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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), iron isomaltoside 1000 (Monofer®, 100 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 con-

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 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

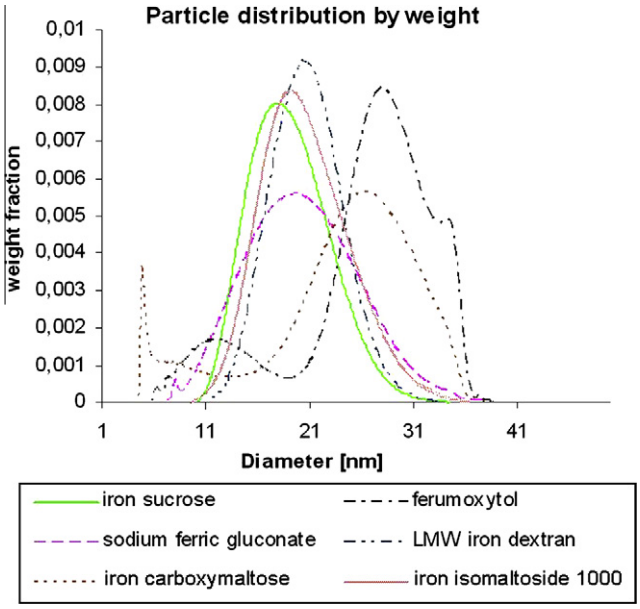

Fig. 1. Weight distribution vs. particle diameter as determined by gel permeation chromatography.

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.

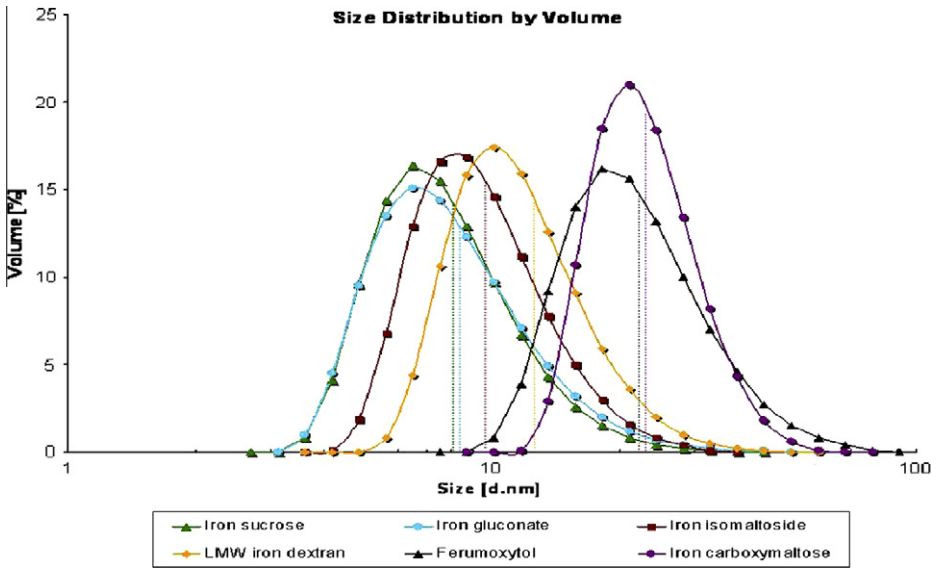

Table 2
Zeta potentials ζ of IV iron polynuclear complexes at different pH values.

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.

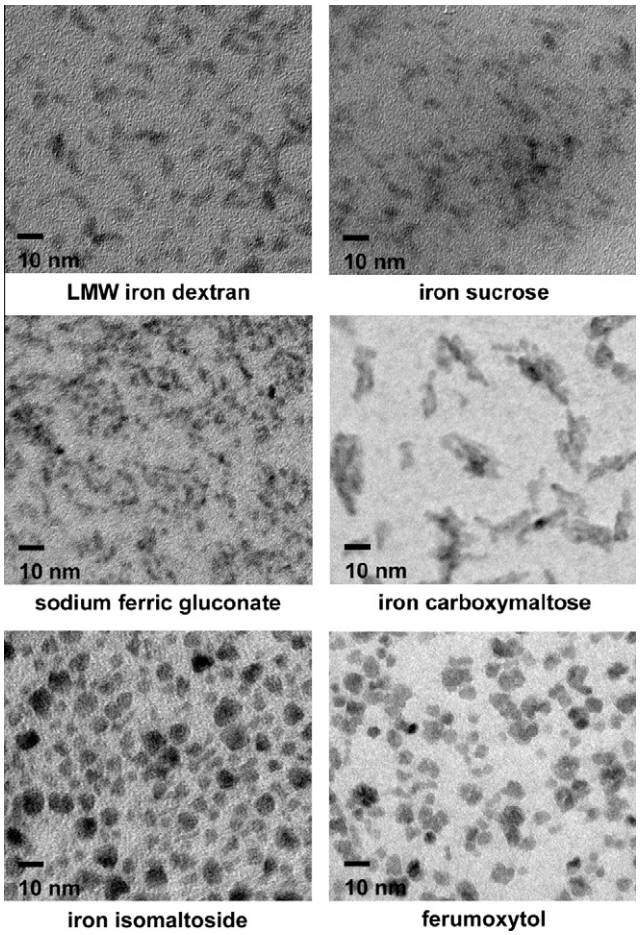

Fig. 3. Transmission electron microscopy images of intravenous iron preparations. Conditions: 1 mg Fe/ml.

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° 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

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