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Anomalous Mössbauer Line Broadening for Nanosized Hydrous Ferric Oxide Cores in Ferritin and its Pharmaceutical Analogue Ferrum Lek in the Temperature Range 295–90 K

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Abstract

Mössbauer spectra of ferritin and its pharmaceutical analogue Ferrum Lek, both containing nanosized hydrous ferric oxides cores in the forms of ferrihydrite and akaganéite, respectively, were measured in a temperature range 295–90 K. An anomalous line broadening with temperature decrease was observed for ferritin below ~150 K and for Ferrum Lek below ~130 K. Some anomalies were also observed below these temperatures for spectral area and quadrupole splitting.

1. Introduction

Iron storage protein ferritin contains nanosized mineral hydrous ferric oxide core in the form of ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$) with varying degrees of crystallinity and the presence of some inorganic phosphates [1]. This core contains up to 4500 iron atoms and the core size may vary from ~4 till ~8 nm. Details about ferritin and its iron core structure can be found in [1–3]. One of commercial pharmaceutical analogues of ferritin produced by Lek, Slovenia is Ferrum Lek which is used for treatment of iron deficiency. Ferrum Lek also contains nanosized mineral hydrous ferric oxide core in the form of akaganéite (β–FeOOH). The size of this core is about 8 nm. Both iron cores are surrounded with a shell: 24 protein subunits in ferritin and polymaltose in Ferrum Lek.

Mössbauer spectroscopy is a good technique to study various iron containing species, and has been widely applied for the study of nanosized hydrous ferric oxide cores in various ferritins and its pharmaceutical analogues such as Imferon, Maltofer®, Ferrum Lek, etc. (see, for instance, [4–15]). Mössbauer spectra of nanosized hydrous ferric oxide cores in these systems at the temperatures above 80 K are mainly doublets reflecting superparamagnetic state. Mössbauer spectra demonstrate appearance of magnetic sextets in addition to doublet when the rate of superparamagnetic relaxation decreases below the critical rate which depends on the particle size [16]. The low temperature region (below 80 K) was studied in details (for review see [13]). In order to elucidate the differences between the iron core structure in ferritin and Ferrum Lek we studied nanosized hydrous ferric oxide cores in human liver ferritin as a natural product and in commercial pharmaceutical product Ferrum Lek using Mössbauer spectroscopy in a temperature range 295–90 K.

2. Materials and Methods

Human liver ferritin was prepared in lyophilized form at the Russian State Medical University, Moscow, Russian Federation (method of ferritin preparation was briefly described in [7]). Powdered sample of 100 mg of protein was packed into the Plexiglas sample holder. Commercial pharmaceutical product Ferrum Lek (Lek, Slovenia) in the form of tablets was used as

ferritin analogue. Each Ferrum Lek tablet contained 100 mg of Fe. Sample was prepared from one third of tablet by powdering and was packed into the sample holder. The sample thickness of Ferrum Lek powder did not exceed 10 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 4096 bits and temperature variable liquid nitrogen cryostat with moving absorber. Details and characteristics of this spectrometer are given elsewhere [17–19]. The ^{57}Co(Rh) source of ~1.0×10^9 Bq was used at room temperature. Spectra were measured in the temperature range between 295 and 90 K in transmission geometry and registered in 4096 channels. Statistical counts for ferritin were in the range of 8.3×10^5 – 2.0×10^6 counts per channel and the signal-to-noise ratios were in the range from 51 to 100 and those for Ferrum Lek samples were in the range of 2.6×10^5 – 6.3×10^5 counts per channel and the signal-to-noise ratios were in the range from 101 to 141.

In order to emphasize changes reflected by our measurements as a function of temperature, first we characterize the observed spectra in a model independent way, by deriving their relative absorption area, the first moment and the second central moment as follows. The *b* baseline that equals to the expected value of counts far enough from resonance can be readily estimated in the case of the present spectra by fitting them to a number of Lorentzians that provides a statistically acceptable fit. Once this is accomplished, the spectra can be normalized as

$$y_i = \begin{cases} \dfrac{b - n_i}{b} & if \quad n_i \le b \\ 0 \quad if & n_i > b \end{cases},$$

where n_i denotes the measured counts in the *i*th channel. The y_i values thus observed are further normalized to give a sum of unity as

$$p_i = \frac{y_i}{\sum_i y_i}.$$

The p_i values can be treated as a probability distribution over the channels, and as such can be

meaningfully characterized by the first moment (mean, *m*, central shift) and the second central moment (variance) and the square root of the latter (standard deviation, σ). Accordingly, we calculate the corresponding quantities as

$$m_{ch} = \sum_i i p_i \,,$$

$$\sigma_{ch} = \sqrt{\sum_i (i - m_{ch})^2 p_i} \,,$$

where the index "ch" indicates that the quantities are expressed in channels. Finally, we convert these quantities to the corresponding velocity values *m* and σ via

$$m = \Delta v \cdot (m_{ch} - n_0) \,,$$

$$\sigma = \Delta v \cdot \sigma_{ch} \,,$$

where Δv and n_0 denote the calibration factor and the zero velocity channel, respectively. With the above notation, the relative absorption area *a* can be given as

$$a = \Delta v \cdot \sum_i \frac{b - n_i}{b} \,.$$

Changes in the quantities *m*, σ, and *a* are expected to reflect corresponding changes in the physical quantities of the ^{57}Fe isomer shift, line width and quadrupole splitting, and the recoilless fraction, respectively.

The spectra were also fitted using the UNIVEM-MS program with a least squares procedure and the Lorentzian line shape. Parameters determined for the measured spectra were: isomer shift, quadrupole splitting, line width and spectrum area. The instrumental (systematic) error for each spectrum point and for the hyperfine parameters were ±0.5 and ±1 channel, respectively [19]. The error of S did not exceed 10 %. 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 the isomer shifts are given relative to that of α–Fe at 295 K.

Additionally we used the standard absorber of sodium nitroprusside (SNP) glued on the alumina foil free from iron (5 mg Fe/cm^2) and potassium ferrocianide (PFC) in the forms of powder

packed into the sample holder and the powder glued on the alumina foil free from iron (the iron surface density of the samples did not exceed 5 mg Fe/cm^2). For the control of cryostat vibrations (that may occur with temperature decrease as a result of nitrogen gas flow increase and also as a result of powdered particles vibration in the samples in spite of close packing) we measured Mössbauer spectra of SNP and both PFC samples at selected temperatures. These spectra were fitted using UNIVEM-MS program. The obtained line widths and total areas versus temperature are shown in Fig. 1. It is clearly seen that there is no anomalous line broadening while approximately linear slope should be considered as a result of both cryostat vibrations and increase of the effective thickness due to increase in the Mössbauer effect probability (*f*-factor). We can point out that in the case of two PFC samples normalized relative spectrum area for glued powder was slightly smaller at 90 K than those for packed powder sample that may be a result of small amount of PFC lost during glued sample preparation. However, these spectral areas for both PFC demonstrated the absence of significant effect of particles vibrations.

3. Results and Discussion

Mössbauer spectra of ferritin and Ferrum Lek sample measured in the temperature range between 295 and 90 K displayed doublet shapes without any magnetic components. First, changes in the spectral shape were evaluated in a model independent way then the spectra were fitted using one quadrupole doublet as the first approximation. Though a fit with one doublet cannot account for all the spectral features, this approach allowed us to derive approximations of the main Mössbauer parameters as functions of temperature and compare the results with model independent analysis. The main unexpected result was an unusual temperature dependence of the line width for both nanosized hydrous ferric oxide cores as shown in Fig. 2a. This was confirmed by the corresponding unusual temperature dependence of the second central moment (Fig. 2b). The anomaly detected concerning the line width was found to be accompanied by a corresponding anomaly in the normalized relative spectral area versus temperature (Fig. 3). Temperature dependences of the

central shift obtained from the model independent analysis and both isomer shift and quadrupole splitting obtained from the one doublet fit are shown in Fig. 4.

It is clearly seen that both the model independent analysis and the one quadrupole doublet fit give similar results indicating unusual temperature dependence of Mössbauer parameters. With decreasing temperature the anomaly starts to develop at a critical temperature T_0 that may be approximated as the temperature of the intersection of straight lines fitted to the "high" and "low" temperature data as shown in Fig. 2. Temperatures at which these anomalies appear to be started are ~130 K for Ferrum Lek with nanosized akaganéite core and ~150 K for ferritin with nanosized ferrihydrite core.

An earlier study of reconstituted horse spleen ferritin dynamics in [20] demonstrated narrowing of Mössbauer line width at around 264 K that was not observed in our case. Another anomaly in the temperature dependence of *f*-factor for ferritin and its analogue polysaccharide iron complex (PIC) Niferex, both containing ferrihydrite core, was observed at a temperature below 60 K in [21]. The authors explained their observation as being the result of magnetostriction which may be a reason of a decrease of *f*-factor at temperatures above 30 K for ferritin and above 60 K for PIC. They excluded the effects of sample thickness and core motions inside protein shell while did not consider cryostat vibrations effect. However, the temperature dependence of the *f*-factor of ferritin obtained in [21] was different from our result shown in Fig. 3b,d. In our case of ferrihydrite cores there is a decrease of spectral area at 90 K in comparison with those at 98 and 115 K. As for akaganéite cores, in Fig. 3a,c we also can see unusual dependence in the temperature range between 130 and 90 K.

One could consider that slowdown of fast magnetic relaxation in the superparamagnetic nanoparticles may be a reason of unusual line broadening in the Mössbauer spectra of nanosized hydrous ferric oxides cores in human liver ferritin and in Ferrum Lek below 160 K. However, recently it was found that the anisotropy energy barrier for human liver ferritin was smaller than that for Ferrum Lek [22], which would imply that the temperature at which the line broadening

starts as the result of slowdown of magnetic relaxation should be higher for Ferrum Lek. In contrast, it is clearly seen in Fig. 2a that unusual line broadening starts for the ferritin at higher temperature than that for the Ferrum Lek. Therefore, we should exclude magnetic relaxation from the possible origins of this anomaly. At the same time, neither cryostat vibrations nor sample thickness can explain the anomalous line broadening shown in Fig. 2a, as they should both display linear temperature dependence in the temperature range 295–160 K, similar to that shown in Fig. 1a.

We hypothesize that the anomalous changes detected in the Mössbauer parameters of ferritin and Ferrum Lek are caused by a phase transition occurring in the range of 120–160 K, which influences the dynamic properties and the average positions of oxygen ions in the oxygen octahedra surrounding the iron ions. At higher temperatures excited vibrational modes of the oxygen octahedra may realize a fast enough relaxation of the electric field gradient (EFG) at the ^{57}Fe nucleus to make only an averaged quadrupole splitting value observable in the Mössbauer spectra. With the temperature decreasing below ~130 K a slow-down of oxygen lattice vibrations may occur that gives gradually rise to the appearance of the different possible EFG levels instead of an averaged one. In the spectra this may lead to an apparent broadening of the lines of the doublets as observed, as well as to a change in the magnitude of the apparent quadrupole splitting as clearly found for Ferrum Lek (Fig. 4).

The anomalies observed in the temperature dependences of the normalized relative spectral area and – to a lesser extent – in that of the isomer shift & central shift below ~130 K refer to an anomalous change in the vibrational state of the ^{57}Fe nuclei, which corroborates the occurrence of a phase transition in this temperature range.

Taking into account some small structural differences between ferrihydrite and akaganéite, we can assume that ferrihydrite cores undergo such kind of microstructural rearrangements at higher temperatures (below ~150 K) while akaganéite cores undergo similar microstructural rearrangements at lower temperatures (below ~130 K).

4. Conclusion

A formerly unknown anomaly has been discovered in the ^{57}Fe Mössbauer parameters of ferritin and its pharmaceutical analogue Ferrum Lek at around T_0 ~150 and ~130 K, resectively. The anomaly is most clearly visualized in an enhanced rate of increase of the Mössbauer line width with decreasing temperature below T_0, as well as in an anomalous temperature dependence of the spectral area just below T_0. In addition, the quadrupole splitting and – to a lesser extent – the isomer shift also reflect the anomaly in around the same temperature interval. The anomaly can be interpreted as being the result of a phase transition influencing the vibrational state of the oxygen octahedron surrounding iron ions, as well as that of the iron ions themselves. The anomalous increase of the line width with decreasing temperature was hypothesized to be caused by the slowing down of oxygen atomic vibrations and an associated slow-down in the relaxation of the electric field gradient at the ^{57}Fe nuclei.

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FIGURE LEGENDS

Fig. 1. Line width (***a***) and relative area normalized to 295 K value (***b***) of Mössbauer spectra of the standard absorber sodium nitroprusside (◆) and potassium ferrocianide powder (●) and glued powder (○) versus temperature.

Fig. 2. Unusual temperature dependence of the line width (***a***) and second central moment (***b***) for the Mössbauer spectra of nanosized hydrous ferric oxides cores in human liver ferritin (△) and in Ferrum Lek (□). Indicated dashed lines represent linear approximations for the data. Arrows indicate suggested temperatures of starting unusual behavior of parameters.

Fig. 3. Anomalous temperature dependence of the relative area normalized to 295 K value obtained from the model independent analysis (***a***, ***b***) and from the one doublet fit (***c***, ***d***) of the Mössbauer spectra of nanosized hydrous ferric oxides cores in Ferrum Lek (□) and in human liver ferritin (△).

Fig. 4. Temperature dependences of the central shift obtained from the model independent analysis (***a***) and isomer shift (***b***) and quadrupole splitting (***c***) obtained from the one doublet fit of the Mössbauer spectra of nanosized hydrous ferric oxides cores in human liver ferritin (△) and in Ferrum Lek (□). Dashed lines indicate different slopes for quadrupole splitting temperature dependences.

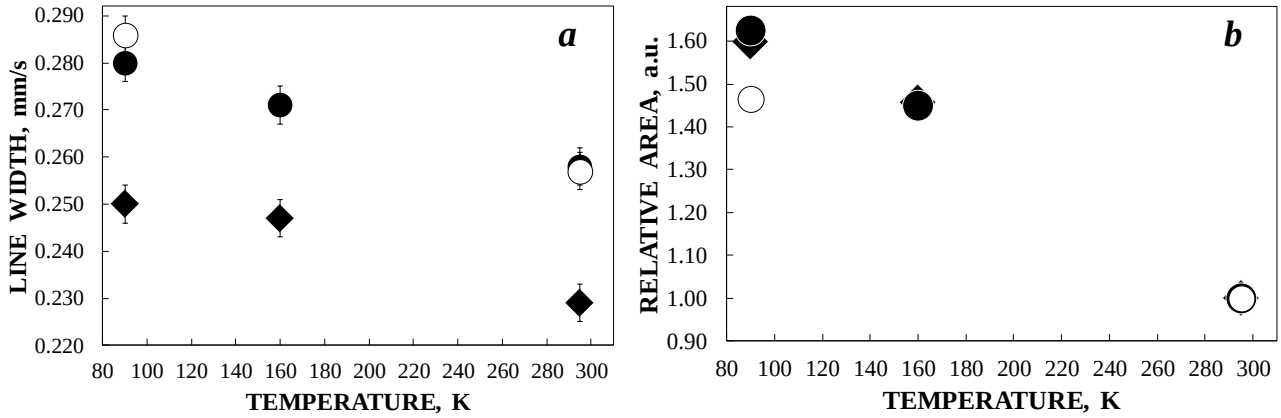

a
b
LINE WIDTH, mm/s
RELATIVE AREA, a.u.
TEMPERATURE, K
TEMPERATURE, K

Fig. 1.

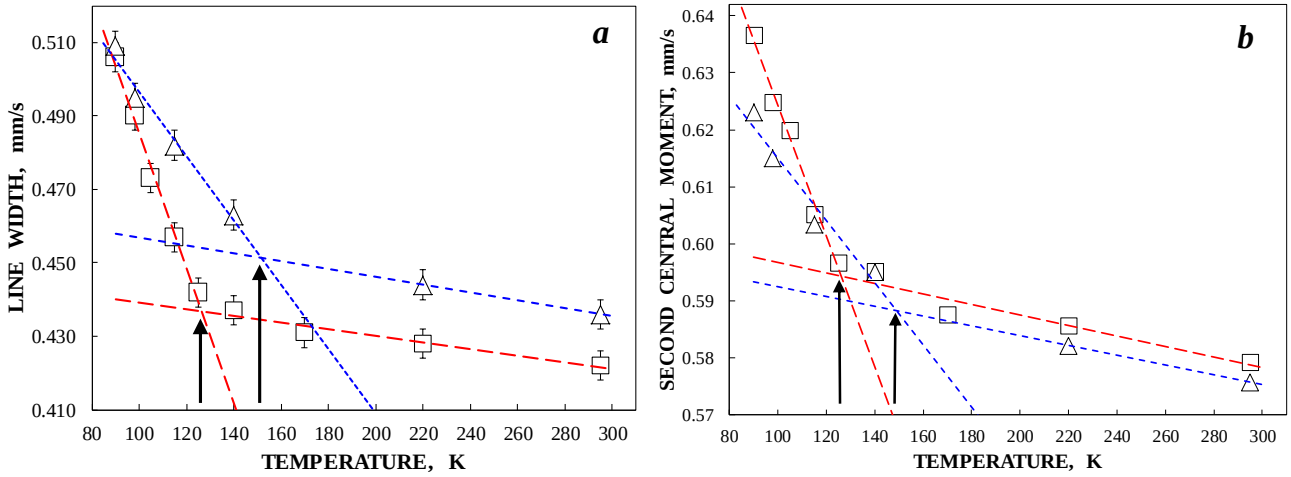
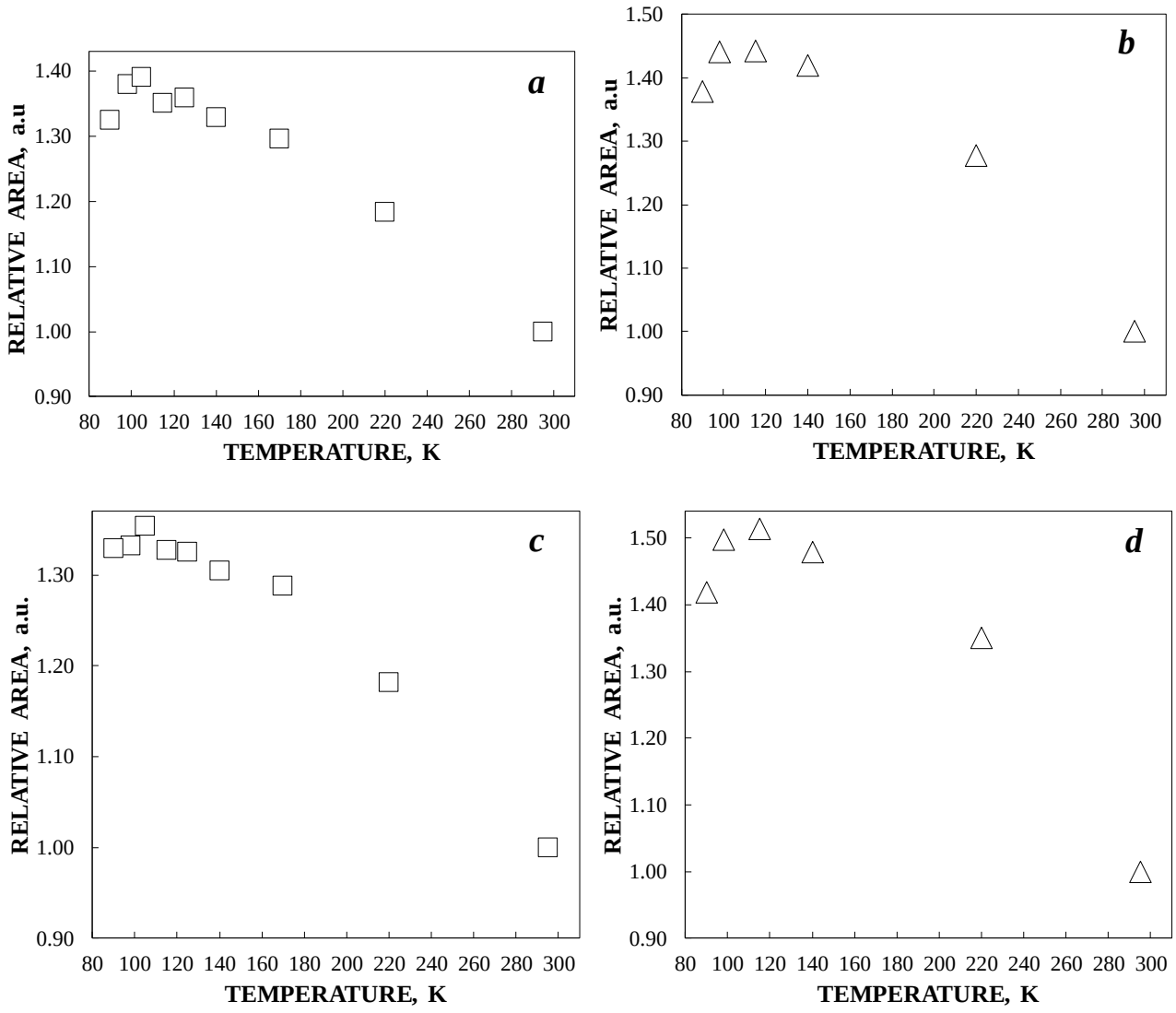

a
b
LINE WIDTH, mm/s
SECOND CENTRAL MOMENT, mm/s
TEMPERATURE, K
TEMPERATURE, K

Fig. 2.


a
b
c
d
RELATIVE AREA, a.u
RELATIVE AREA, a.u.
TEMPERATURE, K
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1.20
1.30
1.40
1.50
80 100 120 140 160 180 200 220 240 260 280 300

Fig. 3.

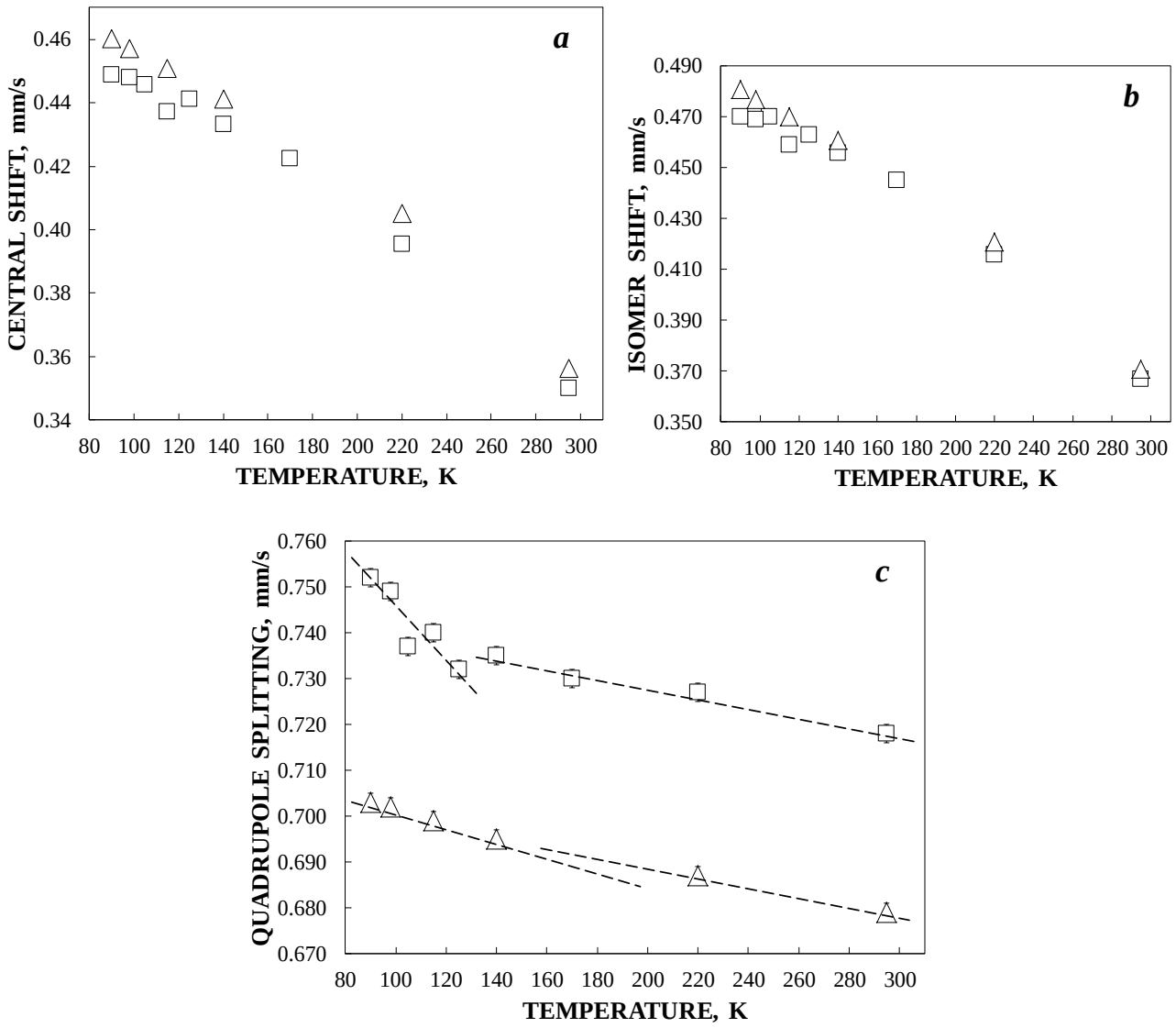
a
CENTRAL SHIFT, mm/s
0.46
0.44
0.42
0.40
0.38
0.36
0.34
80 100 120 140 160 180 200 220 240 260 280 300
TEMPERATURE, K
b
ISOMER SHIFT, mm/s
0.490
0.470
0.450
0.430
0.410
0.390
0.370
0.350
80 100 120 140 160 180 200 220 240 260 280 300
TEMPERATURE, K
c
QUADRUPOLE SPLITTING, mm/s
0.760
0.750
0.740
0.730
0.720
0.710
0.700
0.690
0.680
0.670
80 100 120 140 160 180 200 220 240 260 280 300
TEMPERATURE, K

Fig. 4.