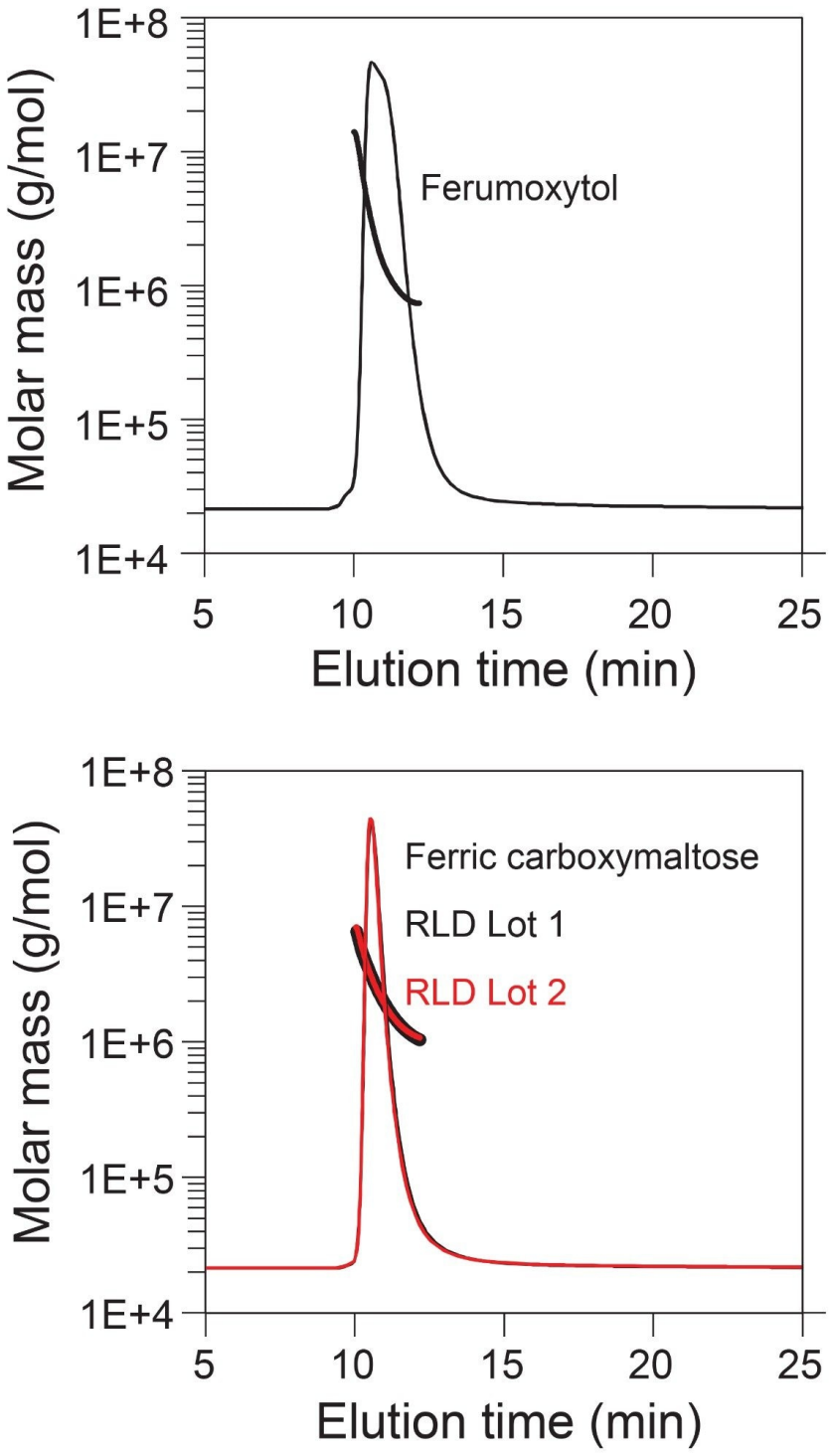

Figure 5. SEC-MALS UV chromatograms and molar mass overlays measured for

polymeric sugar iron products. (Top) Ferumoxytol. (Bottom) Ferric carboxymaltose.

Ferric carboxymaltose is another drug product approved for treating anemia in chronic kidney disease patients. As expected, and similarly to ferumoxytol, SEC-MALS reveals that ferric carboxymaltose also contains particles that have higher molar mass than those in the iron sucrose RLD (Figure 5, bottom panel), and overall the product appears less polydisperse than both ferumoxytol and iron sucrose (Table 1). Similarly to ferumoxytol, iron mass recovery is excellent, at close to 90%, with no discrete peaks from smaller iron species or free iron in the inclusion limit. That said, the particles in ferric carboxymaltose and ferumoxytol are significantly larger than iron sucrose and consequently elute closer to the exclusion limit (Figure 5). Consequently, a larger pore size column like the 450 Ångström GTxResolve™ SEC column, either alone or in tandem with a 250 Å XBridge Premier Protein column, may yield improved separation. As seen for iron sucrose, SEC-MALS is also an excellent tool for lot-to-lot comparisons of these larger colloidal iron products, enabling assessment of multiple attributes from a single experiment. The two lots of ferric carboxymaltose compared here are effectively identical across all the quantified attributes (Figure 5, Table 1).

FFF-MALS is also an excellent choice for these larger colloidal iron products made with polymeric sugars. Specifically, the tunable separation range of FFF-MALS enables separation of small species like the iron sucrose complexes (Figure 1) as well as larger species such as those in ferric carboxymaltose or even larger species that may be filtered out by SEC columns and escape detection in SEC-MALS experiments. For example, FFF delivers greatly improved separation of ferric carboxymaltose compared to the 250 Ångström SEC column (Figure 6), and the more ideal separation in the FFF experiment enables superior quantification of molar mass, dispersity, and conformation. The measured conformation plot slopes suggests that the ferric carboxymaltose complexes are more compact than the iron sucrose complexes (Table 1). Combined, SEC-MALS and FFF-MALS thus provide high-resolution characterization of iron-sugar complexes as well as identification and quantification of smaller complexes, free sugar, or undesired degradation products.

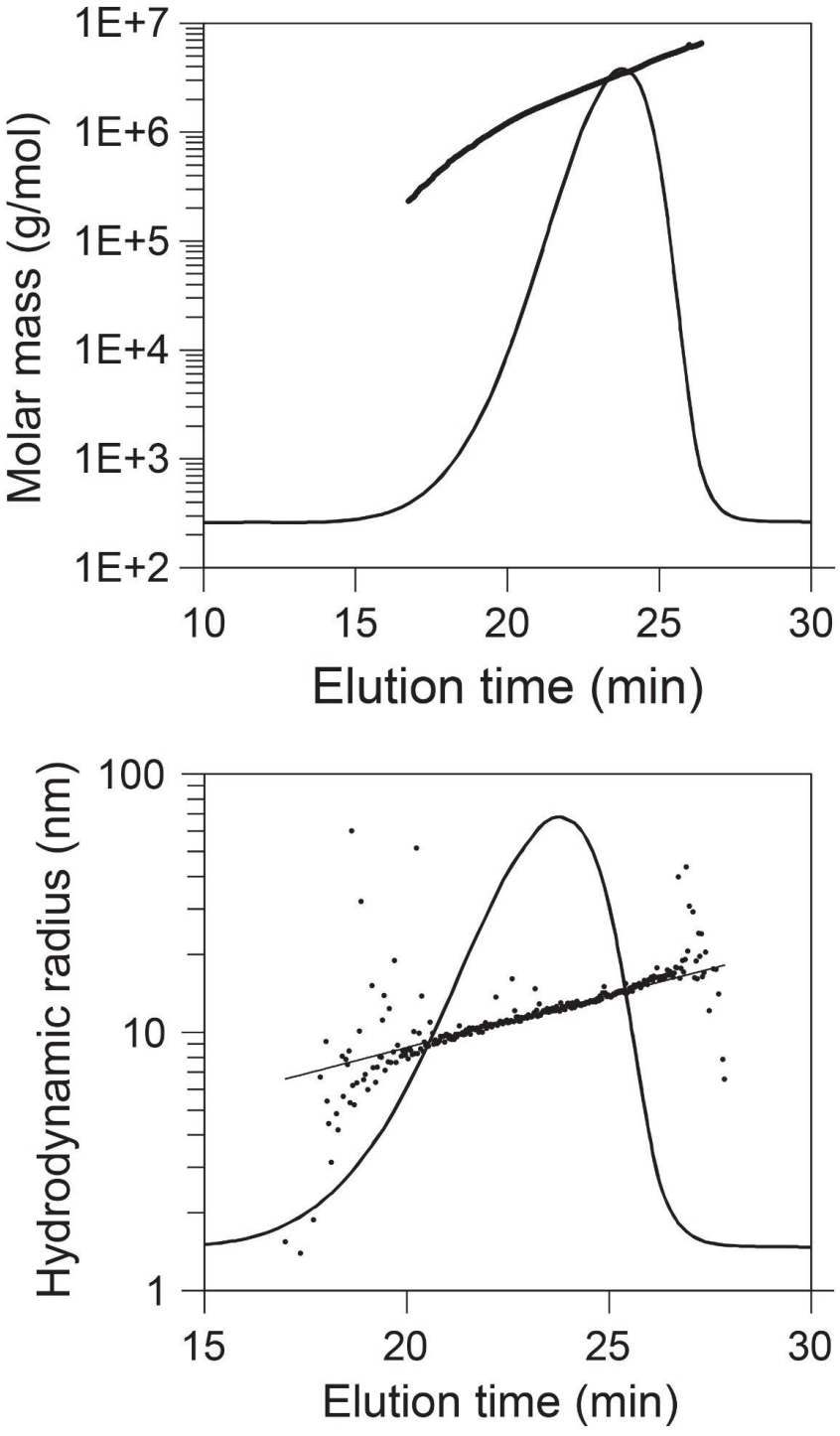

Figure 6. FFF-MALS quantification of ferric carboxymaltose molar mass (top) and size

(bottom). The results are consistent with the corresponding SEC-MALS results, but FFF may offer wider separation range in a single experiment than single-column SEC, without filtering out large species.

Conclusion

Both clinical and biophysical data are required for regulatory approval of colloidal iron drug products, and SEC-MALS and FFF-MALS are excellent orthogonal tools for high-resolution multi-attribute quantification. SEC-MALS offers more comprehensive characterization than FFF-MALS for products that contain small molecules or small co-solutes like iron sucrose, while FFF-MALS is an effective complement not only for larger iron products like ferumoxytol and ferric carboxymaltose, but also for products with smaller particles, such as iron sucrose. These versatile analysis tools may help identify the molecular mechanisms behind the adverse reactions associated with this drug class and facilitate development of safer colloidal iron drug products.

References

1. Krupnik, L. et al. Iron-Carbohydrate Complexes Treating Iron Anaemia: Understanding the Nano-Structure and Interactions with Proteins Through Orthogonal Characterization. *J. Control. Release*. 368, 566 (2024).
2. Lim, W.; et al. Canadian Expert Consensus: Management of Hypersensitivity Reactions to Intravenous Iron in Adults. *Vox Sanguinis.* 114, 363 (2019).
3. Bhandari, S.; et al. Intravenous Irons: From Basic Science to Clinical Practice. *Pharmaceuticals*. 11(3), 82 (2018).
4. Sun, D.; et al. Comparative Evaluation of U.S. Brand and Generic Intravenous Sodium Ferric Gluconate Complex in Sucrose Injection: Physicochemical Characterization. *Nanomaterials.* 8(1), 25 (2018).
5. United States Pharmacopeia. Iron Sucrose Injection. USP-NF (2023).

Featured Products

Arc HPLC System <https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/arc-hplc-system.html>

DAWN MALS Photometers <https://www.wyatt.com/products/instruments/dawn-multi-angle-light-scattering-detector.html>

Eclipse FFF System <https://www.wyatt.com/products/instruments/eclipse-field-flow-fractionation-system.html>

720009610, October 2025

© 2026 Waters Corporation. All Rights Reserved.
Terms of Use Privacy Notice Trademarks Careers Legal and Privacy Notices Cookies Cookie Preferences Do Not Sell My Personal Information