Quadrupole Splitting and Paramagnetic Iron Core Structure in Iron-Dextran Complexes: A Mössbauer Effect Study

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The pharmaceutically important iron-dextran complexes (ferritin models) were studied by Mössbauer spectroscopy in frozen solution at 87 K and in lyophilized form at 297 and 87 K. The quadrupole splittings of the lyophilized forms of iron-dextran complexes measured at 297 and 87 K were slightly different. The quadrupole splittings of several complexes in lyophilized form and frozen solutions measured at 87 K were also different. Slight differences in the quadrupole splitting were found for various iron-dextran complexes. Mössbauer spectra of iron-dextran complexes were better fitted using two quadrupole split doublets. Slight differences in the corresponding quadrupole splittings and doublet ratios were observed for various complexes.

Key words: Iron-dextran Complexes; Mössbauer Spectroscopy; Paramagnetic Iron Core; Quadrupole Splitting.

Introduction

Iron-dextran complexes (IDC) are used as drugs for treatment of iron deficiency anemias. They are pharmaceutically important models of the iron storage protein ferritin. Ferritin consists of a polynuclear hydrous ferric oxide core with about 4500 iron atoms with a multisubunit protein shell [1], while the IDC consist of a FeOOH core with a dextran shell. Several comparative studies of the physical properties of the iron core in ferritin and IDC were made using electron diffraction [2], EXAFS [3] and Mössbauer spectroscopy [4]. These studies showed some differences between the iron cores of ferritin and IDC. Variations of physical properties were also obtained for different IDC's in Mössbauer and positron annihilation [5 - 8] and Mössbauer and X-ray diffraction studies [9, 10]. Our previous Mössbauer study of various IDC's in frozen solution and lyophilized form [5 - 8] did not reveal any differences of the hyperfine parameters for the iron core of the two forms at 87 K. Comparative Mössbauer studies on IDC in frozen solution and the solid state also did not reveal any differences [11]. In the present work we consider new results of further comparative Mössbauer studies of the iron state in various IDC's in frozen solution and in lyophilized form obtained with a new high precision and sensitive Mössbauer spectrometer.

Experimental

Materials

We used industrial pharmaceutical IDC products such as Imferon (Fisons, UK), Imferon (Benger, USA) and Ferridextran (Lečiva, ČSSR). Elaborated IDC's were prepared by adding $FeCl_3$ to a dextran solution with 18% H_2O_2, and heating at 60 °C for 1 h. The pH of the solution was changed to 8.0 by adding Na_2CO_3; the solution was then centrifuged

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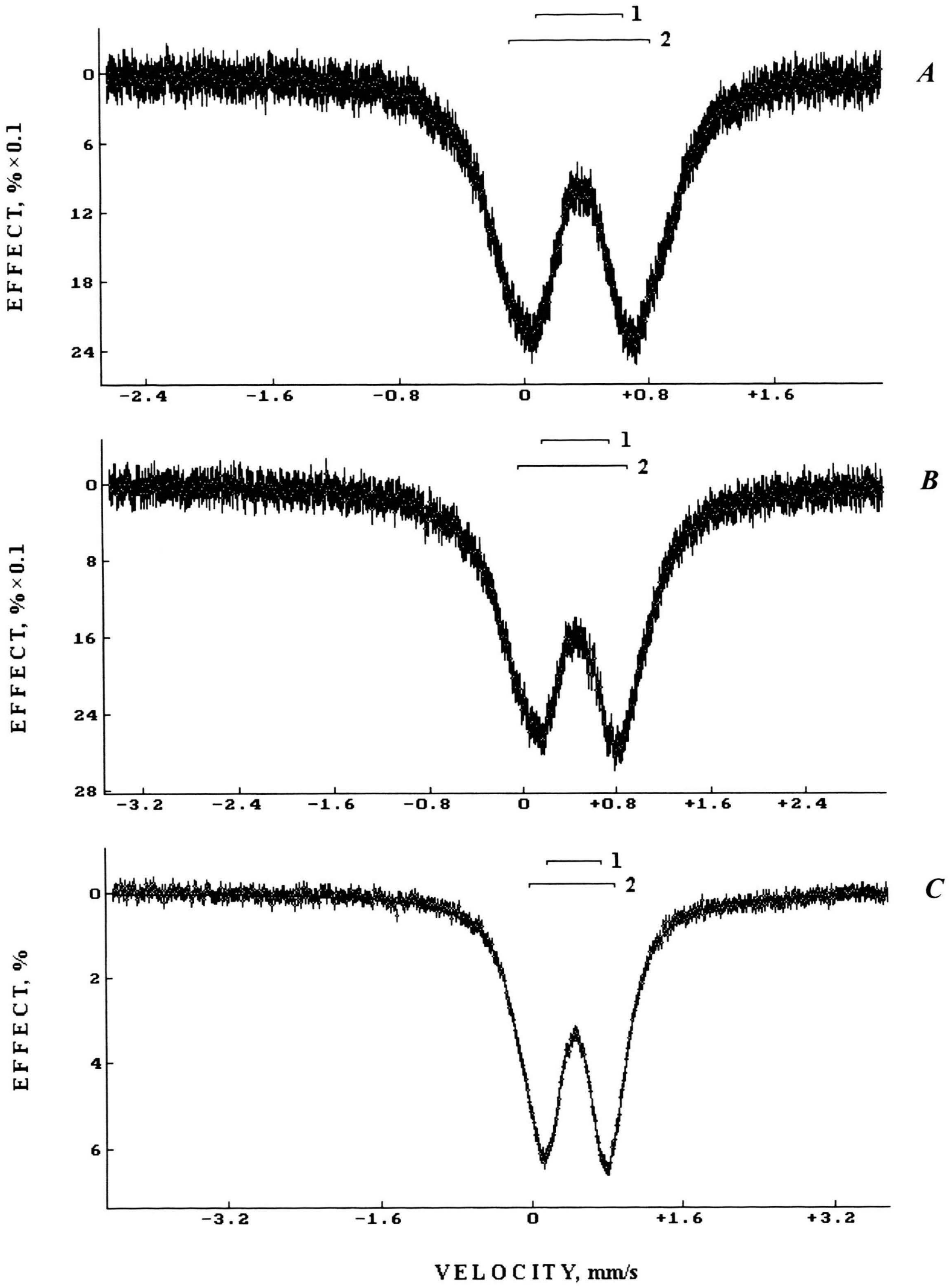

Fig. 1. Mössbauer spectra of IDC3P. A: lyophilized, $T = 297$ K; B: lyophilized, $T = 87$ K; C: frozen solution, $T = 87$ K. Solid lines are results of the least squares fit using two quadrupole doublets (1 and 2) for the iron core. Vertical bars are the statistical errors for each spectral point.

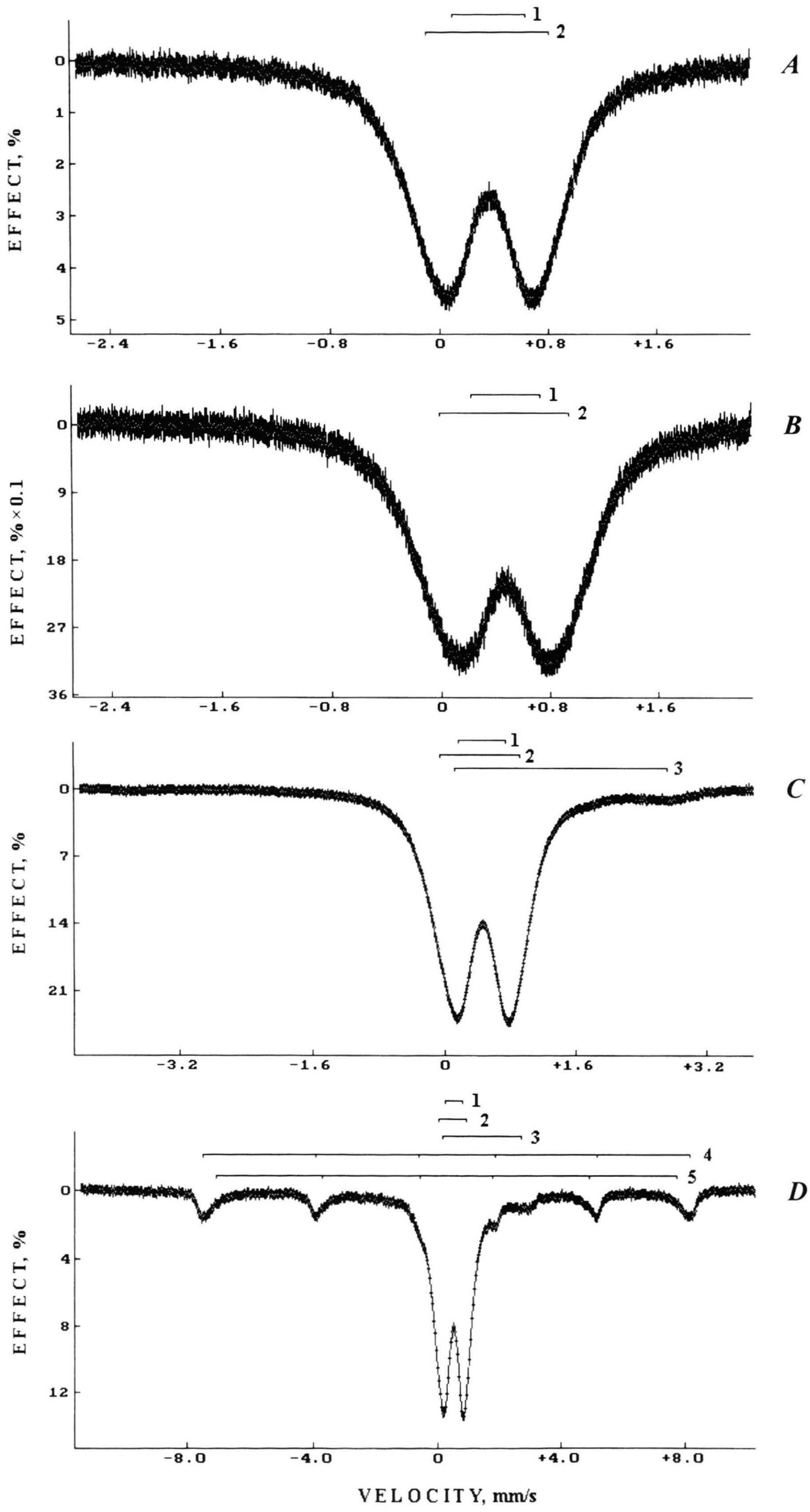

Fig. 2. Mössbauer spectra of Imferon (Fisons, UK), A: lyophilized, T = 297 K; B: lyophilized, T = 87 K; C: frozen solution, T = 87 K; Imferon (Benger, USA), D: frozen solution, T = 87 K. Solid lines are results of the least squares fit. 1 and 2: two quadrupole doublets for the iron core; 4, 5: magnetic components for the iron core; 3: the high spin Fe^{2+} impurity. Vertical bars are the statistical errors for each spectral point.

Table 1. Mössbauer parameters for iron-dextran complexes (fitting with one quadrupole doublet for the iron core); [a] L: lyophilized form, FS: frozen solution; ∗: Fe^{2+} impurity.

Sample	Form [a]	T [K]	δ [mm/s]	ΔE_Q [mm/s]	H_{eff} [kOe]	S [%]	χ^2	
IDC3P	L	297	0.361±0.010	0.729±0.010	—	100	0.37	
	L	87	0.463±0.013	0.746±0.013	—	100	0.38	
	FS	87	0.455±0.016	0.717±0.016	—	100	2.12	
IDC4P	L	297	0.356±0.010	0.719±0.010	—	100	1.76	
	L	87	0.459±0.013	0.737±0.013	—	100	0.30	
	FS	87	0.449±0.016	0.700±0.016	—	96	2.64	
			1.390±0.016	2.333±0.016		4		∗
IDC6P	L	297	0.362±0.010	0.741±0.010	—	100	3.48	
	L	87	0.461±0.013	0.788±0.013	—	96.5	1.07	
			1.431±0.013	2.390±0.013		3.5		∗
	FS	87	0.455±0.016	0.744±0.016	—	92	5.22	
			1.471±0.016	2.550±0.016		8		∗
IDC8P	L	297	0.369±0.010	0.730±0.010	—	100	1.52	
	L	87	0.465±0.013	0.785±0.013	—	100	0.20	
	FS	87	0.460±0.016	0.739±0.016	—	97	9.15	
			1.399±0.016	2.355±0.016		3		∗
Ferri-dextran	L	297	0.350±0.008	0.704±0.008	—	100	2.05	
	L	87	0.457±0.013	0.735±0.013	—	100	0.26	
	FS	87	0.449±0.016	0.729±0.016	—	93	2.73	
			1.497±0.016	2.373±0.016		7		∗
IDC81	FS	87	0.465±0.016	0.713±0.016	—	100	3.81	
IDC87	L	297	0.351±0.008	0.693±0.008	—	100	7.42	
	L	87	0.465±0.016	0.714±0.016	—	100	1.46	
	FS	87	0.456±0.016	0.657±0.016	—	93	2.01	
			1.276±0.016	2.834±0.016		7		∗
Imferon (Fisons, UK)	L	297	0.358±0.010	0.704±0.010	—	100	0.73	
	L	87	0.460±0.010	0.740±0.010	—	100	0.32	
	FS	87	0.457±0.016	0.707±0.016	—	97	14.70	
			1.507±0.016	2.509±0.016		3		∗
Imferon (Benger, USA)	FS	87	0.495±0.042	0.723±0.042	—	71	4.81	
			0.477±0.042	−0.286±0.042	484.6±1.5	12		
			0.501±0.042	−0.316±0.042	456.3±1.5	13		
			1.576±0.042	2.536±0.042	—	4		∗

and filtered with Millipore filters (0.8 μm + 0.45 μm + 0.22 μm). The solutions were concentrated by means of membranes PTGC 000 C5 with a transmission limit for globular proteins of 10000 Da. The obtained IDC's were IDC3P, IDC4P, IDC6P, IDC8P, IDC81 and IDC87. The free iron was chemically determined in the IDC6P solution only. Some IDC solutions were also lyophilized.

Mössbauer Spectroscopy

Mössbauer spectra were measured with a constant acceleration computerized precision spectrometer which was a part of the multi-dimension parametric Mössbauer spectrometer SM-2201 [12]. The noise of the velocity signal of the spectrometer was 1.5×10^{-3} mm/s, the drift of the zero point velocity $\pm2.6\times10^{-3}$ mm/s, the nonlinearity of the velocity signal 0.01%, and the harmonic distortion factor was 0.005% for the frequency band 0 - 1120 Hz. The parabolic distortion of the spectra was at most 0.1% from the statistical rate which was in the range from 5×10^5 to 1.6×10^6 counts per channel. These characteristics of the driving system permitted to increase the sensitivity of the Mössbauer spectrometer and the quality of measured spectra in comparison with our previous data [5 - 8]. A 2.5×10^9 Bq ^{57}Co(Cr) source was used at room temperature.

Mössbauer spectra of IDC in lyophilized form and frozen solutions were measured at 87 K using a liquid nitrogen cryostat, and also in lyophilized form at room temperature. Sodium nitroprusside and α-Fe were used as standard absorbers. The spectra were computer fitted with the least squares procedure using a Lorentzian line shape. The Mössbauer parameters (quadrupole splitting ΔE_Q, isomer shift δ, hyperfine field H_{eff}, subspectrum area S, absorption effect) and the statistical criterion χ^2 were determined. The values of isomer shift are given relative to α-Fe at 295 K.

Results and Discussion

Mössbauer spectra of several IDC in lyophilized form and frozen solution are shown in Figs. 1 and 2. We observed that elaborated IDC, Ferridextran and Imferon (Fisons, UK) had an iron core in the paramagnetic state at both room and liquid nitrogen temperatures. The Mössbauer spectrum of Imferon (Benger, USA) (Fig. 2, D) showed the presence of the paramagnetic and magnetic states of iron in the core at 87 K. An additional quadrupole split component related to an impurity was found in several IDC's. The Mössbauer spectra of all samples were fitted using one and two quadrupole doublets for the iron core spectral components. The Mössbauer parameters are given in Tables 1 and 2, respectively.

Approximation Using one Quadrupole Doublet for the Iron Core

Mössbauer parameters of lyophilized IDC's measured at room temperature demonstrated small differences of the ΔE_Q values. It was found that the ΔE_Q's for the FeOOH core in Ferridextran and Imferon (Fisons, UK) were the same and lower than that of elaborated IDC. It should be noted that the ΔE_Q for lyophilized Imferon (Benger, USA), measured earlier [7] at room temperature, was (0.67±0.01) mm/s. This value is slightly lower than the present ΔE_Q value for lyophilized Imferon (Fisons, UK) which is comparable within the error with the value of

Table 2. Mössbauer parameters for iron-dextran complexes (fitting with two quadrupole doublets for the iron core); [a] L: lyophilized form, FS: frozen solution; [b] fixed parameter; ∗: Fe^{2+} impurity, ∗∗: magnetic components.

Sample	Form [a]	T [K]	δ [mm/s]	ΔE_Q [mm/s]	H_{eff} [kOe]	S [%]	χ^2	
IDC3P	L	297	0.360±0.010	0.918±0.010	—	58	0.19	
			0.364±0.010	0.554±0.010		42		
	L	87	0.450±0.013	0.928±0.013	—	60	0.28	
			0.478±0.013	0.577±0.013		40		
	FS	87	0.441±0.016	0.910±0.016	—	52	1.26	
			0.464±0.016	0.583±0.016		48		
IDC4P	L	297	0.355±0.010	0.966±0.010	—	42	0.57	
			0.357±0.010	0.568±0.010		58		
	L	87	0.454±0.013	0.999±0.013	—	42	0.21	
			0.462±0.013	0.580±0.013		58		
	FS	87	0.437±0.016	0.986±0.016	—	32	1.41	
			0.458±0.016	0.579±0.016		64		
			1.288±0.016	2.491±0.016		4		∗
IDC6P	L	297	0.363±0.010	0.932±0.010	—	57	1.07	
			0.362±0.010	0.566±0.010		43		
	L	87	0.452±0.013	1.065±0.013	—	44	0.43	
			0.473±0.013	0.592±0.013		52		
			1.328±0.013	2.542±0.013		4		∗
	FS	87	0.436±0.016	1.032±0.016	—	33	1.53	
			0.468±0.016	0.604±0.016		58		
			1.390±0.016	2.676±0.016		9		∗
IDC8P	L	297	0.366±0.010	0.921±0.010	—	60	0.44	
			0.372±0.010	0.547±0.010		40		
	L	87	0.461±0.013	0.883±0.013	—	84	0.16	
			0.473±0.013	0.562±0.013		16		
	FS	87	0.446±0.016	0.920±0.016	—	60	1.88	
			0.471±0.016	0.578±0.016		35		
			1.455±0.016	2.223±0.016		5		∗
IDC81	FS	87	0.459±0.016	0.906±0.016	—	55	1.25	
			0.469±0.016	0.554±0.016		45		
IDC87	L	297	0.346±0.008	0.959±0.008	—	36	2.00	
			0.352±0.008	0.570±0.008		64		
	L	87	0.456±0.016	0.912±0.016	—	56	1.11	
			0.473±0.016	0.542±0.016		44		
	FS	87	0.436±0.016	0.941±0.016	—	32	1.43	
			0.455±0.016	0.558±0.016		59		
			1.362±0.016	2.600±0.016		9		∗
Ferri-dextran	L	297	0.349±0.008	0.965±0.008	—	39	0.92	
			0.351±0.008	0.561±0.008		61		
	L	87	0.455±0.013	1.069±0.013	—	34	0.15	
			0.458±0.013	0.565±0.013		66		
Ferridextran	FS	87	0.442±0.016	0.994±0.016	—	44	1.16	
			0.454±0.016	0.532±0.016		48		
			1.481±0.016	2.352±0.016		8		∗
Imferon (Fisons, UK)	L	297	0.353±0.010	0.943±0.010	—	47	0.33	
			0.361±0.010	0.547±0.010	53			
	L	87	0.456±0.010	0.953±0.010	—	60		
			0.465±0.010	0.512±0.010		40		
	FS	87	0.440±0.016	0.975±0.016	—	39	2.36	
			0.466±0.016	0.565±0.016		57		
			1.430±0.016	2.605±0.016		4		∗
Imferon (Benger, USA)	FS	87	0.479±0.042	0.918±0.042	—	41	2.18	
			0.507±0.042	0.553±0.042	—	28		
			0.476±0.042	−0.283±0.042	484.9±1.5	12		∗∗
			0.485±0.042	−0.283±0.042	458.9±1.5	14		∗∗
			1.576^b	2.536^b	—	5		∗

ΔE_Q = 0.67±0.02 mm/s for Imferon (Fisons, UK) obtained in [4]. The ΔE_Q values for other elaborated lyophilized IDC's from [7] coincide with new data for elaborated IDC. We note that the ΔE_Q values for the different Imferon samples from [13] and [3], (0.62±0.05) mm/s and 0.72 mm/s (mean), respectively, were slightly different also. Comparison of Mössbauer hyperfine parameters of ferritin measured at room temperature showed some differences too. The ΔE_Q value for rat liver ferritin was (0.70± 0.02) mm/s [4], while that for placental ferritin was (0.58±0.02) mm/s [14].

Mössbauer spectra of lyophilized IDC samples measured at 87 K showed that an additional quadrupole doublet was observed in the sample of IDC6P. Mössbauer parameters of this component characterize the high spin Fe^{2+} compound. The ΔE_Q values for IDC6P and IDC8P were found to be increased in comparison with data for other IDC's. Moreover, the ΔE_Q's for lyophilized IDC6P, IDC8P, Ferridextran and Imferon (Fisons, UK), measured at 87 K, appeared to be higher than those at room temperature, while the ΔE_Q's for lyophilized IDC3P, IDC4P and IDC87, measured at room temperature and at 87 K, were the same. The previously obtained Mössbauer parameters for lyophilized Imferon (Benger, USA) at 87 K showed an ΔE_Q of (0.68±0.05) mm/s [7], which was lower than that of IDC6P and IDC8P and coincided with the ΔE_Q values for other IDC's within the error's. However, the Mössbauer spectrum of lyophilized Imferon (Benger, USA) was a mixture of doublet and sextet components (78% and 22%, respectively). The value of H_{eff} for the magnetically ordered component was (484.0±1.6) kOe [7, 8].

Mössbauer spectra of IDC's in frozen solutions showed the presence of an additional quadrupole doublet with parameters characteristic of the high spin Fe^{2+} compound for all IDC's except IDC3P and IDC81. Our previous results [8] showed the same additional doublet (4%) for Ferridextran only. New measurements gave 7% of the additional doublet for Ferridextran. The presence of free iron was determined for the IDC6P solution during the preparation of an elaborated IDC only. The Mössbauer study showed that the content of this free iron in IDC6P is two times higher than in other elaborated and industrial IDC's and the same as in Ferridextran solution. However, this additional iron was detected in IDC6P in lyophilized form at 87 K only. It may be the result of a stronger bond of the Fe^{2+} compound with the dextran shell in lyophilized IDC6P than in Ferridextran due to structural differences of the dextran shell. This stronger bond raises the probability of the Mössbauer effect (recoil-free fraction of Mössbauer radiation).

The Fe^{2+} compound is an impurity resulting from the reduction of Fe^{3+} in solution during IDC preparation. Its quantity in IDC solutions depends on the preparation procedure and purification. The Mössbauer spectrum of Imferon (Benger, USA) consisted of a mixture of doublets and a sextet with asymmetric absorption lines, while the spectrum of Imferon (Fisons, UK) consisted of several quadrupole doublets only. Both industrial products contained about 4 - 5% of the Fe^{2+} impurity.

The ΔE_Q values for IDC's frozen solutions varied from (0.657±0.016) mm/s (IDC87) to (0.744±0.016) mm/s (IDC6P) and reflected some structural differences in the core. The higher value of the quadrupole splitting may indicate a higher octahedral site distortion and, therefore, poorer iron core crystallinity [15]. It was also interesting to observe small differences in the ΔE_Q values measured at 87 K for all IDC samples in lyophilized form and frozen solutions except IDC3P and Ferridextran. This result is in disagreement with previous data [7, 8] and indicates that lyophilization may affect the iron core by slightly increasing the octahedral distortion. It should be noted that we fitted the magnetically split component with asymmetrical line shape in the Mössbauer spectrum of the Imferon (Benger, USA) frozen solution using two sextets (see Table 1). The value of H_{eff} for the large sextet was found to be higher than that of the IDC's studied in [9, 11] at 77 K and in agreement with our previous results [7, 8]. The presence of at least two magnetically ordered components in the Mössbauer spectrum of Imferon (Benger, USA) frozen solution may be the result of a different crystallinity level of the FeOOH core and/or a larger iron core size than in Imferon (Fisons, UK).

The low temperature Mössbauer data for ferritin from various sources showed also some differences in the ΔE_Q values. For instance, ΔE_Q = (0.65±0.02) mm/s for placental ferritin at 77 K [14], ΔE_Q = (0.69±0.02) mm/s for human ferritin at 78 K [16], ΔE_Q = (0.75±0.02) mm/s for limpet ferritin at 78 K [16] and ΔE_Q = (0.72±0.02) mm/s for horse ferritin at 90 K [17]. These data indicate that the iron core structure in natural proteins may be different due to the conditions of iron core formation and the protein shell. The iron core structure in IDC may depend on the preparation conditions and dextran shell. It should be noted that Mössbauer spectra of ferritin in the liquid nitrogen temperature region did not reveal any magnetic hyperfine fields, and the iron core in various ferritins was in the paramagnetic state. However, the Mössbauer spectrum of the relevant iron storage protein hemosiderin from patients with secondary hemochromatosis indicates the presence of an additional sextet with asymmetrical absorption lines at 77 K as a result of the microstructural differences between the iron core and that in other hemosiderins (see [18]). The value of H_{eff} for this hemosiderin was found to be (497±5) kOe at 4.2 K. This value was slightly higher than that for Imferon (Benger, USA) observed at 87 K.

Approximation Using two Quadrupole Doublets for the Iron Core

Several reviews on Mössbauer studies of iron oxides and oxyhydroxides pointed out that the paramagnetic room temperature Mössbauer spectra of these materials were better fitted using more than one quadrupole doublet or a distribution of quadrupole splittings [15, 19, 20]. In our previous studies [7, 8] we used two and three quadrupole doublets to fit the paramagnetic Mössbauer spectra of IDC's measured at room and liquid nitrogen temperature. However, a two doublets fit was better for all spectra. Therefore, additionally we fitted the new Mössbauer spectra of IDC's with an approximation of the paramagnetic component related to the iron core, using two quadrupole doublets (the fitting with three or more quadrupole doublets was not satisfactory for all spectra). Mössbauer parameters are given in Table 2. It is clearly seen that the statistical criterion χ^2 was significantly lower for the same spectra. The values of ΔE_Q for both doublets related to the iron core are close to those reported in [21, 22] for the room temperature Mössbauer spectra of β-FeOOH. However, we observe small differences in the ΔE_Q values for both the large and small doublets, respectively, for the studied IDC's. The ratio of both doublets varies for different IDC's as well as for different states (lyophilized and solution) and temperatures. It should be mentioned that the superparamagnetic Mössbauer spectrum of Imferon (Benger, USA) at 87 K was fitted using two doublets and two sextets. These results may reflect small microstructural differences for various IDC's as well as the presence of core regions with greater or smaller octahedral distortion and crystallinity. These regions may be related to surface and interior parts of each iron core. It is possible that lyophilization may affect some IDC's with slight changes of the iron core structure.

We note that Mössbauer spectra of placental ferritin measured at room temperature and at 77 K were also better fitted using two quadrupole doublets (with ΔE_Q of (0.84±0.02) mm/s and (0.38±0.02) mm/s, respectively) [14] with lower quadrupole splitting for both doublets than those obtained for all IDC's.

Conclusion

The measurements with a high precision and sensitive Mössbauer spectrometer revealed new peculiarities of the IDC iron core. In contrast to our previous results [7, 8] it was found that lyophilization leads to a small increase of the ΔE_Q value for the paramagnetic component. This fact indicates an increase of the electric field gradient on the ^{57}Fe nuclei resulting from some structural variations (small octahedral distortions) of the iron core during lyophilization. The obtained differences in the ΔE_Q values for the paramagnetic components of various IDC's indicate that these complexes have slightly different iron core structures in corresponding states and at corresponding temperatures. Moreover, the samples of Imferon from different sources have superparamagnetic and paramagnetic low temperature Mössbauer spectra, respectively, that may be a result of different iron core structures and size. Finally, we observed the Fe^{2+} impurity in the majority of studied samples, while the previous studies indicated the presence of the impurity in Ferridextran only.

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