Iron in ferrous gluconate and Ascofer®

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Abstract. Ferrous gluconate and antianemic medicament Ascofer® were investigated with Mössbauer spectroscopy in order to determine forms of iron ions present in both types of samples. Room temperature spectra gave a clear evidence that two phases of iron were present viz. ferrous (Fe$^{2+}$) as a major one with a contribution of $\sim 85 \pm 5 \%$, and ferric (Fe$^{3+}$) whose contribution was found to be $\sim 15 \pm 5 \%$. Ferrous ions were shown to occupy at least two different sites.

1. Introduction
Standard treatment of iron deficiency diseases such as anemia involves administration of iron supplements. To be absorbed, dietary iron must be in its ferrous (Fe$^{2+}$) form, which is soluble. Consequently, the iron supplements for oral intake are most frequently in form of ferrous salts like ferrous fumarate, sulfate and gluconate. As the subject of our present research we have chosen Ascofer®, a medicine produced by a Polish pharmaceutical firm, Espefa®, based on ferrous gluconate. It is fabricated in form of a dragee having a core and a shell. The core contains 200 mg of ferrous gluconate (23.2 mg elemental Fe) mixed with starch, talc, ascorbic and stearin acids, and propylene glycol. The shell is made up of talc, saccharine, gelatin, magnesium oxide, arabic gum and cochineal red. Among these constituents only ferrous gluconate should contain iron. Ferrous gluconate was already studied with Mössbauer Spectroscopy (MS) in the past by other investigators [1-3], and its spectrum was analyzed in different ways. In particular, authors of Refs. 1 and 2 fitted the measured spectra with one doublet having spectral parameters characteristic of Fe$^{2+}$ ions in the high-spin state, while those of Ref. 3 interpreted their spectra in terms of two doublets ascribed to Fe$^{2+}$ ions occupying two different sites. They also found that the gluconate samples they investigated viz. a commercial one and a freshly prepared in a chemical laboratory were contaminated with $\sim 10$ at% of Fe$^{3+}$ ions. The aim of our present study was to verify which of the two approaches applied in the spectra analysis is more appropriate as well as to see if the forms and amounts of iron present in Ascofer® are similar to those in ferrous gluconate.

2. Experimental

2.1. Samples
Investigated in this study were both particular components of Ascofer® i.e. (a) ferrous gluconate, (b) talc, (c) saccharine, (d) gelatin, (e) magnesium oxide, (f) arabic gum and (g) cochineal red, as well as
Ascofer® itself. Among these components only ferrous gluconate was expected to contain iron. However, the iron was also found to be present in talc. Its room temperature spectrum could have been well fitted with one singlet and one doublet. Spectral parameters of the latter are close to those of FeSO$_4$ [4]. Concerning ferrous gluconate, two samples A and B were investigated in this study. They were fabricated by various manufacturers. In the case of Ascofer® some of the investigated samples had different series numbers i.e. they might be composed of the ingredients of different origin.

2.2. Mössbauer spectra and their analysis

$^{57}$Fe-site Mössbauer spectra were recorded at room temperature in a transmission geometry using a standard spectrometer with a drive working in a sinusoidal mode. Gamma rays of 14.4 keV energy were supplied by a $^{57}$Co/Rh source. The investigated samples were in form of powder that, in the case of Ascofer®, was produced by pounding a dragee in an agate mortar. The samples of ferrous gluconate had a mass of 200 mg and for Ascofer® one dragee was used. All the spectra of ferrous gluconate and those of Ascofer® have had similar shape i.e. asymmetric doublet with a small bump close to the inner part of the left-hand line – see Figs. 1 and 2. The shape reflects the presence of two kinds of Fe ions known from the previous studies [1-3]. The major ferrous phase is represented by two intensive lines from which one is centered at ~0.5 mm/s and the other one at ~2.5 mm/s. The minor ferric phase is also represented by two lines (doublet) from which the left-hand one is hidden under a much more intensive left-hand line associated with the ferrous phase, while the other one can be seen in form of the bump centered at ~0.5 mm/s. In the previous investigations the subspectrum due to the ferrous phase was treated either as one doublet [1] or as two doublets [3]. As one of the aims of the present study was to verify whether or not Fe$^{2+}$ ions occupy two different sites as claimed in [3], the subspectrum was fitted with the quadrupole distribution method based on the one described elsewhere [5] i.e. a priori no assumption on the number of the sites occupied by Fe$^{2+}$ ions was made. The subspectrum representing the ferric phase was treated as one doublet. The asymmetry of the major quasi-doublet, which according to [3] does not originate, as suggested in [1], from Goldanskij-Karyagin effect, was here accounted for by assuming a linear correlation between the isomer shift, $IS$, and the quadrupole splitting, $QS$. Using such procedure all the measured spectra could have been successfully fitted. The values of $IS$ are given relative to a metallic iron.

**Figure 1:** (Online color) (a) $^{57}$Fe Mössbauer spectrum recorded at 295 K on sample A of the ferrous gluconate, and (b) the corresponding distribution of the quadrupole splitting derived from the subspectrum ascribed to Fe$^{2+}$ ions (major doublet in (a)).
3. Results and discussion

3.1. Ferrous Gluconate

The spectra measured on samples A and B and the corresponding distributions of $QS$ are similar. As shown for the sample A in Fig. 1b, the distribution of $QS$ is not symmetric which means that $Fe^{2+}$ ions occupy more than one site. Assuming Gaussian distribution of $QS$ for each site, two Gaussians must have been taken into account in order to correctly fit the overall distribution curve. The centre of each Gaussian gives the value of $QS$ associated with the particular site and its relative area the relative population of this site. Spectral parameters obtained with such procedure are presented in Table 1. As follows from it, in both samples i.e. A and B iron exists in two forms viz. as the ferrous (Fe$^{2+}$) and as the ferric (Fe$^{3+}$). The former with the abundance of $\sim 85 - 88\%$ is the major fraction while the latter is a minor one, and it is an undesired phase, hence can be treated as a contamination. The data displayed in Table 1 also give a clear evidence that Fe$^{2+}$ ions occupy, at least, two different sites that differ in values of $QS$, and abundances. As the origin of the difference is not known, we will assume, following [3] that the two variations of Fe$^{2+}$ ions occupy sites with slightly different symmetry because they have different $QS$-values. The data presented in Table 1 also give evidence that the actual phase composition of the samples A and B is meaningfully different, while the values of the spectral parameters ascribed to Fe$^{2+}$ and Fe$^{3+}$ ions are rather similar in both samples. That means that the difference in the relative phase composition is of a technological origin. Such conclusion can be further supported by the results discussed in [3], according to which the contribution of Fe$^{3+}$ ions in the samples studied by those authors was $\sim 10$ at%. Also the ratio between the abundances of the Fe$^{2+}$ ions present at the two sites seems to be strongly dependent on the technology of the sample production: that determined in this study is equal to $\sim 3$ which is much less than $\sim 5$ as found elsewhere [3].

Table 1 The best-fit spectral parameters as obtained by fitting room temperature spectra recorded on ferrous gluconate samples A and B. Abundances, $<a>$ and $a$ are in [%], isomer shifts, $IS$, quadrupole splittings, $QS$, distribution width, $G$ and line width at half maximum, $\Gamma$ in [mm/s].

| Sample | $<$a$>$<IS$>$ <QS$>$ | Site 1 | Site 2 | Fe$^{2+}$ |
|--------|-----------------|-------|-------|-----------|
|        | average         | a     | G     | a         | G         | a     | IS    | QS    | $\Gamma$ |
| A      | 87.9 1.21 2.93 74.6 3.04 0.28 | 25.4 2.68 0.42 | 12.1 0.38 0.89 0.63 |
| B      | 84.7 1.21 2.90 72.8 3.04 0.32 | 27.2 2.52 0.52 | 15.3 0.41 0.80 0.80 |
3.2. Ascofer®

The $^{57}\text{Fe}$ Mössbauer spectrum of Ascofer® should, in principle, be identical with that of ferrous gluconate recorded in the same conditions. A possible difference between the spectra recorded on different samples of Ascofer® may be caused by use of (a) ferrous gluconate and/or (b) talc of different origins in the process of its production. As mentioned above, the samples A and B of ferrous gluconate differ by ~5% in the relative abundance of $^{57}\text{Fe}^2^+$ ions, hence the difference of this order in the relative amount of these ions can be expected in Ascofer®, too. To verify whether or not this is true, we have recorded room temperature spectra on four different samples produced in 2007. One of them is shown in Fig. 2. The spectral parameters obtained from all four spectra are given in Table 2. It can be readily seen that, in fact, the abundances of $^{57}\text{Fe}^2^+$ ions, $<a>$, fall into two groups: (I) with $<a>$ between ~82-83%, hence close to that found for the ferrous gluconate B, and (II) with $<a>$ between ~88-91%, hence close to that revealed for the ferrous gluconate A. A lack of the exact match may be due to the presence of additional $^{57}\text{Fe}^2^+$ ions in Ascofer® due to talc which, in general, may have a different origin in different series of Ascofer®, and, consequently, the different amount of various Fe-ions.

Table 2 The best-fit spectral parameters as obtained by fitting the room temperature spectra recorded on four samples of Ascofer® all produced in the same year but having different series. Abundances, $<a>$ and $a$ are in [%], isomer shifts, $I_S$, quadrupole splittings, $Q_S$, and line width at half maximum, $\Gamma$ in [mm/s]. Typical errors: 3% in $a$, 0.02 mm/s in $I_S$, $Q_S$ and in $\Gamma$.

| sample | $<a>$ | $<I_S>$ | $<Q_S>$ | Site 1 | Site 2 | Site 3 |
|--------|-------|---------|---------|-------|-------|-------|
|        | a     | IS      | QS      | a     | IS    | QS    |
| 1      | 91.2  | 1.22    | 2.90    | 81.6  | 1.23  | 3.04  |
| 2      | 87.8  | 1.22    | 2.99    | 93.8  | 1.22  | 3.04  |
| 3      | 82.6  | 1.22    | 2.94    | 83.6  | 1.22  | 3.04  |
| 4      | 80.3  | 1.22    | 2.96    | 88.0  | 1.23  | 3.04  |

4. Summary

Using $^{57}\text{Fe}$-site Mössbauer spectroscopy, several samples of pure ferrous gluconate and those of Ascofer® were investigated. In the former iron was found as (1) $^{57}\text{Fe}^2^+$ and (2) $^{57}\text{Fe}^3^+$ ions. The $^{57}\text{Fe}^2^+$ ions constitute a major phase with a population between ~83 and ~85% depending on the origin of gluconate. The $^{57}\text{Fe}^2^+$ ions are distributed at least over two sites with a population of ~73-75% on site 1 and ~17-15% on site 2. In Ascofer, iron exists also in the same forms as in ferrous gluconate. Their relative amount depends on the series which is likely related to the different technological origin of ferrous gluconate used in the production process. In addition, a small amount of iron was also detected in talc which is one of the constituting ingredients of Ascofer®, hence it can slightly bias the quantitative phase analysis of this medicine.

References
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