

# Mössbauer spectroscopy of protein-passivated iron oxide nanoparticles

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**Abstract** Within this work we report a Mössbauer spectroscopic study of uncoated and bovine serum albumin (BSA-) passivated magnetic iron oxide particles. Electron microscopy confirms the successful preparation of the particles with sizes in the 10 to 30 nm range. The analysis of the Mössbauer spectra at high fields shows that only 20% of the iron is present in form of  $\text{Fe}_3\text{O}_4$ -nanoparticles. The majority (80%) of the iron is present as maghemite ( $\gamma\text{-Fe}_2\text{O}_3$ ). Coating with BSA does not influence the particle morphology and does also not affect the magnetite to maghemite ratio.

**Keywords** Maghemite · Magnetite · Nanoparticles · Mössbauer spectroscopy · Bovine serum albumin

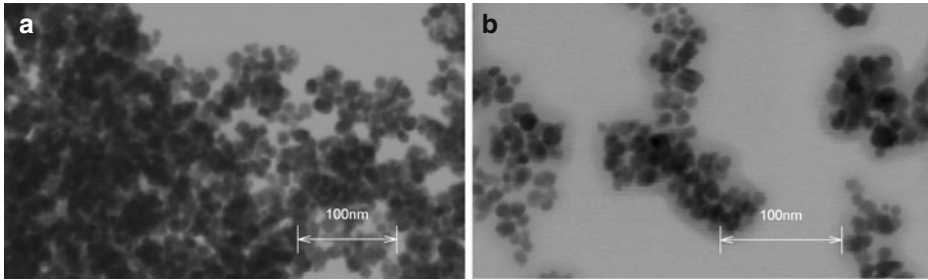
## 1 Introduction

Magnetic iron oxide nanoparticles have potential applications in thermal therapy, as magnetic carriers for medical drugs, as contrast agents in magnetic resonance imaging or as carriers for precious noble metals in reusable catalysts for liquid phase reactions. A major problem which has to be overcome is the lack of long-time stability of the magnetic carrier particles e.g. in catalytic applications. Therefore passivation of the particle surface is often performed by preparing metal nanoparticles with silica or polymer coatings. Recently the preparation of protein-passivated  $\text{Fe}_3\text{O}_4$  nanoparticles with low toxicity and rapid heating properties for thermal therapy has been reported [1]. The preparation of the  $\text{Fe}_3\text{O}_4$  nanoparticles has been done according to Massart's co-precipitation method [1, 2] and Bovine serum albumin (BSA) has been

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**Fig. 1** Transmission electron micrographs of as prepared magnetic iron oxide particles according to Massart's method [1, 2] before (a) and after coating with BSA (b)

used as coating for the iron oxide particles. The protective BSA layer ensures not only high stability and low cytotoxicity, but also relatively easy biofunctionalization by means of attaching targeting molecules via the surface lysine groups of the protein [1]. Within this work we report a Mössbauer spectroscopic study of these BSA-passivated magnetic iron oxide particles, which has at least to our knowledge not been performed up to now.

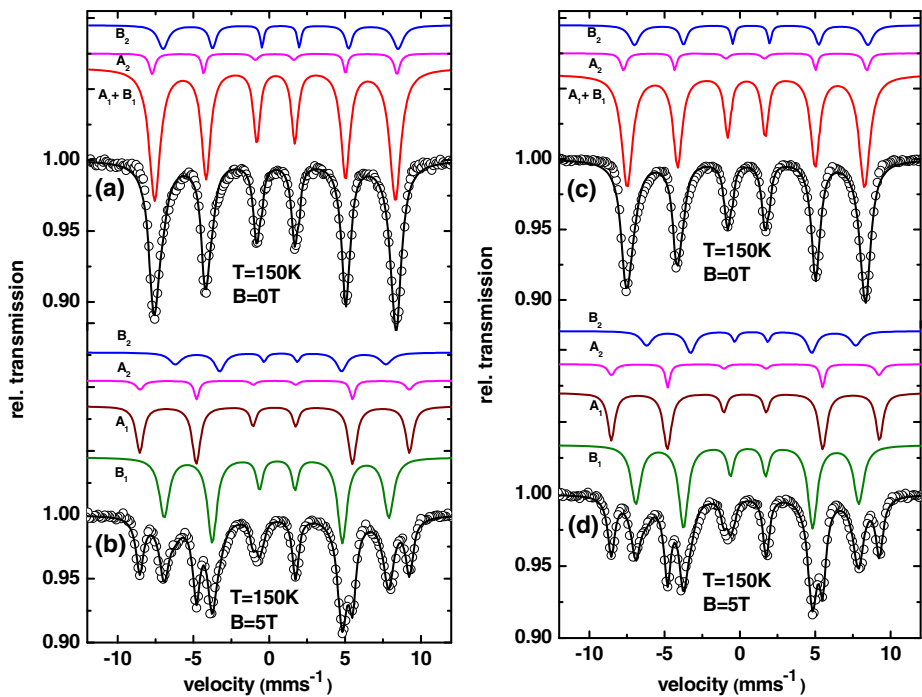
## 2 Materials and methods

Bovine Serum Albumin (BSA) was purchased by Carl Roth GmbH. The magnetic iron oxide particles were prepared by coprecipitation of Fe(II) and Fe(III) chlorides according to Massart's method [2]. With maintaining a molar ratio of  $\text{Fe}^{2+}:\text{Fe}^{3+} = 1:2$ , 0.0575 g  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  and 0.1575 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  were dissolved in 10 ml milliQ water under nitrogen. After 10 min purging with nitrogen, 2.5 ml  $\text{NH}_4\text{OH}$  was added to the solution. The formed black solution was incubated at  $90^\circ\text{C}$  for 20 min and then cooled down to room temperature. The black precipitate was collected with a magnet and washed twice with water. The BSA-coated iron oxide nanoparticles were prepared using the iron oxide nanoparticles and 250 mg BSA, which was each added to 20 ml milliQ water [1]. The mixture was sonicated for 4 h. The particles were collected with a magnet and washed twice with water in order to remove excess BSA. Mössbauer spectra were measured in a closed cycle cryostat equipped with a superconducting magnet (Cryo Industries of America Inc.) and recorded using a conventional spectrometer in the constant-acceleration mode. Isomer shifts are given relative to  $\alpha\text{-Fe}$  at room temperature. The spectra were analyzed by least-square fits using Lorentzian line shapes.

## 3 Results and discussion

Transmission electron micrographs of the as prepared magnetic iron oxide particles are shown in Fig. 1. Both, the uncoated iron oxide particles (Fig. 1a) and the BSA-passivated iron oxide particles (Fig. 1b) have a mean diameter of 12 nm. Obviously the BSA-coating does neither influence the size nor the morphology of the iron oxide particles.

Mössbauer spectra of the uncoated iron oxide nanoparticles obtained at 150 K in zero magnetic field as well in an external field of 5 T are displayed in Fig. 2a and b.



**Fig. 2** (left) Mössbauer spectra of as prepared iron oxide particles obtained at 150 K in zero field (a) and in a field of 5 T perpendicular to the  $\gamma$ -ray (b). (right) Mössbauer spectra of BSA-passivated iron oxide particles obtained at 150 K in zero field (c) and in a field of 5 T perpendicular to the  $\gamma$ -ray (d). The solid lines represent the result of an analysis with symmetrical sextets (intensity ratio 3:2:1 for (a) and 3:4:1 for (b)) and parameters given in Table 1

The majority of the spectral area ( $\sim 80\%$ ) in the high field spectrum consists of two sextets with opposite hyperfine fields (30% for component  $A_1$  and 51% for component  $B_1$ ). The ratio of both spectral areas  $A_1/B_1$  equals about to 0.59, which is the ratio expected for the ferrimagnetic  $\gamma$ - $\text{Fe}_2\text{O}_3$  spinel with  $(\text{AB}_2\text{O}_4)$  structure, if one assumes that all  $\text{Fe}^{3+}$  vacancies are located on the octahedral B-lattice [3]. The isomer shift for  $A_1$  ( $\delta_{A_1} = 0.35 \text{ mms}^{-1}$ ) and  $B_1$  ( $\delta_{B_1} = 0.51 \text{ mms}^{-1}$ ) are both characteristic for  $\text{Fe}^{3+}$  ions. Therefore we conclude that component  $A_1$  represents  $\text{Fe}^{3+}$  ions in the tetrahedral sublattice and component  $B_1$  represents  $\text{Fe}^{3+}$  ions in the octahedral B lattice sites of ferrimagnetic  $\gamma$ - $\text{Fe}_2\text{O}_3$ .

A minority of the spectral area (20%) with the components  $A_2$  and  $B_2$  show indeed characteristic Mössbauer parameters of  $\text{Fe}_3\text{O}_4$ . The isomer shift of component  $B_2$  ( $\delta_{B_2} = 0.75 \text{ mms}^{-1}$ ) is characteristic for  $\text{Fe}^{2.5+} - \text{Fe}^{2.5+}$  ions in the octahedral B lattice sites of  $\text{Fe}_3\text{O}_4$  at temperatures above the Verwey transition ( $T_v \cong 110 \text{ K}$ ) where electron hopping and spin pairing occurs. This is also consistent with the antiparallel alignment of the internal hyperfine field of  $B_2$  with the external field. Component  $A_2$  exhibits  $\delta_{A_2} = 0.35 \text{ mms}^{-1}$  and its internal hyperfine-field aligns parallel with the external field, a behavior which is typical for  $\text{Fe}^{3+}$  ions of the tetrahedral A sublattice of magnetite [4].

**Table 1** Mössbauer parameters of the uncoated and BSA coated magnetic iron oxide nanoparticles as obtained from the analysis of the spectra shown in Fig. 2

B(T)	Component	Rel. area(%)	$\delta(\text{mms}^{-1})$	$\Delta E_Q(\text{mms}^{-1})$	$\Gamma_{1,2,3}(\text{mms}^{-1})$	$B_{\text{hf}}(\text{T})$
Uncoated magnetic iron oxide nanoparticles						
0	A <sub>1</sub> +B <sub>1</sub>	80.2	0.41	0	0.9,0.7,0.5	49.2
	A <sub>2</sub>	6.6	0.35	0	0.5,0.3,0.5	50
	B <sub>2</sub>	13.2	0.75	0	0.8,0.6,0.3	48
5	A <sub>1</sub>	30.6	0.35	0	0.6,0.6,0.5	55.1
	B <sub>1</sub>	51.1	0.51	0	0.8,0.7,0.5	45.9
	A <sub>2</sub>	6.1	0.35	0	0.6,0.4,0.4	55
	B <sub>2</sub>	12.2	0.75	0	1.0,0.8,0.4	43
BSA coated magnetic iron oxide nanoparticles						
0	A <sub>1</sub> +B <sub>1</sub>	80.2	0.42	0	0.9,0.7,0.5	48.8
	A <sub>2</sub>	6.6	0.35	0	0.5,0.3,0.5	50
	B <sub>2</sub>	13.2	0.75	0	0.8,0.6,0.3	48
5	A <sub>1</sub>	29.9	0.35	0	0.6,0.6,0.5	55.2
	B <sub>1</sub>	49.7	0.52	0	0.8,0.7,0.5	45.8
	A <sub>2</sub>	6.8	0.35	0	0.6,0.4,0.5	55
	B <sub>2</sub>	13.6	0.75	0	0.9,0.8,0.4	43

In conclusion this study shows univocally that the main phase of the particles prepared according to Massart's method is not Fe<sub>3</sub>O<sub>4</sub> as in general believed but  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>. Coating with BSA does not influence the particle morphology and does also not affect the magnetite to maghemite ratio.

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