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Research paper

A comparative study of the physicochemical properties of iron isomaltoside 1000 (Monofer®), a new intravenous iron preparation and its clinical implications

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ABSTRACT

The treatment of iron deficiency anemia with polynuclear iron formulations is an established therapy in patients with chronic kidney disease but also in other disease areas like gastroenterology, cardiology, oncology, pre/post operatively and obstetrics' and gynecology. Parenteral iron formulations represent colloidal systems in the lower nanometer size range which have traditionally been shown to consist of an iron core surrounded by a carbohydrate shell. In this publication, we for the first time describe the novel matrix structure of iron isomaltoside 1000 which differs from the traditional picture of an iron core surrounded by a carbohydrate. Despite some structural similarities between the different iron formulations, the products differ significantly in their physicochemical properties such as particle size, zeta potential, free and labile iron content, and release of iron in serum. This study compares the physiochemical properties of iron isomaltoside 1000 (Monofer®) with the currently available intravenous iron preparations and relates them to their biopharmaceutical properties and their approved clinical applications. The investigated products encompass low molecular weight iron dextran (CosmoFer®), sodium ferric gluconate (Ferrlecit®), iron sucrose (Venofer®), iron carboxymaltose (Ferinject®/Injectafer®), and ferumoxytol (Feraheme®) which are compared to iron isomaltoside 1000 (Monofer®). It is shown that significant and clinically relevant differences exist between sodium ferric gluconate and iron sucrose as labile iron formulations and iron dextran, iron carboxymaltose, ferumoxytol, and iron isomaltoside 1000 as stable polynuclear formulations. The differences exist in terms of their immunogenic potential, safety, and convenience of use, the latter being expressed by the opportunity for high single-dose administration and short infusion times. Monofer is a new parenteral iron product with a very low immunogenic potential and a very low content of labile and free iron. This enables Monofer, as the only IV iron formulation, to be administered as a rapid high dose infusion in doses exceeding 1000 mg without the application of a test dose. This offers considerable dose flexibility, including the possibility of providing full iron repletion in a single infusion (one-dose iron repletion).

1. Introduction

Parenteral iron therapy is today widely used for the treatment of iron deficiency anemia. Patients with chronic kidney disease (CKD) also frequently need treatment with parenteral iron preparations in addition to erythropoietin stimulating agents [1]. For renal failure patients on dialysis, the average iron requirements due to blood loss are equivalent to 1–3 g of elemental iron per year [2]. This can easily be accomplished by frequent low dose IV iron administrations, during the regular dialysis sessions.

From initial, generalized use in nephrology parenteral iron therapy has spread in recent years to other disease areas; gastroenterology [3], cardiology [4,5], oncology [6], pre/post operatively [7], obstetrics', and gynecology [8]. However, care providers in these segments have less frequent patient contact, resulting in an increased demand for convenient administration of large IV iron doses in one clinical session.

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Historically, the first parenteral iron preparations were toxic, being administered as an iron oxyhydroxide complex. This problem was circumvented with the introduction of compounds containing iron in a core surrounded by a carbohydrate shell [9]. The currently marketed parenteral iron preparations are considered equally efficacious but vary in molecular size, pharmacokinetics, and adverse reaction profiles. The intravenous iron agents currently available include high molecular weight iron dextran (Dexferrum®), low molecular weight iron dextran (Cosmofer®, Infed®), sodium ferric gluconate (Ferrlecit®), iron sucrose (Venofer®), iron carboxymaltose (Ferinject®/Injectafer®), and ferumoxytol (Feraheme®). High molecular weight iron dextran has been linked to an increased risk of anaphylaxis and anaphylactoid reactions, and it is not available in Europe [10–13]. Although this problem is very much reduced with low molecular weight iron dextran [10–13], there is still a test dose requirement and the infusion of larger doses is hampered by a 4–6 h infusion time. Sodium ferric gluconate and iron sucrose can only be used in moderate iron doses due to the relative weakness of the iron complex [14]. Two new parenteral iron compounds, iron carboxymaltose, and ferumoxytol were recently introduced in the EU and the US markets, respectively. The FDA failed to approve iron carboxymaltose for distribution in the USA due to unexplained hypophosphatemia, an increased number of adverse cardiac events and an imbalance in death rates in the treatment arm compared to the control arm in different RCTs [15].

Although more stable than sodium ferric gluconate and iron sucrose, the administration of iron carboxymaltose and ferumoxytol is still limited to a maximum total dose of 1000 mg and 510 mg, respectively.

The newest IV iron agent Iron isomaltoside 1000 (Monofer®) (e.g., iron oligo isomaltoside (1000) as generic name) is developed and manufactured by Pharmacosmos in Denmark and was introduced in Europe in 2010. The carbohydrate isomaltoside 1000 is a pure linear chemical structure of repeating α1-6 linked glucose units, with an average size of 5.2 glucose units and an average molecular weight of 1000 Da, respectively. It is a nonbranched, nonanaphylactic carbohydrate [16,17], structurally different from branched polysaccharides used in iron dextran (Cosmofer).

The production method and the short nonionic isomaltoside 1000 allows for the construction of a special matrix-like structure with interchanging iron molecules and linear isomaltoside 1000 oligomers. The resulting matrix contains about 10 iron molecules per one isomaltoside pentamer in a strongly bound structure that enables a controlled and slow release of bioavailable iron to iron-binding proteins with little risk of free iron toxicity [18,19]. This allows iron isomaltoside 1000 to be administered safely as a rapid high dose intravenous infusion or bolus injection offering considerable dose flexibility, including the possibility of providing full iron repletion in a single infusion, the so-called one-dose iron repletion.

This article introduces and compares physicochemical properties of iron isomaltoside 1000 (Monofer®) with currently marketed iron formulations. In addition, this comparative study of polynuclear iron formulations currently used in the treatment of anemic disorders includes perspectives on the relevance of these properties with respect to safety, efficacy, and convenience of administration.

2. Materials and methods

2.1. Materials

Sodium ferric gluconate (Ferrlecit®, 12.5 mg Fe/mL in 3.2 mL ampoules; Sanofi-Aventis, Frankfurt, Germany), iron sucrose (Venofer®, 20 mg Fe/mL in 5 mL ampoules; Vifor, München, Germany), low molecular weight iron dextran (CosmoFer®, 50 mg Fe/mL in 2 mL ampoules; Teva, Mörfelden-Walldorf, Germany), iron isomaltoside 1000 (Monofer®, 100 mg Fe/mL in vials; Pharmacosmos, Holbaek, Denmark), iron carboxymaltose (Ferinject®, 50 mg Fe/mL in 2 mL vials; Vifor, München, Germany), and ferumoxytol (Feraheme®, 30 mg Fe/mL, in 17 mL vials; AMAG Pharmaceuticals, Lexington, MA, USA) were obtained from a pharmacy or directly from the manufacturer. The Ferrozine® reaction kit was purchased from Roche Diagnostics GmbH, Mannheim. All iron formulations were used immediately after opening the vial or kept at 4 °C under nitrogen. Solutions were made from double-distilled water.

2.2. Gel permeation chromatography (GPC)

The apparent average molecular weight was analyzed by gel permeation chromatography. Prior to sample analysis, the columns were calibrated using dextran standards. The dextran standards used for GPC calibration were the commercial available Pharmacosmos standards and consisted of Dextran 25, 50, 80, 150, 270, and 410, respectively. The average molecular weights M_w and the peak average molecular weights M_P were 23.000, 21.400; 48.600, 43.500; 80.900, 66.700; 147.600, 123.600; 273.000, 196.300; 409.800, 276.500 for Dextran 25, 50, 80, 150, 270, and 410, respectively. The standards have been evaluated against the Ph.EUR and USP dextran standards.

The detector used in the GPC measurements is a VE 3580 RI detector (Viscotec). Data are collected and calculations are made using the Omnisec 4.1 software from Viscotec.

The hydrodynamic diameter d_h was calculated from the hydrodynamic volume $V_h = M_p \cdot |\eta|$, where the intrinsic viscosity $|\eta|$ is given by the Mark Houwink equation [20]

$$|\eta| = k\overline{M}_v^a$$

where $\overline{M}_v^a$ is the viscosity average molecular weight.

2.3. Dynamic light scattering (DLS) and zeta potential

The size distribution and zeta potential of the whole particle, which can include an iron hydroxide core plus a carbohydrate shell, was determined by DLS. The diluted samples (0.4 mg Fe/mL double-distilled and sterile filtered water) were measured using a Zetasizer Nano S (Malvern Instruments Ltd.; Worcestershire, UK) including a He–Ne Laser with a wavelength of $\lambda = 633$ nm, which illuminated the samples and detects the scattering information at an angle of 173° (Noninvasive Back-scatter technology). Zeta potential measurements were performed at different pH values by addition of 0.1 N HCl or NaOH, respectively. The data were analyzed with the firmware, Zetasizer Software DTSv612 yielding volume distribution data.

2.4. Transmission electron microscopy (TEM)

The dimension of the iron complex nanoparticle core was determined with an EM420 transmission electron microscope (FEI/Philips, Oregon, USA) at 120 kV. All preparations (1 mg Fe/mL, double-distilled water) were deposited onto a hydrophilized copper grid (300 mesh, Ø 3 mm) and were allowed to dry. The median of the geometrical diameter $d_g = \sqrt{(d_s^2 + d_l^2)/2}$ was determined ($n = 50$, d_s = shortest dimension, d_l = longest dimension).

2.5. X-ray diffraction (XRD)

X-ray measurements of dried out solutions (30 °C) were performed with a XRD 3000 TT (Seifert, Ahrensburg, Germany) using Cu radiation ($\lambda = 1{,}54178$ Å, 40 kV, 30 mA) in Bragg Brentano configuration (automatic divergence slit, angular rate 0,18°/min).

The particles mean diameter d was determined from the Scherrer equation: $d = \frac{\lambda}{\beta \cdot \cos\theta}$, where β is the full width at half maximum of the peak at 36° 2θ or 63° 2θ.

2.6. Mössbauer spectroscopy

Mössbauer spectra of iron isomaltoside 1000 were recorded using a conventional spectrometer in the constant-acceleration mode. Isomer shifts are given relative to α-Fe at room temperature. The spectra were measured in a closed cycle cryostat (Cryo Industries of America, USA) at 150 K, equipped with permanent magnets. The magnetically split spectra were analyzed by least-square fits using Lorentzian line.

2.7. Dialysable iron in buffer

The amount of free iron was estimated using the dialysis technique following pH adjustment of each iron dispersion to 7.5. A dispersion volume containing 150 mg of iron (7.5 mL for LMW iron dextran, iron isomaltoside 1000, iron carboxymaltose and ferumoxytol, respectively; 15.0 mL for sodium ferric gluconate, and 11.25 mL for iron sucrose) was added resulting in concentrations of 20.0 mg Fe/mL for all iron products except for sodium ferric gluconate (10.0 mg Fe/mL) and iron sucrose (13.3 mg Fe/mL). Dilutions were made with water and 0.9% sodium chloride solution, respectively. The volumes were added inside the dialysis tubing (12,000–14,000 MWCO, Medicell, London, United Kingdom) and dialyzed for 24 h at 20 °C against 100 mL of water or sodium chloride solution, respectively. The total volume including the dialysis tube was 107.5 mL. Dialysis of each iron agent was performed in duplicate. Iron in the surrounding solution was quantified using ICP-MS (inductively coupled plasma mass spectrometry). The ICP-MS instrument was a Thermo iCap 6000 ICP-OES (Thermo Scientific, Danmark).

Iron is measured at 238,201 nm. The measurement is made axial. Two-point (left–right) baseline correction and external linear calibration curve are used.

The experiments were carried out at room temperature (20–24 °C). In order to evaluate the effect of pH on the level of dialysable, free iron above experiments were conducted also for the high dose IV iron formulations low molecular weight iron dextran, iron isomaltose, iron carboxymaltose, and ferumoxytol without pH adjustment.

2.8. Acid soluble FeOOH

The acidic hydrolysis of the FeOOH in $[FeOOH]_mL_n$ was followed by quantifying the decreasing FeOOH concentration with UV-spectroscopy. The spectrometer used was a Lambda 20 (Perkin Elmer). Readings at 287.3 nm were made from a scan using data interval 1.0 nm, scan speed 249 nm/min, a slit with of 2.0 nm and a smooth with of 2.0 nm.

The absorbance of iron agents (10 mg Fe/l, 10 mm path length) in 0.9% NaCl/0.2375 M HCl was measured at 287.3 nm from $t = 0$ min to $t = 48$ h, unless otherwise specified. Initial absorbance after dilution of the iron preparation at $t \approx 0$ min was set to 1 according to 100% undissolved FeOOH and all other measurements were normalized for this. Ln(normalized data) was plotted against time and fitted with a second degree polynomial ($R^2 > 0.990$). Half-life $t_{0.5}$ was calculated from $f_{\text{polynomial}}(t_{0.5}) = \ln(0, 5)$.

2.9. Ferrozine®-detectable labile iron

The dissolution of iron in serum was determined by the Ferrozine®-method [21–24]. Ferrozine® does not only detect the free iron but also the weakly bound iron in the complex and the transferrin bound iron in serum, this determination allows one to quantify the in vitro labile iron pool of the investigated intravenous iron formulations. By this method, iron is detected in the ferrous as well as the ferric state as the ferric iron is reduced by ascorbate to ferrous iron. Briefly, human serum was incubated with the iron preparation corresponding to theoretical doses of 200 mg and 500 mg iron, leading to a serum concentration of 66.7 μg/mL and 166.7 μg/mL for a person with a body mass of 70 kg, respectively. These serum concentrations are consequences of a blood volume of 0.07 L per kg and a serum fraction of 60% of the blood volume, yielding a total serum volume of approx. 3 L [25]. The experiment was performed at room temperature (22 °C) in 1.5 mL Eppendorf-tubes. Incubations were done for 10 and 45 min, respectively. Thereafter, a 100 μL sample was analyzed by addition of 700 μL reagent 1 containing thiourea (115 mM) and citric acid (200 mM), followed by addition of 350 μL of reagent 2 containing sodium ascorbate (150 mM) and Ferrozine® (6 mM). Absorption of the complex was measured at 562 nm over approximately 60 min using a PERKIN ELMER Lambda 20 (Perkin Elmer Inc., Waltham, MA, USA) UV–Vis spectrometer. The obtained absorbance versus time curve was fitted to a second degree polynomial for each incubation period and the intercept with the ordinate was calculated to receive the comparable theoretical amount of Ferrozine®-detectable iron. The regression coefficient for the polynomial function was always better than 0.995. The labile iron pool was calculated by linear regression analysis of the obtained intercepts from curves at 10 min incubation and 45 min incubation.

2.10. Elucidation of molecular structure of iron isomaltoside 1000

Proton and carbon NMR spectra were obtained on a Bruker 800 MHz NMR instrument as ca. 5% solutions in D_2O at 300 K. Signals were referenced to external dioxane.

The iron isomaltoside 1000 formulation (10.3 mg) was dissolved in D_2O (600 μL). The sample was transferred to a 5 mm NMR-tube and the ^{13}C NMR spectrum was recorded at 20 °C on a Bruker Avance 800 instrument at 201.12 MHz for carbon (799.96 MHz for proton), integrated and compared with the spectrum of the oligosaccharide alone (7.3 mg) in D_2O (600 μL) [26]. Both samples were measured again and the signals integrated after addition of 2.24 mg of methyl β-maltoside as internal reference.

Molecular modeling: First the isomaltodisaccharide was constructed and an MD calculation using the modeling program, MOE (Molecular Operating Environment, Version 2009.10, Chemical Computing Group Inc., Montreal, Canada), at 450 K, stepsize 0,1 fs clearly showed a significant preference for the gt conformation of C-5–C-6 bonds independent of the starting point. The O-1–C-6 of the glycosidic bond had a weak preference for a trans-arrangement and the orientation of the C-1–O-1 bond satisfied the exoanomeric effect.

The isomaltoside pentamer (composed of 5 α1-6 linked glucose molecules) with glucitol at the reducing end was built from disaccharides in their preferred conformation and energy minimized. The molecule was soaked in water (eight layers) and molecular dynamics was performed at the above conditions corresponding to a period of 2 ns. The additive effect of the oligosaccharide repeat to stabilize the preferred conformation when compared to the disaccharide was significant. The resulting structure was re-soaked and was subjected to energy minimization in water.

3. Results

3.1. Overall particle size

3.1.1. Gel permeation chromatography

The distributions calculated from the GPC chromatograms of the iron preparations show homogenous distributions with the

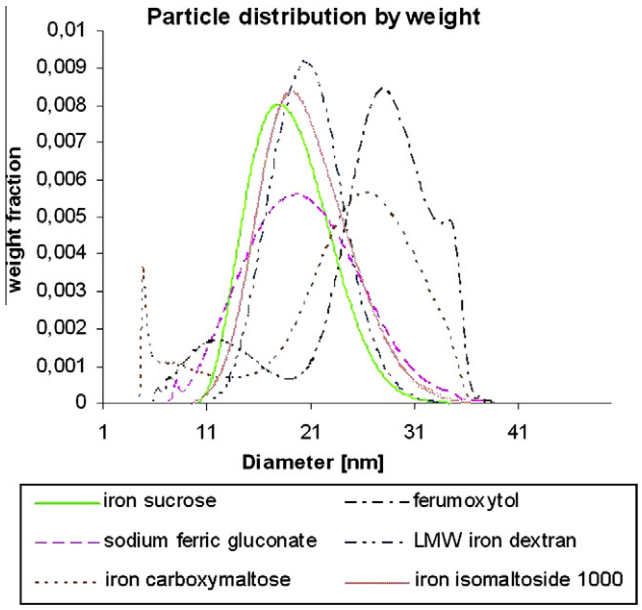

Fig. 1. Weight distribution vs. particle diameter as determined by gel permeation chromatography.

Table 1
Shell /Particle dimensions as determined by gel permeation chromatography (GPC) and dynamic light scattering (DLS).

Iron complex	MW (kDa)	Calculated shell-Ø (nm)[c]	Shell-Ø (nm)	
	GPC		DLS	
Sodium ferric gluconate	164.1	20.3	8.6[a]	0.244[b]
Iron sucrose	140.1	19.1	8.3[a]	0.192[b]
LMW iron dextran	165.0	20.7	12.2[a]	0.149[b]
Iron isomaltoside 1000	150.0	20.5	9.9[a]	0.182[b]
Iron carboxymaltose	233.1	23.8	23.1[a]	0.07[b]
Ferumoxytol	275.7	26.3	23.6[a]	0.143[b]

[a] Median-Ø.
[b] Polydispersity index.
[c] The most frequently found particle diameter in the distribution.

exception of ferumoxytol and iron carboxymaltose which show additional smaller and larger diameter peaks (Fig. 1). The hydrodynamic diameters d_h rise in the order iron sucrose < sodium ferric gluconate < iron isomaltoside 1000 < LMW iron dextran < iron carboxymaltose < ferumoxytol (Table 1). Ferumoxytol was eluted near the exclusion volume, indicating that both its diameter and molecular weight might be underestimated.

3.1.2. Dynamic light scattering (DLS)

The hydrodynamic diameter determined with DLS also measures the carbohydrate shell of the IV iron agents and therefore is larger than iron oxide core diameters determined by TEM or XRD. In Fig. 2 narrow volume distributions of the whole particle diameters are shown. The medians of the hydrodynamic diameters rise from 8.3 to 23.6 nm in the order iron sucrose < sodium ferric gluconate < iron isomaltoside 1000 < LMW iron dextran ≪ ferumoxytol < iron carboxymaltose (Table 1). The zeta potentials of the iron preparations are shown in Table 2. Without pH adjustment, all iron preparations are negatively charged with the exception of iron carboxymaltose. The order of particle charges starting with the most negative iron preparation is ferumoxytol (−43.2 mV) < iron gluconate ≈ iron sucrose < iron isomaltoside 1000 < iron dextran < iron carboxymaltose (+3.7 mV). Acidification of the samples increased the zeta potential of iron carboxymaltose and decreased the negative zeta potential of all other compounds. At a pH value close to the physiological pH, all formulations showed a negative zeta potential, though that for iron carboxymaltose was much smaller.

3.2. Size and structure of core

3.2.1. Transmission electron microscopy (TEM)

TEM images of IV iron agents are shown in Fig. 3. Dark, electron dense, beadlike structures present the cores of the iron oxide complexes, surrounded by a less electron dense matrix, which may be attributed to a carbohydrate fraction. The medians of the geometrical diameter of the core rise from 4.1 to 6.2 nm in the order sodium ferric gluconate < iron sucrose < LMW iron dextran < iron isomaltoside 1000 ≈ ferumoxytol (Table 3). In case of iron carboxymaltose cores tend to cluster and single cores are not definable. The median geometrical core diameter of these clusters is 11.7 ± 4.4 nm.

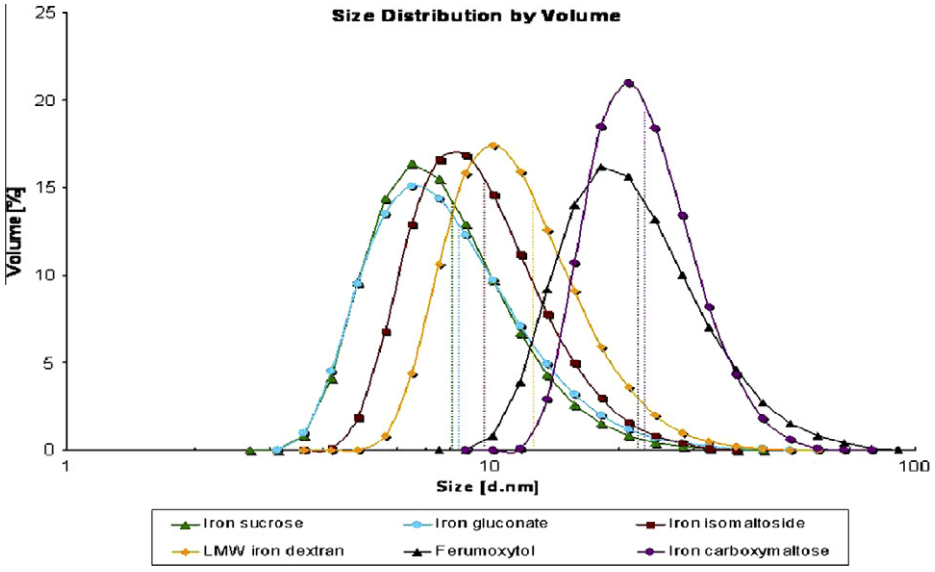

Fig. 2. Volume distribution of the hydrodynamic diameter of intravenous iron preparations as determined by dynamic light scattering (DLS). Conditions: 0.4 mg Fe/ml. Vertical lines assign the median diameter.

Table 2
Zeta potentials ζ of IV iron polynuclear complexes at different pH values.

Iron gluconate		Iron sucrose		LMW iron dextran		Ferumoxytol		Iron isomalto-side 1000		Iron carboxymaltose	
pH	ζ (mV)	pH	ζ (mV)	pH	ζ (mV)	pH	ζ (mV)	pH	ζ (mV)	pH	ζ (mV)
4.35	−16.50	4.49	−14.25	3.02	−3.56	3.39	−11.95	3.3	−3.98	3.26	9.46
7.4	−29.70	7.43	−26.20	6.4[a]	−15.30	6.6[a]	−43.20	6.3[a]	−22.00	5.36[a]	3.68
8.36[a]	−29.10	11.03[a]	−28.15	7.31	−17.25	7.36	−30.55	7.35	−21.05	7.26	−8.52
10.5	−29.60			11.8	−15.75	10.4	−34.40	9.03	−28.95	9.54	−16.35
								11.5	−26.40		

[a] pH in bidistilled sterile-filtrated water, without any pH adjustment.

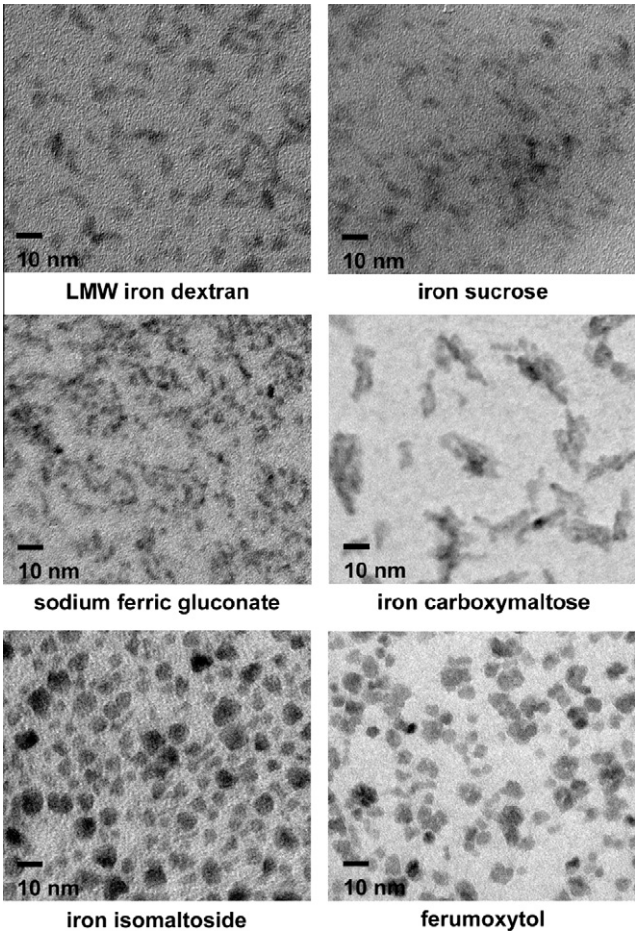

Fig. 3. Transmission electron microscopy images of intravenous iron preparations. Conditions: 1 mg Fe/ml.

3.2.2. X-ray diffraction (XRD)

The particles mean diameters *d* of the cores were determined using the Scherrer equation and are presented in Table 3. The mean diameters of single core complexes are in the range of 3.3–6.4 nm and appear in accordance with diameters measured by TEM.

In Fig. 4, X-ray diffractograms of IV iron agents (upper part of figure) are compared with diffraction data of standard iron oxides from the ICDD (lower part of figure, International Centre for Diffraction Data). Peaks belonging to the carbohydrate fraction are marked with arrows. With the exception of ferumoxytol the IV iron agents show broad regions of high intensities at in part similar angle values of diffraction with similar intensities.

The patterns of iron sucrose and sodium ferric gluconate show a structure similar to 2-line ferrihydrite as there are just two major iron oxide peaks at 36° 2θ and 62° 2θ. Two others at 14° 2θ and 22° 2θ belong to amorphous sucrose [27]. Small reflections at 40° 2θ and 56° 2θ could be a hint that also other structures are mixed in like akaganeite.

The X-ray results of iron carboxymaltose indicate the akaganeite structure with same intensities at same angles except for a minor peak instead of a major peak at 12° 2θ. LMW iron dextran and iron isomaltoside 1000 show a pattern which is similar to akaganeite as well, but conformity is not as good (minor peaks instead of major peaks at 12° 2θ and 35° 2θ, in part missing minor peaks).

The diffractogram of ferumoxytol, which is used as IV iron agent and contrast agent in magnetic resonance imaging as well, is close to pattern of magnetite and maghemite. Sharp peaks in the diffractogram belong to crystalline mannitol in the formulation.

3.3. Ferrous iron content

3.3.1. Mössbauer spectroscopy

The mössbauer spectrum of iron isomaltoside 1000 shows a doublet with an isomer shift δ = 0.44 mm/s and a quadrupole splitting EQ = 0.78 mm/s (Fig. 5). Both parameters are characteristic for iron in the ferric state. There is no indication of iron in the ferrous state as characteristic isomer shifts and splittings are absent.

3.4. Dialysable iron content

3.4.1. Dialysis

The results of the determination of the dialyzable "free" iron content are shown in Table 3. It appears that iron isomaltoside 1000, iron carboxymaltose, and ferumoxytol yield very low free iron contents smaller than 0.002% of the total iron content. This was independent of the liquid used for the dilution and dialysis (water versus sodium chloride solution). Iron dextran yielded free iron contents of 0.1% and 0.2% in water and sodium chloride solution, respectively. The highest free iron content was observed for sodium ferric gluconate yielding more than 1% in sodium chloride dilutions. However, the free iron content in the iron sucrose preparation (0.067% in NaCl and 0.057% in water) was lower than expected. The experiment without pH adjustment showed that only iron carboxymaltose was affected by pH. As depicted in Fig. 6 the content of free iron in iron carboxymaltose increases from below the detection limit (<0.002%) at pH 7.5–0.262% when the experiment is conducted in nonbuffered 0.9% NaCl.

3.5. Labile iron

3.5.1. Acid soluble iron

In acidic solution, FeOOH is dissociated: $FeOOH + 3HCl \rightarrow Fe^{3+} + 3Cl^- + 2H_2O$. In this study, iron formulations $[FeOOH]_mL_n$ with different carbohydrate ligands L were decomposed similarly: $[FeOOH]_mL_n + 3mHCl \rightarrow mFe^{3+} + 3mCl^- + 2mH_2O + nL$. As the molar extinction coefficient of the complex at 287.3 nm ($\varepsilon^{287.3nm}_{[FeOOH]_mL_n} \approx 3000\ M^{-1}\ cm^{-1}$) is substantially higher than the extinction coefficient of Fe^{3+} ($\varepsilon^{287.3nm}_{[FeOOH]mLn} \approx 580\ M^{-1}\ cm^{-1}$) or carbohydrate (negligible), the decreasing FeOOH concentration is approximately proportional to the measured absorbance.

Table 3
Core dimensions as determined by transmission electron microscopy (TEM) and X-ray diffraction (XRD), acidic hydrolysis stability and dialysable iron with and without pH adjustment.

Iron complex	Core-Ø (nm)		$t_{0.5}$ (h)	Dialysable iron[e] (%)		
	TEM	XRD	Acidic hydrolysis	WFI[f]	NaCl[f]	NaCl[g]
Sodium ferric gluconate	4.1[a] ± 1.7	3.4	4.0 ± 0.1	0.789 ± 0.048	1.338[d]	
Iron sucrose	5.0[a] ± 0.8	3.3	4.9 ± 0.1	0.057[d]	0.067[d]	
LMW iron dextran	5.6[a] ± 1.2	4.4	21.0 ± 1.2	0.100 ± 0.0096	0.207 ± 0.0071	0.172[h] ± 0.0048
Iron isomaltoside 1000	6.3[a] ± 1.2	4.2	25.2 ± 1.2	<0.002[c]	<0.002[c]	0.014[h] ± 0.0029
Iron carboxymaltose	11.7[a,b] ± 44	4.3	25.6 ± 1.6	<0.002[c]	<0.002[c]	0.2621[h] ± 0
Ferumoxytol	6.2[a] ± 1.4	6.4	62.4 ± 0.4	<0.002[c]	<0.002[c]	0.005[h] ± 0.0047

[a] Median-Ø.
[b] Median-Ø of an agglomeration of several cores. Single cores are not definable.
[c] Detection limit.
[d] Only one sample.
[e] Calculated from ICP-MS measurements.
[f] Adjustment to pH 7.5.
[g] Without pH adjustment.
[h] Product approved for high dose administration.

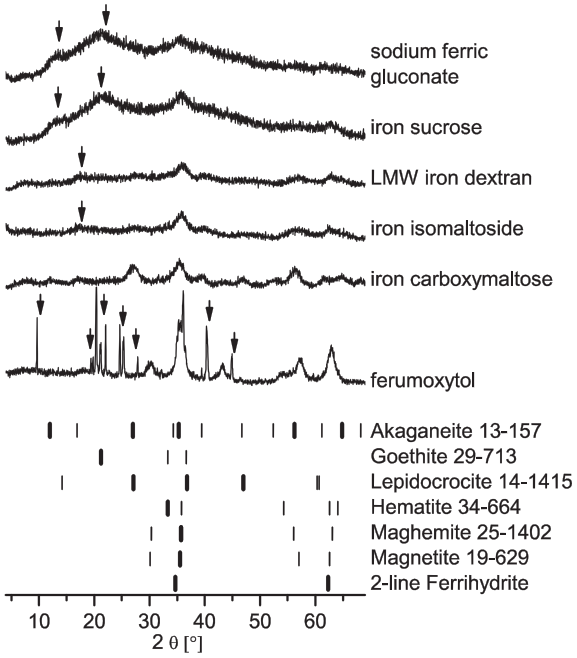

Fig. 4. X-ray spectra of intravenous iron preparations. At the bottom, the spectra are compared with diffraction data of standard iron oxides from the ICDD (International Centre for Diffraction Data). Thick reflexion lines of standard iron oxides: Intensity 70–100%. Thin reflexion lines of standard iron oxides: 30–69% intensity. Peaks belonging to carbohydrate fraction are assigned by arrows.

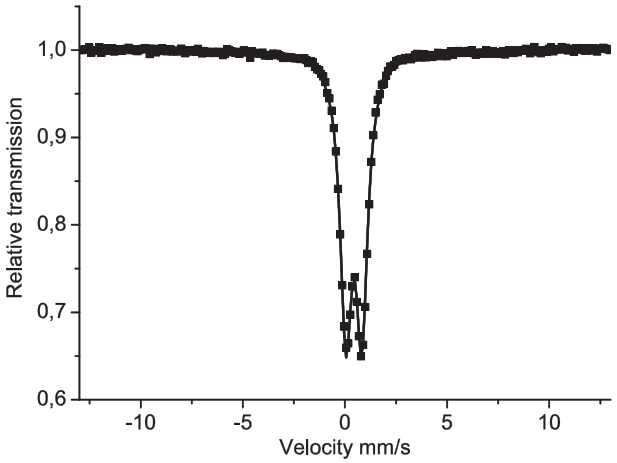

Fig. 5. Mössbauer spectrum of MonoFer as powder measured at 150 K. Measuring points are fitted with a line of Lorentz shape. Isomer shift $\delta = 0.44$ mm/s, quadrupole splitting $E_Q = 0.78$ mm/s, line widths $\Gamma = 0.69$ mm/s.

The rate of hydrolysis is a measure of the relative stability of the FeOOH entity. In Fig. 7, it can be seen that the fraction of FeOOH remaining decreases with time, and in Table 3, the half-times of FeOOH decomposition of the various iron preparations are compared. The complex stability is increasing in the order of iron gluconate < iron sucrose ≪ iron dextran < iron carboxymaltose ≈ iron isomaltoside 1000 ≪ ferumoxytol. There is some indication that the rate of degradation relates to the surface area of the iron complex formulation and decreasing with increasing particle size (Fig. 8).

3.5.2. Ferrozine®-detectable labile iron in human serum

The results of the determination on detectable labile iron with the Ferrozine®-method are shown in Fig. 9. The amount of the labile iron, measured by this test was nearly equivalent to the administered dose of 200 mg and 500 mg, respectively. The products which show the highest fraction of labile, Ferrozine®-detectable iron are, by far, iron gluconate and iron sucrose (3.2 ± 0.4% for iron gluconate, 3.5 ± 0.2% for iron sucrose at a 200 mg dose, respectively). For the different compounds, the fraction of labile

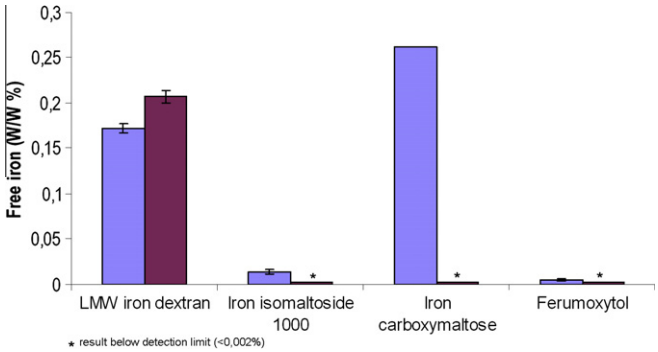

Fig. 6. Comparative free iron content in high dose IV iron products. The detection limit was 0.002%. Red bars indicate free iron content following adjustment of the diluted preparation to pH 7; blue bars indicate results obtained without pH adjustment. Star indicates concentrations below detection limit. SD's are listed in Table 3.

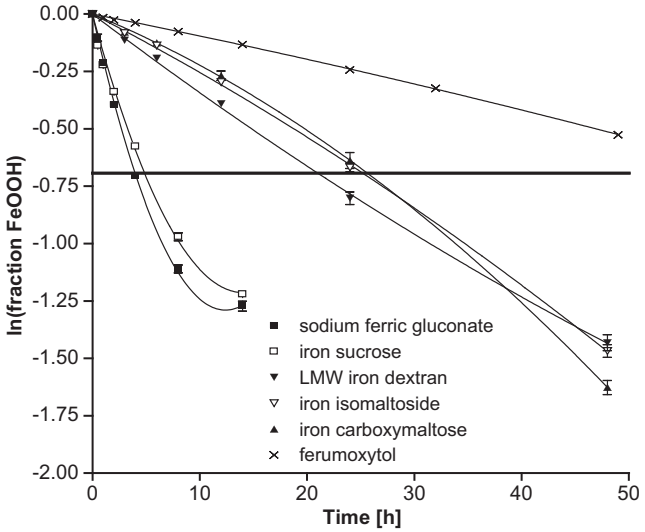

Fig. 7. Acid soluble iron. Concentration: 10 μg Fe/ml, 0.2375 M HCl. At $t = 0$ min, the fraction of FeOOH is 1 according to 100% not hydrolyzed FeOOH. Each point represents the average of three measurements; error bars are sometimes smaller than symbols. Data were fitted with a second degree polynomial ($R^2 > 0.990$). The solid line labels the half-time. SD's are listed in Table 3. For further details refer to methods.

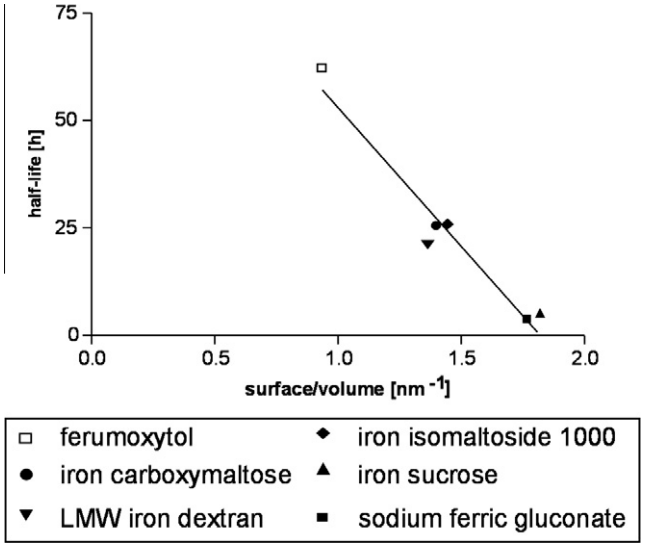

Fig. 8. Half-time of FeOOH hydrolysis against FeOOH core surface.

iron is decreasing in the order of iron sucrose ≈ iron gluconate ≫ iron dextran > iron isomaltoside 1000 ≈ ferumoxytol > iron carboxymaltose.

3.6. Molecular structure of iron isomaltoside 1000

Proton NMR spectra obtained at 800 MHz of 6-O-D-glucityl oligoisomaltoside (isomaltoside 1000) indicate a pure sample of higher oligomeric isomaltoside 1000s with an average polymerization of about 5.2, i.e., less than 1.5% reducing sugar is left (data not shown). The proton NMR data particularly the coupling constant between hydrogen H-5 and H-6 and H-6′ for the internal residues being 3.0 and 5.0 Hz, respectively, indicate the population of the rotational isomers of the hydroxymethyl group is approximately 60/40 for the gt/gg isomers [28]. ^{13}C NMR spectra obtained at

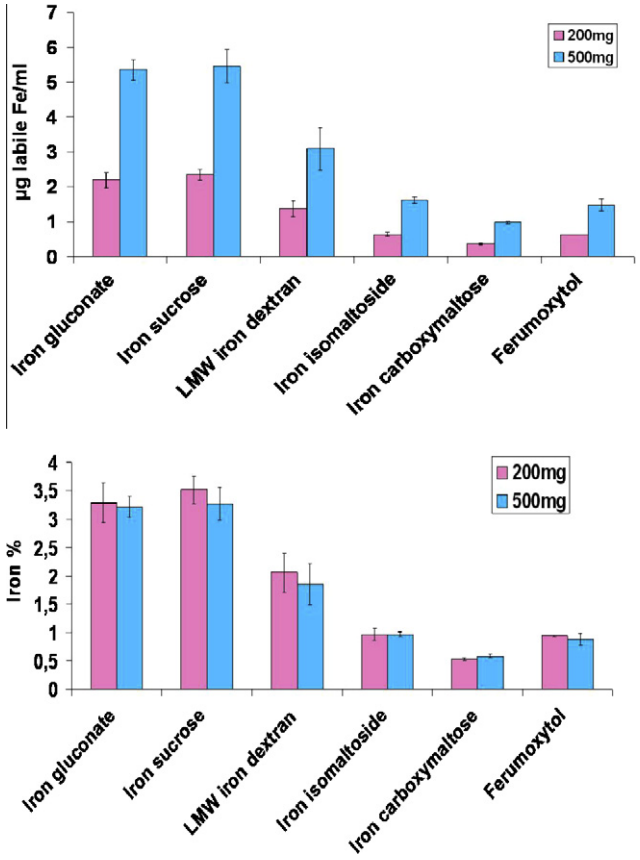

Fig. 9. Comparative labile iron pools of parenteral iron products. Upper diagram: Concentration of the Ferrozine®-detectable labile iron pool in μg/ml. The bars represent the average of at least four measurements. Lower diagram: Ferrozine®-detectable labile iron in percentage of the total used dose. For each measurement, the iron complex was incubated in human serum for 10 and 45 min, respectively. Thereafter, the Ferrozine® reaction was performed and in each case an intercept of a second degree polynomial regression function of the absorption versus time curve with the ordinate was calculated. These intercepts were extrapolated to an incubation time of $t = 0$ by linear regression, yielding the labile iron pool in serum for each intravenous iron product.

200 MHz of isomaltoside 1000 confirmed the above-mentioned conclusions as shown in Fig. 10a. Data of ^{13}C NMR measurements for the iron isomaltoside 1000 complex as prepared described in patent [26] showed line broadening of signals as seen in Fig. 10b. The spectrum demonstrates a significant line broadening of the carbon signals carbon C-1, C-5, and C-6 and a smaller line broadening of C-3 and C-2 all from the "internal" glucose residues, suggesting that the complexation of the iron in the isomaltoside 1000 matrix preferentially takes place in the cavity shown in Fig. 11. While carbon signals in presence of iron were only subjected to some line broadening no signals could be obtained in proton spectra of the complex. The suppression of signal intensity in integrated signals of individual carbon atoms was a measure of complex formation between their attached oxygen atoms and the iron atoms.

The following relative intensities as compared to the free oligosaccharide were measured for central sugar residues in the oligosaccharide-iron complex: C-1: 48% (98 ppm); C-2: 60% (71.6 ppm); C-3: 48% (73.7 ppm); C-4: 67% (69.7 ppm); C-5: 53% (70.4 ppm); C-6: 52% (65.7 ppm). This indicates that iron complex formation occurs primarily via O-3, O-5, and O-6 of these sugar residues.

Molecular dynamics modeling followed by energy minimization of 6-O-D-glucityl isomaltopentaoside isomaltoside 1000 using the MOE program resulted in the minimum energy conformation which, with all the rotational isomers around the 6-position in

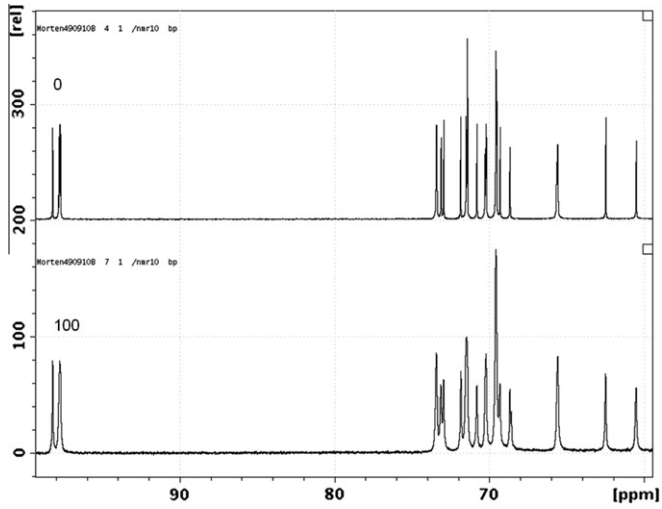

Fig. 10. (a/b) 200 MHz carbon-13 NMR spectra of isomaltoside (upper) and isomaltoside/iron complex (lower) prepared according to Ref. [26].

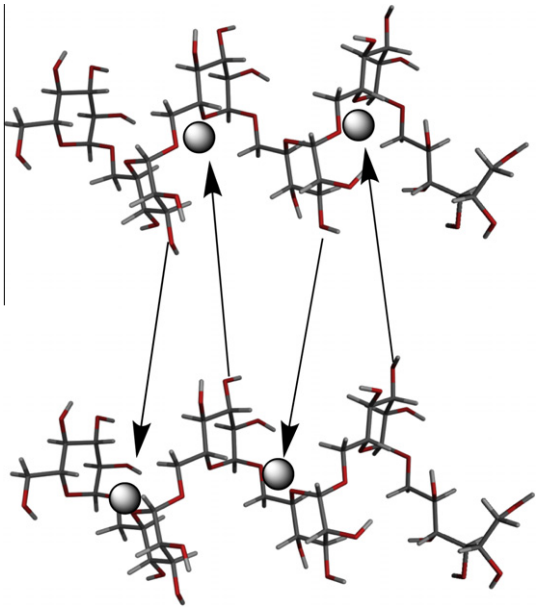

Fig. 11. Schematic representation of possible complexation site based on carbon-13 NMR line broadening studies.

the "gt" conformation, show that the structure repeats itself every second glucose residue leaving O-5 and O-6 from each residue and O-3 from residues in a neighboring chain positioned to interact and chelate with iron atoms. This allows chains to stack through iron oxygen interactions. Every second residue is to the same face of the molecule available for further complexation with iron (Fig. 12).

4. Discussion

4.1. Structure

Iron isomaltoside 1000 contains isomaltoside 1000, a pure linear chemical structure of repeating α1-6 linked glucopyranose residues. It is an unbranched, nonanaphylactic carbohydrate with an average size of 5.2 glucose units and an average molecular weight of 1000 Da, respectively, structurally different from the branched dextran polysaccharides present in iron dextran. Low molecular weight dextran has a molecular weight around 5000 and on average one α-1-3 branch point per 32 glucose residues. A computer model using the same approach as described in the experimental part of one of such structures is shown in Fig. 13 and is clearly very different from the isomaltoside 1000 shown in Fig. 12. Isomaltoside 1000 consists predominantly of 3–5 glucose units and is prepared from oligomers used for prevention of dextran-induced anaphylactic reaction. Hence, this preparation does not contain dextran, and therefore, there is no requirement for a test dose. Analysis by XRD does not show sharp diffraction peaks for any of the iron preparations, indicating structures with little crystallinity, which is the consequence of small crystal size and structural disorder. The iron oxyhydroxide in iron isomaltoside 1000 seems to consist of a "mixed layer" similar to Akaganeite.

The core of iron sucrose has a structure close to 2-line Ferrihydrite, possibly mixed with layers of Akaganeite. Earlier investigations already have identified the iron oxyhydroxide core of iron sucrose as 2-line Ferrihydrite (X-ray diffraction, SEAD) [29,30] and Akaganeite (X-ray diffraction, Mössbauer) [31], respectively.

The iron carboxymaltose pattern is in accordance with Akaganeite. The consideration that the distance between latticed planes at small diffraction angles is close to 1 nm indicates that a 5 nm core is just a few lattice planes wide and that the formation of a long-range order, which is characteristic for crystals, is hardly possible.

In the case of sodium ferric gluconate, earlier investigations already have identified the iron oxyhydroxide core as Akaganeite (X-ray diffraction, Mössbauer) [31]. LMWID and Ferumoxytol structures resemble akaganeite (LMWID) and magnetite and maghemite (Ferumoxytol), respectively, an observation which has not been reported before.

The visual appearance of the iron formulations as viewed by TEM varied considerably as shown in Fig. 3, both in terms of size and shape. Some preparations are well defined in terms of spherically shaped particles (iron isomaltoside 1000) whereas others display irregularly shaped particles, varying in size. Results of the instrumental size analysis of the parenteral iron preparations demonstrated that these results are also partly dependent on the method of determination. In this work, four different methods for size analysis were applied, two of them measuring the hydrodynamic diameter (GPC, DLS) and the other two the diameter of the iron core (XRD and TEM). The latter methods yielded generally smaller particle sizes, except for iron isomaltoside, where more or less identical diameters were obtained by DLS and TEM, which supports the formation of a matrix-type structure and thus confirms structural dissimilarities between iron isomaltoside and the other iron complexes. Discrepancies between DLS and GPC might be due to dilution effects, since higher concentrations of iron preparations tend to form clusters which may result in higher diameters of the respective iron preparation. Another explanation for the diverging results based on DLS vs. GPC can be given by the zeta potential of the investigated preparations. The highest differences were observed for iron gluconate, iron sucrose, LMW iron dextran, and iron isomaltoside 1000 (Table 1). This may be due to their negative charge at small particle size, which may interact with the spherical silica particles containing polar diol groups of the column material yielding shorter retention times and thus higher hydrodynamic radii for GPC. Ferumoxytol, which is also negatively charged, has a much higher diameter, thus the charge per unit surface area is lower leading to a lower interaction with the column material. It is noteworthy that, except for iron carboxymaltose, the zeta potentials of all iron formulations indicated that they carried a negative charge in the pH-range 3–11 (Table 2). Iron carboxymaltose underwent a change in sign from positive zeta potential at acidic and neutral pH-conditions to negative potential at alkaline pH. Interestingly, an FDA Advisory Committee meeting on safety of iron

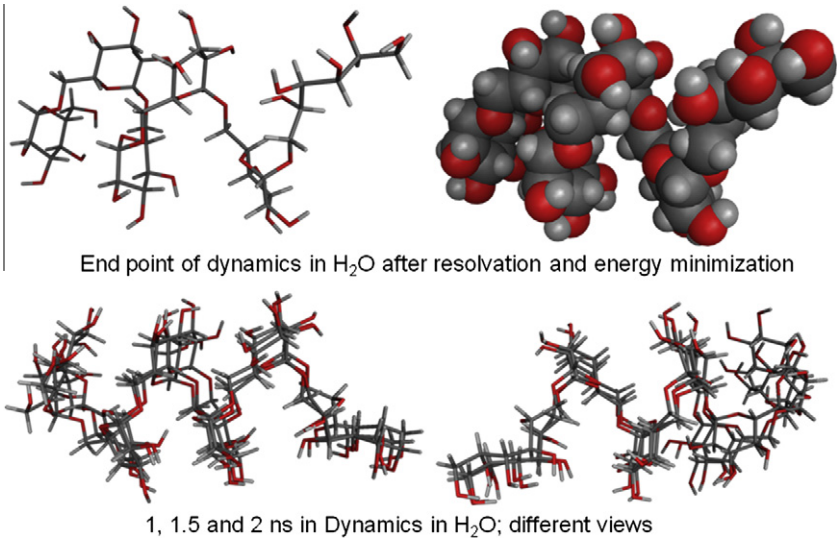

Fig. 12. End point of MD simulation.

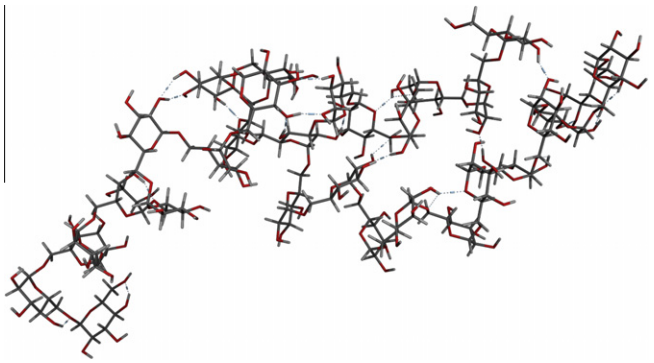

Fig. 13. computer model of low molecular weight dextran , 5000 Daltons, and one branch point. main chain 23 residues, side chain 9 residues.

carboxymaltose pointed out the existence of an imbalance in the occurrence of clinically important hypophosphatemia following high dose intravenous iron carboxymaltose, whereas this effect was not observed in a control group receiving oral iron and is not documented for other intravenous iron products [15]. Maybe the difference in Zeta potentials and thereby the positive charge on iron carboxymaltose can be a lead in explaining the mechanism behind iron carboxymaltose induced hypophosphatemia. If iron carboxymaltose carries a positive charge when it is in the circulation, negatively charged phosphate could potentially be trapped by electrostatic interaction with iron carboxymaltose.

It is also noteworthy to mention that with the exception of iron carboxymaltose and ferumoxytol all parenteral iron preparations followed a monomodal size distribution. As determined by GPC, both iron carboxymaltose and ferumoxytol showed an additional peak at smaller particle sizes and ferumoxytol in addition contained a shoulder indicating some fraction of the particle size having distinct higher molecular weight than the average number given (Fig. 1).

Detailed investigations presented in this manuscript using ^{13}C NMR and molecular modeling studies strongly suggest a spheroidal matrix structure for iron isomaltoside 1000 in which iron atoms are bound and dispersed [approx 10 iron atoms per oligosaccharide molecule]. This seems to be a much more likely description of the true iron isomaltoside 1000 molecular assembly than that of an iron core surrounded by a carbohydrate shell. Comparison of TEM images and DLS curves shows that these give more or less identical diameters for iron isomaltoside whereas for the other iron complexes DLS diameters strongly overestimate the diameters given by TEM. This also supports the results that a real matrix is formed and shows that iron isomaltoside is quite different from the other products since all the other TEM/DLS comparisons seem to suggest a core shell structure. A possible explanation for this unique molecular assembly could be given by the short, linear, and nonionic isomaltoside 1000 structure combined with proprietary Pharmacosmos production technology. This enables the production of a matrix structure composed of interchanging layers of linear isomaltoside 1000 with iron atoms placed in cavities between, and within, the oligosaccharide molecules (Figs. 11 and 12).

Integration of the ^{13}C NMR spectra of iron isomaltoside 1000 and the parent oligosaccharide against an internal reference indicate that more than 90% of the iron sample signals are visible.

Future studies should investigate the underlying mechanism for the existence of these structural differences in the iron carbohydrate complex.

4.2. Free iron

Free iron was determined following dialysis of the diluted iron preparations In general, the free iron fractions were observed to be low, although for sodium ferric gluconate a free iron content of 1.33% was found. This coincides with the known low stability of this iron complex [32]. Surprisingly, the free iron content of the other low stability complex iron sucrose was less than 0.1%, a value which is not reflected by the lability of the complex. Generally, iron sucrose is considered as a semi-robust moderately strong complex, whereas iron gluconate is labile and weak. Other investigators have found similar results for iron sucrose [25]. The underlying reason for this observation is currently unknown, an interaction of the complex with the dialysis membrane may not be ruled out For iron isomaltoside 1000, iron carboxymaltose and ferumoxytol free iron content was below the detection limit of the method (<0.002%) when the experiment was done in a pH 7.4 buffered 0.9% NaCl solution. Interestingly, the content of free iron in iron carboxymaltose jumps from <0.002% to 0.262% when the experiment is conducted in standard saline, which does not contain a buffer. This indicated that the dialyzable iron in iron carboxymaltose is sensitive to pH. It may be worth noting that dilutions of IV iron formulations for drip infusion are prepared in nonbuffered

physiological saline solutions. On the other hand, for a 1000 mg iron dose, this will represent the release of a maximum of 2.62 mg free iron over 24 h, only 40% of the capacity of transferring to bound iron, such that the probability of increasing free iron in blood seems low.

4.3. Labile iron

Labile iron is not identical to free iron in the iron products, since it can be considered as weakly bound iron within the nanoparticle which is readily mobilized by chemical reactions or in the presence of iron complex forming agents, such as proteins in blood plasma. In acidic solutions, labile bound iron may be mobilized in the presence of H_3O^+ ions from the oxyhydroxide structure. Iron sucrose and sodium ferric gluconate released their iron content at the highest rate, followed by a group consisting of iron dextran, iron carboxymaltose, and iron isomaltoside 1000, whereas ferumoxytol was most resistant toward iron mobilization at acidic pH (Fig. 7). One explanation for these observed differences may be a reasonable inverse correlation between the size of the iron complex/oxyhydroxide particle size as determined by TEM and the release rate, which was lowest for ferrumoxytol (highest half-time) and highest for iron sucrose (lowest half-time) (Figs. 7 and 8). As depicted in Fig. 9, the amount of labile iron determined by diluting the iron formulations with human serum (Ferrozine®-method) show similar results to those obtained in the experiments on release of iron under acidic conditions and results from our investigations on labile and free iron. The percentage of labile iron released as shown in this study is independent on the iron dose employed and covers free as well as labile bound iron to iron-binding proteins such as transferrin and other serum proteins.

Overall, these results demonstrate that labile iron content in all parenteral iron products designed for rapid and high dose administration [iron isomaltoside 1000, iron carboxymaltose and ferumoxytol] is less than 1% of the administered iron dose. Therefore, these parenteral iron products can be considered as optimized dosage forms with respect to burst release of iron from the carbohydrate iron complex.

4.4. Immunogenic properties of the carbohydrate

Similar to the risk of free iron reactions, anaphylactic or anaphylactoid reactions have traditionally been a concern when using IV iron compounds [9]. On the one hand, it has been assumed that antibody-mediated anaphylactic reactions caused by circulating dextran antibodies occur more frequently with iron dextran products. On the other hand, iron induced anaphylactoid reactions can occur with all IV iron preparations, but they are generally not thought to be mediated through an immune response [9]. Today there is only a test dose requirement for Dexferrum (high Mw iron dextran), CosmoFer (low Mw iron dextran), and Venofer (iron sucrose)—and for Venofer this only applies within Europe. All the newly introduced high dose IV preparations (Monofer [iron isomaltoside 1000], Ferinject®/Injectafer® [iron carboxymaltose], Feraheme [ferumoxytol]) are based on carbohydrates with a reduced immunogenic potential and no test doses are required.

In the case of Monofer, the carrier carbohydrate isomaltoside 1000 is based on a chemical modification of oligomers known to prevent dextran-induced anaphylactic reactions. The ability of isomaltose oligomers (5 glucose units) to prevent or block anaphylaxis to dextrans was first reported by Coulson and Stevens [33] in the 1960s, but intensive research by Richter et al. [34–36] in the 1970s and 1980s revealed its unique role as a specific monovalent hapten against circulating anti-dextran antibodies. Studies by Richter et al. documented that even in animals maximally sensitized against dextran isomaltose oligomers of 1430 Da or lower were nonanaphylactogenic and desensitizing [16].

The term hapten is defined as a substance capable of binding to specific antibodies without inducing anaphylaxis or induction of antibody formation. Thus, by binding to the receptor sites on circulating anti-dextran IgG, isomaltose oligomers block and prevent these sites from participation in the formation of large immune complexes exhibiting multiple (polyvalent) IgG-specific epitopes, thereby avoiding classical anaphylactic reactions.

In later multinational clinical trials involving over 5 million patients, it was shown that a pre-injection of isomaltose oligomers was able to reduce the risk of anaphylaxis to polyvalent clinical dextran from ca. 1 in 3000 to less than 1 in 200,000 patients [33,35,37–39]. Therefore, isomaltose oligomers are well-documented inhibitor haptens of dextran anaphylaxis with a convincing clinical record that establishes their non-anaphylactic nature, thus providing the rationale for eliminating test dosing when administering Monofer.

4.5. Clinical consequences

The efficacy of IV iron is directly related to the amount of iron administered, but differences in core size and carbohydrate chemistry determine pharmacological and biologic differences between the different iron formulations. These include clearance after injection, iron release in vitro, early evidence of iron bioactivity in vivo, and maximum tolerated dose and rate of infusion, as well as effects on oxidative markers, propensity for inducing hypophosphatemia (Ferinject®/Injectafer®), and propensity to cause transient proteinuria (Venofer®) or hepatic damage (Ferrlecit®) following administration [40–45]. Thus, efficacy, safety, and convenience of dosage should be taken into account when selecting an IV iron compound.

The efficacy of all IV iron preparations for treating anemia has been consistently proved in a variety of clinical settings with a very low rate of severe ADEs, e.g. [5], although iron dextran complexes may cause well-known dextran-induced anaphylactic reactions, which are significantly more frequent with high molecular weight iron dextran (HMWID) than with low molecular weight iron dextran (LMWID) [13]. The risks of total ADEs (OR 3.2, 95%CI 2.7–3.8) and life-threatening ADEs (OR 3.4, 95%CI 2.0–5.9) were significantly increased among recipients of HMWID compared with LMWID. Nevertheless, it is worth noting that there were no significant differences in mortality rates between LMWID and iron gluconate (OR 0.3, 95%CI 0.1–1.3) or iron sucrose (OR 0.2, 95%CI 0.1–1.0), although life-threatening ADEs were significantly more frequent among recipients of LMWID [13]. In addition, excluding HMWID, the rates of life-threatening ADEs associated with IV iron (1.4 per million doses), including iron-related deaths (0.3 per million doses) [13], are much lower than that of ABT-related (allogenic blood transfusion) severe side effects (10 per million units) and ABT-related deaths (4 per million units) [46].

Therefore, with the exception of HMWID (increased rates of severe ADEs and deaths), the acute safety differences among IV iron products are small and clinically irrelevant when given at the recommended doses, though whenever possible a product based on a carbohydrate with reduced immunogenic activity should be preferred (comparator trials are needed to be certain). In this regard, the new IV iron formulations (Monofer®, Ferinject®/Injectafer® and Feraheme®) are all based on carbohydrates with reduced immunogenic properties thereby avoiding the need for a test dose (reduction of treatment time). At least Monofer® and Ferinject® are based on a carbohydrate with documented reduced immunogenic activity. Feraheme® was also supposed to be based on a carbohydrate with documented reduced immunogenic activity but recently published case reports have shown that dextran sensitive patients can react to Feraheme® as well [47].

Table 4
Summary of clinical properties of IV iron formulations.

Product	CosmoFer®[a] (low Mw iron dextran)	Ferrlecit®[b] (iron gluconate)	Venofer®[a] (iron sucrose)	Ferinject®[a] (iron carboxymaltose)	Feraheme®[c] (ferumoxytol)	Monofer®[a] (iron isomaltoside 1000)
Carbohydrate	Dextran (branched polysaccharides)	Gluconate (monosaccharides)	Sucrose (disaccharides)	Carboxymaltose (branched polysaccharides)	Carboxymethyl dextran (branched polysaccharides)	Isomaltoside 1000 (unbranched linear oligosaccharides)
Maximum single dose	20 mg/kg	125 mg	200 mg	15 mg/kg single dose limit: 1000 mg	510 mg	20 mg/kg
Maximum single-dose administration in a 80 kg man	1600 mg	125 mg	200 mg	1000 mg	510 mg	1600 mg
Maximum single-dose administration in a 60 kg woman	1200 mg	125 mg	200 mg	900 mg	510 mg	1200 mg
One dose iron repletion (TDI)	Yes	No	No	No	No	Yes
Infusion within 1 h	No	NA	NA	Yes	Yes	Yes
Test dose required	Yes	NA	Yes/No*	No	No but must wait 60 min after injection	No
Iron concentration (mg/ml)	50	12.5	20	50	30	100
Vial volume (ml)	2 and 10	5	5	2 and 10	17	1, 5 and 10

[a] eMC, Summary of Product Characteristics (SPC) electronic Medicines Compendium.
[b] Bridgewater, NJ; Sanofi Aventis, Inc., US Ferriedt Prescribing information 2010.
[c] AMAG Pharmaceuticals, Feraheme Prescribing information.
* Test dose in Eroupe (yes) but not in US (no).

All IV preparations may cause anaphylactoid reactions caused by labile iron which are characterized by nausea, hypotension, tachycardia, chest pain, dyspnoea (lung edema), and bilateral edema of the hands and feet, and should not be misread as anaphylaxis [40]. Hence, formulation stability and free or labile iron content determine the maximal dose and maximal speed of infusion. Accordingly, large doses of iron isomaltoside 1000 (20 mg/kg), iron carboxymaltose (15 mg/kg, max 1000 mg), LMWID (20 mg/kg), and ferumoxytol (510 mg) can be administered in a single session, as they are all strong and robust formulations, with very low content of free or labile iron (Table 4). However, Ferinject® may cause unexplained hypophosphatemia, and its free iron content looks very sensitive to pH (Table 3, Fig. 6) and dilution (according to the Ferinject® SPC), whereas the administration of large doses of InFed/CosmoFer is hampered by an extended infusion time (4–6 h). In contrast, a much lower single dose is allowed for ferric gluconate (125 mg) or iron sucrose (200 mg), as they are more labile and weak formulations. Thus, correction of iron deficiency with these IV compounds is a time- and resource-consuming option (8–12 sessions to administer 1500 mg iron). A summary of the clinically relevant product characteristics is given in Table 4.

5. Conclusions

The analyzed polynuclear iron formulations are all characterized by a nanosized structure resembling Lepidocrocite, Akaganeite, Ferrihydrite, Magnetite or Maghemite, or mixture of these depending on the product. The homogenicity of the products varied a lot with iron isomaltoside 1000 displaying very well defined spherically shaped particles. With the exception of iron carboxymaltose and ferumoxytol, all parenteral iron preparations followed a monomodal size distribution.

^{13}C NMR and molecular modeling studies indicate that iron isomaltoside 1000 is a iron carbohydrate matrix structure contrary to the classical iron core–carbohydrate shell description. The high dose IV iron products are all characterized by a low content of labile and free iron. The content of free iron measured in physiological saline was the lowest in ferumoxytol and iron isomaltoside 1000 followed by low Mw iron dextran and iron carboxymaltoside which had the highest content of free iron.

In conclusion, iron isomaltoside 1000 (Monofer®) is a new IV iron source that is based on iron (III) and chemically modified isomalto-oligosaccharides. In contrast to the polysaccharides in iron dextrans, the carbohydrate isomaltoside 1000 is linear and unbranched with a low immunological activity. Hence, a test dose is not necessary. Compared to the existing IV iron preparations iron isomaltoside 1000 contains strongly bound iron in an iron carbohydrate matrix, with a very low content of labile and free iron. This enables Monofer, as the only IV iron formulation, to be administered as a rapid high dose infusion in doses over 1000 mg. This allows flexible dosing including high and rapid iron repletion, offering convenient one visit iron therapy for a wide range of patients.

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