Hyperfine Interact
DOI 10.1007/s10751-013-0953-z

Comparative study of Ascofer® and ferrous gluconate using Mössbauer spectroscopy with a high velocity resolution

M. I. Oshtrakh · E. G. Novikov · S. M. Dubiel · V. A. Semionkin

Abstract A comparative study of ferrous gluconate as well as fresh and outdated tablets of Ascofer® was carried out using Mössbauer spectroscopy with a high velocity resolution. The obtained results revealed the presence of three ferrous and one ferric component in all investigated samples which may be related to ferrous gluconate molecules and ferric contamination and/or aging effect.

Keywords Mössbauer spectroscopy · Pharmaceutical products · Ferrous gluconate · Ascofer®

1 Introduction

^{57}Fe Mössbauer spectroscopy has turned out to be useful for an additional characterization of the quality of iron containing pharmaceutical products. Some successful examples of studies of various iron containing medicaments can be found in [1–9]. These observations are important in order to better control the iron state in such medicaments because their pharmaceutical effect in the body is related to the form and valence of iron. It was

Proceedings of the 32nd International Conference on the Applications of the Mössbauer Effect (ICAME 2013) held in Opatija, Croatia, 1–6 September 2013

M. I. Oshtrakh (✉) · E. G. Novikov · V. A. Semionkin
Department of Physical Techniques and Devices for Quality Control, Institute of Physics and Technology, Ural Federal University, Ekaterinburg 620002, Russian Federation
e-mail: oshtrakh@gmail.com

M. I. Oshtrakh · E. G. Novikov · V. A. Semionkin
Department of Experimental Physics, Institute of Physics and Technology, Ural Federal University, Ekaterinburg 620002, Russian Federation

S. M. Dubiel
Faculty of Physics & Applied Computer Science, AGH University of Science & Technology, 30-059 Kraków, Poland

Published online: 07 November 2013

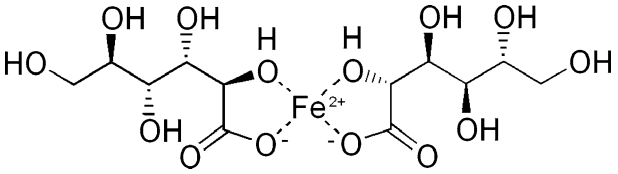

Fig. 1 Structural formula of the ferrous gluconate

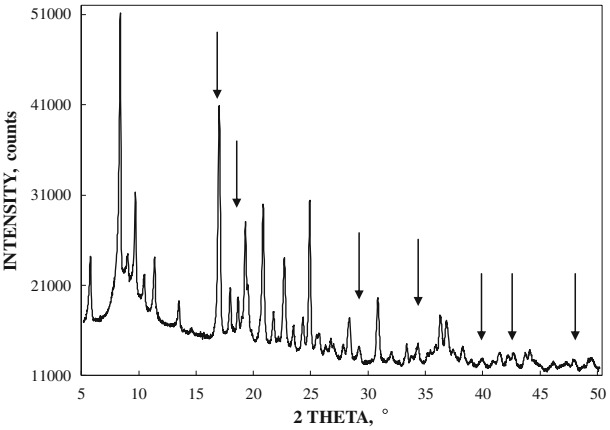

Fig. 2 X-ray diffraction pattern of the ferrous gluconate sample provided by Espefa. Arrows indicate peak positions for iron-gluconate from the PDF2 reference code 00-005-0257

shown earlier that Mössbauer spectroscopy with a high velocity resolution gives better accuracy in the hyperfine parameters evaluation, hence it enables revealing small variations in their values. The latter may be of importance with regard to the structure of the iron containing compound. Furthermore, it also provides more accurate fits of complicated spectra [10, 11], including those of pharmaceutical products [12, 13]. Ascofer® - a product of the Polish pharmaceutical firm, Espefa, based in Kraków - is a medicament used for a treatment of iron deficiency. The iron-carrying compound of Ascofer® is the ferrous gluconate ($C_{12}H_{24}FeO_{14}$, its structural formula is shown in Fig. 1). The ferrous gluconate is used as an iron supplement in various pharmaceutical products. Samples of Ascofer® and the ferrous gluconate - supplied by Espefa - were studied previously using the Mössbauer spectroscopy with a low velocity resolution [14–16]. In the present work we continued the study of these pharmaceutical products by means of Mössbauer spectroscopy with a high velocity resolution which demonstrates higher analytical possibilities [10].

2 Experimental

A fresh ferrous gluconate powder and also a fresh and two outdated (since 2006 and 2004, respectively) tablets of Ascofer® provided by Espefa (Kraków, Poland) were studied. Each tablet of Ascofer® contains 23.2 mg of Fe. About half of each tablet was powdered. The ferrous gluconate powder was used for the X-ray diffraction (XRD) analysis using a XRD–7000 powder diffractometer (Shimadzu) operating at 40 kV and 30 mA with Ni-filtered

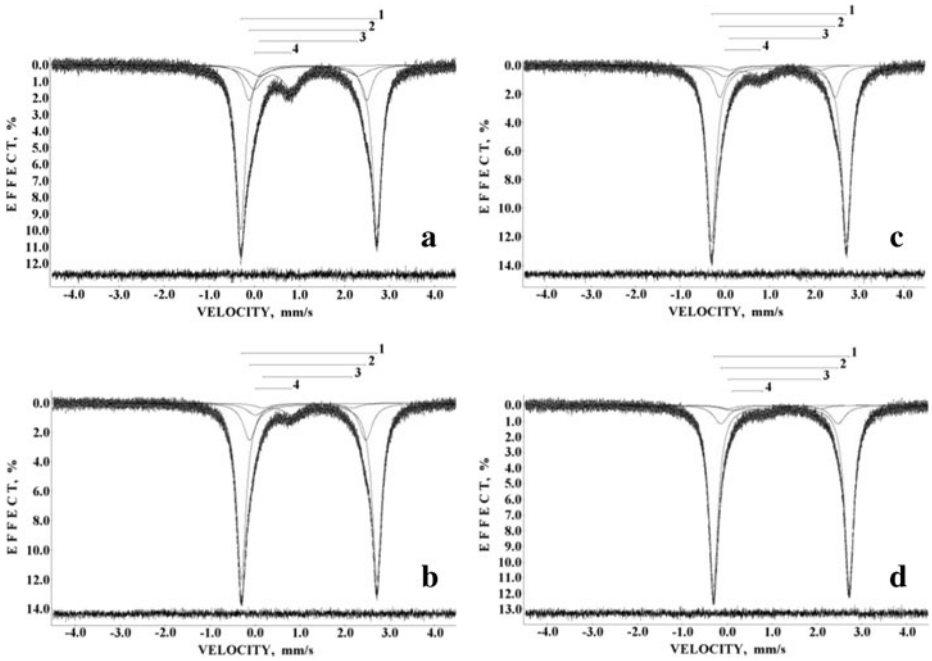

Fig. 3 Mössbauer spectra of the ferrous gluconate (**a**), the fresh Ascofer® (**b**), the outdated Ascofer® since 2006 (**c**), and the outdated Ascofer® since 2004 (**d**), measured in 4096 channels at 295 K. 1–4 are the results of the best fit. Differential spectra are shown below each spectrum

CuK_α radiation. For the Mössbauer study the powder of each sample was placed into a Plexiglas sample holder with a diameter of 2 cm and tightly closed by a cover to exclude particle vibration during measurement. The thickness of the samples was less than 6 mg Fe/cm^2.

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 using quantification with 4096 steps. Details and characteristics of this spectrometer and the system were given elsewhere [17–19]. A 1.8×10^9 Bq $^{57}Co(Rh)$ source of the 14.4 keV gamma radiation (Ritverc GmbH, St. Petersburg) was used at room temperature. The Mössbauer spectra were recorded at 295 K in transmission geometry in 4096 channels with the moving absorber placed in a cryostat. The spectra were measured in the velocity range of about ± 5 mm/s with statistics from 3.4×10^5 to 3.6×10^5 counts per channel. A signal-to-noise ratio for the obtained spectra was in the range between 73 and 82. The spectra were next computer fitted with the least squares procedure using the UNIVEM-MS program with Lorentzian line shape. Spectral parameters such as: isomer shift, δ, quadrupole splitting, ΔE_Q, line width, Γ, relative subspectrum area, S, and the statistical quality of the fit, χ^2, were determined. The quality of the fit was evaluated using the differential spectrum, the χ^2 value and the physical meaning of the parameters. An instrumental (systematic) error for each spectrum point was of the order of ± 0.5 channels (the velocity scale), the instrumental (systematic) error for the hyperfine parameters was of the order of ± 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. Values of δ are given relative to α–Fe at 295 K.

Table 1 Mössbauer parameters obtained from the best fits of the Mössbauer spectra of the ferrous gluconate and Ascofer® samples measured at 295 K in 4096 channels

Sample	Γ, mm/s	δ, mm/s	ΔE_Q, mm/s	S, %	Component[a]
Ferrous gluconate	0.251 ± 0.005	1.208 ± 0.002	3.022 ± 0.002	61.0	Ferrous (1)
	0.296 ± 0.020	1.185 ± 0.002	2.602 ± 0.007	15.5	Ferrous (2)
	0.540 ± 0.028	1.207 ± 0.009	2.189 ± 0.068	8.6	Ferrous (3)
	0.439 ± 0.009	0.400 ± 0.006	0.807 ± 0.012	14.9	Ferric (4)
Ascofer® (fresh)	0.255 ± 0.005	1.208 ± 0.002	3.021 ± 0.002	69.0	Ferrous (1)
	0.348 ± 0.015	1.176 ± 0.002	2.586 ± 0.006	19.6	Ferrous (2)
	0.459 ± 0.058	1.172 ± 0.012	1.984 ± 0.059	3.5	Ferrous (3)
	0.455 ± 0.016	0.408 ± 0.007	0.799 ± 0.013	7.8	Ferric (4)
Ascofer® (outdated, 2006)	0.254 ± 0.005	1.207 ± 0.002	3.022 ± 0.002	73.0	Ferrous (1)
	0.304 ± 0.013	1.170 ± 0.002	2.580 ± 0.005	15.8	Ferrous (2)
	0.383 ± 0.050	1.117 ± 0.014	2.025 ± 0.048	2.9	Ferrous (3)
	0.499 ± 0.019	0.390 ± 0.011	0.789 ± 0.022	8.3	Ferric (4)
Ascofer® (outdated, 2004)	0.252 ± 0.005	1.209 ± 0.002	3.023 ± 0.002	80.3	Ferrous (1)
	0.383 ± 0.024	1.164 ± 0.003	2.606 ± 0.012	12.4	Ferrous (2)
	0.406 ± 0.076	1.050 ± 0.027	2.061 ± 0.035	2.5	Ferrous (3)
	0.566 ± 0.046	0.454 ± 0.021	0.687 ± 0.035	4.7	Ferric (4)

[a]Numbers in parenthesis correspond to numbers of components in Fig. 3

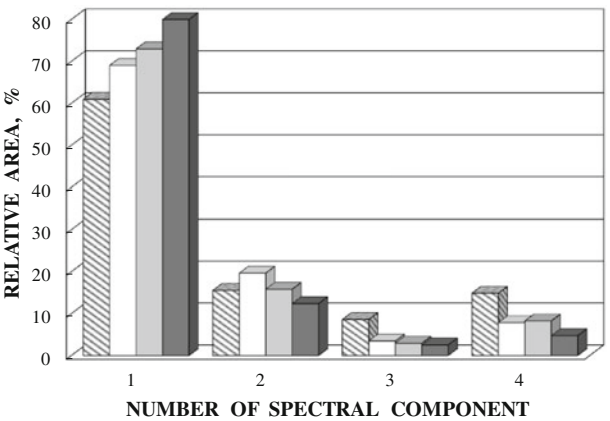

Fig. 4 A comparison of relative areas of four different spectral components as obtained in the best fit of Mössbauer spectra of the ferrous gluconate (▨), fresh Ascofer® (□), outdated Ascofer® since 2006 (▩) and outdated Ascofer® since 2004 (■). Numbers of the components are the same as in Fig. 3 and Table 1

3 Results and discussion

A typical X-ray diffraction pattern for the ferrous gluconate is shown in Fig. 2. It is clearly seen that the XRD pattern demonstrates the presence of various peaks while only some of these were identified as ferrous gluconate using the data from the PDF2

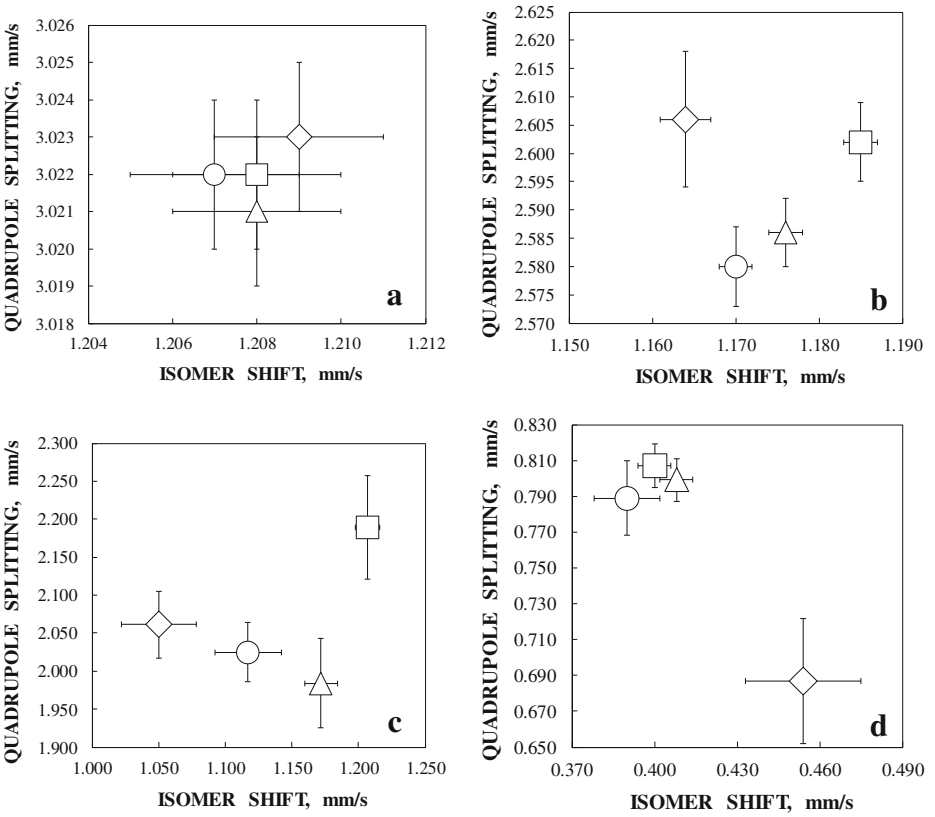

Fig. 5 Plots of the Mössbauer hyperfine parameters of four different spectral components obtained for the best fit of the Mössbauer spectra of the ferrous gluconate (□), fresh Ascofer® (△), outdated Ascofer® since 2006 (◯) and outdated Ascofer® since 2004 (◇); **a** – the main ferrous component 1, **b** – the minor ferrous component 2, **c** – the minor ferrous component 3 and **d** – the minor ferric component 4

reference code 00-005-0257. Unfortunately, this is the only reference code for iron gluconate in the PDF2 data base. We can suppose that the sample of ferrous gluconate contains unknown salts and organic components. However the producer, Espefa, did not supply their sample with an exact chemical composition. In this case the Mössbauer study could be very important for quality control of Ascofer® and its iron-carrying compound.

The Mössbauer spectra of the ferrous gluconate and the fresh and the outdated Ascofer® samples are displayed in Fig. 3. These spectra are similar in shape and could be analyzed in terms of several quadrupole doublets. The best fit of these spectra reveals the presence of 4 quadrupole doublets with different relative areas and hyperfine parameters corresponding to ferrous and ferric compounds. The Mössbauer parameters are given in Table 1.

The existence of components 1–3 found in the ferrous phase agrees with previous findings, albeit obtained with a different approach [15, 16]. The three components, which differ mostly by the value of the quadruple splitting, may be either related to three different iron sites in the molecule of the iron gluconate (an option which is at cross with the

current knowledge of this structure – see Fig. 1), or they reflect some molecule's modifications/distortions (molecular isomerism, hydration, etc.). A minor ferric component was also found in all spectra in addition to the ferrous components. It is noteworthy to observe that the relative area of the main ferrous component viz. 1 increased while that of the ferrous component viz. 2 decreased with the increase of age of the Ascofer® tablets, which is clearly seen in Fig. 4. In contrast, the relative area of the ferrous component 3 remained unchanged with the Ascofer® aging. As for the relative area of the minor ferric component, it was found to have decreased in the case of the most aged Ascofer® sample. The origin of the decrease of the relative amount of the ferric component with aging is not clear yet because the outdated samples did not belong to the same production series. As shown previously [16], the relative contribution of the ferric phase ranged between ∼9 and ∼18 %, depending on the sample's production series. On the other hand, a decrease of its contribution could also have an aging origin [16]. An unexpected transformation of the ferrous component 2 into 1 observed with Ascofer® aging could be understood in terms of some instability of the structure of the initial ferrous gluconate molecules.

It was also of interest to make a comparison between the Mössbauer hyperfine parameters obtained for the spectral components in the studied samples. Thus, Fig. 5 illustrates a relationship between the quadrupole splitting and the isomer shift. The main components in all spectra have the same hyperfine parameters while for the minor components small variations of the parameters beyond the errors can be seen. If we consider the ferrous components 2 and 3 as a result of ferrous gluconate molecule modifications, these results may indicate small structural variations in these compounds. In the case of the ferric component, it is noteworthy to observe different values of the hyperfine parameters for the most aged Ascofer® tablet in comparison to other samples.

4 Conclusions

The present study of the ferrous gluconate and Ascofer® tablets containing ferrous gluconate using Mössbauer spectroscopy with a high velocity resolution enabled the following conclusions to be drawn:

(a) Iron is present as ferrous (major component) and ferric (minor component) phases in the investigated samples.
(b) The ferrous phase is not homogenous as it is composed of three components differing from each other in the value of the quadrupole splitting and their relative contribution.
(c) The relative amounts of both ferrous and ferric components in the Ascofer ® samples are characteristic of the sample.
(d) A significant difference of the relative amount of the ferric component was revealed in the most outdated sample of Ascofer®. Its origin could not be uniquely identified in this study.
(e) The Mössbauer spectral parameters found for the ferric component in the oldest sample of Ascofer® show values which are distinctively different from the corresponding ones found for other samples.

Acknowledgments The authors wish to thank Dr. A.V. Chukin for XRD measurement (Ural Federal University, Ekaterinburg). This work was supported by the basic financing from the Ministry of Education and Science of Russian Federation, and by the Ministry of Science and Higher Education, Poland.

References

1. Yang, C.-Y., Bryan, A.M., Theil, E.C., Sayers, D.E., Bowen, L.H.: J. Inorg. Biochem. **28**, 393–405 (1986)
2. Matzanke, B.E., Bill, E., Butzlaff, C., Trautwein, A.X., Winkler, H., Hermes, C., Nolting, H.-F., Barbieri, R., Russo, U.: Eur. J. Biochem. **207**, 745–755 (1992)
3. Coe, E.M., Bowen, L.H., Bereman, R.D.: J. Inorg. Biochem. **58**, 291–296 (1995)
4. Knight, B., Bowen, L.H., Bereman, R.D.: J. Inorg. Biochem. **64**, 225–229 (1996)
5. Funk, F., Long, G.J., Hautot, D., Büchi, R., Christl, I., Weidler, P.G.: Hyperfine Interact. **136**, 73–95 (2001)
6. Oshtrakh, M.I., Milder, O.B., Semionkin, V.A.: Anal. Chim. Acta. **506**, 155–160 (2004)
7. Oshtrakh, M.I., Milder, O.B., Semionkin, V.A.: Hyperfine Interact. **156/157**, 273–277 (2004)
8. Oshtrakh, M.I., Milder, O.B., Semionkin, V.A.: J. Pharm. Biomed. Anal. **40**, 1281–1287 (2006)
9. Oshtrakh, M.I., Milder, O.B., Semionkin, V.A.: J. Radioanal. Nucl. Chem. **269**, 547–533 (2006)
10. Oshtrakh, M.I., Semionkin, V.A., Grokhovsky, V.I., Milder, O.B., Novikov, E.G.: J. Radioanal. Nucl. Chem. **279**, 833–846 (2009)
11. Oshtrakh, M.I., Semionkin, V.A., Milder, O.B., Novikov, E.G.: J. Mol. Struct. **924–926**, 20–26 (2009)
12. Oshtrakh, M.I., Semionkin, V.A., Milder, O.B., Novikov, E.G.: Hyperfine Interact. **190**, 67–74 (2009)
13. Oshtrakh, M.I., Novikov, E.G., Dubiel, S.M., Semionkin, V.A.: Hyperfine Interact. **197**, 287–294 (2010)
14. Kulgawczuk, D.S., Ruebenbauer, K., Sepiol, B.: J. Radioanal. Nucl. Chem. Artic. **131**, 43–50 (1989)
15. Gozdyra, R., Dubiel, S.M., Cielak, J.: J. Phys. Conf. Ser. **217**, 012146 (2010)
16. Dubiel, S.M., Cielak, J., Gozdyra, R.: J. Mol. Struct. **991**, 171 (2011)
17. Oshtrakh, M.I., Semionkin, V.A., Milder, O.B., Novikov, E.G.: J. Radioanal. Nucl. Chem. **281**, 63–67 (2009)
18. Semionkin, V.A., Oshtrakh, M.I., Milder, O.B., Novikov, E.G.: Bull. Rus. Acad. Sci. Phys. **74**, 416 (2010)
19. Oshtrakh, M.I., Semionkin, V.A.: Spectrochim. Acta, Part A: Molec. Biomolec. Spectrosc. **100**, 78 (2013)