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A Study of an Iron Dextran Complex by Mössbauer Spectroscopy and X-Ray Diffraction

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ABSTRACT

An injectable iron dextran complex used as a hematinic has been studied using Mössbauer spectroscopy (18–295 K) and x-ray powder diffraction. The iron is 100% high-spin Fe^{3+} as determined by Mössbauer spectroscopy. While different samples of the complex showed different magnetic ordering temperatures, all the spectra can be adequately fitted to a distribution of doublets at room temperature and to a distribution of sextets at low temperatures. The sextet distribution is broad and asymmetric due to the relatively large distribution of particle sizes. There is a range of temperatures over which both sextet and doublet coexist. The x-ray diffraction patterns of the different samples gave similar patterns with broad, weak peaks at 5.07, 3.24, 2.51, 2.28, 1.94, 1.63, and 1.49 Å. These values are consistent with cell contracted akaganéite, β-FeOOH. In one sample, the peaks are slightly shifted and are consistent with "normal" akaganéite.

INTRODUCTION

Iron is probably the most important transition metal involved in biological systems, being vital to both plants and animals. The importance of iron in living systems, especially its role in oxygen (hemoglobin) and electron (cytochromes) transport, has been known for some time [1]. Iron is essentially insoluble under physiological conditions and hence is carried and stored by proteins (transferrin and ferritin, respectively) [2].

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Ferritin consists of a large protein coat (apoferritin) encasing a core of hydrous iron oxide. The core is approximately 40–80 Å in diameter and can contain up to 4500 iron atoms. The core has a chemical composition estimated as $(FeOOH)_8(FeO \cdot H_2PO_4)$ and has properties similar to the mineral ferrihydrite, $5Fe_2O_3 \cdot 9H_2O$ [3].

Polyols and polysaccharides can form complexes with iron preventing hydrolysis and solubilizing the metal at approximately neutral pH [4]. Examples include fructose [5], citrate [6], sorbitol [7], and various sugars [8]. Soluble and relatively stable iron complexes are of great interest as a treatment of anemia, an illness that can be caused by a deficiency in essential body iron [4]. Iron dextrans have been used both as an oral iron supplement [9], and as an injectable source of iron [10]. These complexes are capable of maintaining relatively high concentrations of iron in a soluble, nontoxic form at physiological pH, and can act as effective donors of iron into the body system [11].

The family of iron dextrans have been found to have some physical properties in common with the iron storage protein ferritin and this, in part, might explain their effectiveness. Imferon was an iron dextran pharmaceutical, where EXAFS [12, 14] and Mössbauer [13, 14] studies have shown a similarity between Imferon and horse spleen ferritin. However, Imferon was found to be more similar in its electron diffraction pattern to β-FeOOH, akaganéite, than to ferritin. Towe indicates that apart from small ($< 2\%$) changes in d-spacing [15], all the x-ray diffraction peaks of ferrihydrite are repeated in ferritin and β-FeOOH. The latter has more peaks: 7.46, 3.30 Å (strong), and 5.25 and 1.64 Å (medium). Imferon has peaks at 3.30 and 1.64 Å, but not 7.46 and 5.25 Å. Towe, on that basis, concluded that Imferon is more like akaganéite than ferrihydrite. However, akaganéite has a Mössbauer spectrum that is a sextet above 135 K and has several distinct components at low temperatures [16], while the Mössbauer spectra of ferrihydrite and Imferon show doublets at 77 K [14]. A recent Mössbauer spectroscopic study on Imferon indicates, in contrast, that at 87 K the spectrum consists of a doublet/sextet mixture, although the relative proportions were not given [17]. This result was ascribed to a different source of the sample. Iron dextrans have been shown by Mössbauer studies to have Fe^{3+} in a distorted octahedral environment of oxygens [9], as is the case in ferritin, Imferon, and akaganéite.

To date, relatively few studies of the chemical and physical properties of the iron dextran complexes have been carried out. The fact that these complexes have important biological functions and in addition might serve as useful models for the iron core of ferritin makes further investigation of this particular class of compounds important.

EXPERIMENTAL

Samples

Four separate iron dextran samples were obtained from Abbott Laboratories and used without modification. The samples differed slightly in preparation, but all samples except A included heating the solid in suspension up to 100°C, followed by autoclaving. There were also slight differences in the procedure employed for drying the samples.

The elemental analyses for the samples under study are shown in Table 1.

TABLE 1. Elemental Analyses of Iron Dextran Samples

Element	Sample A	Sample B	Sample C	Sample D
chloride	1.84%	2.06%	0.4%	1.56%
iron	12.11%	17.28%	12.38%	15.08%

The analyses were obtained from Galbraith Laboratories (Knoxville, TN) and are accurate to 1% of the value.

X-Ray Diffraction

X-ray diffraction patterns were obtained on the iron dextran samples using CuK-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 decrease the background.

Mössbauer Spectroscopy

Spectra were obtained at various temperatures using a ~ 25 mCi ^{57}Co(Rh) source. Results at 77 K were obtained by cooling the absorber in a constant temperature cryostat, using liquid nitrogen as coolant. The spectra collected below 77 K were obtained with the absorber in a closed-cycle helium cryostat (maintained to within ≈ 0.1 K). In both cases the source was at room temperature. The velocity was calibrated by laser interferometry [18].

Absorbers were composed of mixtures of the iron dextran samples (approximately 40 mg) and powdered sucrose pressed flat into a uniform thickness. The samples were mounted in brass rings having an inside area of 1.75 cm^2. A benzene/styrofoam mixture was used to seal the sample within the ring and aluminum foil was used as the backing.

The spectra were fitted, after subtraction of the Fe in the spectrometer windows and in the aluminum foil used, to a distribution of doublets at room temperature, mixtures of doublets and sextets at intermediate temperature, and a distribution of sextets (and doublets if needed) at lower temperature [19]. All lines were assumed to be of Lorentzian shape, with the full width at the half maximum fixed at 0.35 mm/s. The fitting program allowed the isomer shift, quadrupole splitting, and peak area ratios to vary.

RESULTS

X-Ray Powder Diffraction

There are several peaks observable in the x-ray diffraction patterns; one of the most noticeable features is a broad peak at approximately 2.5 Å, which is characteristic of many iron oxides. Also characteristic is the peak at ~ 1.5 Å. These result from the local 2D close-packed arrangement of the anions (Fig. 1). Comparison of these peaks with those reported for other iron (oxy)hydroxides shows that the major mineral in this sample is most similar to cell contracted akaganéite, β-FeOOH [20] for samples B and C. The x-ray diffraction patterns

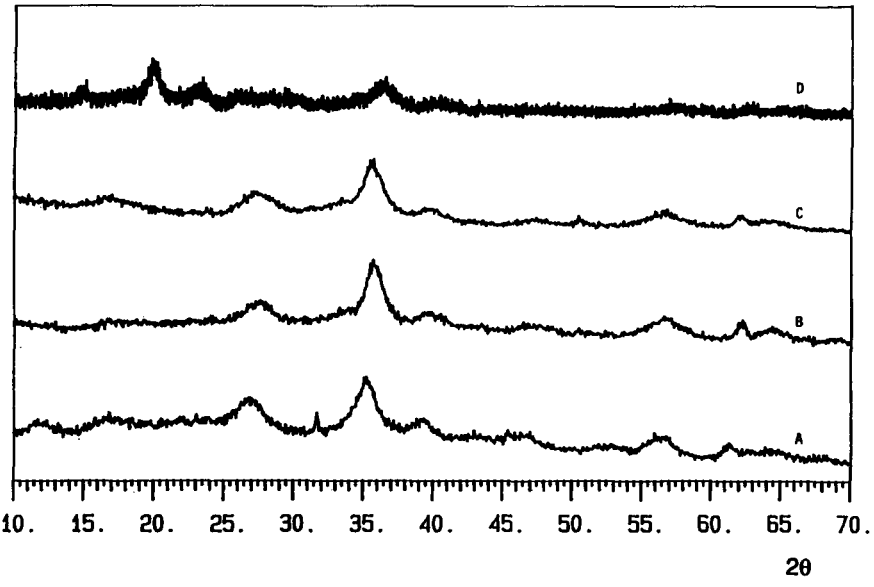

FIGURE 1. X-ray powder diffraction patterns for samples A, B, C, and D.

do not match as closely to "normal" akaganéite (tetragonal FeOOH, β form) [16], except for sample A for which it is a good match. The major peak at 7.41 Å is absent in the cell contracted form, and the peak at 3.33 Å is shifted [20], as observed for B and C. The patterns for A, B, and C do not match well with ferrihydrite, $Fe_5O_7(OH) \cdot 4H_2O$. Sample D is quite different and will be discussed later. The x-ray diffraction powder data are shown in Table 2.

Mössbauer Spectra

The spectra for all the samples at 295 K are all 100% doublet at room temperature, and there is negligible variation in isomer shift (Table 3). In all cases the spectra (Fig. 2) were fit to a distribution of doublets, from Q.S. = 0.2 to 1.6 mm/s, so that a comparison could be made between the mean and maximum quadrupole splittings for these samples with values reported in the literature [9]. It can be seen that the values for the quadrupole splittings vary significantly between sample A and the others. For samples B and D a single doublet gives a better fit.

The Mössbauer spectra for samples B and D consist entirely of a sextet and hence the samples are totally magnetically ordered at 77 K (Table 4). Differences in isomer shifts between the tables are due to temperature changes. Sample B has the highest field value indicating that it is the most crystalline. Samples A and C are a mixture of doublet and sextet, characteristic of small superparamagnetic particles which are not fully ordered at this temperature. They are less ordered than B and D.

In an effort to examine potential smaller differences in these samples, Mössbauer spectra were obtained down to 18 K (Table 5) in order to determine the ordering temperatures (Fig. 3). At 18 K the spectra for all samples show 100% sextet, but are broadened with an asymmetric distribution of fields tailing

TABLE 2. X-Ray Diffraction Data for the Samples Studied

2θ(°)	I	d(Å)	2θ(°)	I	d(Å)
	Sample A			Sample B	
12.00	w, br	7.38	17.50	vw,i	5.07
16.75	w, br	5.29	27.50	m, br	3.24
26.75	m, br	3.33	35.80	s, sh	2.51
*31.75	m, v.sh	2.82	39.60	m, br	2.28
35.25	s, sh	2.55	47.00	w, br	1.93
39.00	w, br	2.31	56.70	m, br	1.63
46.50	vw, br	1.95	62.20	m, sh	1.49
52.50	vw, br	1.74	64.40	m, br	1.45
56.25	m, br	1.64			
61.50	w, m	1.51			
64.00	vw, br	1.46			
	Sample C			Sample D	
17.00	w, br	5.22	14.70	w	6.01
27.50	m, br	3.24	19.80	w	4.48
35.75	s, sh	2.51	23.40	w, i	3.80
39.75	w, br	2.27	36.30	m, br	2.47
47.50	vw, br	1.91	40.60	w, i	2.22
50.50	w, sh	1.81	57.20	w, i	1.61
56.50	m, br	1.63	62.75	w, i	1.48
62.25	w, m	1.49			
64.00	vw,i	1.46			

s = Strong, m = Medium, w = Weak, br = Broad, sh = Sharp, i = Ill-defined.
* = NaCl.

towards lower values (Fig. 3). This asymmetry is measured by the difference between the maximum and average fields and is greater for sample A than for B and C (Table 5). This is consistent with a relatively large distribution of particle sizes for A [21]. The narrower the temperature range over which the doublet and sextet forms coexist, the more uniform the size of the particles. For sample A the range is very large, which indicates a sample with a nonuniform distribution of particle size, but a distribution which is on average smaller than B and C.

DISCUSSION

From the x-ray diffraction results it appears that the coarsely crystalline component of the core of the iron dextran is most similar to that of cell contracted

TABLE 3. 295 K Data

Sample	I.S. (mm/s)	Q.S. (mm/s) mean/max
A	0.37	0.67/0.68
B	0.36	0.79/0.71
C	0.36	0.75/0.67
D	0.36	0.78/0.64

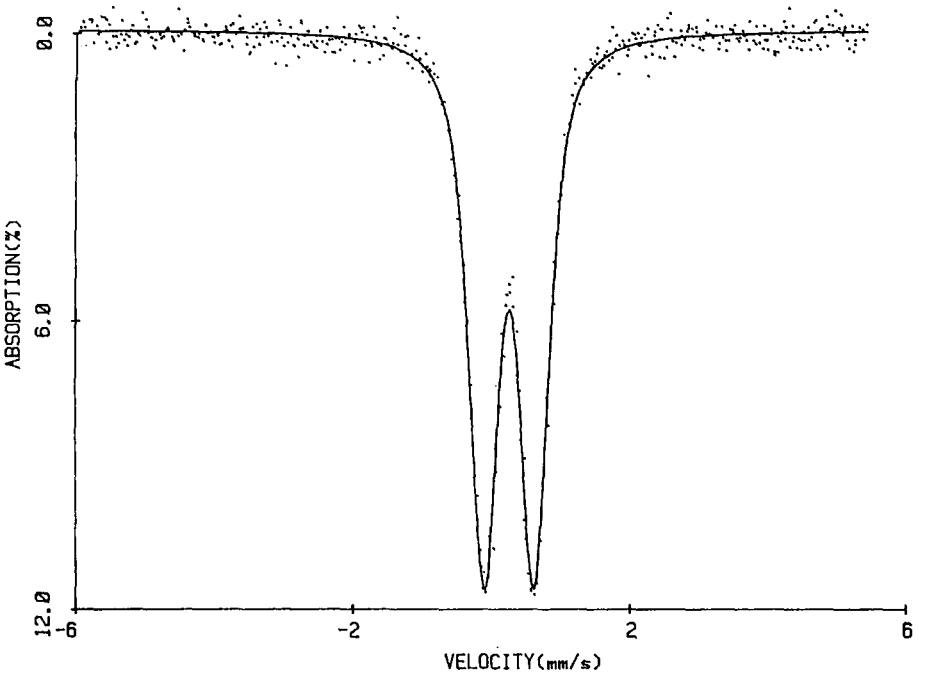

FIGURE 2. Doublet for sample C at room temperature.

akaganéite in the cases of B and C, which exhibit very similar patterns. Sample A is slightly different in that it best matches "normal" akaganéite. Sample A also has a peak associated with crystalline NaCl (2.82 Å). The pattern for sample D is dissimilar to the others, and appears to contain peaks corresponding to a mixture of materials. The peaks at 6.01, 3.80, and 2.47 Å may be due to the presence of lepidocrocite (γ-FeOOH). The peak at 4.48 Å is consistent with goethite (α-FeOOH). The peaks at smaller d-spacing can all be assigned to a mixture of lepidocrocite and goethite, although consistent with akaganéite as well.

It was found by Chandy that when solid "normal" akaganéite was heated, it changed to an orthorhombic form, a cell contracted akaganéite [20]. On initial heating the diffraction lines were found to shift to higher angles, indicating a

TABLE 4. 77 K Data

Sample	%D/S	I.S. (mm/s)	Q.S. (mm/s)	Field (kOe) max/ave
A	72%S	0.47	−0.19	424/354
	28%D	0.45	0.72	—
B	100%S	0.47	−0.11	475/395
C	87%S	0.47	−0.09	465/389
	13%D	0.48	0.63	—
D	100%S	0.48	−0.09	468/352

TABLE 5. Low Temperature Data for Samples A–C

Sample	Temp. (K)	%D/S	I.S. (mm/s)	Q.S. (mm/s)	Field (kOe) max/ave
A	18	100%S	0.47	−0.15	452/419
	50	84%S	0.46	−0.19	442/395
		16%D	0.51	0.99	—
B	18	100%S	0.45	−0.10	465/452
C	19	100%S	0.48	−0.08	489/461

contraction of the tetragonal cell until at 320°C the orthorhombic structure was fully formed. The final product on heating to about 390°C was hematite.

β-FeOOH has the tetragonal α-MnO_2 structure, which contains channels where Cl^- and H_2O molecules can be accommodated. The contraction of the tetragonal cell to an orthorhombic one can be explained by a loss of some of the contents of the channels on heating. It has been reported that Cl^- cannot be washed out of β-FeOOH with water, and that large anions, especially Cl^-, are essential for the formation of the open tetragonal structure and help stabilize it [22]. This would explain the presence of chloride in the iron dextran complexes (from $FeCl_3$ starting material) (Table 1).

As mentioned above, akaganéite has a tetragonal unit cell. Ferritin, ferrihydrite, and α-FeOOH are based on hexagonal close-packed oxygens, while in γ-FeOOH the oxygens are cubic close-packed. However, all have Fe^{3+} octahedrally coordinated to oxygen.

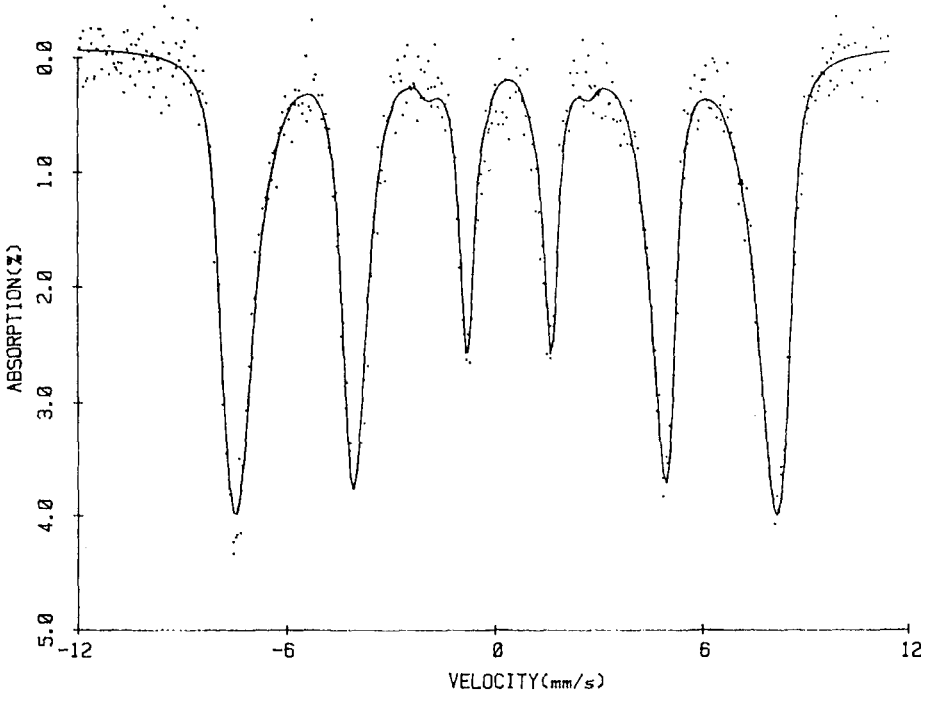

FIGURE 3. Sextet for sample C at 19 K.

The 295 K Mössbauer data (Table 3) for all the samples of iron dextran can be compared to those of ferritin, Imferon, and akaganéite (Table 6) [14, 16]. The present iron dextran Mössbauer spectra have isomer shifts similar to Imferon and ferritin. However, the quadrupole splitting values are quite distinct. For samples B, C, and D the mean quadrupole splitting value is larger than the maximum. The same behavior is observed for Imferon, but the Q.S. values are lower for Imferon. For sample A the mean and maximum quadrupole splittings are very similar, indicating a symmetric distribution, as is observed for ferritin, although again the Q.S. values are higher. All the samples are quite different from akaganéite, which is best fit to two doublets at R.T.

The sextet spectrum for akaganéite is unsymmetrical, with the second and sixth lines having noticeably lower absorptions compared to the first and fifth lines, indicating at least two distinct sites [16, 23]. The sextet spectra for the iron dextrans are broadened, but although the individual peaks are asymmetric and require a distribution of fields, the peaks are symmetric about the center, indicating only one distinct type of site, in contrast to akaganéite.

Although sample A most closely resembles "normal" akaganéite by x-ray powder diffraction it is not like akaganéite in its Mössbauer spectra, being part doublet at 77 K and having a very low field at 18 K. This could be due to the small particle size, caused by the fact that the sample in solution did not undergo a heating process. Higher temperatures are known to promote the formation of larger and more uniform crystals.

Sample B orders over a similar temperature range to akaganéite [16], i.e., 180–295 K. It has the highest ordering temperature of the samples A, B, and C. These properties may be due to the large Cl/Fe ratio, although its x-ray powder diffraction pattern indicates peak shifts due to cell contraction.

Sample C has an x-ray powder pattern very similar to B, but is different when using Mössbauer spectroscopy. At 77 K sample B is completely ordered, but sample C is not. Sample A is also partially ordered at this temperature, but has a lower percent sextet and field values than sample C. This can be explained by the fact that sample C has been through a heating process and therefore is more crystalline, with larger particles than A.

Sample D orders over a broad range of temperatures (80–295 K) consistent with a mixture of materials, as shown by the results from x-ray diffraction. The probable reason for this sample being a mixture of iron oxyhydroxides, is that the solution was evaporated to dryness by forced heating to obtain the solid. It is possible that the akaganéite formed in sample D converted to the other two oxyhydroxides, lepidocrocite and goethite, on heating. Sample A was precipitated by alcohol, and samples B and C were freeze-dried.

TABLE 6. Literature Values of 295 K Mössbauer Data

Compound	Isomer Shift	Q.S.(mean/max)	Reference
Ferritin	0.36	0.64/0.62	14
Imferon	0.36	0.72/0.61	14
Akaganéite	0.38	0.55	16
	0.39	0.95	16

CONCLUSIONS

The physical characteristics of these iron dextran samples have some similarities to those reported for Imferon. However, small but real differences are evident in the detailed behavior of the Mössbauer spectra over the temperature range 18–77 K. These differences apparently are related to minor variations in the preparation procedure and specifically, in this case, to the heating of solutions of the iron salt and dextran and also the chloride content of the final product.

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