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Factors influencing safety and efficacy of intravenous iron-carbohydrate nanomedicines: From production to clinical practice

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Abstract

Iron deficiency is an important subclinical disease affecting over one billion people worldwide. A growing body of clinical records supports the use of intravenous iron-carbohydrate complexes for patients where iron replenishment is necessary and oral iron supplements are either ineffective or cannot be tolerated by the gastrointestinal tract. A critical characteristic of iron-carbohydrate drugs is the complexity of their core-shell structure, which has led to differences in the efficacy and safety of various iron formulations. This review describes parameters influencing the safety and effectiveness of iron-carbohydrate complexes during production, storage, handling, and clinical application. We summarized the physicochemical and biological assessments of commercially available iron carbohydrate nanomedicines to provide an overview of publicly available data. Further, we reviewed studies that described how subtle differences in the manufacturing process of iron-carbohydrate complexes can impact on the physicochemical, biological, and clinical outcomes of original product versus their intended copies or so-called iron "similar" products.

Key words: Iron-carbohydrate complex; Physicochemical characterization; Iron deficiency; Nanomedicine; Colloidal nanoparticles; Critical quality attribute

From colloidal suspensions to nanoparticles: a brief introduction

Nanoparticles (NPs) are revolutionizing nanomedicine by providing diverse therapeutic and diagnostic tools, including biological sensing, drug delivery, imaging, cell separation, and tissue repair.[1–3] Nano-sized particles provide a combination of unique properties and mobility, which allows particles to interact with different cells types.[4,5] Among various nanomedicines, iron oxide and oxyhydroxide NPs have gained extensive attention over the past years owing to their distinctive physicochemical properties. More than 50 years prior to the appearance of the term nanomedicines in the 1990s, colloidal NPs had already been employed for drug delivery purposes.[6] One example is iron oxyhydroxide particles, also known as iron-carbohydrate complexes, which were developed before they were even recognized as a class of nanomedicine to help treat iron deficiency.[7] Accordingly, in 1949, the first nano-sized colloidal particle-based product was introduced (iron sucrose; Venofer®, Switzerland) to be administered as a replacement medication for oral iron supplements for the treatment of iron deficiency.[8]

As specified by the World Health Organization (WHO), iron deficiency anemia is recognized as one of the most prevalent nutrient deficiencies affecting 1.6 billion people globally.[9–11] Iron deficiency has various causes, and severe cases can occur due to blood loss, poor nutrition, Chronic Kidney Disease (CKD), and Inflammatory Bowel Disease (IBD). Patients undergoing gastric bypass surgery and chemotherapy are also at risk of developing iron deficiency.[12–15] In contrast to other nutrient deficiencies, the occurrence of iron deficiency is common in both developed and developing countries.[16,17] Iron is one of the most abundant metallic elements in the human body,

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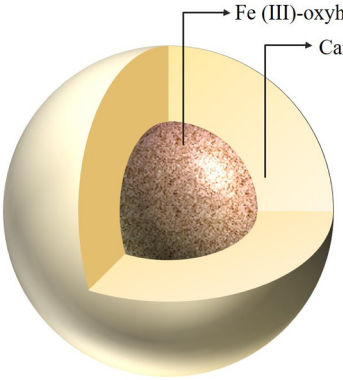

Trade name	Carbohydrate shell
InFeD	Low molecular weight dextran
Ferrlecit	Gluconate
Venofer	Sucrose
Ferinject	Carboxymaltose
FeraHeme	Polyglucose sorbitol carboxymethyl ether
Monofer	Isomalto-oligosaccharides
Maltofer	Polymaltose

Figure 1. Schematic representation of iron-carbohydrate complexes.

and the total amount of normal body iron content is between 200 and 300 mg in adult females and around 600-1000 mg in adult males.[9,18] Notably, iron is an essential component of many cells since iron-containing enzymes are crucial for various metabolic processes, mainly including synthesis of Hemoglobin (Hb) for oxygen transport, cellular respiration by redox enzymes, and cellular proliferation.[1,15] Consequently, iron deficiency can lead to severe and harmful effects on both cells and tissues.[14,19–20]

Conventional oral iron supplements in the form of ferrous iron might not be considered as an ideal therapeutic approach for the correction of iron deficiency[16,21] as they may cause gastrointestinal intolerance, prolonged iron store repletion time, and impaired absorption of iron.[9,14] Conversely, intravenous iron-carbohydrate complexes provide an alternative approach that can lead to higher Hb levels as well as faster replenishment of the body iron stores. This is particularly important for patients with absolute iron deficiency who require a rapid iron replacement.[22,23] In this regard, an increasing number of intravenous iron complexes have been developed and considered for the treatment of iron deficiency over the past decade.[11,22] However, until now, there is no precise information on the uptake mechanism of iron-carbohydrate complexes. In general, following intravenous administration of these complexes, they enter the bloodstream and mix with plasma, which triggers their uptake into the reticuloendothelial system (RES).[8,24] The removal of the complexes from the circulating plasma takes place by resident phagocytes of the liver, spleen and bone marrow. At this stage, the carbohydrate shell starts to be degraded, which facilitates dissociation from the iron core. The released iron is either incorporated by ferritin into intercellular iron stores, or is released from the cell to be taken up by extracellular iron-binding proteins, such as transferrin.[9,25–26]

Currently available intravenous iron-carbohydrate complexes are: low molecular weight iron dextran (InFeD®, Allergan Sales, LLC), sodium ferric gluconate (Ferrlecit®, Sanofi-Aventis Canada Inc.), iron sucrose (Venofer®, Vifor Pharma Ltd), ferric carboxymaltose (Ferinject®, Vifor Pharma Ltd), ferumoxytol [polyglucose sorbitol carboxymethyl ether] (FeraHeme®, AMAG Pharmaceuticals Inc.), iron isomaltoside (Monofer®, Pharmacosmos Ltd), and iron polymaltose (Maltofer®, Vifor Pharma Ltd).[9,11,22] Structurally, all intravenous iron-carbohydrate complexes are NPs consisting of spheroidal polynuclear Fe (III)-oxyhydroxide/oxide cores shielded by carbohydrate shells with an overall diameter between 8 and 30 nm (Figure 1).[21,25,27] The carbohydrate shell surrounding the iron plays crucial roles in stabilizing the iron core, slowing down the release of bioactive iron, protecting the particles from further aggregation, as well as sustaining the particles in a colloidal suspension.[10,23] Since the advent of intravenous iron complexes, various types of carbohydrates have been selected for these nanomedicines, including dextran, gluconate, sucrose, carboxymaltose, isomalto-oligosaccharide, and polyglucose sorbitol carboxymethyl ether.[11,22]

Despite structural similarities, iron complexes differ in their physicochemical properties, including particle size, surface charge (measured as zeta potential), chemical nature of the carbohydrate shell, total iron content (Fe(II) + Fe(III)), and labile iron content,[28,29] (i.e. ionic iron loosely bound to the core).[3,14,21,30] Differences in coating of iron complexes can also induce diverse pharmacological and biological effects. Observed effects may concern an impact on the clearance rate of NPs, the release rate of bioactive iron from the NPs, as well as other pharmacokinetic (pK) parameters and the body distribution of the NPs, which in turn influences the infusion rate applied and the maximum tolerated dose.[19,26] Accordingly, the safety and efficacy of intravenous iron-carbohydrate complexes with different carbohydrate coatings may be affected by their respective pharmacokinetic profile and their stability.[21] High-molecular weight iron dextran, whose clinical administration has been halted, is an excellent example for the importance of evaluating the effect of carbohydrate coating on the safety of iron complexes using a combination of physicochemical characterization methods and biological studies.[22] Clinical trials revealed that in contrast to currently available low-molecular weight iron dextran (96,000 Da) the high-molecular weight formulation (265,000 Da) induces serious and life-threatening anaphylactic reactions. These results have been found to be related to the large size of dextran in the latter formulation.[10,26,31]

With the above-mentioned differences between various intravenous iron-carbohydrate complexes, comprehensive knowledge of their physicochemical characteristics is crucial to improve safety and efficacy of the currently available products as

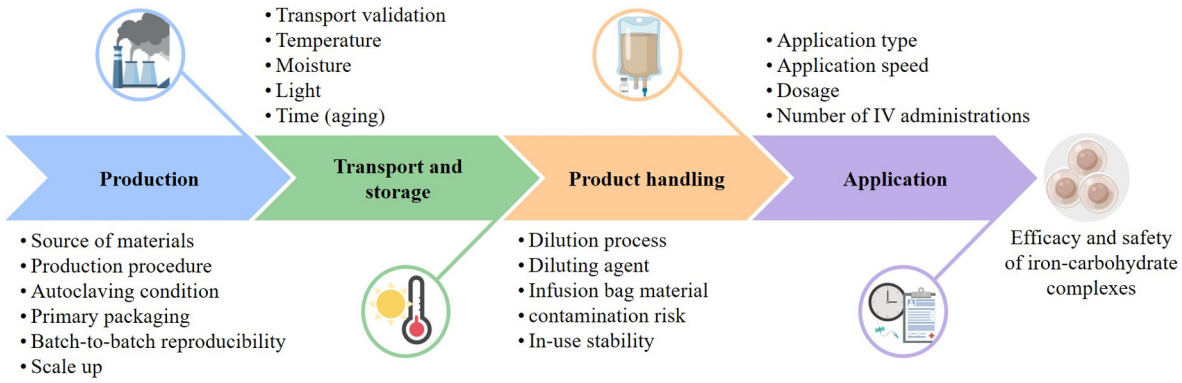

Figure 2. Fishbone diagram presenting factors influencing safety and efficacy of intravenous iron-carbohydrate complexes during production, storage, transport, handling and ultimately clinical applications.

well as for the formulation of new iron preparations in the future. Therefore, the objective of this review is to identify potential factors that can affect quality attributes of iron carbohydrate-based nanomedicines for parenteral iron deficiency treatment. Here, we explore and assess the current knowledge of the physicochemical properties as well as biological responses of commercially available iron complexes while focusing on most important parameters influencing the safety and efficacy of these nanomedicines from production to clinical application.

Factors influencing intravenous iron products safety and efficacy

As detailed in Figure 2, several factors throughout production, storage, transport, handling, and eventually clinical applications might influence the safety and stability of different iron-carbohydrate complexes. Influencing factors and their corresponding parameters will be discussed in detail in subsequent sections.

Production

Intravenous iron-carbohydrate complexes belong to a group of pharmaceutical compounds known as Non-Biological Complex Drugs (NBCDs).[32] As their name indicates, these compounds are highly complex, and their production process mainly determines their physicochemical properties.[33] The manufacturing process of iron-carbohydrate complexes involves several key steps. As mentioned in Figure 2, the source of primary materials, especially for the iron core could be of importance to minimize potential elemental contaminations and needs to be considered as a quality attribute of iron complexes.[9,30,34–35] The manufacturing process for iron-carbohydrate complexes is initiated through the reaction of water-soluble iron (III) salts, such as ferric chloride, nitrate, and sulfate with a weak base, followed by precipitation of iron (III)-oxyhydroxide cores in the reaction mixture.[9,36] The reaction between the produced iron core and carbohydrate solution can then occur under specific conditions (temperature, pH, reaction time, etc.). Following the synthesis of the iron complexes, isolation of the product is achieved by addition of a water-soluble organic solvent to the reaction mixture to precipitate the iron-carbohydrate complexes.[36] The process is then finalized by a purification step, such as filtration, centrifugation, lyophilization, or distillation to obtain a proper product for use as a parenteral iron complex.[9] Notably, any slight variations in the production process of iron complexes such as pH, temperature, bases, material sources, and reaction time might influence the quality of the final product.[37] Physicochemical properties that can be affected due to variations in the manufacturing process include molecular weight distribution, particle size distribution, the valence state of iron, surface charge, and crystallinity.[25,33] Accordingly, small changes in the physicochemical characteristics of iron complexes could alter parameters such as, *in vivo* stability, iron release rate, cellular uptake, and tissue distribution, which might intrigue unexpected *in vivo* responses both in terms of safety and efficacy.[38,39]

During production, the other two factors that influence the safety and efficacy of iron-carbohydrate complexes are autoclaving conditions and the primary packaging material (Figure 2). In 2014, Shah et al investigated the effect of thermal stress on the molecular weight of iron sucrose (Venofer) autoclaved at 121 °C and 15 bars, while applying different steaming cycle times of 0.5, 1, and 3 h. A notable increase in the molecular weight as a function of autoclaving time was reported due to colloidal aggregation of iron sucrose particles. This observation ultimately infers the impact of autoclaving conditions on the chemical stability of iron complexes.[33] For the packaging of the finished product, glass containers are the most commonly used material. The interior glass surface is usually coated with a layer of silicone polymer or silicon dioxide to increase its stability.[40] Glass has been widely used for the storage of different parenteral drugs over an extended period. However, there are concerns about the occurrence of glass delamination and unwanted interactions between the glass and pharmaceutical products. These depend on several factors, including glass composition, properties of the pharmaceutical solutions, and storage conditions.[41] Since intravenous iron products have complex formulations, ongoing research for evaluating the safety and stability of primary packaging material is of high importance to investigate any drug degradation and increased risk of toxicological effects. Further, a study published by Hauser et al indicated the impact of employing different production procedures on batch-to-batch reproducibility of the size of iron dextran particles and crystalline morphology.[2] Therefore, with

Table 1
The availability of research publications [reference number] evaluating the impact of storage conditions and product handling on physicochemical properties of commercially available intravenous iron complexes.

Influencing factors	Product storage				Product handling			
	Light	Time (aging)	Temperature	Moisture	Dilution	Diluent	Bacterial growth	Infusion bag
LMWID†								
Iron sucrose			[33]			[33]	[51]	[33]
Sodium ferric gluconate		[52][53]	[52][53]		[52]	[53][54]		[52][53]
Ferumoxytol								
Iron isomaltoside								
Iron polymaltose	[55]	[55]	[55]		[55]			[55]
Ferric carboxymaltose						[56]	[56]	[56]
SPIONs		[57]	[57]					

† Low Molecular Weight Iron Dextran

respect to scaling-up strategies, ensuring a high batch-to-batch reproducibility of the manufactured iron complexes is imperative to minimize possible variations in physicochemical properties of the final parenteral product.

Notably, intravenous iron similars that are intended copies of their originators have been manufactured in recent years in some countries and are accessible in the global market.[42–44] However, underestimating the high complexity of iron preparations along with non-disclosed proprietary and confidential information on the manufacturing process of originator products has led to the production of iron similars with slightly different structures and biological responses [42] which will be discussed in more detail.

Product transport and storage

Post-manufacturing, intravenous iron-carbohydrate complexes are exposed to various mechanical stresses and environmental conditions (Figure 2). The stability and mechanical strength of the carbohydrate shell surrounding the iron core might affect the vulnerability of iron complexes to damage during transport.[45,46] Additionally, the physicochemical properties of nano-sized particles are prone to change when stored under different conditions, such as temperature, relative humidity, and light.[33,47] Another complication that may occur after the manufacturing is associated with the aging of these products, which can also accelerate under incorrect storage conditions.[45] The aging process of non-coated NPs is generally known to be slow. However, when compared to the non-coated NPs, the aging process of coated particles may lead to different results with respect to their toxicity and physicochemical properties (i.e. size, crystal type, surface charge).[47,48] Therefore, correct storage conditions can play an essential role in preserving the physicochemical properties of iron-carbohydrate complexes and ensuring their medication safety.[45]

Our investigation into the post-manufacturing parameters exposed very few accessible studies focusing on the effect of storage and handling on the safety and efficacy of iron complexes. To provide more insight into this gap in the research literature, we have gathered the currently available scientific references focusing on influencing parameters during storage and handling of iron-carbohydrate complexes in Table 1. As shown, particularly the lack of publicly available studies with respect to an impact by light and moisture was noted. We also widened the scope of our investigation by exploring the availability of such knowledge concerning another group of intravenous iron coated nanoparticles, superparamagnetic iron oxide nanoparticles (SPIONs). SPIONs exhibit a unique capacity to interact with an external magnetic field, which makes them attractive to be used as a contrast enhancement agent for *in vivo* Magnetic Resonance Imaging (MRI).[2,49,50] Although SPIONs have been investigated intensively using both physicochemical and biological assessments, Table 1 shows that the data on the influence of storage and transport conditions on the safety and stability of these NPs are also inaccessible.

As an example, molecular weight distribution of sodium ferric gluconate has been examined using high-performance gel permeation chromatography to evaluate the impact of temperature and time on particle stability.[53] Their molecular weight has been shown to remain stable at 50 °C for 30 days. The same result has been reported for samples subjected to a higher temperature within one week. However, at 70 °C particles have exhibited signs of degradation followed by particle aggregation over an extended time. Overall, the degradation of sodium ferric gluconate and changes in molecular weight of particles have been observed at relatively high temperatures, indicating the possibility of alteration in physicochemical properties of iron complexes in response to changes in storage time and temperature. The influence of storage time (0-12 weeks) and temperature (4-45 °C) on physicochemical properties and cellular uptake of SPIONs with hybrid coating has also been investigated in a study performed by Zaloga et al. [57] Based on their observation a reduction in saturation magnetization (Ms) of SPIONs has been reported to be dependent on both storage time and temperature, most likely owing to the oxidation of the iron (magnetite/maghemite) core. Since a high Ms is crucial for targeted molecular imaging, the decrease of this parameter over time can negatively influence the diagnostic efficacy of SPIONs.[57,58] This phenomenon has shown to induce a visible color change of the suspension from black-brown to reddish-brown over an increase in storage temperature and time. Despite oxidation of the iron core, no notable differences in particles size and surface charge have been observed. The particles' uptake by human T-lymphoma cells has also been reported to be reduced, which has been suggested to be a consequence of oxidative stress observed upon cellular uptake.[57]

Product handling

As depicted in Figure 2, handling procedures are the third factor impacting the stability of intravenous iron complexes. There are several primary constituents to be considered: dilution process, diluting agent, and the infusion bag material. Intravenous iron complexes are administered using two methods, either by slow injection of the undiluted product or by intravenous infusion of a diluted iron preparation.[54] The dilution ratio, as well as the nature of the diluent, can influence the stability of the carbohydrate shell in iron complexes, ultimately affecting the amount of labile iron content.[33] There are many concerns regarding the link between the amount of released labile iron from various iron complexes and the occurrence of adverse reactions in patients. Since the maximum tolerable amount of labile iron is stated to be ≤8 mg,[29] high amounts of labile iron released from degraded or destabilized iron-carbohydrate complexes as a result of improper handling, cannot be fully taken up by the RES system. This can then induce severe toxicity, such as oxidative stress and denaturation of plasma components.[55,59] It is important to establish a dilution procedure, which has no or little effect on iron product stability and safety during handling.[29,56] Besides, since the preparation of intravenous products in hospitals can pose a potential risk of microbial contamination, it is crucial to maintain strict aseptic techniques to minimize the risk of infections when handling sterile starting products for intravenous administration.[60] Further, with the effect of storage conditions on the stability of iron-carbohydrate complexes discussed earlier, iron preparations need to be correctly stored before and after the dilution process in order to ensure in-use stability for clinical applications.

Previous studies have demonstrated that vein inflammation also known as phlebitis could be minimized by diluting the iron dextran product in 0.9% NaCl rather than 5% dextrose.[54] In a study published in 2010, no visible changes in the molecular weight of diluted sodium ferric gluconate (Nulecit®, intended copy product) in 0.9% NaCl compared to the undiluted product were reported.[52] In another study by Yang et al, the effect of different diluents on the stability of sodium ferric gluconate has been evaluated. Based on their results, the molecular weight of diluted sodium ferric gluconate in sucrose versus sodium gluconate remained stable.[53] Additionally, the stability of sodium ferric gluconate diluted with alkaline buffers at different pH levels has been tested. At pH 8 and 9, a consistent molecular weight of sodium ferric gluconate has been reported. However, when the products were diluted at pH 10 and 11, small molecular species (36,500-56,300 Da) were generated, indicating the decomposition of the polynuclear iron core. Overall, this study suggests acceptable colloidal stability of sodium ferric gluconate over variations in storage and handling conditions employing several physicochemical characterizations, such as particle size analysis, ultracentrifugation, dialysis, zeta potential, and osmolality analysis.[53] With respect to iron sucrose, experiments conducted by Shah et al have demonstrated colloidal iron nanoparticle recovery at the formulation pH of 10.5 after being destabilized at low pH (>1.2).[33]

For handling of diluted iron complexes, the material chosen for the infusion bag needs careful consideration. So far, polypropylene (PP) infusion bags are chiefly used in clinical practice for intravenous infusion. However, until now, merely a few studies have tested the stability of diluted iron complexes when stored in infusion bags of different materials.[55,56] To the best of our knowledge, among various intravenous iron complexes, the physicochemical properties of ferric carboxymaltose and iron polymaltose are the only products that have been tested for their stability in infusion bags after having been diluted.[55,56] Overall, ferric carboxymaltose (Ferinject) diluted in 0.9% NaCl examined by their molecular weight has been reported to maintain its stability for 72 h at 30 °C in both PP bottle and PP bags. Furthermore, only slight changes in the iron content (5%) of diluted products in PP bottle or PP bags have been reported, which suggested no detectable absorption of iron at the surface of the PP containers.[56] Diluted iron polymaltose (Ferrosig™) has also shown high stability for up to 28 days in polyvinyl chloride infusion bags at room temperature and under refrigerated conditions. No sign of discoloration, precipitation, or changes in pH of the diluted iron polymaltose was observed throughout their experiments using different storage conditions. Notably, over this period, the diluted Ferrosig only lost 0.3% of its total complexed iron, which indicates low probability to cause toxic reactions in patients.[55]

As was summarized, in response to different storage and handling conditions, measuring the molecular weight of the iron complexes provides a sensitive parameter to examine degradation or aggregation of these NPs.[56] However, as identified in Table 1, more in-depth physicochemical and biological characterizations are required to overcome the current lack of detailed systematic studies comprising the influence of transport, storage and handling conditions on all available intravenous iron-carbohydrate complexes.

Application

As shown in Figure 2, at the final step of clinical application, several factors, such as choice of iron formulation, dosage, administration method (infusion versus injection), administration speed, and the number of intravenous sessions could vary widely, affecting the safety and efficacy of iron-carbohydrate complexes.[12,61–62] Intravenous products can be administered as push injection and/or as infusions. Administration time can vary between fast push and slow infusions depending on the type of product.[8,63] Since intravenous iron-carbohydrate complexes differ in their pharmacokinetic properties such as uptake by the RES and particle degradation, the choice of administration method, speed and the number of intravenous sessions must be arranged according to the information provided by the manufacturer and careful consideration is required based on the patient's medical history.[8,29,64] From a safety perspective, hypophosphatemia and hypersensitivity reactions are the two main side effects associated with IV iron supplementation.[65–69] In a clinical analysis published in 2017, the occurrence of these two complications has been assessed for 231 patients with IBD following treatment with isomaltose versus ferric carboxymaltose. This head-to-head comparison suggested the higher occurrence rate of hypophosphatemia under ferric carboxymaltose therapy, while the use of isomaltoside was associated with a

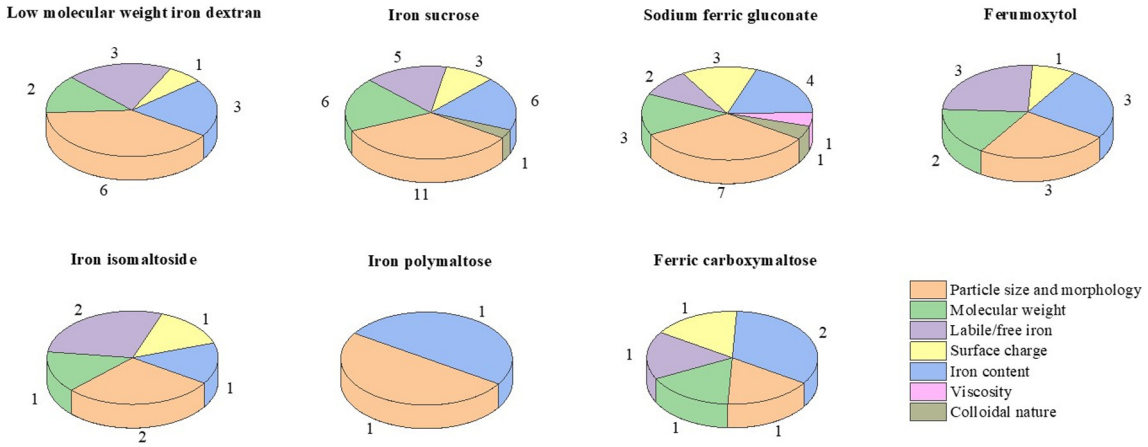

Figure 3. Pie charts illustrating the number of scientific publications on physicochemical characterization of commercially available intravenous iron-carbohydrate complexes relevant to assess the products after the manufacturing process.

higher risk of hypersensitivity reactions. Since the same dosage of iron was administered during this evaluation, the differences in the observed side effects have been suggested to be associated with differences between the structure and the iron release mechanism of the two iron formulations.[70]

The correct dose to achieve a normal Hb level for an individual patient must be thoughtfully selected by the clinical staff, especially when considering the administration of follow-on products, so-called iron similars. In a retrospective study conducted in 2011, correction of iron deficiency in hemodialysis patients has been studied by substituting an original iron sucrose product with an iron sucrose similar (ISS).[44] A significant reduction of Hb levels was reported after the switch to the ISS. Accordingly, a 35% increase in iron dose was needed to reach similar Hb levels as with the original iron sucrose. Although iron similars are known to be less costly in comparison to their originators, comparable clinical trials suggest that this could be invalidated owing to the increased dosage requirement.[44,71–72]

The patient's condition might also influence the final decision concerning the number of required intravenous sessions and the choice of administration method. For instance, the multiple low doses of intravenous iron formulations, such as iron sucrose and sodium ferric gluconate might not be a significant challenge for patients who are already undergoing chemotherapy or hemodialysis. However, a single infusion of maximum iron dose using other formulations such as iron isomaltoside, ferric carboxymaltose, iron dextran, and ferumoxytol can reduce the necessity for multiple office visits and importantly increase convenience to both physicians and patients.[73] Altogether, data collected from clinical practice have so far highlighted subtle, though important differences in pharmacokinetic and pharmacodynamic behavior of various intravenous iron complexes.

Characterization approaches: physicochemical, biological, and clinical assessment

Until now, physicochemical characterizations including analysis of particle size, chemical structure, molecular weight, colloidal nature, labile or free iron, surface charge, total iron content, and viscosity are the main assessment tools of manufactured iron-carbohydrate complexes. However, as depicted in Figure 3, there is a clear imbalance in the numbers of scientific publications on the different physicochemical characteristics of seven commercially available iron complexes. The majority of scientific publications are accessible for iron sucrose, with just a few publicly available on iron polymaltose. Among the different physiochemical characteristics mentioned above, only information on particle size and morphology is available for all products. On the other hand, except for iron sucrose and sodium ferric gluconate, information on their colloidal nature and viscosity is missing for most iron complexes.

For size analysis, dynamic light scattering (DLS), which allows for the determination of the hydrodynamic diameter (z-average) of NPs and quantifies the distribution of particle size by the polydispersity index (PDI), is commonly applied.[2,10,39,74] DLS is known to be a quick and straightforward method when it comes to sample preparation. However, as experimental conditions can affect the reported size distribution,[39,75] imaging methods such as (scanning) transmission electron microscopy (STEM) and atomic force microscopy (AFM) are usually performed in addition to DLS measurements to provide more robust information on particle core size, shape, and agglomeration status.[10,26,39] Based on the results proposed by Verhoef et al, the application of STEM for four different iron complexes (sodium ferric gluconate, iron sucrose, iron carboxymaltose, and iron isomaltoside 1000) has demonstrated the presence of spherical iron cores for all preparations except for iron carboxymaltose, which has exhibited rod-shaped iron cores.[10] This observation is in good agreement with other studies, where TEM and AFM analyses have also confirmed iron complexes uniformity in size, especially for iron sucrose preparations.[24,26,27,74] Existing methods to evaluate iron core crystallinity and the presence of Fe (II) and Fe (III) within the core structure are X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and Mössbauer spectroscopy.[23,27,39] While XRD and FTIR analyses of iron sucrose, sodium ferric gluconate, iron dextran, and iron polymaltose have demonstrated

akaganeite (β-FeOOH) as the core structure.[27,76–77] Mössbauer spectra of ferumoxytol have shown a maghemite structure with Fe (II) content <1%.,[2378] These observations could emphasize the relevance of further pre-clinical studies to examine the effect of higher Fe (II) content observed for iron sucrose (10-15%) in comparison to other products since it can lead to oxidation stress (formation of reactive oxygen species (ROS)).[21,30] The molecular weight distribution of iron-carbohydrate complexes is another essential quality attribute, which is in direct relation to the rate of iron released from the core after intravenous administration.[33,39] Differences in the composition of the carbohydrate shell can intensively affect the overall molecular weight of the iron complexes and have mainly been evaluated by gel permeation chromatography.[19,26] Regarding differences in the molecular weight of various carbohydrate shells, studies have mentioned the following trend for the manufactured iron-carbohydrate complexes: iron dextran >> ferumoxytol > iron carboxymaltose > iron isomaltoside 1000 > iron sucrose > sodium ferric gluconate.[19,23,26]

Essential physicochemical characterization is linked to the colloidal nature (lyophilic or lyophobic) of iron-carbohydrate complexes. Iron sucrose preparations have shown to maintain their stability without any significant signs of aggregation when both dialysis experiments and incubation in solutions of monovalent electrolyte (1 M of NaCl) for up to 24 h were employed.[33] Several studies have confirmed the lyophilic nature of iron sucrose and sodium ferric gluconate, which prove low sensitivity of these two colloidal NPs to added electrolytes and indicate high colloidal stability.[33,53,79] Moreover, for measuring the amount of labile iron released from intravenous iron complexes colorimetric assays, such as Bleomycin, MAK025 iron, and Ferrozine assays have been suggested to be employed.[39,80] Between sodium ferric gluconate, ferumoxytol and iron sucrose, the lowest amount of detectable free iron has been reported for ferumoxytol. In fact, ferumoxytol with a superparamagnetic iron oxide core and polyglucose sorbitol carboxymethyl ether shell was initially developed as a contrast agent for MRI.[81] As mentioned earlier, unlike other intravenous iron complexes with akageneite (β-FeOOH) core structure, ferumoxytol has been reported having maghemite (γ-Fe_2O_3) core with very low Fe (II) content. A maghemite core with high Fe (III) content renders the iron core more stable against reduction, which explains the noticeable decrease in the amount of released labile iron from ferumoxytol in comparison with other iron complexes.[21,23,78]

Given the differences between various formulations of iron-carbohydrate complexes, similar pharmacokinetic profiles and pharmacologic properties cannot be assumed for all products.[74,82] Accordingly, biological (pre-clinical) studies are required to obtain a general overview of their toxicological effects, efficacy, and uptake mechanism. Several *in vitro* and *in vivo* studies have shown that iron complexes can induce oxidative stress,[13,42,83] boost bacterial growth,[51] cause pro-inflammatory reactions,[42,84] and increase the secretion of specific cytokines.[1,2] Some of these biochemical reactions are considered to occur owing to the binding of iron complexes to interacting proteins of the complement system[85]; however, the role of the iron core in the activation of the complement system is not fully identified yet. A study performed by Hempel et al confirmed the carbohydrate shell surrounding the iron core is the primary structural element in provoking immunotoxicity and inflammatory reactions.[86] It is not entirely understood whether oxidative conditions are linked to the physicochemical and structural properties of different iron-carbohydrate complexes.[13,59,83,87,88] *In vitro* evaluations had confirmed a significant increase in ROS when iron sucrose, iron dextran, ferric gluconate, and ferric carboxymaltose were cultured with peripheral blood mononuclear cells isolated from both healthy volunteers and patients with CKD.[13,83,89]

Despite current knowledge concerning the role of macrophages in capturing released iron by phagocytosis, the mechanism by which iron is released from various iron complexes to participate in iron replenishment is poorly understood.[28] A recent *in vitro* study has confirmed elevated sensitivity of the THP-1 macrophage cell line in response to ferric carboxymaltose compared with iron sucrose.[89] Overall, studies suggest that after exposure to iron formulations, different types of macrophages in the body can inflict inconsistent responses.[90] Iron-carbohydrate complexes can also differ in their iron loading capacity, which indicates the ability of transferrin protein to bind serum iron.[91] Iron polymaltose and iron dextran have been shown to provide high iron loading capacity in comparison to sodium ferric gluconate and iron sucrose.[90] The observed differences between the bioavailability and absorption of several iron complexes are related to a variety of factors, such as particle size, molecular weight, surface charge, and type of carbohydrate surrounding ferric iron.[25,74,90] Notably, the low efficacy in the iron loading of lower molecular weight complexes may also be caused by their accelerated renal clearance.[90]

Clinical trials are the final step for the characterization of intravenous iron complexes, which permits excluding clinically significant differences that may not be identified by non-clinical approaches. So far, a significant number of clinical studies have been conducted on these products; however, several factors can affect the clinical outcomes of such studies and make it difficult to draw a certain decision in choosing a particular intravenous iron complex with the highest efficacy and with the least side effects.[92] Since several diseases such as chronic kidney disease, heart failure, gastrointestinal disease, and cancer can be the underlying reason for iron deficiency, reported adverse reactions and effectiveness of an iron product may be influenced by the patient's medical history.[64,65,93–94] Accompanying factors such as age, gender, genotypic variations, and the previous exposure of the patients to intravenous iron complexes, can also affect the patient's reaction to the intravenously administered iron supplements and this needs to be accurately stated in clinical trials.[95–96] To indicate efficacy of the particular iron product, as well as occurrence of iron overload after intravenous administrations measurement of NBTI, LDL oxidation, analysis of hemoglobin, C-reactive protein, ferritin, and plasma phosphate, are among the most common clinical laboratory tests.[97] A notable limitation of clinical trials is the short-term nature of conducted studies; therefore, tracking of long-term adverse events has not been thoroughly investigated for most available intravenous iron formulations[4] and could be of importance to patients with chronic kidney failure, who require long-term dialysis and frequent iron infusion.[98–99] The comprehensive study published in 2014 provided a systematic review into the overall safety profile of commonly used intravenous iron

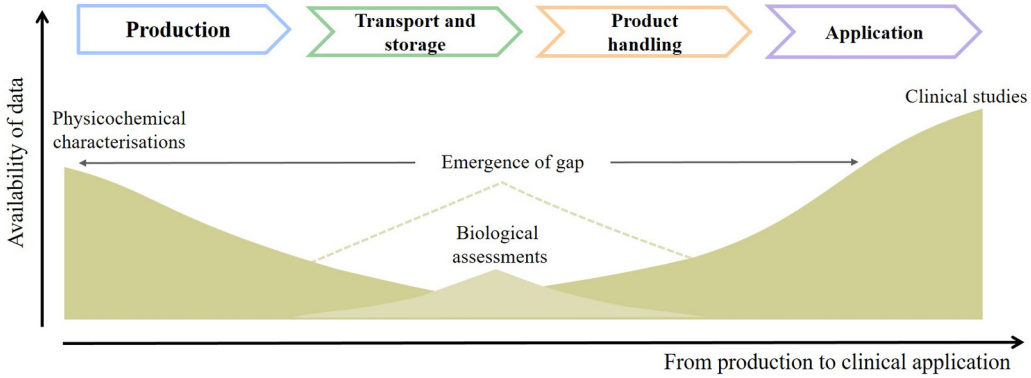

Figure 4. The evaluation of nanoparticles can be divided into the physicochemical characterization to verify the impact of manufacturing, storage and handling parameters, followed by biochemical analysis to assess nanoparticle-cell interactions, and ultimately safety and functionality evaluations based on clinical data. The current research gap indicates a weak correlation between physicochemical characteristics and clinical outcomes. Further biological assessments of iron complexes can effectively bridge the gap between pre-clinical and clinical studies (depicted by dashed lines).

complexes analyzing a total of 103 trials from 1966 through 2013.[100] In this study, the high safety of iron complexes especially in terms of newer preparations has been recommended. The authors have also stated the necessity of a head-to-head comparison of different intravenous iron complexes focusing on patients with a specific health condition, which could provide valuable insight into the safety and efficacy of varying iron formulations.[100]

Overall, as shown in Figure 4, there exists a relatively poor correlation between the currently available physicochemical characterization methods, biological assessments, and clinical data. The largest research gap exists for biological assessments, which are highly important to bridge between physicochemical characterization and clinical outcomes of these complex structures. *In vitro* investigations are vital in terms of providing a platform for direct application of the iron-carbohydrate complexes into cell culture media, which enables a sensitive and controllable approach for several evaluations including the analysis of oxidative stress as well as broadening the knowledge on the mechanism by which the iron complexes are taken up by macrophages.[13] Notably, most physicochemical evaluations have failed to indicate differences between intended copies and originator products, which might represent weaknesses of available characterization tools to recognize subtle structural variations.

The effect of the manufacturing process on iron-carbohydrate complexes shows that small modifications in the manufacturing process might cause several concerns about the safety and curative equivalence between these products and their originators.[38,42,44,70] A study published by Toblli et al has shown variations between physicochemical properties of six iron sucrose similars (ISSs) and Venofer. They have reported dissimilarities in both stability and molecular structure of ISSs versus Venofer, particularly linked with titratable alkalinity, turbidity point, and molecular weight distribution.[14] Furthermore, biological assessments have indicated functional deviations between originator iron complexes and their intended copy products. However, concerning ISSs, a higher level of induced oxidative stresses has been notified causing less certainty regarding their safety.[14,42,83,101] Since carbohydrate iron complexes have complex chemical structures, a thorough evaluation is required to ensure that the intended copy products replicate those of the original. Meanwhile, the differences between reported results based on current available characterization approaches make it challenging to conclude on the safety and efficacy of several intravenous preparations.[102]

Summary and outlook

Pharmacokinetic and pharmacodynamic properties of nanomedicines are not solely defined by their chemical composition but also by the physical nature of their NPs. This is also reflected in various regulatory documents such as FDA's considerations for product involving the application of nanotechnology, where it is stated that a product is engineered to 1) have at least one dimension in the nanoscale range and 2) exhibit properties that are attributable to this dimension. Hence, we have to assume that the chemical composition, as well as the size, morphology and surface characteristics of the NPs upon administration, is decisive for the nanomedicine's pharmacological action.

Despite a great number of publications on the physicochemical properties of commercially available intravenous iron products, few studies are available to represent the impact of production procedure on iron complexes. Since detailed production protocols are being kept confidential by manufacturers, a comprehensive physicochemical comparison that can give strong conclusion on the impact of each manufacturing parameter including temperature, pH, material source, scale-up procedure, autoclaving condition, and primary packaging is hardly accessible to researchers.

Here, we mainly reviewed publications describing parameters affecting intravenous iron complexes after production (Figure 3). Understanding these influencing factors is necessary to ensure the particles' ability to resist alterations induced by transport, storage and handling processes.[45,46,57] Such studies should be based on clinically relevant critical quality attributes (CQAs). Understanding of the mechanisms by which the physicochemical properties of the nanomedicines impact their biological behavior is therefore adamant. By today, these factors are not fully

understood. Further research in finding relevant and sensitive non-clinical models for evaluating iron NPs with different physicochemical characteristics is needed to assess the CQAs responsible for product stability and finally the efficacy and safety profiles of parenteral iron products. In relation to clinical trials published on intravenous iron complexes, the database is currently expanding at a rapid pace as demonstrated in Figure 4. However, the conclusion reached in our investigation indicated a weak correlation between physicochemical characterizations and pre-clinical studies as well as pre-clinical versus clinical data.[14,89]

Although nanomedicines storage and handling are known to be challenging, owing to the complexity of these products, published research and information on physicochemical stability are missing for most intravenous iron complexes (Table 1). Such studies are needed to contribute to the knowledge of potential changes in physicochemical properties during their journey from manufacturing to application to the patient. For healthcare professionals, such information would be highly relevant to establish appropriate clinical practice protocols ensuring product quality during storage and handling for best clinical outcome. The lack of reliable and transparent consensus emphasizes the need for additional data to address the identified knowledge gap between the manufacturing and developmental step and clinical administration. This could clarify the differences between iron complex products and ultimately reflect on both the choice of the iron complex as well as the decision to switch between various products in clinical practice.

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