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Comparative Study of Nanosized Iron Cores in Human Liver Ferritin and its Pharmaceutically Important Models Maltofer® and Ferrum Lek Using Mössbauer Spectroscopy[1]

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Abstract—Studies of human liver ferritin and its pharmaceutically important models Maltofer® and Ferrum Lek were carried out using Mössbauer spectroscopy with a high velocity resolution at 295 and 90 K and Mössbauer spectroscopy with a low velocity resolution at 40 and 20 K. The Mössbauer spectra fits using a multicomponent model confirm the hypothesis of the complicated heterogeneous structure of nanosized iron cores in the investigated samples.

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INTRODUCTION

Iron ions play an important role in various biological processes and many vitally important biological molecules with enzymatic, transport, and storage functions contain iron [1]. The main iron storage protein in living systems is water-soluble ferritin, which consists of a nanosized iron core covered by a protein shell. The outside diameters of the protein sphere and iron core are 12 and 6–8 nm, respectively. Ferritin's iron core is a polynuclear composition of ferric oxyhydroxide in the form of ferrihydrate with approximate formulae ($5Fe_2O_3 \cdot 9H_2O$) or $(FeOOH)_8(FeO : OPO_3H_2)$. It is now being studied intensively using different techniques, but its real structure is still unclear [2–6]. Iron deficiency in the body results in the widespread over the world anemia disease: more than a billion people have deficit of iron, while more than 700 million have iron deficiency anemia [7]. Ferritin models appeared to be among the most effective medicines for treating anemia. Their effectiveness could be related to their similarity to biological analog (the ferritin molecule) [8, 9]. These pharmaceuticals contain iron cores in the form of β-FeOOH surrounded with various polysaccharides, e.g., dextrin, dextran, and polymaltose.

The model of the ferritin iron core was previously assumed to have two different areas: an internal antiferromagnetic core with Neel temperature $T_N \approx 310$ K and an external amorphous layer with lower T_N values—a so-called core–shell model [10]. According to this model, Mössbauer spectra were fitted using only two components: paramagnetic components at high temperatures and magnetic ones at low temperatures [11–14]. However, some authors fitted Mössbauer spectra of ferritin and its models using more than two components [14–17]. In addition, a multiphase model of the iron core containing 60–80% ferrihydrite, 15–25% magnetite and/or maghemite, and 1–10% hematite was recently considered in [18]. A comparative analysis of ferritin and its models using Mössbauer spectroscopy in a wide temperature range was performed in this work with the aim of further investigating structural peculiarities in the nanosized iron cores.

EXPERIMENTAL

A lyophilized human liver ferritin was obtained from the Russian State Medical University, Moscow (the procedure for preparing the ferritin was described elsewhere [19]). In this work, 100 mg of protein powder was placed in a plexiglass sample holder with a diameter of 20 mm. Commercially available samples of Maltofer® (Vifor Inc., Switzerland) and Ferrum Lek (Lek, Slovenia) were used as ferritin models. These pharmaceuticals represent a composition of trivalent iron with polymaltose in tablets with 100 mg of iron per each tablet. Samples of Maltofer® and Ferrum Lek were ground into powder using 1/3 of a tablet and placed in the plexiglass sample holders. The thicknesses of the Maltofer® and Ferrum Lek samples for Mössbauer measurements were ~10 mg Fe cm^{-2}.

The Mössbauer spectra were measured at 295 and 90 K using an automated precision Mössbauer spectrometric system with a high velocity resolution (registration in 4096 channels), built on the base of an SM-2201 spectrometer and an upgraded cryostat with a moving absorber and operating range of 295–85 K (the cryostat was developed by the Research Institute

[1] This article was translated by the authors.

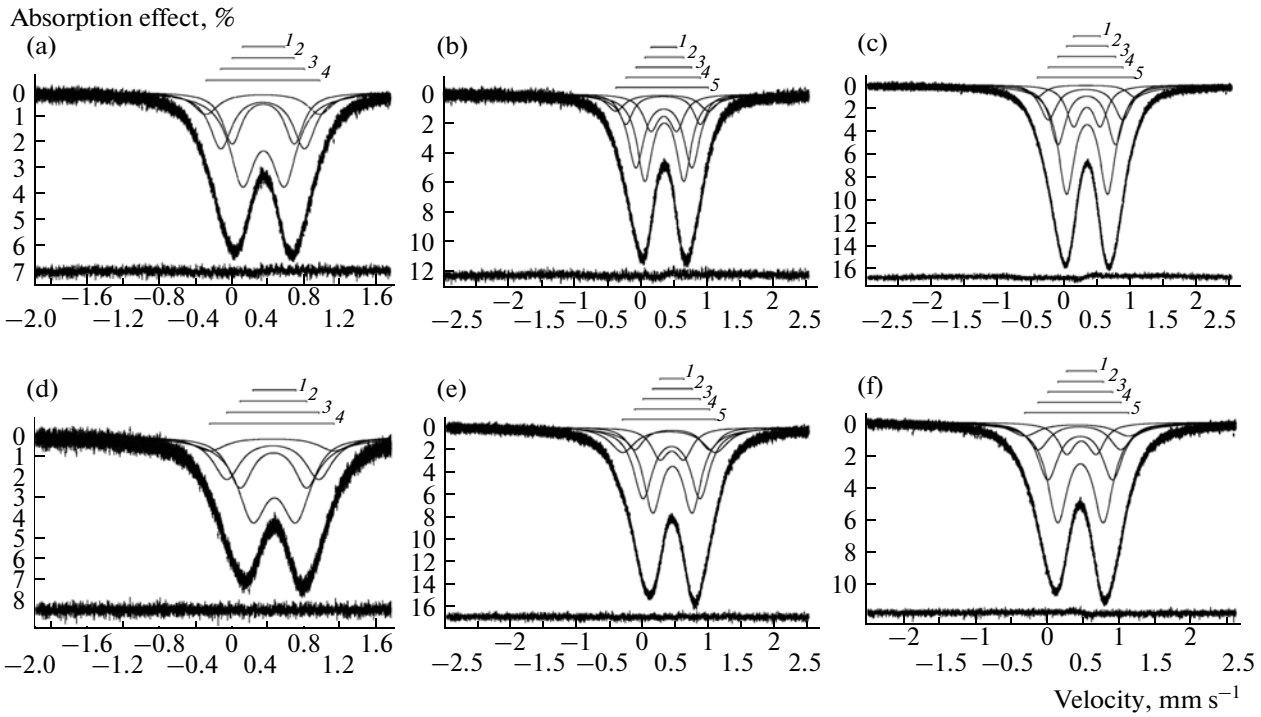

Fig. 1. Mössbauer spectra of human liver ferritin (a, b) and its pharmaceutically important models Maltofer® (c, d) and Ferrum Lek (e, f) measured at 295 K (a, c, e) and 90 K (b, d, f) with a high velocity resolution (in 4096 channels). Indicated components are the results of the best fits.

of Physics, Southern Federal University, Rostov-on-Don) [20–22]. The Mössbauer source, ^{57}Co in a rhodium matrix of about ~1.8×10^9 Bq (RITVERC GmbH, St. Petersburg), was used at room temperature. A standard absorber of sodium nitroprusside (SNP) with an effective thickness of 5 mg Fe cm^{-2} was used to calibrate the spectrometer's velocity scale (line width, 0.229 ± 0.003 mm s^{-1}; absorption effect, ~12%). The time of measuring each spectrum was between two and four days, and the statistical counting rates were in the range of 3.1×10^5 to 1.1×10^6 counts per channel in the case of registration in 4096 channels. The velocity resolution was ~0.0012 mm s^{-1} per channel and the signal-to-noise ratios were in the range from 96 to 134.

The Mössbauer spectra of the studied samples were measured with a low velocity resolution (registration in 256 channels for both direct and reverse motions) at 40 and 20 K using a KFKI type spectrometer with a triangular velocity reference signal and an APD closed cycle refrigerator. The measurement time for each spectrum was between one and two days, and the statistical counting rates were in the range from 1.6×10^5 to 1.6×10^6 counts per channel in the case of registration in 256 channels. The velocity resolution was ~0.11 mm s^{-1} per channel and the signal-to-noise ratios were in the range from 31 to 55. For their analysis spectra registered in the direct motion were used only (without folding). The same standard absorber of sodium nitroprusside (SNP) with an effective thickness of 5 mg Fe cm^{-2} was used to control the spectrometer (line width, 0.23 ± 0.05 mm s^{-1}; absorption effect, ~3.5%).

All measured spectra were computer fitted using the UNIVEM-MS program (the program was developed by the Research Institute of Physics, South Federal University, Rostov-on-Don) with a least squares procedure and the Lorentzian line shape. The parameters determined for each spectra were isomer shift δ, quadrupole splitting ΔE_Q, magnetic hyperfine field H_{eff}, line width Γ, relative subspectrum area S, and statistical criterion χ^2. The differential spectrum, χ^2, and a physical sense of the spectral parameters were used to choose the best fits. During Mössbauer parameters evaluation, two types of errors were considered: instrumental and calculated. The instrumental (systematic) error for each spectrum point was ±0.5 channel (the velocity scale); the instrumental (systematic) error for the hyperfine parameters was ±1 channel; and for the line width it was ±2 channels in mm s^{-1} [22]. Provided the error calculated during the fitting procedure (fitting error) for these parameters exceeded the instrumental (systematic) error we used the former one and vice versa. The error in determining the relative error did not exceed 10%. All of the values for the isomer shifts are given relative to α-Fe at 295 K.

RESULTS AND DISCUSSION

The Mössbauer spectra of human liver ferritin, Maltofer® and Ferrum Lek measured with a high

velocity resolution at 295 K were similar doublets with different absorption effects and thus with different amounts of iron per sample (Fig. 1). Since the Mössbauer spectra of ferric hydrous oxides have non-Lorentzian line shapes [23, 24], the spectra of the investigated samples were fitted using the heterogeneous iron core model described in [16, 25, 26] with several quadrupole doublets. It should be noted that the spectra of each sample measured at 295 and 90 K were fitted using consistent models, so some parameters (the intensities and/or line widths) for the corresponding components were fixed. The best fits of these spectra were obtained with different numbers of components: 4 quadrupole doublets were used for the ferritin spectra and 5 quadrupole doublets were used for both iron–polymaltose complexes Maltofer® and Ferrum Lek. The Mössbauer parameters obtained within the best fit are given in the Table. A comparison of relative areas of corresponding components in the Mössbauer spectra of the investigated samples measured at 295 and 90 K is shown in the Fig. 2. It is clearly seen that the majority of relative area values for corresponding components are similar within the error. However, we observed an increase in the line width for corresponding components with temperature decrease to 90 K that in particular may be related to the cryostat vibrations occurring with liquid nitrogen infusion. The best fits of the Mössbauer spectra of studied samples using several paramagnetic components confirmed the complicated heterogeneous structure of nanosized iron cores. These components can be attributed to various areas/layers of the iron cores with different degrees of crystallinity, the density of atoms packing, the presence of one or several crystallites in the iron core, the presence of impurities, or with other factors that affecting the formation of the iron core in ferritin and its models. One can assume that the quadrupole doublets with the lowest values of ΔE_Q obtained during the fitting of the spectra measured at 295 and 90 K are related to ^{57}Fe nuclei in the internal areas/layers of the iron core, demonstrating the high degree of crystallinity and packing density. The quadrupole doublets with the highest values of ΔE_Q (the 4th doublet in the ferritin spectrum and the 5th doublet in the Maltofer® and Ferrum Lek spectra) could characterize the external amorphous layer of the iron core. The fitting of the ferritin Mössbauer spectra using 4 quadrupole doublets could thus indicate the complicated heterogeneous structure of the ferritin iron core and the presence of different internal areas or layers. The described complicated structure of the iron core could result from the process of its formation.

Despite the Mössbauer spectra of iron-polymaltose complexes Maltofer® and Ferrum Lek being fitted using 5 quadrupole doublets, the values of the Mössbauer parameters were found to be different. This finding could indicate that the areas/layers in the nanosized iron core correspond to components of different sizes, degrees of crystallinity, density of atoms packing,

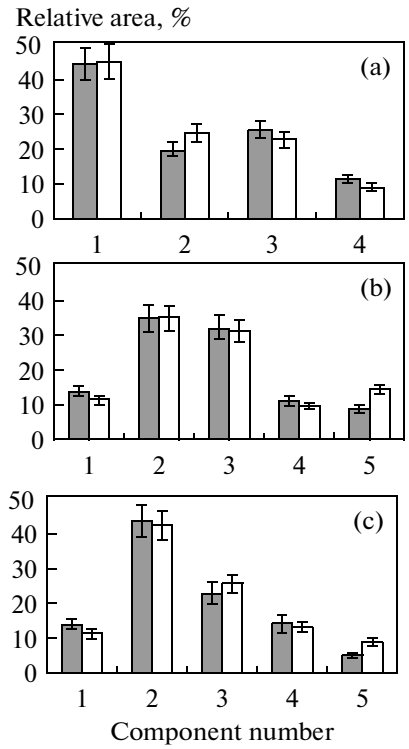

Fig. 2. Histograms of the relative areas of the components in the Mössbauer spectra of human liver ferritin (a) and its models Maltofer® (b) and Ferrum Lek (c) measured at 295 K (■) and 90 K (□). Numbers of spectral components are corresponding to those in Fig. 1.

and so on that might be related to different manufacturing processes.

The Mössbauer spectra of the human liver ferritin, Maltofer® and Ferrum Lek measured at 40 and 20 K with a low velocity resolution are shown in Fig. 3. The spectrum of the ferritin sample demonstrates the superparamagnetic state of the iron cores with a minor magnetic component. In contrast, magnetically split components prevail in the spectra of Maltofer® and Ferrum Lek, while the paramagnetic component drops substantially with the temperature decrease from 40 to 20 K. The magnetic anisotropy energies for the iron cores in the ferritin and in Maltofer® and Ferrum Lek are therefore different. Assuming that the magnetic anisotropy constants for the iron cores in the studied samples are similar, one may conclude that the iron core volume in ferritin is somewhat smaller than that in the pharmaceutical models Maltofer® and Ferrum Lek. The Mössbauer spectra of Maltofer® and Ferrum Lek measured at 40 and 20 K were fitted better using 5 magnetic sextets and 2 quadrupole doublets and 7 magnetic sextets and 1 quadrupole doublet, respectively. The values of the Mössbauer parameters obtained within the best fit are shown in the Table. It should be noted that the Mössbauer parameters for the spectra of iron–polymaltose complexes showed some

Mössbauer parameters obtained from the best fits of the Mössbauer spectra of ferritin, Maltofer®, and Ferrum Lek, measured with a high velocity resolution at 295 and 90 K and with a low velocity resolution at 40 and 20 K

Sample	T, K	No.[a]	δ, mm s^{-1}	ΔE_Q, mm s^{-1}	H_{eff}, kOe	Γ, mm s^{-1}	S, %
Ferritin	295	1	0.370 ± 0.001	0.464 ± 0.002		0.337 ± 0.002	44
		2	0.368 ± 0.001	0.686 ± 0.002		0.254 ± 0.003	20
		3	0.361 ± 0.001	0.925 ± 0.002		0.298 ± 0.003	25
		4	0.362 ± 0.002	1.258 ± 0.004		0.332 ± 0.007	11
Maltofer®	295	1	0.354 ± 0.002	0.389 ± 0.005		0.274 ± 0.005	14
		2	0.362 ± 0.002	0.590 ± 0.002		0.272 ± 0.003	35
		3	0.356 ± 0.002	0.844 ± 0.002		0.291 ± 0.003	32
		4	0.345 ± 0.002	1.122 ± 0.004		0.248 ± 0.007	11
		5	0.330 ± 0.002	1.397 ± 0.008		0.322 ± 0.010	8
Ferrum Lek	295	1	0.354 ± 0.002	0.402 ± 0.002		0.258 ± 0.003	14
		2	0.357 ± 0.002	0.621 ± 0.002		0.303 ± 0.003	44
		3	0.352 ± 0.002	0.872 ± 0.002		0.287 ± 0.003	23
		4	0.334 ± 0.002	1.125 ± 0.003		0.304 ± 0.004	15
		5	0.338 ± 0.002	1.464 ± 0.005		0.270 ± 0.007	4
Ferritin	90	1	0.483 ± 0.001	0.480 ± 0.003		0.399 ± 0.004	44
		2	0.474 ± 0.004	0.737 ± 0.004		0.330 ± 0.006	24
		3	0.466 ± 0.001	1.020 ± 0.003		0.362 ± 0.008	23
		4	0.457 ± 0.004	1.376 ± 0.014		0.427 ± 0.019	9
Maltofer®	90	1	0.463 ± 0.002	0.364 ± 0.007		0.312 ± 0.007	11
		2	0.472 ± 0.002	0.599 ± 0.002		0.341 ± 0.005	35
		3	0.459 ± 0.002	0.865 ± 0.002		0.352 ± 0.004	31
		4	0.468 ± 0.002	1.136 ± 0.007		0.328 ± 0.013	9
		5	0.420 ± 0.002	1.409 ± 0.009		0.464 ± 0.009	14
Ferrum Lek	90	1	0.483 ± 0.002	0.411 ± 0.005		0.286 ± 0.004	11
		2	0.471 ± 0.002	0.636 ± 0.002		0.340 ± 0.004	42
		3	0.467 ± 0.002	0.903 ± 0.003		0.346 ± 0.005	25
		4	0.456 ± 0.002	1.152 ± 0.006		0.367 ± 0.011	13
		5	0.417 ± 0.003	1.449 ± 0.014		0.494 ± 0.014	9
Maltofer®	40	1	0.19 ± 0.12	−0.12 ± 0.12	178.3 ± 3.5	0.78 ± 0.24	15
		2	0.64 ± 0.12	−0.42 ± 0.12	313.6 ± 3.5	0.78 ± 0.24	14
		3	0.44 ± 0.12	−0.11 ± 0.12	401.8 ± 3.5	0.78 ± 0.24	32
		4	0.41 ± 0.12	0.63 ± 0.12		0.54 ± 0.24	28
		5	0.45 ± 0.12	1.38 ± 0.22		0.73 ± 0.24	11
Ferrum Lek	40	1	0.26 ± 0.12	−0.19 ± 0.12	194.6 ± 5.8	1.55 ± 0.27	18
		2	0.68 ± 0.12	−0.44 ± 0.12	336.2 ± 5.7	1.55 ± 0.24	23
		3	0.45 ± 0.12	−0.16 ± 0.12	401.8 ± 3.5	0.90 ± 0.24	26
		4	0.43 ± 0.12	0.66 ± 0.12		0.57 ± 0.24	25
		5	0.42 ± 0.12	1.34 ± 0.12		0.68 ± 0.24	8
Ferritin	20	1	0.38 ± 0.12	−0.69 ± 0.22	344.6 ± 8.0	0.96 ± 0.39	10
		2	0.42 ± 0.12	0.48 ± 0.12		0.51 ± 0.24	72
		3	0.43 ± 0.12	0.94 ± 0.12		0.41 ± 0.24	18
Maltofer®	20	1	0.05 ± 0.12	0.29 ± 0.12	81.9 ± 3.5	1.04 ± 0.24	10
		2	0.45 ± 0.12	−0.07 ± 0.12	318.9 ± 3.7	1.18 ± 0.24	17
		3	0.41 ± 0.12	−0.06 ± 0.12	367.7 ± 3.5	0.65 ± 0.24	15
		4	0.46 ± 0.12	−0.17 ± 0.12	394.8 ± 3.5	0.58 ± 0.24	16
		5	0.43 ± 0.12	−0.10 ± 0.12	418.0 ± 3.5	0.60 ± 0.24	28
		6	0.40 ± 0.12	0.80 ± 0.12		0.85 ± 0.24	14
Ferrum Lek	20	1	0.66 ± 0.12	−0.59 ± 0.12	177.6 ± 3.5	0.78 ± 0.24	10
		2	0.71 ± 0.12	−0.49 ± 0.12	306.3 ± 3.5	0.78 ± 0.24	9
		3	0.43 ± 0.12	−0.03 ± 0.12	353.8 ± 3.5	0.78 ± 0.24	17
		4	0.45 ± 0.12	−0.18 ± 0.12	388.2 ± 3.5	0.57 ± 0.24	18
		5	0.44 ± 0.12	−0.15 ± 0.12	415.2 ± 3.5	0.62 ± 0.24	33
		6	0.42 ± 0.12	0.89 ± 0.12		0.78 ± 0.24	13

Note: [a] Numbers of components correspond to the numbers of the components in the Mössbauer spectra in Figs. 1 and 3

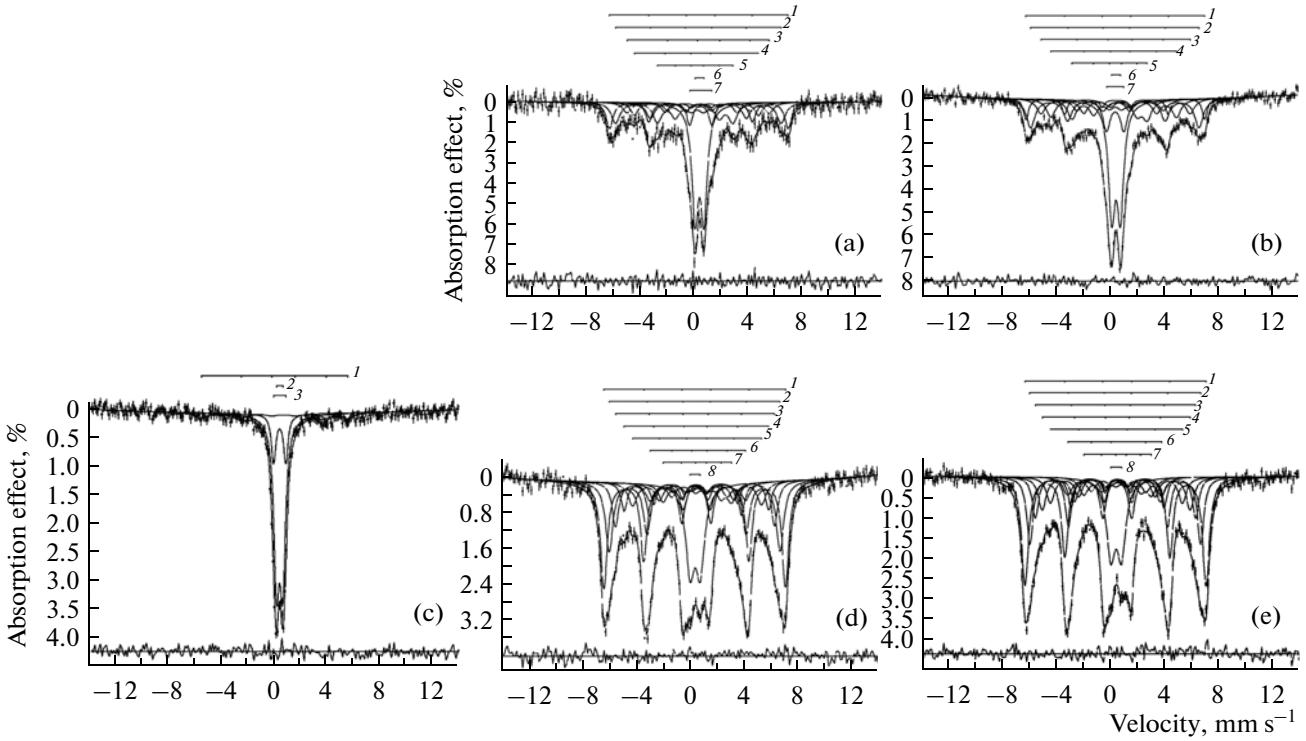

Fig. 3. Mössbauer spectra of human liver ferritin (c) and its models Maltofer® (a, d) and Ferrum Lek (b, e) measured at 40 K (a, b) and 20 K (c, d, e) with a low velocity resolution (in 250 channels). The indicated components are the results from best fits.

differences that could also be a result of the different manufacturing processes of Maltofer® and Ferrum Lek. The presence of several components in the Mössbauer spectra of Maltofer® and Ferrum Lek confirms that its iron cores have complicated heterogeneous structures. For instance, the magnetically split components in the spectra measured at 20 K with higher values of H_{eff} could be related to ^{57}Fe nuclei in the internal areas/layers of the iron core with a higher degree of crystallinity.

CONCLUSIONS

Our study of human liver ferritin and its pharmaceutically important models Maltofer® and Ferrum Lek using Mössbauer spectroscopy in the temperature range from 295 to 20 K demonstrated the complicated heterogeneous structure of the nanosized iron cores in the studied samples, which could be used for the subsequent development of the core–shell model. The components of the Mössbauer spectra obtained within the best fit could indicate the presence of crystallites or various areas/layers with different sizes, degrees of crystallinity, density of atoms packing, the possible presence of impurities, or other factors that influence the formation of the iron core in ferritin and its models.

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REFERENCES

1. Aisen, P. and Listowsky, I., *Ann. Rev. Biochem.*, 1980, vol. 49, p. 357.
2. Theil, E.C., *Ann. Rev. Biochem.*, 1987, vol. 56, p. 289.
3. Theil, E.C., *Ann. Rev. Nutr.*, 2004, vol. 24, p. 327.
4. Proulx-Curry, P.M. and Chasteen, N.D., *Coord. Chem. Rev.*, 1995, vol. 144, p. 347.
5. Massover, W.H., *Micron.*, 1993, vol. 24, no. 4, p. 389.
6. Bauminger, E.R., Harrison, P.M., Hechel, D., Nowik, I., and Treffry, A., *Hyper. Interact.*, 1994, vol. 91, p. 835.
7. Danielson, B.G. and Geisser, P., and Shneider W., *Iron Therapy,* St. Gallen: Vifor Int., 1996.
8. Allen, L.H., *J. Nutr.*, 2002, vol. 132, p. 813.
9. Liu, T.-C., Lin, S.-F., Chang, C.-S., et al., *Int. J. Hematol.*, 2004, vol. 80, p. 416.

10. Brooks, R.A., Vymazal, J., Goldfarb, R.B., Bulte, J.W., and Aisen, P., *Mag. Res. Med.*, 1998, vol. 40, p. 227.
11. Papaefthymiou, G.C., Viescas, A.J., Horn, R., et al., *Hyper. Interact.*, 2005, vol. 165, p. 333.
12. Bou-Abdallah, F., Carney, E., Chasteen, N.D., et al., *Biophys. Chem.*, 2007, vol. 130, p. 114.
13. Papaefthymiou, G.C., *Nano Today*, 2009, vol. 4, p. 438.
14. Papaefthymiou, G.C., *Biochim. Biophys. Acta*, 2010, vol. 1800, p. 886.
15. Funk, F., Long, G.J., Hautot, D., Buchi, R., Christl, I., and Weidler, P.G., *Hyper. Interact.*, 2001, vol. 136, p. 73.
16. Oshtrakh, M.I., Milder, O.B., and Semionkin, V.A., *Hyper. Interact.*, 2008, vol. 181, p. 45.
17. Oshtrakh, M.I., Semionkin, V.A., Milder, O.B., and Novikov, E.G., *J. Mol. Struct.*, 2009, vols. 924–926, p. 20.
18. Jung, J.H., Eom, T.W., Lee, et al., *J. Mag. Mag. Mater.*, 2011, vol. 323, p. 3077.
19. Oshtrakh, M.I., Semionkin, V.A., Prokopenko, P.G., et al., *Int. J. Biol. Macromol.*, 2001, vol. 29, p. 303.
20. Oshtrakh, M.I., Semionkin, V.A., Milder, O.B., and Novikov, E.G., *J. Radioanal. Nucl. Chem.*, 2009, vol. 281, p. 63.
21. Semionkin, V.A., Oshtrakh, M.I., Milder, O.B., and Novikov, E.G., *Bull. Rus. Acad. Sci. Phys.*, 2010, vol. 74, no. 3, p. 475.
22. Oshtrakh, M.I. and Semionkin, V.A., *Spectrochim. Acta A: Molec. Biomolec. Spectrosc.*, 2012, vol. 100, p. 78.
23. Murad, E. and Johnston, J.H., in *Spectroscopy Applied to Inorganic Chemistry*, Long, G.J., Ed., New York: Plenum Publ., 1987, vol. 2, p. 507.
24. Murad, E., Bowen, L.H., Long, G.J., and Quin, T.G., *Clay Miner.*, 1988, vol. 23, p. 161.
25. Oshtrakh, M.I., Alenkina, I.V., Dubiel, S.M., and Semionkin, V.A., *J. Mol. Struct.*, 2011, vol. 993, p. 287.
26. Alenkina, I.V., Oshtrakh, M.I., Klepova, Yu.V., et al., *Spectrochim. Acta A: Molec. Biomolec. Spectrosc.*, 2012, vol. 100, p. 88.