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The ^{57}Fe hyperfine interactions in human liver ferritin and its iron-polymaltose analogues: the heterogeneous iron core model

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Abstract Human liver ferritin and its iron-polymaltose pharmaceutical analogues Ferrum Lek, Maltofer® and Ferrifol® were studied using Mössbauer spectroscopy at 295 and 90 K. The Mössbauer spectra were fitted on the basis of a new model of heterogeneous iron core structure using five quadrupole doublets. These components were related to the corresponding more or less close-packed iron core layers/regions demonstrating some variations in the ^{57}Fe hyperfine parameters for the studied samples.

Keywords Mössbauer spectroscopy · Hyperfine interactions · Ferritin · Iron-polymaltose complexes · Iron core

1 Introduction

Ferritin molecules are responsible for the iron storage in the body. Mammalian ferritin consists of a 24-subunits protein shell with a size of ∼12 nm and a cavity inside the shell with a diameter of ∼8 nm (for review of ferritin structure and functions see, for instance, [1–4]). The cavity contains a nanosized iron core in the form of ferrihydrite ($5Fe_2O_3{\times}9H_2O$) or ferric hydrous oxide complex with some inorganic phosphates with an approximate formula $(FeOOH)_8(FeO{:}OPO_3H_2)$. The largest amount of iron atoms may be up to 4500, however,

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normal mammalian ferritin core contains of about 2500 iron atoms. The structure of ferritin iron core is a subject of numerous studies some of which have been discussed briefly in [5, 6] (see references therein). The core structure was considered as being in crystalline and amorphous forms, as monocrystalline or polycrystalline forms or consists of a mixture of these structures, as one crystallite, several crystallites, multidomain and polyphasic iron core, and so on. Basing on these structures different iron core models were used to fit the ferritin Mössbauer spectra. For instance, the core-shell model was used in [7, 8] to fit spectra using two components, the polyphasic model was considered in [9, 10] with three spectral components. Some ferritin analogues are used and developed as medicaments for treatment the iron deficiency anemia. These analogues consist of a polysaccharide shell surrounding the nanosized iron core in the form of akaganéite (β-FeOOH). The structure of the iron core in ferritin analogues is also unclear. For instance, in the study of iron-dextran complexes the akaganéite core was considered as a structure with two non-equivalent Fe^{3+} sites to reach an agreement with the results of Mössbauer spectra fit using two components [11]. Therefore, further study of the iron core structure in ferritin and its analogues is of interest. For this aim we applied Mössbauer spectroscopy with a high velocity resolution (with a high discretization of the velocity reference signal up to 2^{12}) to reveal small differences in the ^{57}Fe hyperfine parameters for ferritin and its pharmaceutical analogues for detailed analysis of the iron cores.

2 Experimental

Human liver ferritin in lyophilized form was provided by Prof. P.G. Prokopenko (Russian State Medical University, Moscow). The method of ferritin preparation was given in [12]. Commercial samples of fresh Ferrum Lek (Lek, Slovenia), Maltofer® (Vifor Inc., Switzerland) and Ferrifol® (CTS Chemical Industries Ltd., Israel) tablets were used. Each tablet contains 100 mg Fe, therefore, 1/3 of each tablet was powdered for the study. Samples for Mössbauer spectroscopy were placed into Plexiglas sample holders with diameter of 20 mm and height of 5 mm. Samples powders were close packed in the sample holders to exclude particles vibrations. Ferritin sample had a weight of 100 mg with ∼20 % of iron abundance. Samples of ferritin analogues had a thickness of ∼10, ∼8 and ∼8 mg Fe/cm^2 for Ferrum Lek, Maltofer® and Ferrifol® , respectively.

Mössbauer spectra were measured using an automated precision Mössbauer spectrometric system built on the base of the SM-2201 spectrometer with a saw-tooth shape velocity reference signal formed by the digital-analog converter using discretization of 2^{12} (quantification using 4096 steps) and temperature variable liquid nitrogen cryostat with moving absorber (the best temperature control indicates the temperature stability of ±0.1 K). Details and characteristics of this spectrometer and the system were considered in [13–15]. The (1.8–1.0)×10^9 Bq ^{57}Co in rhodium matrix source (Ritverc GmbH, St. Petersburg) was used at room temperature. The Mössbauer spectra were measured in transmission geometry with moving absorber in the cryostat at 295 and 90 K and recorded in 4096 channels. Statistical count rates for the ferritin spectra at 295 and 90 K were 2.7×10^6 and 2.0×10^6 counts per channel with the signal-to-noise ratios 95 and 99, respectively, while those for the Ferrum Lek, Maltofer® and Ferrifol® spectra were in the range of 1.0×10^5 – 6.3×10^5 counts per channel with the signal-to-noise ratios in the range from 60 to 133. The measurement time for one spectrum varied between one and two weeks.

The spectra were computer fitted with the least squares procedure using UNIVEM-MS program with a Lorentzian line shape. The spectral parameters such as isomer shift, δ,

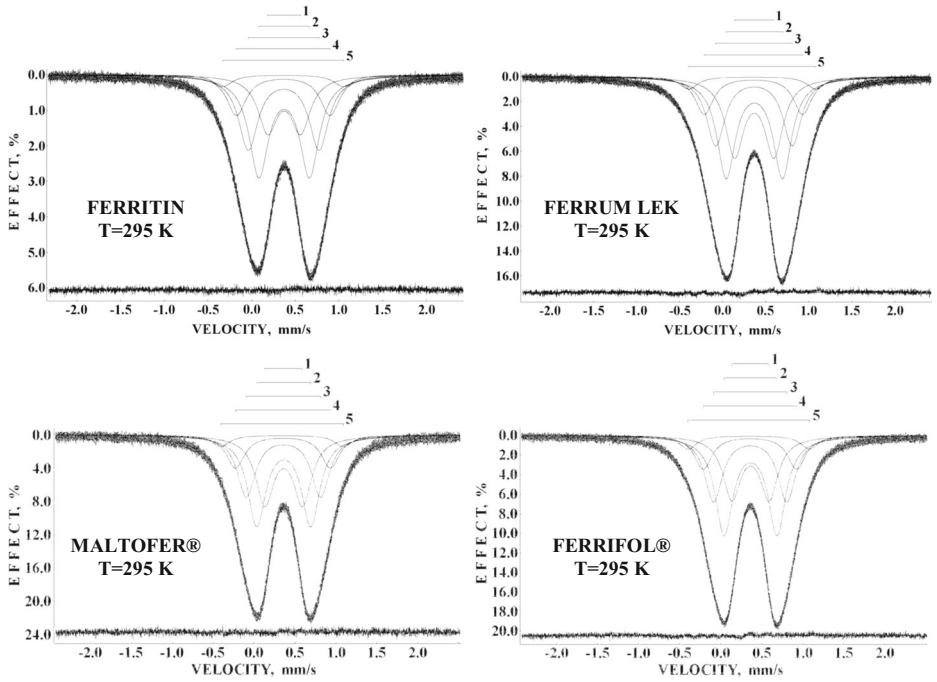

Fig. 1 Mössbauer spectra of human liver ferritin and its iron-polymaltose analogues Ferrum Lek, Maltofer® and Ferrifol® measured at 295 K in 4096 channel. 1–5 are the results of the fits within the new heterogeneous iron core model. The differential spectra are shown below

quadrupole splitting, ΔE_Q, line width, Γ, relative subspectrum area, A, and statistical criterion, χ^2, were determined. An 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. If an error calculated with the fitting procedure (fitting error) for these parameters exceeded the instrumental (systematic) error we used the larger error instead. Criteria of the best fit were differential spectra, χ^2 values and physical meaning of parameters. The standard absorber of sodium nitroprusside (SNP) with a thickness of 5 mg Fe/cm^2 was used for velocity scale calibration. The Mössbauer spectra of SNP measured at different time between samples measurements demonstrated pure Lorentzian line shape with Γ =0.229±0.002 mm/s. Velocity resolution in the spectra was ∼0.001 mm/s per channel. Values of δ are given relative to α–Fe at 295 K.

3 Results and discussion

Mössbauer spectra of human liver ferritin and its iron-polymaltose analogues measured at 295 and 90 K are shown in Figs. 1 and 2, respectively. These spectra are similar two-peak patterns which cannot be fitted well using one quadrupole doublet (see [16]) that may be a result of the heterogeneous iron core structure. Considering various fits of the Mössbauer spectra of ferritin and its analogues within the heterogeneous iron core model we used a superposition of several quadrupole doublets to reach the best fits using free variations of parameters or some constraints [5, 6, 16]. Here we used the recent approach to

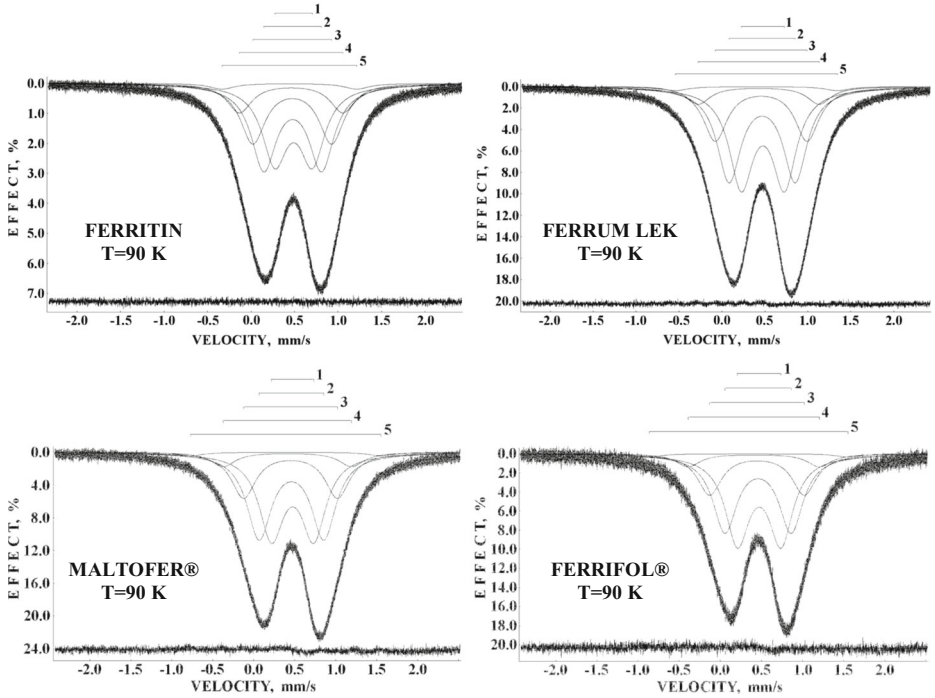

Fig. 2 Mössbauer spectra of human liver ferritin and its iron-polymaltose analogues Ferrum Lek, Maltofer® and Ferrifol® measured at 90 K in 4096 channel. 1–5 are the results of the fits within the new heterogeneous iron core model. The differential spectra are shown below

fit the Mössbauer spectra considered in [6]. This model suggests the heterogeneous iron core with different layers/regions which are homogeneous within each layer/region. If we omit from consideration any interlayer/interregional interactions and the borders between layers/regions we can relate each spectral component to corresponding layer/region. In this case we can use the same line width for each quadruple doublet with its variations during the fit. Comparison of the fits of the measured spectra using different number of quadrupole doublets demonstrated that 5 quadrupole doublets were enough to reach good differential spectra and χ^2 values within the standard deviation σ with those for the 6-doublet fits. The results of the 5 quadrupole doublets fit are shown in Figs. 1 and 2.

The differences in ΔE_Q values obtained for 5 quadrupole doublets may be related to some variations in corresponding microenvironments of the ^{57}Fe. The different layers/regions can be considered for ferrihydrite and akaganéite nanoparticles. The Fe–O packing may vary from the most close-packed to the less close-packed in these layers/regions. The smallest quadrupole splitting can be assigned to the most close-packed layer/region while the largest one can be associated with the less close-packed layer/region, the latter may be probably related to the surface of nanoparticle. A comparison of the ^{57}Fe hyperfine parameters for each component (layer/region) for human liver ferritin and its iron-polymaltose analogues Ferrum Lek, Maltofer® and Ferrifol® at both 295 and 90 K are shown in Fig. 3. It is clearly seen that at 295 K the ^{57}Fe hyperfine parameters for all spectral components for human liver ferritin were different from those of iron-polymaltose analogues while ΔE_Q and δ values for Ferrum Lek, Maltofer® and Ferrifol® were close

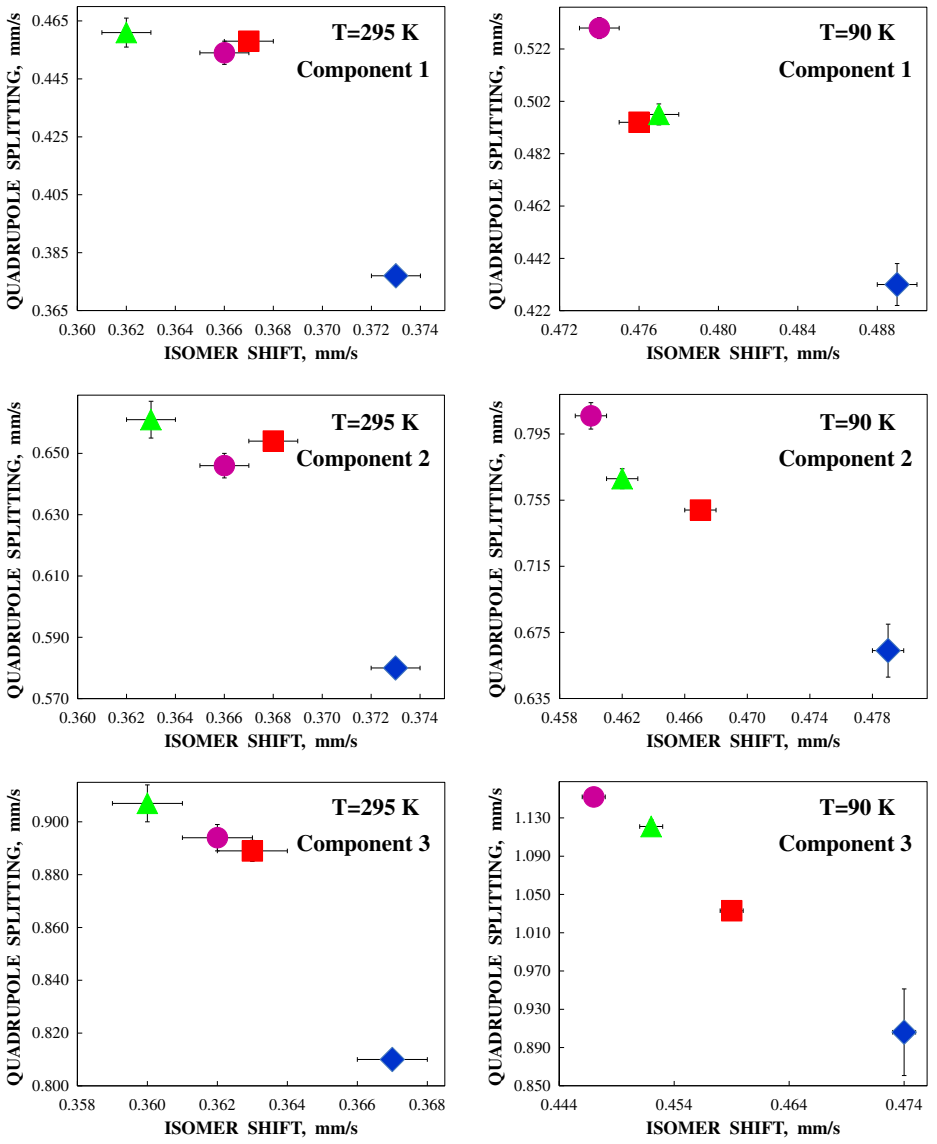

Fig. 3 The plots of the ^{57}Fe hyperfine parameters for different spectral components obtained within the new heterogeneous iron core model fits of the Mössbauer spectra of human liver ferritin (◆), Ferrum Lek (■), Maltofer® (▲) and Ferrifol® (●) measured at 295 and 90 K

for corresponding components. However, the differences in the ^{57}Fe hyperfine parameters for ferritin, Ferrum Lek, Maltofer® and Ferrifol® at 90 K appeared to be very clear and demonstrated small variations of ΔE_Q and δ values for corresponding spectral components of these samples. The latter may indicate any different changes in the local microenvironments in the iron cores in human liver ferritin, Ferrum Lek, Maltofer® and Ferrifol® at low temperature.

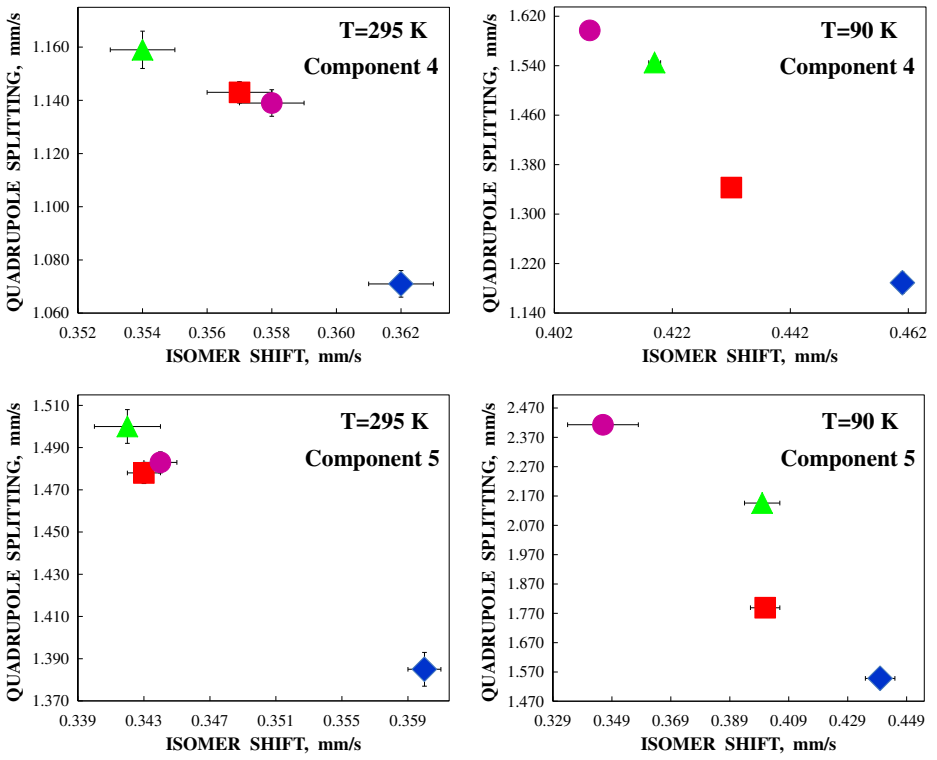

Fig. 3 (continued)

Earlier we have shown that the Mössbauer spectra of ferritin and fresh samples of Ferrum Lek and Maltofer® measured in a large velocity range at 90 K had no magnetic components; however, at lower temperatures ferritin analogues demonstrated magnetic and paramagnetic components while ferritin remained paramagnetic at 20 K [5]. Moreover, the observed features of the Mössbauer spectra of ferritin, Ferrum Lek and Maltofer® in the temperature range ∼160–90 K cannot be related to slowdown of magnetic relaxation and cryostat vibrations [6, 17]. Therefore, the low temperature (at 90 K) differences in the ^{57}Fe hyperfine parameters may be a result of some variations in the iron cores of akaganéite nanoparticles in the studied iron-polymaltose complexes and ferrihydrite nanoparticles in human liver ferritin. Therefore, we compared the relative areas of spectral components for human liver ferritin, Ferrum Lek, Maltofer® and Ferrifol® at 295 and 90 K (see Fig. 4). The relative areas for all corresponding spectral components were close for all samples at 295 K. It was found that the relative areas of component 1 increased with temperature decrease while those for component 2 appeared to be almost the same as at 295 K. In contrast, the relative areas of spectral components 3–5 decreased in different ways with temperature decrease. The largest change was observed for the component 5 which may be associated with surface layer/region in the iron cores. Using suggestion about low temperature structural rearrangement [6, 17, 18] (phase transition [19]) in the iron cores we can suppose the following. The degree of Fe–O packing can be changed with temperature decrease with increase of the most close-packed layer/region volume and consequent decrease of the less close-packed

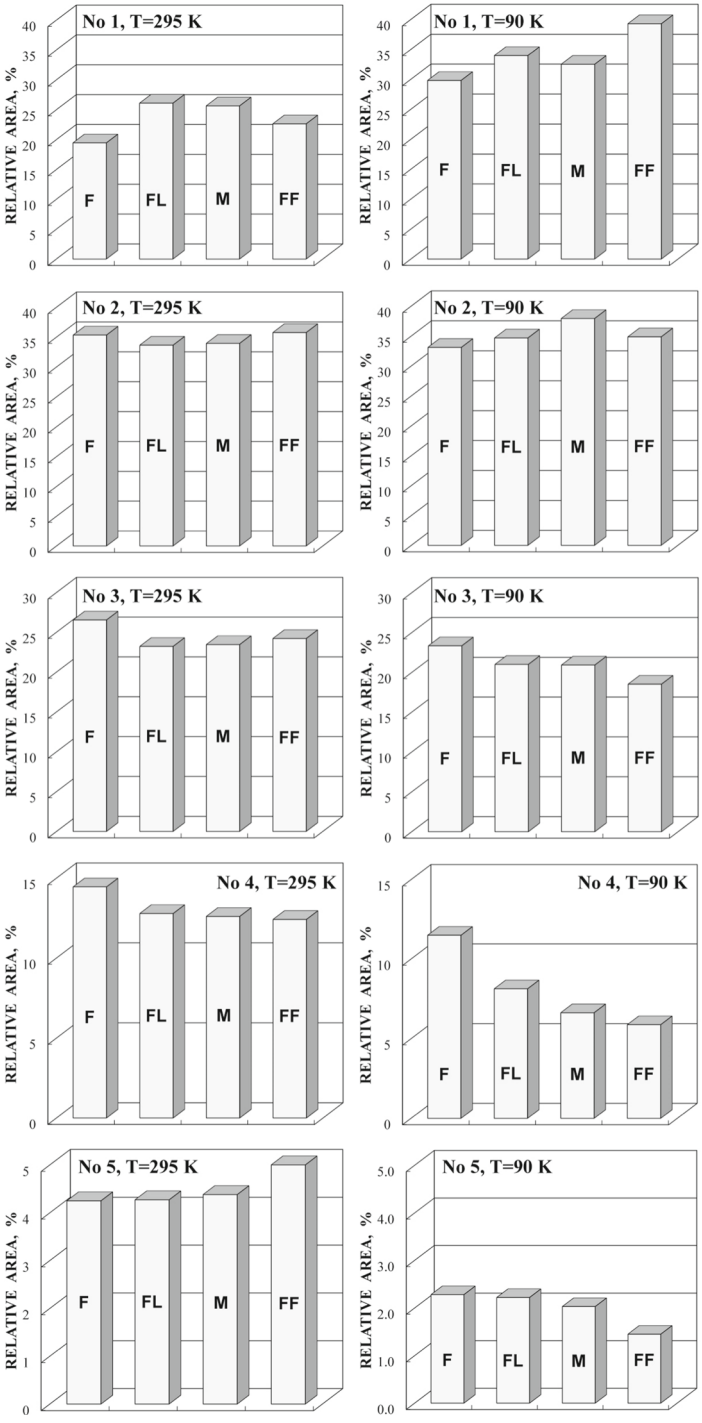

Fig. 4 Comparison of the relative areas for spectral components 1–5 indicated in Figs. 1 and 2 for human liver ferritin (**F**), Ferrum Lek (**FL**), Maltofer® (**M**) and Ferrifol® (**FF**) at 295 and 90 K

layers/regions volumes. In this case the possible differences in the akaganéite cores in iron-polymaltose complexes related to some differences in the manufacturing processes could be reflected by small variations in the ^{57}Fe hyperfine parameters and relative areas of the corresponding Mössbauer spectra components at low temperature.

4 Conclusion

Investigation of human liver ferritin and its iron-polymaltose analogues Ferrum Lek, Maltofer® and Ferrifol® using Mössbauer spectroscopy with a high velocity resolution at 295 and 90 K demonstrated that these spectra could be fitted well using the heterogeneous iron core model with different core layers/regions accounting for homogeneous local microenvironment of the ^{57}Fe in each layer/region.

The Mössbauer spectra of all samples were fitted well within this model using 5 quadrupole doublets with the same line widths varied during the fit. These quadrupole doublets were related to the more or less close-packed layers/regions in the iron cores on the basis of a suggestion about association of smaller quadrupole splitting with a larger degree of Fe–O packing.

A comparison of the ^{57}Fe hyperfine parameters for revealed spectral components demonstrated clear difference between quadrupole splitting and isomer shift values for human liver ferritin and its iron-polymaltose analogues while at 295 K these values for Ferrum Lek, Maltofer® and Ferrifol® were not so clear distinguished as at 90 K. The observed variations in the relative areas of spectral components were considered as a result of the low temperature structural rearrangements in the ^{57}Fe local microenvironments in the corresponding layers/regions in both nanosized ferrihydrite and akaganéite cores.

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