BIOTRANSFORMATION AND METABOLISM OF MAGNETIC NANOPARTICLES IN AN ORGANISM FROM MÖSSBAUER SPECTROSCOPY

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Abstract. Mössbauer spectroscopy is a powerful method for investigation structural, magnetic and thermodynamic properties of magnetic nanoparticles, in particular those delivered in a body. Multiform temperature- and field-dependent Mössbauer absorption spectra of fine particles provides the researcher with rich information about physical characteristics inherent to such particles staying in different environment. With that the $^{57}$Fe gamma-resonant spectroscopy is efficiently used to study spin states, electronic and dynamical properties of iron-containing proteins in a living organism. For quantitative estimates of characteristics of the magnetic nanoparticles it is required to define a model of the magnetic dynamics. We have developed such a model and performed consistent least-square fitting procedure for the set of temperature- and magnetic field-dependent spectra as well as magnetization curves of nanoparticles injected into mice. This allowed us to reliably evaluate changes in the nanoparticles characteristics and the chemical transformation of the iron to paramagnetic ferritin-like forms in mouse’s organs as a function of time after injection of nanoparticles. In fact, the approach allows the researcher to quantitatively characterize biotransformation and metabolism of magnetic nanoparticles injected into a body.

1. Introduction

A multitude of techniques are used to characterize structural, magnetic and thermodynamic properties of magnetic nanomaterials, among which one of the most informative is the Mössbauer spectroscopy. Today one of the most promising applications of this method seems to be an analysis of absorption curves of iron-containing magnetic nanoparticles injected into a living organism. The main puzzle in such investigation is to decompose the spectra into partial contributions of exogenous iron atoms in nanoparticles and endogenous iron atoms included, for example, in ferritin of the organism [1]. The temperature evolution of the spectral shape for initial nanoparticles is similar with the conventional behavior of the spectra of an ensemble of single-domain particles with magnetic anisotropy. With that a significant lines broadening in the spectra of the mouse liver and spleen after injection of nanoparticles as well as an additional doublet of lines are observed. The doublet parameters are typical for Mössbauer spectra of iron in ferritin-like proteins [2]. The formal analysis generally used for such spectra and based on the consideration of continuous distributions of the hyperfine field $H_{hf}$ at iron
nuclei [3], results in only qualitative treatment. Meantime, the most accurate model for analysis the temperature-dependent Mössbauer spectra of single-domain particles is the multi-level relaxation model that takes into account their magnetic anisotropy and diffusion of uniform magnetization [4]. This model was successfully used to fit self-consistently the experimental spectra of an ensemble of magnetic nanoparticles at various temperatures [5, 6], including nanoparticles injected into a living organism [1]. Moreover, there is another highly informative, long ago known and very easy method, i.e., measurements of the gamma-resonant spectra of nanoparticles in a weak static magnetic field. But an adequate theory for describing the field-dependent shape of the spectra has been developed only in the recent past [7]. This formalism can be extended within the multi-level relaxation model [4] on the basis of a model of magnetic dynamics developed in [7].

The main purpose of this paper is to demonstrate the advantages of the theoretical approach to the self-consistent treatment of the temperature- and field-dependent Mössbauer spectra and magnetization curves for characterizing the biotransformation and metabolism of magnetic nanoparticles in an organism by the example of behavior of nanoparticles injected into mice.

2. Samples and experiment
We have used industrial ferrofluid “fluidMAG-ARA-250” (Chemicell Gmbh, Germany). The suspension of the magnetic nanoparticles was injected into mice. On the expiry of fixed intervals of time the mice were destroyed, organs pulled out and lyophilized. The dried initial ferrofluid and lyophilized mouse’s organs were then trituted and the powder samples were prepared for Mössbauer exploration. The $^{57}\text{Fe}$ gamma-resonant absorption spectra of the samples were measured at liquid nitrogen and room temperatures on a conventional spectrometer in the linear-speed regime and transmission configuration with a $^{57}\text{Co}$(Rh) radioactive source. The spectra of the samples were also taken in the cross (with respect to the gamma-beam direction) magnetic field of the intensity $H = 3.4$ kOe using a permanent magnet. Magnetization curves for the samples were measured on the magnetometer LakeShore in a field up to 5 kOe. The $^{57}\text{Fe}$ Mössbauer spectra and magnetization curve of initial nanoparticles as well as the spectra of mouse liver two days after injection are shown in figures. 1 and 2.
3. Formalism for calculating the relaxation Mössbauer spectra

Nowadays the most accurate model for accounting relaxation processes in the ensemble of single-domain particles is founded on the quantum-mechanical description of nanoparticle with the volume \( V \) and the uniform magnetization \( M_0 \) (or the total spin \( S \)) which vector \( \mathbf{M} \) changes its orientation randomly in the anisotropy field \([4]\), so that we have the following expression for the particle energy (figure 3):

\[
E = -KV \cos^2 \theta = -KV m^2/S^2 = E_m. \tag{1}
\]

Here, \( K \) is the axial magnetic anisotropy constant, \( \theta \) is the angle between the magnetization vector and the easy magnetic axis of the particle and \( m \) is one of \( 2S+1 \) possible spin projections onto this axis. The populations of the energy levels \( E_m \) are determined by Gibbs’ distribution:

\[
W_m = e^{-E_m/k_B T} \sum_k e^{-E_k/k_B T}, \tag{2}
\]

where \( T \) is the absolute temperature and \( k_B \) is the Boltzmann constant. The transitions between these states can be specified under the assumption that relaxation is associated with the transverse components of the random magnetic field \([4]\).
Calculations of the Mössbauer spectra within the multi-level relaxation model can be performed in terms of the stochastic approach which results in the following formula for the absorption spectrum for gamma-quantum with energy $E_{\gamma} = \hbar \omega$ [1,4]:

$$\sigma(\omega) = -\frac{\sigma^2}{4} \Gamma_0 \sum_\alpha \left| C_\alpha \right|^2 \langle W | \hat{A}^{-1}_\alpha (\omega) | 1 \rangle.$$ (3)

Here, $\omega \equiv \omega - E_0 / \hbar$, $E_0$ is the energy of the resonance transition, $\Gamma_0 \equiv \Gamma_0 / \hbar$ is the natural line width, $\alpha = (m_e, m_g)$ specifies hyperfine transition with the nuclear spin projections $m_e$ and $m_g$ onto the $\mathbf{H}_{hf}$ direction for the excited (e) and ground (g) states, $C_\alpha$ determines the intensity of $\alpha$-th hyperfine transition, $\langle W \rangle$ is the row vector of the equilibrium populations of the stochastic states and $| 1 \rangle$ is the column vector with all components equal to unity. The operator

$$\hat{A}_\alpha(\omega) = \omega + i \Gamma_0 / 2 - \hat{\omega}_\alpha + i \hat{P}$$ (4)

is defined by the diagonal matrix of hyperfine interaction

$$(\hat{\omega}_\alpha)_{mm'} = \delta_{mm'} \omega_m m / S,$$ (5)

where

$$\omega_\alpha = (g_e m_e - g_g m_g) \mu_N \mathbf{H}_{hf} / \hbar$$ (6)

specify the resonance transitions between the ground and exited states ($g_{g,e}$ is their g-factors, $\mu_N$ is the nuclear magneton), and the tridiagonal relaxation matrix

$$P_{mm \pm 1} = -P_{mm \pm 1} f_{mm \pm 1}, \quad P_{mm} = -P_{mm-1} - P_{mm+1},$$ (7)

$$p_{mm+1} = P_{m+1} = D [S(S+1) - m(m+1)],$$ (8)

$$f_{ij} = \begin{cases} \exp \left[ - (E_j - E_i) / k_B T \right], & E_j > E_i \\ 1, & E_j < E_i \end{cases},$$ (9)

specified by the diffusion constant $D$ [4].
The quantitative analysis of experimental spectra requires averaging over particles sizes in the sample studied, e.g., over the Gaussian distribution of diameters:

\[ P(d > 0) \propto \exp\left(-\frac{(d/\bar{d} - 1)^2}{2\gamma_d^2}\right) \]  \quad (10)

In this case, the resulting spectra is expressed as

\[ \bar{\sigma}(\omega) = \int \sigma(\omega) x^3 P(x) dx / \int x^3 P(x) dx \]  \quad (11)

Here, we have taken into account different number of atoms in the particles with different sizes.

4. Calculating the Mössbauer spectra in a magnetic field

Recently we have developed a model for describing nanoparticle’s magnetic dynamics in the external magnetic field \( \mathbf{H} \) [7], when the particle’s energy is given by the expression:

\[ E(\theta, \phi, \Theta) = -KV \cos^2 \theta - HMV , \]  \quad (12)

where \( \theta \) and \( \phi \) are the polar and azimuth angles in respect with the particle’s easy axes, \( \Theta \) is the angle between the directions of this axes and the external magnetic field. For the stationary states of the particle we have choosen the precession orbits \( C_E \) of the magnetization vector’s end in the effective field \( \mathbf{H}_{\text{eff}}(\theta, \phi, \Theta) = -\nabla E(\theta, \phi, \Theta) / M_0 \) (figure 4). These orbits correspond with the fixed values \( E \equiv E_i \) of the particles energy and can be caracterized by the mean values of magnetization \( \mathbf{M}_{\Theta, E} \equiv \mathbf{M}(\Theta, E) \), which can be calculated as the curve integrals along the trajectories \( C_E \):

\[ \mathbf{M}_{\Theta, E} = \int \frac{M_{z,x} dm_z}{c_x \sqrt{1 - m_x^2 - m_z^2}} / \int \frac{dm_z}{c_x \sqrt{1 - m_x^2 - m_z^2}} , \quad \mathbf{M}_{\Theta, E} = 0 , \]  \quad (13)

\[ m_{z,c} \equiv M_{z,c}(E, \Theta)/M_0 . \]  \quad (14)

**Figure 4.** Precession orbits of the magnetization vector’s end (constant energy levels) for a single-domain particle with the axial magnetic anisotropy in a magnetic field \( \mathbf{H} \) with different orientations.
The Mössbauer spectra of nanoparticles in this case can be described within a similar stochastic approach with operators of more general type [1,7]:

$$\sigma(\omega, \Theta) = -\frac{\Gamma_0^2}{4} \Im \sum_{\eta} \text{Sp} \left( \hat{V}_\eta \langle \hat{W} | \hat{A}^{-1}(\omega, \Theta) | \mathbf{1} \rangle \hat{V}_\eta^* \right).$$  \tag{15}$$

Here, \( \hat{V}_\eta \) is the operator for the interaction of the gamma-quantum with a given polarization \( \eta \) and the nucleus, \( \langle \hat{W} | = \langle W | \otimes \mathbf{1}_\eta , | 1 \rangle = | 1 \rangle \otimes \mathbf{1}_\eta \), where \( \mathbf{1}_\eta \) is the identity operator in the space of \((2I_g+1)(2I_e+1)\) nuclear variables \((m_e, m_g)\), where \( I_{g,e} \) are the nuclear spins for the ground and excited states.

The superoperator

$$\hat{A}(\omega, \Theta) = \omega + i\Gamma_0 / 2 - \hat{L}_{\text{hf}}(\Theta) + i\hat{\mathbf{P}}(\Theta)$$  \tag{16}$$
is defined by the Liouville operator of hyperfine interaction that is diagonal over the stochastic states:

$$\langle i | \hat{L}_{\text{hf}}(\Theta) | j \rangle = \hat{L}_{\mu,i}(\hat{M}_i(\Theta)) \delta_{ij}$$  \tag{17}$$

and the relaxation matrix

$$\hat{\mathbf{P}} = \hat{P} \otimes \mathbf{1}_\eta ,$$  \tag{18}$$
$$\hat{P}_{ii} = -\sum_{j \neq i} \hat{P}_{ij} .$$  \tag{19}$$

[1] Here, the superoperator \( \hat{L}_{\mu,i}(\hat{M}_i(\Theta)) \) is determined by the matrix elements of the Hamiltonians of hyperfine interaction for the ground and excited states:

$$\left( \hat{L}_{\mu,i} \right)_{m_e, m'_e; m_g, m'_g} = \hat{H}_{m_e, m'_e}^{(e)} \delta_{m_g, m'_g} - \hat{H}_{m_g, m'_g}^{(e)} \delta_{m_e, m'_e} ,$$  \tag{20}$$
$$\hat{H}_{\mu,i}^{(e)}(\Theta) = -g_{\mu,i} \varepsilon N \hat{I}_{\text{hf}}(\hat{M}_i(\Theta)) / M_0 ,$$  \tag{21}$$

where \( H_{\text{hf}}^{(0)} \) is the hyperfine field at extremely low temperature.

The resulting spectrum is defined by averaging the partial spectra over the chaotic orientations of the anisotropy axes:

$$\sigma(\omega) = \int \sigma(\omega, \Theta) \sin \Theta d\Theta .$$  \tag{22}$$

5. Slow diffusion limit

When the external field increases the particle’s energy levels move apart and the stochastic transitions between them become less. So, in a field strong enough one can neglect the stochastic averaging in the basis expression \( 15 \). As a result, the absorption spectrum is determined by the Gibbs distribution of effective hyperfine field over the equilibrium population of stochastic states [6]:

$$\sigma(\omega, \Theta) = \sum_i L_i(\omega, \Theta) W_i(\Theta) \tag{23}$$

where
$$L_{\tau}(\omega, \Theta) = \frac{\sigma_{\tau} \Gamma_{\tau}^2}{4} \sum_{\alpha} \left\{ \frac{C_{\alpha} \int F_{\alpha}^{(i)}(\Theta) \, d \Theta}{(\omega - \omega_{\alpha}^{(i)}(\Theta))^2 + \Gamma_\alpha^2 / 4} \right\},$$  \hspace{1cm} (24)$$

$$\omega_{\alpha}^{(i)}(\Theta) = \left( m_{\alpha} \omega_{\delta} - m_{\delta} \omega_{\alpha} \right) \bar{M}_i(\Theta) / M_0.$$  \hspace{1cm} (25)

Here, $F_{\alpha}^{(i)}(\Theta)$ are the polar-angular distributions of the radiation intensity, averaged by the azimuth angle and the polarization. This intensities for unpolarized radiation have the form:

$$F_{m_{\gamma}^{(i)}, m_{\delta}^{(i)}}(\Theta) = \frac{3}{2} \left( 1 - \beta_i(\Theta) \right), \quad F_{m_{\gamma}^{(i)} \pm 1, m_{\delta}^{(i)}}(\Theta) = \frac{3}{4} \left( 1 + \beta_i(\Theta) \right),$$  \hspace{1cm} (26)

$$\beta_i(\Theta) = \cos^2 \theta_j \cos^2 \bar{\Omega}_i(\Theta) + \sin^2 \theta_j \sin^2 \bar{\Omega}_i(\Theta) / 2,$$  \hspace{1cm} (27)

where $\theta_j$ is the angle between the directions of gamma-ray beam and applied magnetic field, $\bar{\Omega}_i(\Theta)$ is the angle between the field direction and the averaged magnetization $\bar{M}_i(\Theta)$ for the $i$-th stationary state.

Note, that the consideration described above can be easily used to calculate the average magnetization of the ensemble of magnetic nanoparticles in a field [7]:

$$M(H) = \int \sin \Theta d\Theta \sum_i W_i(\Theta) \bar{M}_i(\Theta) / H.$$  \hspace{1cm} (28)

6. Results of data treating

Using the worded formalism we have managed a least-squares fitting procedure to analyze simultaneously the whole set of experimental data for initial nanoparticles including the Mössbauer spectra measured at different temperatures and in a magnetic field as well as the magnetization curve on the basis of the same multi-level relaxation model. The results of the self-consistent fitting are shown in figure 1 (left panel) and figure 2 by solid lines. This allows us to reliably characterize the sample with initial nanoparticles with the following estimates (in brackets we point errors in the least significant digit of the parameters values, by ‘and’ we divide values for $T = 78$ K and 300 K): the magnetic characteristic, (i) the mean magnetic anisotropy energy $K\bar{V} / k_B = 350(40)$ K, the average particle diameter $\bar{d} = 10.6(1)$ nm, the relative Gaussian width of particle’s size distribution $\sigma_d / \bar{d} = 0.21(3)$, the critical field of magnetization reversal $H_C = 1.62(8)$ kOe, $D$ (mm/s) = 0.47(7) and 0.95(5), and the Mössbauer characteristics, (ii) the $^{57}$Fe concentration $n_{NP} \cdot 10^{18} = 6.3(4)$ sm$^{-3}$, the isomer shift $\delta$ (mm/s) = 0.475(3) and 0.335(5), $H_{hf}$ (kOe) = 524(1) and 491.5(8).

A similar fitting procedure has been applied to the gamma-resonant spectra of the samples of mouse liver at different stages of biodegradation (figure 1, right panel). The only difference is a necessity to include additional variable parameters of a quadrupolar doublet, indicating the contribution of ferritin-like specimens: the partial area, the isomer shift $\delta_i$, and the quadrupolar splitting $2q_i$. The following principal parameters of the sample of mouse liver two days after nanoparticles injection have been evaluated: nanoparticles characteristics, (i)
\( \frac{K \vec{V}}{k_b} = 50(20) \text{ K}, \ \sigma_{\text{f}} / \bar{a} = 0.7(1), \ H_C = 1.17(3) \text{ kOe}, \) and the ‘ferritin’ characteristics (ii) \( \delta_f \) (mm/s) = 0.37(2) and 0.47(4), \( 2q \) (mm/s) = 0.54(3) and 0.46(7).

In particular, such an analysis allowed us to quantitatively characterize biodegradation and biotransformation of the initial particles in ferritin-like forms in a mouse organism, what is represented in figure 5 by the graph of time-evolution of \(^{57}\text{Fe}\) concentrations in nanoparticles and iron-containing proteins.

![Figure 5. Time-evolution of the \(^{57}\text{Fe}\)-concentrations in nanoparticles and ferritin-like proteins.](image)

### 7. Summary

Thus, a powerful technique for describing biotransformation and metabolism of magnetic fine particles in a living organism is developed and realized on the base of self-consistent treatment of a minimal set of experimental data, which include three Mössbauer spectra measured at different temperatures and in a magnetic field as well as the magnetization curve.

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