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Identification of Iron in Oral Iron Pharmaceuticals: Mössbauer Spectroscopy Study

M. Kądziołka-Gaweł[a,*] and M. Wojtyniak[b]

[a] *University of Silesia in Katowice, Institute of Physics, 75 Pułku Piechoty 1, 41-500 Chorzów, Poland*
[b] *Silesian University of Technology, Institute of Physics, Konarskiego 22B, 44-100 Gliwice, Poland*

Doi: 10.12693/APhysPolA.146.222 *e-mail: mariola.kadziolka-gawel@us.edu.pl

Iron deficiency is one of the best-known forms of nutritional disorders. The ferric and ferrous compounds are usually used in its treatment: ferric pyrophosphate, ferrous fumarate, and ferrous gluconate. They are administered orally and are relatively well tolerated. Selected iron oral pharmaceutical products were studied using ^{57}Fe Mössbauer spectroscopy and X-ray fluorescence method. Using X-ray fluorescence, we can determine the content of elements in the tested products, particularly Fe. Mössbauer spectroscopy allows us to determine the oxidation state of Fe and the relative content of these ions in the investigated oral iron pharmaceuticals. The X-ray fluorescence results show that most investigated products contained much higher Fe values than those declared in the product leaflets. The results of ^{57}Fe Mössbauer spectroscopy research show that ferric pyrophosphate, ferrous fumarate, and ferrous gluconate are the main Fe-containing compounds in the investigated products.

topics: ^{57}Fe Mössbauer spectroscopy, X-ray fluorescence (XRF) method, iron, pharmaceutical products

1. Introduction

Iron plays a significant role in the living systems. Iron is a biochemically active element of such cofactors as heme, centers iron-sulfur, 1- or 2-atomic iron centers, which in turn decide the activity and functions of many proteins necessary for the proper operation of critical biological processes. Iron deficiency is one of the best-known nutritional disorders [1]. It occurs when there is a negative balance between iron absorption and iron requirements and losses. Iron deficiency is caused not only by iron-deficient diets but also by the low iron bioavailability of the diet. Iron is a component of many food products, so with a bit of willingness, there is no problem in providing the body with the right amount of it. The most iron is found in meat, cereal products, vegetables, and fruit. Pregnant women, infants, young children, and adolescents have higher iron requirements and thus have a greater risk of developing its deficiency [2]. Iron deficiency causes anemia and other pathological changes in the body. The usual treatment for these anemias is the administration of oral hematinics, the common ones being ferrous fumarate, gluconate, and sulfate [3]. These are effective and usually relatively well tolerated, except for some gastrointestinal side effects, including constipation, diarrhea, and epigastric pain [3–5]. Therefore, knowledge of the iron valence state is fundamental because it may be related to the effect and toxicity of pharmaceutical products.

The most sensitive technique for the analysis of the iron state is ^{57}Fe Mössbauer spectroscopy (nuclear resonance technique). This technique is very sensitive to the local atomic structure, its local deformation, and atomic or lattice defects when treating the Fe nucleus as a probe of its local surroundings [6]. Based on the results of this method, we can obtain information on, for example, iron oxidation states, the local iron microenvironment, and the relative shares of iron-containing components. This technique has already been used to identify iron and its state in various pharmaceutical products [3, 7–15]. However, more and more Fe pharmaceuticals are available on the market, and they can be easily purchased without a prescription from a doctor. Moreover, the choice of a product is often determined by its price. For this reason, knowledge about the content of individual elements, particularly iron, and its form in such products is essential for our health. Therefore, the study presents research on selected oral iron pharmaceuticals using Mössbauer spectroscopy and X-ray fluorescence.

2. Materials and methods

The following commercially available oral iron pharmaceuticals were selected for research: Ascofer® (ESPEFA, Poland) and Floradix® (SALUS Haus, Germany) containing ferrous gluconate $C_{12}H_{24}FeO_{14}$, Actiferol Fe® (POLSKI LEK, Poland) and Liposomal Iron (Dr. Jacob's Medical GmbH, Germany) containing ferric pyrophosphate $Fe_4(P_2O_7)_3$, GymBeam Iron (GymBeam, Germany), Biorythm^{TM} Fe (Stada Pharm, Poland), and BICAPS® ferr C (Formeds, Poland) containing ferrous fumarate $C_4H_2FeO_4$. According to the product leaflets, the investigated pharmaceuticals contained the following amounts of Fe in daily dose: Ascofer® — 23.2 mg, Floradix® — 14 mg, Actiferol Fe® — 30 mg, Liposomal Iron — 8 mg, GymBeam Iron — 14 mg, Biorythm^{TM} Fe — 28 mg, BICAPS® ferr C — 28 mg. Approximately 10% of the product packaging content was tested, equivalent to three tablets or three daily doses.

The chemical composition of all samples was determined by X-ray fluorescence (XRF) with a ZSX Primus II Rigaku spectrometer. The spectrometer, equipped with the 4 kW, 60 kV Rh anode and wavelength dispersion detection system, allowed for the analysis of the elements from Be to U. No external standards were necessary. Only the internal standards coupled with the fundamental parameters were implemented. The samples for the analysis were prepared in the form of pressed tablets. According to the manufacturer, the global uncertainty in determining the content of individual elements is less than 1 %. Other studies [16, 17] also show that such uncertainty for major elements is standard for this measurement technique, regardless of the spectrometer model. Of course, the uncertainties may be up to 15% for trace elements [16].

TABLE I

Content of elements in the investigated oral pharmaceutical products. The relative uncertainty of the content of individual elements is less than 1%.

Element concentrations [wt.%]								
C	N	O	Mg	Si	P	Fe	K	Sum
Actiferol Fe®								
34.3	0.0	62.3	0.0	0.0	0.9	2.1	0.0	99.6
Liposimal Iron								
36.6	0.0	54.6	0.2	1.8	2.6	2.5	0.8	99.1
Biorythm^{TM} Fe								
30.7	0.0	59.5	0.4	1.7	0.0	7.0	0.0	99.3
BICAPS® ferr C								
30.4	0.0	61.6	0.0	0.0	0.0	7.9	0.0	99.9
GymBeam Iron								
39.5	0.0	57.8	0.5	0.0	0.0	2.0	0.0	99.3
Floradix®								
30.0	6.0	53.4	0.4	2.7	0.8	2.4	2.6	98.3
Ascofer®								
23.0	0.0	58.6	2.2	3.1	0.0	12.6	0.0	99.5

The ^{57}Fe Mössbauer transmission spectra were recorded at room temperature using an Integrated Mössbauer Spectroscopy Measurement System (designed by Wacław Musiał and Jacek Marzec) and a linear arrangement of RITVERC source ^{57}Co:Rh, a multichannel analyzer, an absorber, and a detector. A gas proportional counter LND-45431 was used as a gamma-ray detector. The escape peak of 2 keV and 14.4 keV gamma-ray pulses were selected with a multichannel analyzer. The spectrometer velocity scale was calibrated at room temperature with a 25 μm thick α-Fe foil. All Mössbauer measurements were carried out on powdered samples. The Mössbauer spectra were evaluated by least-square fitting of the lines using the MossWinn 4.0i program.

3. Results and discussion

Table I shows the content of elements in the investigated oral pharmaceutical products determined based on measurements using X-ray fluorescence analysis (XRF). This table compares elements with content greater than 1 wt.% in at least one sample. Of course, the analyzed samples contained elements like Na, S, Cl, Ca, Ti, Mn, or Zn, but their total concentration was much smaller than 1% of the total weight (Table I). Only Floradix® contained larger amounts of Na, S, and Cl, and each of them had approximately 0.5 wt.% content. The large amounts of O and C are mainly related to the content of ferric pyrophosphate, ferrous gluconate, and ferrous fumarate in tested oral pharmaceutical products, but also to vitamin C ($C_6H_8O_6$), with which supplements such as Floradix®, Liposomal Iron, Biorythm^{TM} Fe, and BICAPS® ferr C are additionally enriched. The high amount of B vitamins (including B_1, B_2, B_6, and B_{12}) results in high content of nitrogen in Floradix®.

Taking into account the mass of the pill or daily portion of the investigated supplement and the percentage of iron determined from XRF measurements, the weight of iron in the pill was calculated. Thus, the investigated pharmaceuticals contained the following amounts of Fe given in daily doses: Ascofer® — 32 mg, Floradix® — 22 mg, Actiferol Fe® — 32 mg, Liposomal Iron — 13 mg, GymBeam Iron — 10 mg, Biorythm^{TM} Fe – 34 mg, BICAPS® ferr C – 36 mg. Comparing the obtained Fe weight contents with the values declared by the manufacturer, it can be noticed that only Actiferol Fe® and Biorythm^{TM} Fe contain weights close to the declared ones. The Fe content in GymBeam Iron and Liposomal Iron is lower than declared in the product leaflet, and in the

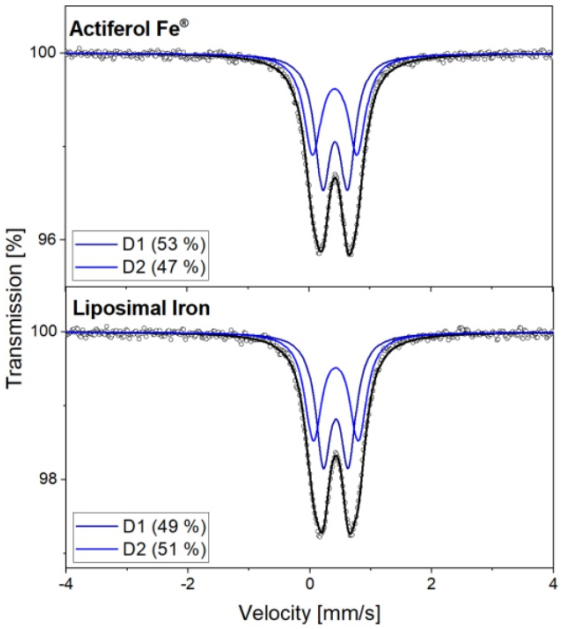

Fig. 1. ^{57}Fe Mössbauer spectra of the Actiferol Fe® and Liposomal Iron containing ferric pyrophosphate. Fitted subspectra (colored lines) and their contributions are shown in each spectrum.

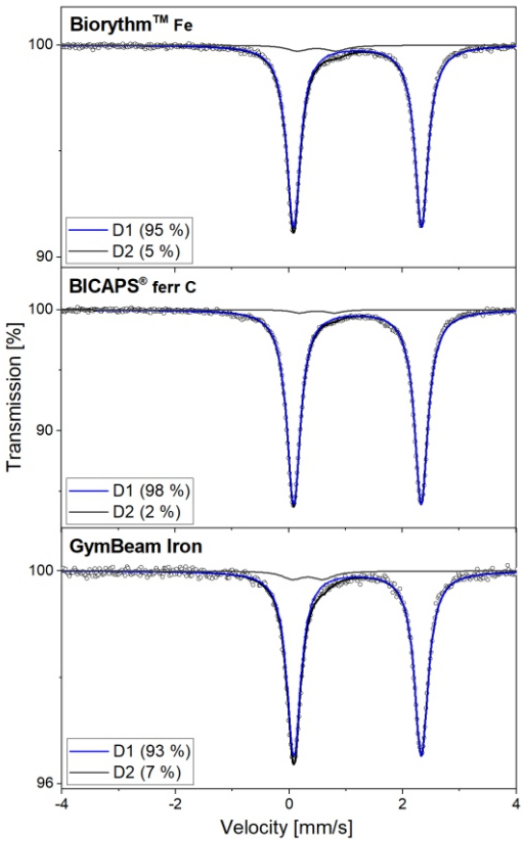

Fig. 2. ^{57}Fe Mössbauer spectra of the Biorythm^{TM} Fe, BICAPS® ferr C, and GymBeam Iron containing ferrous fumarate. Fitted subspectra (colored lines) and their contributions are shown in each spectrum.

remaining ones — much higher; what is more, the Fe content significantly exceeds its reference intake value.

Mössbauer spectra, fitted components, and their contributions to the investigated oral pharmaceutical products are presented in Figs. 1, 2, and 3 for products containing ferric pyrophosphate, ferrous fumarate, and ferrous gluconate, respectively. The spectra obtained for tablets of the same pharmaceutical were similar, so only one spectrum was selected for presentation. The hyperfine parameters of all matched components are summarized in Figs. 4, 5, and 6 for supplements containing ferric pyrophosphate, ferrous fumarate, and ferrous gluconate, respectively.

^{57}Fe Mössbauer spectra of the Actiferol Fe® and Liposomal Iron containing ferric pyrophosphate were fitted with two ferric doublets, D1 and D2 (Fig. 1). The concentration in the spectrum of the first doublet ranged from 48% to 53%; the rest was the second doublet. The isomer shift values of both doublets are almost identical, and the average is equal to 0.425(1) mm/s. However, the doublets differ in quadrupole splitting values; the average value for D1 is 0.408(4) mm/s, and for D2, 0.727(5) mm/s (Fig. 4). The obtained hyperfine parameters for these doublets are consistent with those presented in the literature [13] and indicate two octahedral ferric iron sites in ferric pyrophosphate $Fe_4(P_2O_7)_3$. These two sites have the same coordination but with different symmetry of the site, one with a higher symmetry and the other with a lower symmetry or a higher distortion [13].

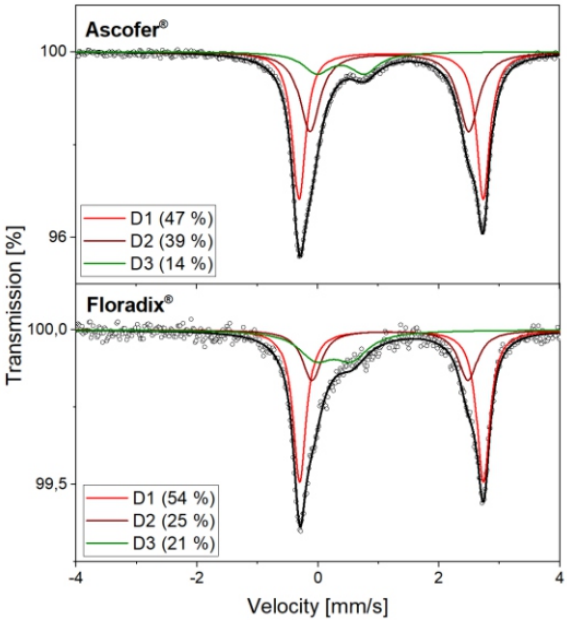

Fig. 3. ^{57}Fe Mössbauer spectra of the Ascofer® and Floradix® containing ferrous gluconate. Fitted subspectra (colored lines) and their contributions are shown in each spectrum.

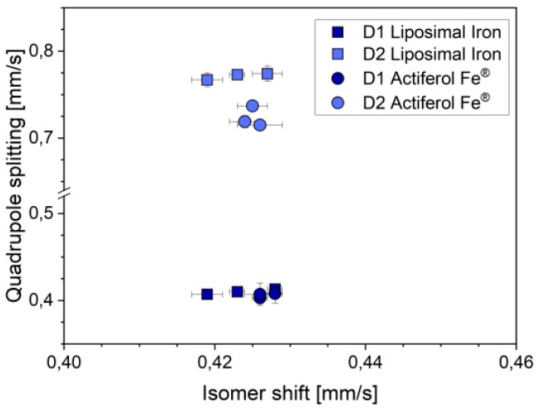

Fig. 4. Relationship between the hyperfine parameters of the fitted components (D1 and D2) for the Actiferol Fe® and Liposomal Iron.

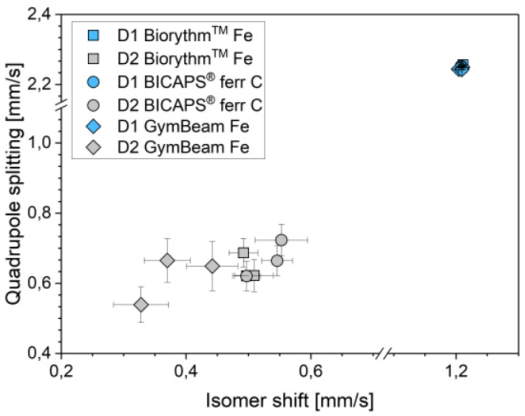

Fig. 5. Relationship between the hyperfine parameters of the fitted components (D1 and D2) for the Biorythm™ Fe, BICAPS® ferr C, and GymBeam Iron.

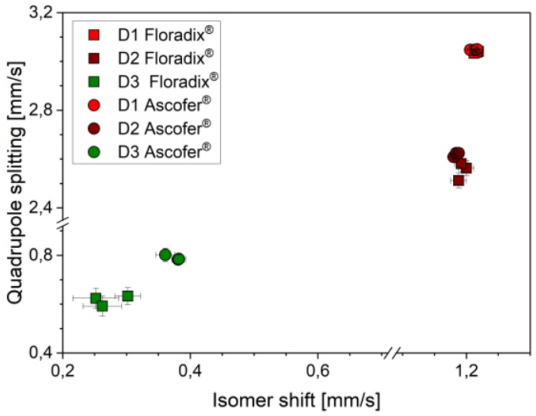

Fig. 6. Relationship between the hyperfine parameters of the fitted components (D1, D2, and D3) for the Ascofer® and Floradix®.

Figure 2 shows the ^{57}Fe Mössbauer spectra of selected iron fumarate pharmaceutical products. All spectra were fitted with two doublets. The hyperfine parameters of the D1 main doublet (Fig. 5) indicate the presence of Fe^{2+} ions in ferrous fumarate [3, 8, 10, 14]. The contribution of this component in the spectrum is 95% for Biorythm^{TM} Fe, 98% for BICAPS® ferr C, and 93% for GymBeam Iron. The second weak doublet D2 with a subspectral area between 2 % and 7% is associated with Fe^{3+} ions in octahedral coordination. This component is probably related to specific phases of impurities that may arise during manufacturing [3] or the result of the oxidation of Fe^{2+} ions to Fe^{3+} in ferrous fumarate in this process or as a result of inappropriate temperature conditions for product storage. On the other hand, research shows that ferrous fumarate is a compound stable up to temperatures of 200°C [18] and resistant to long-term exposure to temperatures of $\sim$ 40°C and humidity [19]. Therefore, the first hypothesis is the most probable.

Figure 3 compares the selected ^{57}Fe Mössbauer spectra for investigated oral pharmaceuticals containing ferrous gluconate. These spectra were fitted with three doublets, and their hyperfine parameters (Fig. 6) indicate that iron in Ascofer® and Floradix® supplements exists in two oxidation states: Fe^{2+} (D1 and D2 doublets) and Fe^{3+} (D3 doublet).

In Ascofer®, the concentration of the first doublet ranges from 46% to 47%, the second from 39% to 40%, and the rest is the third doublet. However, in Floradix®, the concentration of D1 ranges from 52% to 54%, of D2 from 24% to 25%, and the remaining significant share is related to doublet D3. The hyperfine parameters of the ferrous doublets (Fig. 6) are related to iron ions in iron gluconate [7, 9, 14, 15] and indicate that Fe^{2+} in this compound has two different sites with slightly different symmetry because they have different quadrupole splitting values. The doublet associated with Fe^{3+} ions was always observed as a minor component in the Mössbauer spectra of iron gluconate. Its origin is unknown, and some authors consider this additional component an impurity or a result of ferrous gluconate oxidation and the formation of ferric gluconate [7, 9, 14, 15]. The origin of this component may also be related to the pill coating containing the substance E-172, which denotes iron oxides and hydroxides. The latest research indicates that ferrous and ferric components are also observed at low temperatures (below $\sim$ 30 K) and are transitioning into a magnetic state at low temperatures [12]. Taking into account the above results and the fact that an additional component associated with Fe^{3+} ions will occur in this supplement regardless of whether the pill has a shell containing E-172, we can assume that this component is related to the oxidation of Fe^{2+} ions in ferrous gluconate to Fe^{3+} ions, probably during the production of products containing it.

4. Conclusions

Using X-ray fluorescence and ^{57}Fe Mössbauer spectroscopy methods, selected oral pharmaceutical products containing iron in ferric pyrophosphate, ferrous fumarate, and ferrous gluconate were investigated. The tested products are readily available without a prescription. They can be purchased in pharmacies, where you can get professional advice, as well as in drugstores and many online stores. That is why it is essential to constantly verify their elemental composition and the content of individual elements in the daily dose of the product.

The XRF results show that most of the investigated products contained much higher Fe values than declared in the product leaflet. These contents were even several dozen percent higher. However, we should remember that only a randomly selected series of these products were tested. Therefore, the quantitative composition of available pharmaceutical products should be verified more often. Importantly, the investigated materials contained no harmful elements, only those included in the compounds declared in the product leaflets. The results of ^{57}Fe Mössbauer spectroscopy research show that iron ferric pyrophosphate and ferrous fumarate are the main Fe-containing compounds in the investigated products. Their content is 100% in the case of ferric pyrophosphate and more than 93% in the case of ferrous fumarate. The content of ferrous gluconate in the investigated products was smaller than 85%. A significant part of the spectrum is the phase containing Fe^{3+} ions. The origin of this ingredient is unclear, but considering the results obtained and literature data, we can assume that it results from the oxidation of Fe^{2+} ions in ferrous gluconate to Fe^{3+} ions, probably during the production of products containing it.

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