Mössbauer spectroscopy of frozen solutions as a stepwise control tool in preparation of biocompatible humic-stabilized feroxyhyte nanoparticles

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Abstract Mössbauer spectroscopy of frozen aqueous solutions was demonstrated as an efficient stepwise control technique in the new one-pot synthesis of biocompatible feroxyhyte (δ'-FeOOH) nanoparticles in situ stabilized by humic substances. Formation of ultradispersed $Fe(OH)_2$ as an intermediate product and its interaction with humic substances were ascertained. The interaction of the surface of $Fe(OH)_2$ with humic substances was considered as a starting point in the mechanism of in situ stabilization and further growth control of humic-stabilized feroxyhyte nanoparticles.

Keywords Mössbauer spectroscopy · Frozen solutions ·
Superparamagnetic iron oxide nanoparticles · Humic substances ·
In situ stabilization

Abbreviations

SPION	superparamagnetic iron oxide nanoparticles
FAS	frozen aqueous solutions
HS	humic substances

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XRD   X-ray diffraction
SPM   superparamagnetic
RFBR   Russian Foundation for Basic Research

1 Introduction

In the past decade, biocompatible superparamagnetic iron oxide nanoparticles (SPION) have attracted growing attention due to their unique perspectives in biosensing, targeted drug delivery, magnetic resonance imaging and immunosorbent assay [1].

The different synthetic routes in aqueous systems (e.g. precipitation or coprecipitation) are more appropriate for production of biocompatible SPION from biomedical and safety points of view. However, the as-prepared nanoparticles suffer from a broad size distribution, irregular crystalline shapes and strong aggregation because of difficulties in the control of nucleation and growth processes [2]. In order to solve these problems, one-pot techniques are widely used for the preparation of commercial SPION. In this case, the nanoparticles form in situ in the presence of stabilizers (natural and synthetic macromolecules, surfactants, etc.) limiting growth of the SPION and stabilizing their hydrosol [3]. Nevertheless, there are serious difficulties in controlling the phase composition of the nanoparticles in fast reactions of multicomponent systems, especially in the case of synthesis of iron oxides which are susceptible to various interphase transformations. Hence a facile and informative technique for stepwise controlling the processes of in situ stabilized SPION formation still remains a great challenge.

Mössbauer spectroscopy of frozen aqueous solutions (FAS) is widely used for studies of structure and magnetic behavior of iron solvated complexes [4–6], Fe^{2+} and Fe^{3+} porphyrins (hemes and hemins) [7–9], haem proteins [10] and red blood cells [11], etc. It is also used to study chemical reactions such as reduction of iron(VI) in aqueous medium [12] and formation of iron oxyhydroxides and oxides in salt solutions [13]. Thus, quick freezing is an especially powerful approach to study fast processes because it allows to sense and identify intermediates and metastable phases.

In the present work, Mössbauer spectroscopy of FAS was suggested as a stepwise control tool in the one-pot synthesis of magnetic feroxyhyte (δ'-FeOOH) nanoparticles stabilized by humic substances (HS). HS present themselves natural polyelectrolytes which can be used as stabilizers to prevent nanoparticle agglomeration and precipitation [14, 15].

2 Experimental

High-purity $FeCl_2 \cdot 4H_2O$ was purchased from ABCR GmbH&Co (Germany). Potassium humate was purchased from Sakhalinsky Humates (Russia). According to manufacturer's information, potassium humate was produced by alkaline extraction from leonardite. The chemical composition of the humate is given in Online Resource 1. All other reactants were of analytical grade. All solutions were prepared using deionized water.
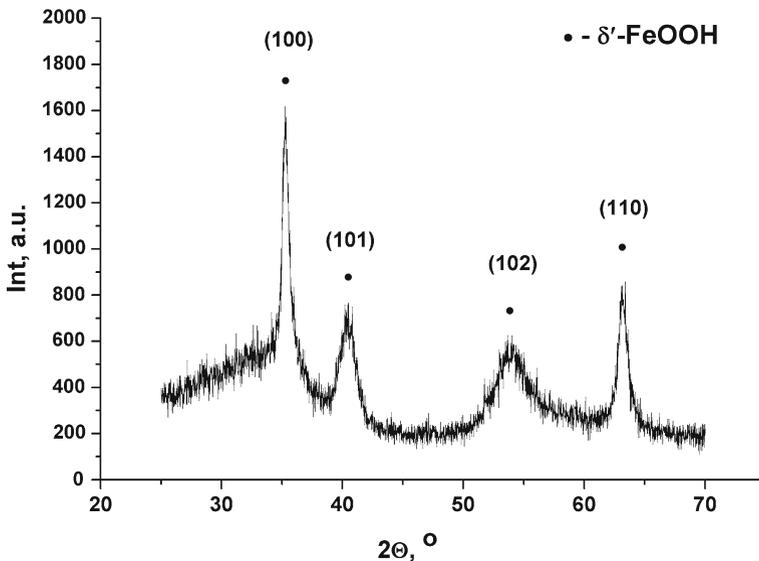
Fig. 1 XRD pattern of the feroxyhyte nanoparticles synthesized by the one-pot synthesis in presence of potassium humate solution

The feroxyhyte nanoparticles were synthesized using one-pot technique involving quick oxidizing of 0.1 M $FeCl_2$ solution by 30 % H_2O_2 at pH 8 in presence of 100 mg/L of HS. At the key points of the synthesis aliquot samples of reaction mixture were taken and rapidly frozen by immersion into liquid nitrogen for further low-temperature Mössbauer studies. A series of samples obtained without HS was used as a blank.

Mössbauer spectra were registered using a MS1104EM express Mössbauer spectrometer (MosTek, Rostov-on-Don) in transmission geometry with a ^{57}Co/Rh sources (activity 3.5 and 10 mCi, Cyclotron, Obninsk) at 78 K. Isomer shifts were measured using α-Fe as a standard. Temperature was maintained within ± 1 K. The spectra were fitted using Univem MS 5.01 software program (MosTec Ltd., Russia).

X-ray powder diffraction (XRD) data were collected in a step scan mode at room temperature using a Rigaku D/Max 2,500 diffractometer with a rotating anode (Cu-Kα radiation, θ–2 θ Bragg–Brentano geometry, 25–70° 2 θ range, 0.02° step). For phase identification, the ICDD PDF2 database [16] was used.

3 Results and discussion

The product synthesized by the one-pot synthesis in the presence of potassium humate solution demonstrates mainly XRD peaks corresponding to δ'-FeOOH (ICDD PDF2 entry #13-87, Fig. 1). Micrographs of the nanoparticles obtained are shown in Online Resource 2.

The curve schematically illustrating the pH changes during the synthesis and the points in which aliquots of reaction mixture were taken for Mössbauer spectroscopy analysis of FAS is presented in Fig. 2.
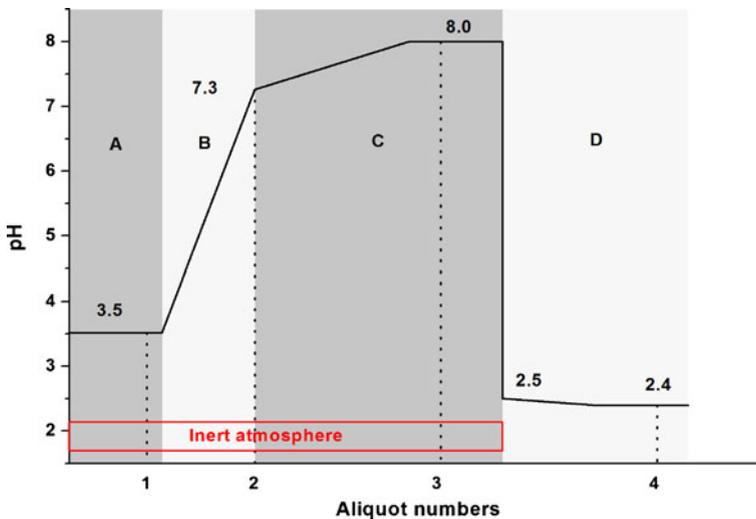
Fig. 2 pH changes during the one-pot synthesis of humic-stabilized feroxyhyte nanoparticles.
Stage A initial 0.1 FeCl$_2$ solution; *stage B* gradual addition of alkaline solution of potassium humate;
stage C addition of 0.1 M NaOH up to pH 8 and a little delay before starting the oxidation; *stage D*
rapid addition of 30 % H$_2$O$_2$ and equilibration. The *dotted lines* show the points in which the aliquot
samples of this reaction mixture were frozen for a Mössbauer study. Blank HS-free samples were
taken at the same points with the exception of the aliquots 1 and 2

The Mössbauer spectrum of a frozen light-greenish FeCl$_2$ solution (stage A,
Fig. 2; aliquot 1, Fig. 3) was fitted by two doublets with isomer shifts of 1.37 mm/s
and 1.35 mm/s and quadrupole splittings of 3.27 mm/s and 2.78 mm/s, respectively
(components A1–A2, Table 1). Component A1 corresponds to Fe(H$_2$O)$_6^{2+}$ ion in a
cubic ice phase [17]. Component A2 can be ascribed to a hydrate of ferrous chloride
with distorted octahedral structure Fe(H$_2$O,Cl$_5$)$^{3-}$ [18], which can crystallize out
during freezing of the solution.

After addition of a potassium humate alkaline solution (stage B, Fig. 2), the
synthetic mixture became dark brown. However it did not have significant effect
on the Mössbauer spectrum of the frozen reaction mixture (aliquot 2, Fig. 3); the
parameters of components B1 and B2 are similar to the ones of doublets A1 and
A2 (Table 1). Thus there is neither interaction between HS and ferrous ions nor
formation of solid phases at this stage of the synthesis.

During gradual addition of NaOH (stage C, Fig. 2), the pH changed very slowly
and a light-colored suspension appeared in the reaction mixture. The Mössbauer
spectrum of the reaction mixture taken after the pH has been set to 8.0 (aliquot 3,
Fig. 3) consists of two doublets with similar parameters (components C1–C2, Table 1)
which are close those of Fe(OH)$_2$ [19]. As long as the spectrum of the respective
humic-free reaction mixture (blank 3, Fig. 3) contains only one component C*1 with
isomer shift of 1.29 mm/s and quadrupole splitting of 3.01 mm/s, component C1 was
ascribed to bulk Fe(OH)$_2$ whereas component C2 was attributed to an interaction of
HS with the surface of Fe(OH)$_2$.

After addition of 30 % H$_2$O$_2$, the reaction mixture became reddish-brown and
its composition changed dramatically. The Mössbauer spectrum of the frozen aliquot
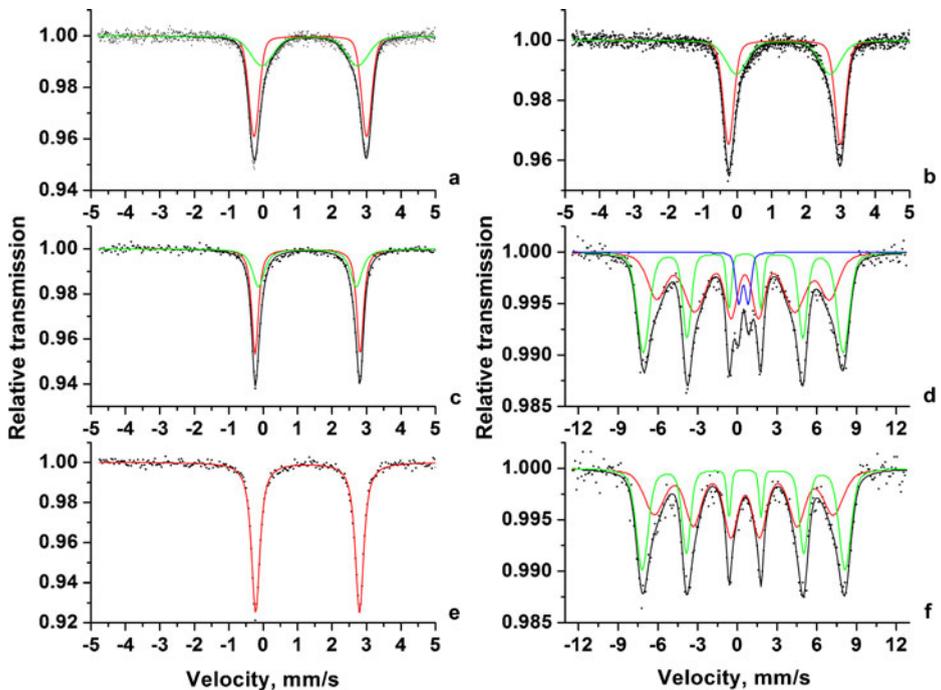
Fig. 3 The Mössbauer spectra of frozen reaction mixtures registered at 78 K: **a** aliquot 1, **b** aliquot 2, **c** aliquot 3, **d** aliquot 4, **e** blank 3, **f** blank 4. *Black dots* represent experimental spectra, *black curves* are the calculated spectra, and *colored curves* indicate components of the calculated spectra

taken after equilibration (aliquot 4, Fig. 3) was fitted by a superposition of two sextets and one doublet. Isomer shifts and quadrupole splittings of the sextets (components D1–D2, Table 1) coincide with the ranges published elsewhere (samples δ-FeOOH [20] and δ-FeOOH [21], Table 1) for magnetically ordered (ferrimagnetic) feroxyhyte. The existence of two different magnetic components in feroxyhyte at liquid nitrogen temperature is usually ascribed to random occupation by Fe^{3+} ions of the octahedral (with higher internal field) and tetrahedral (with lower internal field) positions in the oxygen-hydroxide lattice of δ′-FeOOH [21]. The internal magnetic field decrease of about 5–15 % is expected for small particles below the blocking temperature [22]. The doublet (component D3, Table 1) also observed in [23] was ascribed to a residual superparamagnetic (SPM) state of SPIONs with lower blocking temperature. At the same time the spectrum of a humic-free sample (blank 4, Fig. 3) has only two sextets (components D*1–D*2, Table 1) indicating the ferrimagnetic state of feroxyhyte nanoparticles and the absence of any residual superparamagnetic phases due to a larger particle size. The higher internal magnetic fields of both the sextets in comparison with a humic-stabilized sample also corroborate the increase of particle sizes. This leads us to a suggestion that the presence of HS in the reaction mixture prevents excessive growth of feroxyhyte nanoparticles, decrease their blocking temperature and thus preserves them in a SPM state. These results are concordant with our previous temperature-dependent Mössbauer study of dry

Table 1 Hyperfine parameters of frozen samples of the reaction mixture; δ – isomer shift relative to α-Fe, Δ – quadrupole splitting or shift, Γ_{exp} – the full line width at half height, H_{in} – internal magnetic field (Oe)

Sample / temperature	Component	δ mm/s	Δ mm/s	Γ_{exp} mm/s	H_{in} kOe	S, %
Experimental data		±0.03 mm/s	±0.03 mm/s	±0.03 mm/s	±5 kOe	±1 %
Aliquot 1/78 K	A1	1.37	3.27	0.36		63
	A2	1.35	2.76	0.78		37
Aliquot 2/78 K	B1	1.37	3.25	0.37		61
	B2	1.33	2.73	0.73		39
Aliquot 3/78 K	C1	1.29	3.02	0.24		62
	C2	1.28	2.83	0.41		38
Aliquot 4/78 K	D1	0.50	-0.13	1.65	405	53
	D2	0.51	-0.13	1.11	470	41
	D3	0.46	0.71	0.54		6
Blank 3/78 K	C*1	1.29	3.01	0.30		100
Blank 4/78 K	D*1	0.54	-0.10	1.81	420	56
	D*2	0.53	-0.11	1.07	475	44
Literature data						
$\delta-$FeOOH [20]/ 77 K	R1	0.45 ± 0.11	0 ± 0.1		533 ± 51	
$\delta-$FeOOH [21]/ 80 K	R2	0.69 ± 0.07	-0.15 ± 0.05		525 ± 5	
	R3				505 ± 5	

feroxyhyte nanoparticles showing a pronounced decrease of the blocking temperature in humic-stabilized samples [24].

As long as the Mössbauer spectra of the reaction mixture at stage C show a specific interaction between ultradispersed $Fe(OH)_2$ with HS (Fig. 3; Table 1), we conclude that this interaction is a starting point in the mechanism of in situ stabilization and growth control of feroxyhyte nanoparticles by HS in the described one-pot synthesis. The $Fe(OH)_2$ nanocrystals become entrapped into the HS "matrix" and $Fe(OH)_2$-HS conglomerates are formed. These conglomerates operate as growth domains in the reaction mixture; Fe^{2+} and OH^- ions diffusing into the limited volume of such domains become distributed among several embryocrystals. Such an ion transport would be also affected by a partial negative charge of the HS branches catching cations and repelling anions. These factors lead to slower growth of $Fe(OH)_2$ particles of the domain in comparison with non-embedded embryocrystals. As long as rapid oxidation of ferrous hydroxide embryocrystals by 30 % H_2O_2 leads to topotactic $Fe(OH)_2$ to δ'-FeOOH transition, the small feroxyhyte nanoparticles formed remain stabilized by the HS "matrix" [24].

4 Conclusions

A new Mössbauer spectroscopy study of FAS has shown that the ultradispersed $Fe(OH)_2$ formed as an intermediate product in the synthesis of feroxyhyte nanoparticles interacts with HS. According to the results obtained, this stage is a key point in the mechanism of in situ stabilization by HS which leads to prevention of excessive growth of the ultradispersed solid phase during further $Fe(OH)_2$ oxidation and

promotes formation of SPM feroxyhyte nanoparticles with lower size and blocking temperature.

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