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Physical and Chemical Characterization of Therapeutic Iron Containing Materials: A Study of Several Superparamagnetic Drug Formulations with the β-FeOOH or Ferrihydrite Structure

FELIX FUNK[1,*], GARY J. LONG[2], DIMITRI HAUTOT[2], RUTH BÜCHI[1], ISO CHRISTL[1] and PETER G. WEIDLER[1]

[1] *Institute of Terrestrial Ecology, Swiss Federal Institute of Technology Zurich, CH-8952 Schlieren, Switzerland; e-mail: funk@viforint.ch*
[2] *Department of Chemistry, University of Missouri-Rolla, Rolla, MO 65409-0010, USA*

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Abstract. The effectiveness of therapeutically used iron compounds is related to their physical and chemical properties. Four different iron compounds used in oral, intravenous, and intramuscular therapy have been examined by X-ray powder diffraction, iron-57 Mössbauer spectroscopy, transmission electron microscopy, BET surface area measurement, potentiometric titration and studied through dissolution kinetics determinations using acid, reducing and chelating agents. All compounds are nanosized with particle diameters, as determined by X-ray diffraction, ranging from 1 to 4.1 nm. The superparamagnetic blocking temperatures, as determined by Mössbauer spectroscopy, indicate that the relative diameters of the aggregates range from 2.5 to 4.1 nm. Three of the iron compounds have an akaganeite-like structure, whereas one has a ferrihydrite-like structure. As powders the particles form large and dense aggregates which have a very low surface area on the order of 1 $m^2\,g^{-1}$. There is evidence, however, that in a colloidal solution the surface area is increased by two to three orders of magnitude, presumably as a result of the break up of the aggregates. Iron release kinetics by acid, chelating and reducing agents reflect the high surface area, the size and crystallinity of the particles, and the presence of the protective carbohydrate layer coating the iron compound. Within a physiologically relevant time period, the iron release produced by acid or large chelating ligands is small. In contrast, iron is rapidly mobilized by small organic chelating agents, such as oxalate, or by chelate-forming reductants, such as thioglycolate.

Key words: colloidal iron oxyhydroxides, therapeutic use, X-ray diffraction, Mössbauer, BET surface area, dissolution kinetics.

1. Introduction

Iron oxyhydroxide particles of desired size and chemical properties are of increasing interest in technological applications, such as catalysts, pigments, ferrofluids, recording media, and magnetic resonance imaging contrast agents. In addition, they

* Corresponding author. Present address: Vifor (International) Inc., Rechenstrasse 37, CH-9001 St. Gallen, Switzerland.

have a potential importance in medical applications. Although iron is the second most abundant metal in the Earth's crust, iron deficiency is the most common mineral deficiency disease worldwide. More than one billion people have iron deficiency and about 700 million people have iron deficiency anemia [1].

The low bioavailability of non-haem iron reflects its distinct tendency to hydrolyse and polymerize unless it is strongly complexed [2]. Nutritional iron deficiency may be treated, depending on the risk situation, either by nutrient fortification or by pharmaceutical supplementation. In the latter approach, iron can be administered by either an oral or a parenteral route. The most often used oral preparation is ferrous sulphate, whereas an iron dextran complex and an iron sucrose complex are the preferred forms for parenteral administration [1, 3]. The choice of an administrative route and the specific drug to be used depends upon several factors, including the diagnosis of the cause and impact of the iron deficiency, the effectiveness, safety and economy of the different treatments, and the compliance, toxicity and side effects of a specific drug formulation.

Iron(III) oxyhydroxide complexes with (poly)saccharides form the basis for a group of drugs designed for oral as well as parenteral administration [1]. However, the specifications for the drugs meeting the pharmacokinetical factors required for the different administrative pathways differ widely. The specifications may be achieved through, in some cases, the selection of different carbohydrate coatings or, in other cases, by synthetic procedures which can change the physical and chemical properties of the iron oxyhydroxide complexes.

In this paper we report an investigation of a series of iron(III) oxyhydroxide complexes which are kept in solution as colloidal particles by protection with different carbohydrate coatings. These complexes are prepared specifically for administration by different routes, i.e., by oral, intravenous, or intramuscular routes. In order to acquire information about the structure, size, and surface areas of these iron oxyhydroxy complexes, they have been characterized by powder X-ray diffraction, iron-57 Mössbauer spectroscopy, transmission electron microscopy, BET surface area measurements, and potentiometric titrations. The physico-chemical properties of the complexes have been related to their chemical stability and lability in terms of their dissolution by acid, chelating agents and reducing agents. The results of these studies provide insight into the effectiveness of iron release from the different iron preparations under different conditions, conditions which mimic physiological compartments, such as blood, stomach or intestine. In addition, these studies provide a foundation for assessing the properties necessary for improving preparations for specific drug administrative routes.

2. Experimental

2.1. MATERIALS

The following iron compounds were investigated: Ferrum Hausmann® intramuscular (iron(III) hydroxide dextran complex, Dexfer®; lots 375009A1, solution sam-

ple, and 521119M, powder sample), in the following called iron dextran; Ferrum Hausmann® intramuscular (high molecular weight iron(III) hydroxide complex with polymaltose, Amylofer®; lots 545009A1, solution sample, and 612209M, powder sample), called iron dextrin; Maltofer® (low molecular weight iron(III) hydroxide complex with polymaltose; lots 654009M, drops solution sample, and 512219M, powder sample), called iron polymaltose; and Ferrum Hausmann® i.v. (iron(III) hydroxide sucrose complex, Venofer®; lot 630209, solution sample), called iron sucrose. Human apo-transferrin was purchased from Sigma Chemicals Co., St. Louis, MO, USA. All other chemicals were of the highest purity commercially available. Solutions were prepared using double distilled water.

2.2. POWDER X-RAY DIFFRACTION

Powder X-ray diffraction patterns were obtained on a SCINTAG XDS 2000 diffractometer using Cu K_α 0.15418 nm radiation. This X-ray diffractometer used is a Bragg–Brentano camera equipped with a Peltier cooled, lithium drifted silicon detector. The powder samples of iron dextrin, iron dextran and iron polymaltose were suspended in acetone, gently ground in an agate mortar and transferred with a pipette onto a glass slide and then air dried. The concentrate of iron sucrose was air dried on a glass slide. The diffraction patterns were recorded over a 2θ angular range of 15 to 95° with a step of 0.03° in 2θ and a 10 second counting time per step at room temperature for iron dextrin, iron dextran, iron polymaltose and iron sucrose. For the akaganeite reference sample this range extended over 5 to 134° 2θ.

The diffraction patterns were evaluated, when possible, with the Rietveld method [4]. In this method a least-squares refinement is carried out until the best fit is obtained between the observed and calculated powder diffraction pattern, where the calculated pattern is based on a refined model for the crystal structure or structures, i.e., the unit cell lattice and atomic positional parameters, and the diffraction optics and instrumental factor [5]. For these refinements to be successful the information content of the diffraction pattern must be sufficient, i.e., the patterns should contain many diffraction lines with small line widths at high diffraction angles. The parameters containing information on the quality of the refinement are cited in Table I. R_{wp} and S are a measure of the agreement between the calculated diffraction patterns of the mineral phases in the powder mixture and the measured diffraction profile. Both values are equal to 1.0 in the ideal case. The Bragg factor, R_B, expresses the agreement between the model used for each single phase and the measured diffraction pattern. The Durbin–Watson statistical parameter, D_{wd}, whose maximum is 2.0, reflects the goodness of refinement in total. The space group was I $4/M$, and Z was equal to 8. The chemical formula used in the refinement was equal to FeOOCl with Cl occupying 5% of the possible sites, hence, a formula weight of 89.63 gram per mol can be deduced.

The background was refined with a polynom of 3rd order.

Table I. Crystallite size, strain and quality of fit parameters

Compound	MCL^a (nm)	Strain^b Δdd^{-1}	Quality of fit				
			R_{wp}	S	R_B	D_{wd}	Number of reflections
iron dextrin	4.1	0.004	13.8	3.9	9.6	1.0	198
iron polymaltose	1.9	0.013	12.0	3.6	4.8	1.1	182
iron dextran	1.8	0.010	12.0	3.7	5.1	1.1	189
akaganeite	5.2	0.006	21.4	5.8	13.1	0.3	366

[a] MCL = mean coherence length.
[b] d = lattice spacing.

The mean coherence length (MCL), the average size of the crystals, was calculated with the method of Williamson and Hall [6]. From this method one also obtains information about the internal stress and strain experienced by a crystal. LaB_6 (NIST # 660) was used as an internal line width standard.

2.3. MÖSSBAUER SPECTRA

The Mössbauer spectral absorbers contained 44 mg cm^{-2} of powder and the spectra were measured between 4.2 and 295 K on a constant-acceleration spectrometer which utilized a room temperature rhodium matrix cobalt-57 source and was calibrated at room temperature with α-iron foil. The resulting paramagnetic spectra have been fit with the distribution method of Le Caër [7]. These fits, which used 20 component doublets and a fixed component linewidth of 0.23 mm s^{-1}, revealed no correlation between the quadrupole splitting and the isomer shift. An attempt to fit the spectra containing both superparamagnetic and antiferromagnetic spectral components with the method of Le Caër [7] and with the method of Wivel and Mørup [8] proved unsuccessful. As a result of the failure of these methods to fit the spectra with a distribution model, the spectra have been fit with the minimum number of sextets and doublets needed to reproduce the observed spectral absorption profile. In these fits the components within each magnetic sextet have the same linewidth and an area ratio of 3 : 2 : 1 : 1 : 2 : 3.

The estimated approximate errors for the hyperfine parameters are ±2 kOe for the hyperfine fields, ±0.01 mm s^{-1} for the isomer shifts, ±0.02 mm s^{-1} for the quadrupole splittings, and ±2 percent for the relative areas of the superparamagnetic and antiferromagnetic components. The errors in the parameters derived from the completely superparamagnetic spectra are somewhat smaller.

2.4. TRANSMISSION ELECTRON MICROGRAPHS

Transmission electron micrographs were obtained on a PHILIPS CM30ST electron microscope. Sample material was suspended in ethanol with the help of an ultrasonic bath. Subsequently the drops of the suspension were transferred onto Cu-grids and air dried.

2.5. BET SURFACE AREAS

The N_2 adsorption isotherms were measured with a Micromeritics Gemini 2360 device. Prior to the N_2 adsorption the samples were heated for 24 h at 90°C in a steady stream of nitrogen. Higher temperatures were not applied because of the possible dehydroxylation and subsequent change in the mineral structure of the iron oxyhydroxides [9]. For the calculation of the specific surface area we used the BET equation [10].

2.6. POTENTIOMETRIC TITRATIONS

The charging behavior of iron oxides was determined by potentiometric acid-base titration. Experiments were performed at 25±1°C in a thermostated room using automatic precision titration equipment [11]. Four burettes (Dosimat 605, Metrohm, Herisau, Switzerland), a glass electrode (Metrohm) and an AgCl reference electrode (Metrohm) were connected to a personal computer by a Microlink MF18 interface (Biodata, Manchester). The burettes were filled with CO_2-free deionized water, 0.05 mol dm^{-3} HCl, ~0.05 mol dm^{-3} KOH, and 2 mol dm^{-3} KCl. The KOH and KCl solutions were prepared under a nitrogen atmosphere using CO_2-free deionized water. To keep the solutions CO_2-free during the experiments, all burettes were connected to the atmosphere through a glass tube filled with NaOH on granulated activated carbon. All experiments were carried out in a 250 cm^3 glass vessel, which was continuously flushed with water-saturated, CO_2-free nitrogen gas. Before starting an experiment, solutions were acidified to remove residual CO_2. Typically, experiments were performed by titrating with base, the forward titration, prior to acid titration, the back titration. During the entire titration cycle the ionic strength was held constant within ±1% by adding either water or salt solution to correct for dilution effects resulting from the addition of acid or base. After one titration cycle the ionic strength was adjusted to the next higher level by adding salt solution. In this way a series of forward and backward titrations at different ionic strengths were obtained within a single experiment.

The solution was stirred for two minutes after each addition of titrant. Acid and base dose sizes were automatically adjusted to yield data in steps of about 20 mV. Electrode readings were recorded, if the electrode drift was less than 0.05 mV min^{-1} or after a maximum drift time of 20 min.

Exact base concentration and electrode parameters were obtained by blank titrations of the electrolyte solution by using a least-square fitting routine. The calcu-

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