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An Investigation into the Size of an Iron Dextran Complex

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ABSTRACT

A study was carried out of the solution particle size and the iron core size of an iron dextran complex used as a hematinic. The solution sizes were determined by quasi-elastic laser light scattering, and the core size were determined from the line widths obtained from X-ray powder diffraction patterns. The results show that the range of particle sizes in solution (i.e., the diameter of the particle including the iron core and the dextran coat) was approximately 10–40 nm. The iron core (excluding the dextran outer coat) was determined to be approximately ∼ 5–6 ($\pm$1) nm.

INTRODUCTION

Iron dextran complexes are of great interest as they are extremely useful in the treatment of anemia, the illness which can be caused by a deficiency in body iron [1]. This class of compounds is particularly effective as it can maintain relatively high concentrations of iron in a soluble, nontoxic form at physiological pH [2].

The iron core in the iron dextran complexes have been shown to have some similar properties to the iron storage protein ferritin [3]. This may in part explain their efficacy. There has been considerable discussion over the nature of the ferritin core, with different techniques leading to seemingly contradictory conclusions. Several techniques have suggested that the ferritin core, estimated as $(FeOOH)_8(FeO \cdot H_2PO_4)$, is most similar to the mineral ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$) [4]. EXAFS [5, 6] and Mössbauer spectrocscopy [6, 7] have also shown a similarity between horse spleen ferritin and Imferon [3]. Imferon was probably

the most well-known iron dextran pharmaceutical; however, electron diffraction has shown that it is more similar to the mineral akaganéite (β-FeOOH) [8].

Our previous work has shown that the 5% iron dextran supplied by Abbott Laboratories has a core similar to cell-contracted akaganéite by X-ray powder diffraction, but is distinct from akaganéite by Mössbauer spectroscopy [9]. By EXAFS, it has been found that these complexes closely resemble ferrihydrite [10], but this is due to the short-range nature of the technique. The actual structure is somewhere in between that of akaganéite and ferrihydrite, and can appear to closely resemble both, depending on the technique used as the structural probe.

We have undertaken a study of the size of the solution particles and the core, as studied by laser light scattering and X-ray powder diffraction, respectively. Ferritin is known to consist of a large protein coat (apoferritin) encasing a core of hydrous iron oxide. Ferritins are found in all five living kingdoms, and are involved in iron storage, iron transport, and iron detoxification. From variable temperature Mössbauer spectroscopy, the core is approximately 4–9 nm in diameter, the most common size being ~ 7 nm [11]. The core can contain up to 4500 iron atoms. A ferritin molecule consists of 24 subunits that self-assemble to form an approximately spherical shell. The external diameter of the molecule is approximately 12 nm. The surfaces of the ferritin complexes are obviously different from those of the iron dextrans, as the ferritin cores are surrounded by a protein coat, thought to be linked via bridging carboxylates [12]. Iron dextran complexes, although having a carbohydrate coat, are also linked to the core via carboxylate groups [13].

EXPERIMENTAL PROCEDURE

Samples

The iron dextran samples were supplied by Abbott Laboratories and used without modification. The complex was prepared by adding a solution of dextran to a base-neutralized $FeCl_3$ solution, followed by heating up to 100°C and then sterilizing by autoclaving. The solutions are 5% Fe by weight aqueous solution made from the powdered complex and in the form used as a pharmaceutical.

Measurement of Particle Sizes

The solution particle sizes were determined at 25°C by quasielastic light scattering (QLS) using an Innova 70 argon ion laser source (Coherent, Palo Alto, CA). Light was scattered from samples placed in a temperature-controlled, filtered decalin bath positioned in a BI-200 (Brookhaven) goniometer system. Scattered light was impinged on an EMI 9863/350 photomultiplier tube. The signal was analyzed by a BI-8000AT digital correlator having 128 real-time data channels and allowing geometric spacing of sample times. Intensity-weighted mean diameters were obtained either by the method of cumulants [14] or by the CONTIN size distribution analysis method [15, 16]. In these experiments, the solutions were diluted in order to allow the passage of a laser beam (514.5 nm) and the scattering was measured at an angle of 90°. The solutions were diluted with filtered (2 μm) distilled water and transferred to dust-free tubes in order to minimize contamination from dust particles.

X-Ray Powder Diffraction

X-ray diffraction patterns were obtained on the iron dextran samples using Cu K-α radiation and a Rigaku D/Max-B Series diffraction system equipped with a graphite monochromator. Scans were made from 10° to 70° 2θ at 0.5°/min at 35 kV and 35 mA. Due to low peak intensity, samples were dispersed with acetone on an oriented quartz plate to minimize background.

The mean crystallite dimension (MCD) of a crystalline powder in a direction perpendicular to a set of planes may be obtained from the X-ray diffraction peak widths of a line corresponding to those planes. The value is calculated from the Scherrer equation [17], with K = 0.9 and β, the corrected line width at half maximum. The instruction line broadening value (b) was determined by using a powdered 5 μm quartz sample, and found to be 0.22°. The width at half height (β_0) of the X-ray reflection was measured in 2θ° and the ratio b/β_0 was obtained. The ratio was compared with the correction plots provided by Klug and Alexander [17]. Using this corrected b/β ratio and the measured peak width, β_0, the peak broadening, β, due only to particle size effect, was calculated. The sharpest and most intense peak for these samples, found at $\approx 35.5°$ 2θ, was chosen for this study. This peak can be assigned as the (211) line if the sample is assumed to have the structure of β-FeOOH. If the structure is ferrihydrite, this is the (110) line according to Towe and Bradley [18]; however, this line is less intense and is therefore less likely.

STEM

Scanning transmission electron microscopy of sample 6 was performed with a Hitachi H800 STEM, using a 200 keV beam accelerating voltage and an LaB$_6$ electron source.

RESULTS

The solution particle parameters are shown in Table 1. The average effective diameter obtained for the samples was 17 nm, the same as the average mean particle diameter. The samples were relatively polydisperse, having a somewhat broad range of particle sizes, which has also been previously observed in the asymmetry of the peaks of the Mössbauer spectra, and is also reflected in the large range of temperatures over which the doublet and sextet coexist [9]. The mean crystallite dimension results obtained are shown in Table 2. The average core size is about 5–6 ($\pm$1) nm, which is of the same order of magnitude as the ferritin core.

TABLE 1. Quasi-Elastic Light Scattering Data for 5% Iron Dextran Solutions

Sample	Effective Diam. (nm)	Mean (nm)
A	27.3	24
B	13.9	16
C	9.9	12

TABLE 2. Mean Crystallite Dimension Data for Iron Dextran Samples

Sample No.	β_0	β	$\theta(°)$	θ (rad)	MCD (nm)
1*	1.422	1.3	17.7	0.31	6
2	1.5616	1.5	17.9	0.31	5
3	1.747	1.7	18.2	0.32	5
4	1.706	1.6	17.7	0.31	5
5	1.4695	1.4	17.6	0.31	6
6	1.517	1.4	17.8	0.31	6

*Powder used to make up Solution A.

The scanning transmission electron micrograph (Figure 1) shows superlattice structures, i.e., nanoscale structures with periodic sequences of different materials, in this case ironoxyhydroxide (β-FeOOH-like) particles in a carbohydrate (dextran) matrix. The micrographs show a lattice repeat unit of 7–9 Å. The lattice repeat unit for crystalline 6-line ferrihydrite was observed to be 9.4 Å [19], which are interpreted as sheets of Fe-atoms parallel to (110). No evidence was found for the electron dense planes parallel to (001) as predicted by Towe and Bradley [18]. Detailed interpretation is rendered impossible by the lack of continuous lattice rows, caused by imperfections. From the X-ray powder diffraction data, the poor crystallinity of these types of compounds is well known. Also, from EXAFS data, it has been shown from the Debye–Waller factors that the iron dextran complexes are more disordered than 2000 Fe horse-spleen ferritin in the second shell [10].
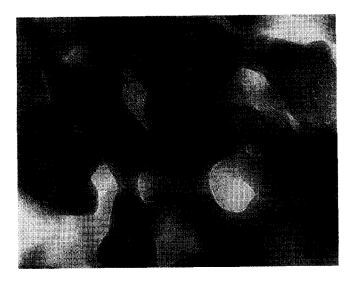

├───────┤   Scale bar = 50 nm

FIGURE 1.   Scanning transmission electron micrograph of iron dextran.

As can be seen from the results, the core size appears to remain fairly constant, despite slight differences in the syntheses of these complexes. These types of complexes are very difficult to synthesize consistently, as even slight variations in, for example, temperature, stirring speed, base concentration, aging, etc., can have a dramatic effect on the final product [20]. It is also possible that the different aging of the solutions could lead to the variations observed in the effective diameters of the solution particles. It has previously been observed that, on aging, the particles in solution become bigger, and hence larger effective diameters are measured [21]. This is thought to be caused by re-solution of the smaller colloidal particles and aggregation of the larger ones. In some cases, the aggregation eventually leads to precipitation. It is possible that aggregation has occurred in our case since solution A is the most aged and has the largest effective diameter.

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