First study on iron complexes in blood and organ samples from thalassaemic and normal laboratory mice using Mössbauer spectroscopy

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Abstract Measurements of iron complexes and iron stores in the body are crucial for evaluation and management of chelation therapy targeted against iron accumulation or overload in blood and organs. In this work, blood and tissue samples from one normal and one thalassaemic laboratory mouse were studied using $^{57}$Fe Mössbauer spectroscopy at 78 K for the first time. In contrast to human patients, these laboratory mice did not receive any medical treatment, thus the iron components present in the samples are not altered from their natural state. The Mössbauer spectra of blood, liver and spleen samples of the thalassaemic mouse were found to differ in shape and iron content compared with corresponding spectra of the normal mouse. These results demonstrate a basis for further exploitation of the thalassaemic mouse model to study thalassaemia and its treatment in more detail using Mössbauer spectroscopy.

Keywords Mössbauer spectroscopy · Thalassaemia · Mice · Blood · Liver · Spleen

Introduction

Beta-thalassaemia (Mediterranean anaemia) is a form of inherited autosomal recessive blood disorder characterised by reduced or absent β-globin chain synthesis. This anomaly prevents production of normal levels of adult haemoglobin and leads to generation of unstable α-globin chain aggregates, which in turn limit effective production of red blood cells (RBCs), leading to mild or severe anaemia.

Haemoglobin is a highly specialised metalloprotein which is responsible for oxygen transportation from the lungs to tissues. It is found in abundance in RBCs. Each RBC contains about 300 million molecules of haemoglobin, each formed by two pairs of identical subunits, the globin chains (Cappellini et al. 2014). Each chain consists of a protein part, and a haem group made of an organic compound called protoporphyrin and a central iron atom (Berg et al. 2002).

In healthy humans, iron that is not used by the body is attached to transferrin, an iron-binding glycoprotein found in the serum (Crichton and Charloteaux-Wauters 1987) circulating in the blood. It is also stored as ferritin and haemosiderin in liver, spleen and bone marrow (Yutaka et al. 2008). Ferritin is a spherical molecule with an 8-nm central cavity which holds 2000–4500 iron(III) oxy-hydroxide atoms (Chasteen and Harrison 1999). It is an iron storage protein which keeps iron in a soluble and non-toxic form, while haemosiderin is an insoluble form of tissue storage iron. As the iron level in the body increases beyond normal, the number of ferritin and haemosiderin molecules increases, as does the iron core size with the number of iron ions in the core.

In thalassaemia and haemochromatosis, iron can exist in forms not bound to transferrin (non-transferrin-bound iron, NTBI) or other traditional binding proteins such as...
haem, ferritin and haemosiderin (Patel and Ramavataram 2012), due to increased transferrin saturation (Yutaka et al. 2008; Eleftheriou 2003). NTBI can catalyse Fenton and Haber–Weiss chemical reactions (Patel and Ramavataram 2012; Eleftheriou 2003; Evans et al. 2008), and can therefore produce harmful hydroxyl radicals which are potentially toxic to cells (Evans et al. 2008; Brissot et al. 2012) and can cause extensive damage to body tissues (Eleftheriou 2003).

Patients with severe anaemia are heavily dependent on frequent blood transfusions to ensure oxygen transport, which leads to iron overload (Cappellini et al. 2014). Furthermore, patients with mild anaemia may also suffer from iron overload due to increased gastrointestinal absorption of iron from the diet (Berg et al. 2002), since there is no active mechanism to excrete iron from the body (Yutaka et al. 2008).

Even with the latest treatments, patients face life-threatening complications, bone deformities, poor growth, hepatosplenomegaly and cardiovascular illness which reduce life expectancy. Chelating agents, such as desferrioxamine and deferiprone, are used to remove excess iron from the body. Accurate, preferably non-invasive, measurements of iron complexes and stores in the body are crucial for evaluation and management of chelation therapy.

$^{57}\text{Fe}$ Mössbauer spectroscopy (MS) (Vertes, Korecz and Burger 1979; Frauenfelder 1963; Goldanskii and Herber 1968; Greenwood and Gibb 1971; Gutlich, Link and Trautwein 1978; Gutlich et al. 2011) can characterise these iron complexes and in particular provide information such as the iron electronic structure, magnetic structure, hyperfine interactions, valence/spin state, local microenvironment/iron stereochemistry and symmetry of environment, iron bonding, number of resonant nuclei, and dynamics. This is in contrast to chemical or clinical markers, which are mainly dedicated to detecting specific iron complexes. Accurate characterisation of iron complexes in the body may lead to improvement of iron chelating agents.

Previous Mössbauer studies characterised iron complexes in blood and organs of humans and some animals. In particular, by comparing RBCs from healthy people and patients with thalassaemia, a new component was identified in the spectra of patients, being considered to be ferritin-like iron (Abreu et al. 1989; Xuanhui et al. 1988; Jiang et al. 1994). Other studies considered differences in the haem iron electronic structure in both oxy- and deoxy-haemoglobin, in normal adult, foetal and leukaemic RBCs. The $\alpha$- and $\beta$-subunits of oxy-haemoglobin (Oshtrakh 1998; Oshtrakh et al. 2011a, b) in normal human RBC samples have been presented. When heart, spleen and liver tissues from healthy individuals and patients with thalassaemia were compared using $^{57}\text{Fe}$ MS, an increased quantity of ferritin and haemosiderin was found in the thalassaemic samples (Kaufman et al. 1980; Bell et al. 1984; Rimbert et al. 1985; Pierre et al. 1998; Chua-anusorn et al. 1994).

Small variations of $^{57}\text{Fe}$ MS parameters were observed in concentrated normal RBC samples between the oxy-haemoglobins ($\alpha$- and $\beta$-subunits) of a human, a rabbit and a pig, which are related to well-known small structural variations in haem iron stereochemistry (Oshtrakh et al. 2011a, b). Further, ferritin and haemosiderin were identified in dietary-iron-loaded and parenteral-loaded liver and spleen samples from rats (Chua-anusorn et al. 1999). It was also found that haemosiderin levels increased with rat age and hence duration of iron overload (Chua-anusorn et al. 1999). Also, the effect of the iron chelating drug desferrioxamine (Desferal) was studied, revealing that it could remove a major part of iron aggregated from iron overload in rat myocardial cells (Bauminger et al. 1987).

Due to the severe complications of thalassaemia, patients receive blood transfusions and iron chelators from a young age. These alter the various forms of iron and their concentration in the body. Therefore, characterisation of the effect of thalassaemia alone on such iron complexes is not possible using human samples. Thalassaemic mice have been used in various fields of research, since they develop iron deposits very early (Yang et al. 1995) and are therefore suitable candidates to study iron accumulation in organs using $^{57}\text{Fe}$ MS. Direct comparison between normal and thalassaemic mice may identify iron complexes arising due to thalassaemia and reveal mechanisms of iron accumulation or overload in blood and organs. This could lead to design of more appropriate binding agents and production of more efficient iron chelators, reducing the effects of iron overload in patients.

Thus, in this work, Mössbauer spectra of blood, liver and spleen samples from one normal and one thalassaemic laboratory mouse were acquired and compared to investigate whether this thalassaemic mouse model can be used to characterise iron complexes associated with thalassaemia.

### Materials and methods

#### Experimental set-up

A complete Mössbauer spectroscopy equipment provided by WissEl (WisseEl 2011) was utilised for spectral measurements in transmission geometry. A 50-mCi $^{57}\text{Co/Rh}$ source was driven at constant acceleration in triangular mode generated by a digital function generator with velocity of $\pm4\text{ mm/s}$. The 14.41-keV $\gamma$-rays of the $^{57}\text{Fe}$ nucleus were detected using a xenon (Xe)-filled proportional counter (LND 4546). The sample was placed inside a dedicated holder then inserted into a cryostat (Ice$^\text{Bath}$, ICE Oxford). The
cryostat was filled with liquid nitrogen (LN) and operated at vacuum of $7 \times 10^{-7}$ mbar. A thermocouple attached to the sample holder was used to measure the sample temperature. The temperature was held stable, within $\pm 0.5$ K, using a controller and a heater attached to the cryostat's heat exchanger.

The Mössbauer spectrum of each biological sample was acquired for about 15 days to obtain statistics of about $1 \times 10^6$ counts per channel. The spectra were registered in 1024 channels (forward and backward movements) using a triangular velocity signal. For analysis, the spectra were folded into 512 channels and the peaks fitted using Lorentzian distributions. Folding and analysis were performed using the IGOR-WinNORMOS software package from WissEl.

**Calibration**

Velocity was calibrated using the four inner peaks of a 99.85% purity $\alpha$-Fe foil (Goodfellow Cambridge Limited) with thickness of 8 μm at room temperature (RT, 298 K). The isomer shift ($\delta$) value of the $\alpha$-Fe foil relative to the $^{57}$Co/Rh source at RT was found to be $-0.110$ mm/s, consistent with the source manufacturing specification (chemical shift of source relative to $\alpha$-Fe of 0.108 mm/s) and corresponding literature (Gutlich et al. 2011).

MS is very sensitive to vibrations that might arise from vacuum pumps and/or evaporation of LN inside the cryostat, so it is crucial to ensure that the resonant absorption line widths remain stable and within acceptable values (<0.30 mm/s) at the desired working conditions. For this reason, the stability of the system against vibrations was carefully investigated by conducting measurements using a 10 mg/cm$^2$ natural-abundance Fe powder sample (<10 μm, ≥99%, Sigma-Aldrich) before and after each biological measurement.

The Mössbauer spectrum of each biological sample was measured with a controller and a heater attached to the cryostat's heat exchanger. From such measurements, the line width ($\Gamma$) for the $\alpha$-Fe foil (RT) and the iron powder sample (RT and 78 K), as well as the corresponding reference values (RT) (Maddock 1998) for expected peak positions. As can be seen, the measured positions of the four Fe absorption peaks were reproduced with accuracy of a few $\sim 10^{-3}$, while their widths were measured with resolution [full-width at half-maximum (FWHM)] of about 0.25–0.28 mm/s. Figure 1 shows the spectra, fitted simultaneously using four Lorentzian distributions, measured from the Fe powder sample at RT (a) and 78 K (b), together with the residuals of the fits. The overall instrumental error, as derived from the Fe powder sample measurements, was found to be <0.03 mm/s. In the following, isomer shift $\delta$ values and peak positions are given relative to $\alpha$-Fe foil at RT.

Finally, background measurements were carried out using an empty sample holder, with statistics of $6 \times 10^6$ counts per channel before folding. The results showed no significant MS absorption (<0.1%) due to iron traces in the set-up environment such as the sample holder or detector window.

**Sample holders**

Custom-made holders for the bio-samples were fabricated by the thermo-forming method using copper moulds and polypropylene (PP) sheets with thickness of 0.75 mm. Cylindrical holders with different diameters (10 and 7 mm) were used for blood, liver and spleen samples, to better fit the corresponding sample volume within the holder. The holder volume was 1, 0.5 and 0.5 ml for blood, liver and spleen samples, respectively. A 2-mm-thick collimator made of copper was attached to each holder.

The absence of atoms such as oxygen, nitrogen and chlorine in the polypropylene molecule $[\text{(C}_3\text{H}_6)_n]$ compared with other thermoplastics results in high transparency to low-energy X- and γ-rays. Table 2 presents the measured FWHM, the net count rate (rate), and the percentage transmission ($T$) without a holder, with an existing standard Plexiglas (PMMA) holder, and with our custom-made polypropylene (PP) holder at $^{57}$Co/Rh energies of 6.36 and 14.41 keV, respectively. The corresponding

| Sample | Line number ($i$) | 1    | 2    | 3    | 4    | 5    | 6    |
|--------|-------------------|------|------|------|------|------|------|
| Reference values (RT) | $X(i)$ [mm/s] | -5.328 | -3.083 | -0.839 | 0.839 | 3.083 | 5.328 |
| $\alpha$-Fe foil (RT) | $X(i)$ [mm/s] | -3.082 | -0.840 | 0.840 | 3.082 | -     | -     |
| Fe powder (RT) | $X(i)$ [mm/s] | -0.281 | 0.274 | 0.274 | 0.281 | -     | -     |
| Fe powder (78 K) | $X(i)$ [mm/s] | -3.045 | -0.751 | 0.973 | 3.265 | -     | -     |

The statistical errors for $X(i)$ and $\Gamma(i)$ are ±0.002 and ±0.004 mm/s, respectively. The instrumental error is <0.03 mm/s.
spectra are shown in Fig. 2. The transmission was measured using an Amptek XR-100CR Si-PIN X-ray detector due to its high energy resolution. As presented in Table 2, the X-ray at 6.36 keV could not be transmitted through the PMMA holder. Even more importantly, the transmission through our PP holder at the energy of interest of 14.41 keV was significantly enhanced (96%) compared with that of the standard PMMA holder (56%).

Table 2 Measured FWHM, net count rate (rate), and transmission (T) without a holder and with a Plexiglas (PMMA) or polypropylene (PP) holder at 57Co/Rh source energies of 6.36 and 14.41 keV (Fig. 2a–c)

| Material | $E_\gamma = 6.36$ keV | $E_\gamma = 14.41$ keV |
|----------|----------------------|----------------------|
|          | FWHM | Rate (cps) | Trans (%) | FWHM | Rate (cps) | T (%) |
| No holder | 0.155 | 124 | 100 | 0.20 | 209 | 100 |
| PMMA holder | – | – | – | 0.22 | 117 | 56 |
| PP holder | 0.153 | 96 | 77 | 0.21 | 201 | 96 |

Mouse model and sample preparation

Blood samples and wet tissues of liver and spleen samples from one normal (C57BL/6J) and one thalassaemic mouse were investigated in this study. This thalassaemic mouse model (Yang et al. 1995) has both $b_1$ and $b_2$ adult mouse globin genes deleted. Mice heterozygous for the deletion ($Hbb^{th-3}/Hbb^{wt}$) were used for the experiment, as homozygous mice die perinatally. Heterozygous $Hbb^{th-3}/Hbb^{wt}$ mice appear normal, but show haematologic indices characteristic of severe thalassaemia, exhibit tissue and organ damage typical of the disease, and show spontaneous iron overload in spleen, liver and kidneys.

The two mice were age- and sex-matched and were raised in similar conditions with the same diet provided to them. Blood samples were collected in plain Eppendorf tubes by retro-orbital bleeding of anaesthetised animals. Heparin was added to prevent coagulation. Following euthanasia of the animals, the liver and spleen were isolated and washed with phosphate-buffered saline solution to remove excess blood traces. All samples were then placed in the custom-made holders at the Department of Physics of the University of Cyprus and stored in a dewar at LN temperature.

Results and discussion

Blood

Figure 3a, b show the MS spectra at 78 K of the blood samples from the normal and thalassaemic mouse, respectively. The spectrum of the normal sample (Fig. 3a) with signal-to-noise (S/N) ratio of 23 was fitted using two quadrupole doublets, representing the $\alpha$ and $\beta$-chains of oxyhaemoglobin, yielding a normalised $\chi^2$ value of 0.93 for the quality of the fit. The parameters extracted for the two quadrupole doublets were then used to fit the spectrum of the thalassaemic sample (Fig. 3b), allowing only the area of the doublets to vary. In this case, a higher $\chi^2$ value of 1.22 was calculated, which along with the corresponding fit residuals shown, indicates a non-satisfactory fit. This could be due to more iron-containing complexes present in this sample. Because of the low S/N ratio (~10) of this spectrum (Fig. 3b), further, more detailed analysis is not useful.
A decreased absorption effect is observed in the spectrum (Fig. 3b) of the thalassaemic sample due to reduced haemoglobin level. This indicates extreme anaemia, as expected in a thalassaemia major patient without any treatment (Eleftheriou 2003).

Table 3 presents the Mössbauer parameters for the fit in Fig. 3a. The values of these parameters are similar to those measured in other studies for normal RBC samples of a human, a rabbit and a pig (Oshtrakh 1998). Also, the relative area of both the α- and β-subunits is comparable in amount, in agreement with the equal distribution of iron nuclei in each type of subunit of the haemoglobin tetramer (Oshtrakh 1998).
A ferritin component was identified in a previous MS study, at about 80 K, from RBC samples of patients with β-thalassaemia intermedia (Bauminger et al. 1979), where the ratio of ferritin to haemoglobin iron varied from 3 to 50% (Bauminger et al. 1979). Due to the fact that thalassaemia patients undergo long-term medical treatment, a ferritin component could not be observed in the MS spectra from RBC samples of patients with thalassaemia major (Jiang et al. 1994). However, a ferritin-like component can appear in the MS spectrum of a blood sample from a β-thalassaemic mouse, since it has not undergone medical treatment like thalassaemic patients. Due to the low S/N ratio of the corresponding spectrum from the thalassaemic mouse, such a ferritin-like component was not observed in this work, but it will be possible in future samples from laboratory mice fed a diet enriched in $^{57}$Fe.

Liver and spleen

Figure 4a and b show the MS spectra at 78 K of the liver samples from the normal and thalassaemic mouse, respectively. The spectrum of the thalassaemic sample (Fig. 4b) with S/N ratio of 16 was fitted using a single doublet ($\chi^2 = 0.99$), while the spectrum of the normal sample (Fig. 4b) with S/N ratio of 5 was not fitted. The MS spectrum of the normal sample presents lower absorption than the thalassaemic sample due to the lower iron concentration. Note that the thalassaemic mouse did not receive any transfusion or iron chelation treatment.

Table 3 presents the Mössbauer fitting parameters for the thalassaemic sample. They are in agreement with those for ferritin-like characteristics, as obtained, at the same temperature, from dietary-iron-loaded and parenteral-loaded liver samples of rats (Chua-anusorn et al. 1999). In the same study (Chua-anusorn et al. 1999), a small sextet component was observed in the spectra of almost half the liver samples. Even though the sextet signal-to-noise ratio was low, its parameters were in fair agreement with the corresponding ones obtained from a human thalassaemic liver sample (Pierre et al. 1998). According to Bell et al. (1984), at 80 K, the haemosiderin magnetic transition can be observed. Rimbert et al. (1985) observed such a sextet component at 80 K only in the spectra of haemodesiderosis liver samples from regularly transfused thalassaemic patients but not in the spectra of normal human liver samples or of iron-overload rat samples from excessive intestinal iron absorption. We also measured a spectra in the velocity range of ±8 mm/s but could not observe such

| Sample          | Sub-spectrum | $\Gamma$ (mm/s) | $\delta$ (mm/s) | $\Delta E_Q$ (mm/s) | Area (%) |
|-----------------|--------------|-----------------|-----------------|---------------------|----------|
| Normal blood    | A            | 0.24 ± 0.03     | 0.28 ± 0.01     | 2.23 ± 0.02         | 51       |
|                 | B            | 0.40 ± 0.03     | 0.28 ± 0.01     | 1.90 ± 0.07         | 49       |
| Thalas. liver   |              | 0.63 ± 0.02     | 0.46 ± 0.01     | 0.71 ± 0.01         | 100      |
| Normal spleen   |              | 0.58 ± 0.01     | 0.47 ± 0.01     | 0.69 ± 0.01         | 100      |
| Thalas. spleen  | A: ferritin-like | 0.56 ± 0.01     | 0.47 ± 0.01     | 0.69 ± 0.01         | 97       |
|                 | B: deoxy-Hb  | 0.24 ± 0.06     | 0.86 ± 0.02     | 2.27 ± 0.03         | 3        |

The instrumental error is <0.03 mm/s. The statistical error is also shown.

![Fig. 4 Mössbauer spectra of liver samples from a one normal and b one thalassaemic mouse measured at 78 K and plotted on the same scale. The thalassaemic sample is fitted using a doublet. The residuals of the fit are also shown](image-url)
a sextet component. This may be due to the smaller iron core in mice in comparison with those in the above-cited literature.

Figure 5a and b show the MS spectra at 78 K of the spleen samples from the normal and thalassaemic mouse, respectively. The spectrum of the normal sample (Fig. 5a, S/N = 27) was fitted using a single symmetrical doublet ($\chi^2 = 1.04$). Its Mössbauer parameters, given in Table 3, are in agreement with those of a ferritin component observed in the corresponding MS spectra, at the same temperature, of human spleen samples (Chua-anusorn et al. 1994). Also, Chua-anusorn et al. (1994) did not observe any sextet component in any of 24 normal human samples.

The corresponding spectrum of the spleen sample for the thalassaemic mouse (Fig. 5b, S/N = 39) was fitted using two sub-doublets ($\chi^2 = 1.01$): a large component (97%) with a slightly asymmetric doublet of a ferritin-like component and another small component (3%) of deoxy-haemoglobin, probably due to an amount of blood remaining within the spleen. Table 3 presents the Mössbauer parameters for the fit. The extracted values are in agreement with those reported from dietary-iron-loaded and parenteral-loaded spleen samples of rats (Chua-anusorn et al. 1999) and also from thalassaemic human spleen samples (Chua-anusorn et al. 1994). A sextet component was observed by Pierre et al. (1998) but not by Chua-anusorn et al. (1999). We also did not observe such a sextet component after measuring our spectrum in the velocity range of ±8 mm/s. This might be due to a smaller iron core in mice and/or a lower blocking temperature for this sextet component. Both spectra of the thalassaemic liver and spleen samples show increased ferritin-like iron relative to the normal ones, because thalassaemic tissues exhibit increased iron deposition due to iron overload.

**Conclusions**

Mössbauer spectra of blood, liver and spleen samples from a normal and a thalassaemic laboratory mouse were acquired at 78 K.

The MS spectrum of the normal blood sample was well fitted using two sub-doublets with the same area, representing the $\alpha$- and $\beta$-subunits of oxy-haemoglobin. The decreased absorption effect observed in the spectrum of the thalassaemic sample is due to the reduced haemoglobin in the sample, which indicates extreme anaemia, as expected in thalassaemia major patients without any treatment (Eleftheriou 2003).

An increased amount of ferritin-like iron was observed in the thalassaemic liver and spleen samples compared with the normal ones, as expected since thalassaemic tissues exhibit increased iron deposition due to iron overload. However, the absorption effect in these samples should be treated with some caution, since the sample population consisted of only one mouse in each group.

The Mössbauer fitting parameters obtained in this work are in good agreement with those reported in literature. Based on our results, normal and thalassaemic mice exhibit iron complexes similar to humans, in both blood and organs. In this sense, our thalassaemic mouse model represents a promising candidate to study thalassaemia using MS.

Future studies will include enrichment of mice with $^{57}$Fe through their diet to increase the absorption in the MS spectra, thus enabling characterisation of iron complexes. $^{57}$Fe-enriched blood and organ samples of normal and thalassaemic mice can then be compared at various ages to investigate the rate of iron accumulation.
Direct comparison of normal and thalassaemic mice samples may reveal iron complexes due to thalassaemia which are not affected by blood transfusion and iron chelators. This would provide deeper insight into the iron complexes associated with thalassaemia, also potentially aiding design of more appropriate iron binding agents.

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Compliance with ethical standards

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

References

Abreu M, Sanchis M, Penalver J, Kanter F (1989) Application of Mössbauer spectroscopy to the study of hemoglobinopathies. Preliminary experience. Sangre 34:325
Bauminger E, Cohen S, Ofer S, Rachmilewitz E (1979) Quantitative studies of ferritin-like iron in erythrocytes of thalassaemia, sickle-cell anemia, and hemoglobin Hammersmith with Mössbauer spectroscopy. Proc Natl Acad Sci USA 76(2):939–943
Bauminger E, Lancou T, Link G, Pinson A, Hershko C (1987) Iron overload in cultured rat myocardial cells. Hyperfine Interact 33:249
Bell S, Weir M, Dickson D, Gibson J, Sharp G, Peters T (1984) Mössbauer spectroscopic studies of human haemosiderin and ferritin. Biochim Biophys Acta 787:227–236
Berg J, Tynmoczko J, Stryer L (2002) Biochemistry, 5th edn. W.H. Freeman, New York. ISBN-13:978-0716746843
Brissot P, Ropert M, Le Lan C, Loréal O (2012) Non-transferrin bound iron: a key role in iron overload and iron toxicity. Biochim Biophys Acta 1820(3):403–410
Cappellini M, Cohen A, Porter J, Taher A, Viprakasit V (2014) Guidelines for the management of transfusion dependent Thalassaemia, 3rd edn. Thalassaemia International Federation Publications, Nicosia, Cyprus. ISBN:978-9963-717-06-4
Chasteen N, Harrison P (1999) Mineralization in ferritin: an efficient means of iron storage. J Struct Biol 126:182–194
Chua-anusorn W, Webb J, Macey D, Yansukon P, Pootrakul P (1994) Mössbauer spectroscopic study of the forms of iron in normal human liver and spleen tissue. Hyperfine Interact 91:905–910
Chua-anusorn W, Webb J, Macey D, de la Motte Hall P, Pierre T (1999) The effect of prolonged iron loading on the chemical form of iron oxide deposits in rat liver and spleen. Biochem Biophys Acta 1454:191–200
Crichton R, Charleotaux-Wouters M (1987) Iron transport and storage. Eur J Biochem 164(3):485–506
Eleftheriou A (2003) About Thalassaemia, Thalassaemia International Federation Publications, Nicosia, Cyprus. ISBN:9963-623-40-9
Evans R, Rafique R, Zarea A, Rapisarda C, Cammack R, Evans P, Porter J, Hider R (2008) Nature of non-transferrin-bound iron: studies on iron citrate complexes and thalassemic sera. J Biol Inorg Chem 13:57–74
Frauenfelder H (1963) The Mössbauer effect. Benjamin, New York
Goldanskii V, Herber R (1968) Chemical applications of Mössbauer spectroscopy. Academic, New York
Greenwood N, Gibb T (1971) Mössbauer spectroscopy. Chapman and Hall, London
Gutlich P, Link R, Trautwein A (1978) Mössbauer spectroscopy and transition metal chemistry. Springer, Berlin
Gutlich P, Bill E, Trautwein A (2011) Mössbauer spectroscopy and transition metal chemistry. Fundamentals and Applications. Springer, Berlin
Jiang K, Ma W, Ortailli I, Pedrazzi G, Zhang X, Izzi G (1994) Some comments on the effectiveness of the therapy for β-thalassemia. Hyperfine Interact 91:859–863
Kauffman K, Papaethymiou G, Frankel R, Rosenthal A (1980) Nature of iron deposits on the cardiac walls in β-thalassemia by Mössbauer spectroscopy. Biochem Biophys Acta 629:522
Maddock A (1998) Mössbauer spectroscopy principles and applications of the techniques. Horwood Chemical Science Series, ISBN 10:1898563160
Oshtrakh M (1998) The features of Mössbauer spectra of hemoglobin in relation to the quadrupole splitting and heme iron stereochemistry. Z Naturforsch 53a:608–614
Oshtrakh M, Kumar A, Kundu S, Berkovsky A, Semionkin V (2011a) Study of human, rabbit and pig oxyhemoglobinins using high velocity resolution Mössbauer spectroscopy in relation to their structural and functional variations. J Mol Struct 993:292–296
Oshtrakh M, Berkovsky A, Kumar A, Kundu S, Vinogradov A, Konstantinova T, Semionkin V (2011b) Heme iron state in various oxyhemoglobinins probed using Mössbauer spectroscopy with a high velocity resolution. Biomol 24:501–512
Patel M, Ramavataram D (2012) Non transferrin bound iron: nature, manifestations and analytical approaches for estimation. Ind J Clin Biochem 27(4):322–332
Pierre T, Chua-anusorn W, Webb J, Macey D, Pootrakul P (1998) The form of iron oxide deposits in thalassemic tissues varies between different groups of patients: a comparison between Thai β-thalassemia/hemoglobin E patients and Australian β-thalassemia patients. Biochem Biophys Acta 1407:51–60
Rimbert J, Dumas F, Kellershohn F, Girot R, Brissot P (1985) Mössbauer spectroscopy study of iron overloadeed livers. Biochimie 67:663–668
Vertes A, Korecz L, Burger K (1979) Mössbauer spectroscopy. Academia Kiada, Budapest
WisssEl—Wissenschaftliche Elektronik GmbH (2011) http://www.wissel-instruments.de. Accessed 23 Nov 2016
Xuanhui G, Nanming Z, Xiufang Z, Naifei G, Youwen H, Rongxin Yutaka K, Ohtake K, Torimoto Y, Kato J (2008) Body iron metabolism and pathophysiology of iron overload. Int J Hematol 89(1):7–15