

## SHORT COMMUNICATION

Magnetically Modified Bentonite as a Possible Contrast Agent  
in MRI of Gastrointestinal Tract\*

H. BARTONKOVA, M. MASHLAN\*\*, I. MEDRIK, D. JANCIK, and R. ZBORIL

*Research Centre of Nanomaterials, Palacký University, Svobody 26, 771 46 Olomouc, Czech Republic  
e-mail: miroslav.maslan@upol.cz*

Received 9 November 2006; Revised 10 April 2007; Accepted 16 April 2007

A composite of iron oxide nanoparticles and mineral matrix has been studied by XRD, Mössbauer spectroscopy, and TEM. Magnetite and superparamagnetic magnetite have been identified by Mössbauer spectroscopy in the nanocomposite. A relationship between the hyperfine parameters and iron oxide particle size has been confirmed by TEM. The optimal concentration of “magnetite—bentonite” composite, when the MRI signal is fully reduced, was found for using this composite as a negative contrast agent.

**Keywords:** magnetite, bentonite, nanoparticles, MRI, Mössbauer spectroscopy

The role of magnetic resonance imaging (MRI) as an important medical diagnostic method has been increasing recently. MRI shows very good sensitivity to tissue differences. On the other hand, it is an expensive, but risk-free procedure. The application of MRI in gastrointestinal (GI) tract is partially limited due to various problems including the necessity to use oral contrast agents to improve the image contrast. Contrast agents work by increasing (positive agents) or decreasing (negative agents) the signal intensity from the GI tract [1].

Most negative contrast agents are superparamagnetic substances based on iron oxides, *e.g.* suspension of miscible superparamagnetic iron oxide nanoparticles in the form of their aggregates coated with siloxane [1, 2] and superparamagnetic ferrite crystals incorporated into monodisperse polymer particles [1, 3, 4].

A new composite of superparamagnetic iron oxide nanoparticles and bentonite, which can be used as an oral contrast agent for MRI of the GI tract, has been synthesized and characterized in this study. Nanoparticles of superparamagnetic magnetite or maghemite guarantee the contrast effect and bentonite acts as in-

ert matrix reducing absorption of iron and as an agent improving dispersion of iron oxide particles. Bentonite is clay formed frequently from the alteration of volcanic ash, consisting predominantly of smectite minerals, usually montmorillonite. Bentonite exhibits strong colloidal properties and its volume increases several times in contact with water, creating a gelatinous and viscous fluid [5].

Magnetic modification of natural bentonite is a two-step process. The first step is based on adsorption of iron cations on a mineral surface. The second step involves their conversion to magnetic iron oxide, maghemite, or magnetite [6].

A suspension of bentonite in distilled water (5 g L<sup>-1</sup>) was prepared by putting fine grained mineral on the surface of water, allowing it to settle down onto the bottom of the vessel and fairly stirring the vessel content. To the suspension containing 1 g of bentonite, iron(II) sulfate solution (0.85 g FeSO<sub>4</sub> · 7H<sub>2</sub>O in 10 mL of distilled water) was added. The mixture was stirred for two hours with a magnetic stirrer. Solutions of potassium nitrate (0.5 g KNO<sub>3</sub> in 10 mL of distilled water) and potassium hydroxide (0.7 g KOH in 10 mL of distilled water) were added in this or-

\*Presented at the XVIIIth Slovak Spectroscopic Conference Spišská Nová Ves, 15—18 October 2006.

\*\*The author to whom the correspondence should be addressed.

**Table 1.** Assignment of Prepared Samples

Bentonite to iron mass ratio	1 : 0	20 : 1	5 : 1	1 : 2	0 : 1
Sample assignment	I	II	III	IV	V

der to the stirred suspension. Dark green precipitate, containing  $\text{Fe}(\text{OH})_2$ , was obtained. Then, the mixture was heated up to  $90^\circ\text{C}$ . When the color of the precipitate changed to dark brown, heating was stopped and the suspension was stirred until it cooled to room temperature. The composite was separated by filtration and air-dried at room temperature. The clay to iron mass ratio was 5 : 1 in this composite (sample III, Table 1). Other samples with different bentonite/iron mass ratios were prepared as listed in Table 1.

An X'Pert PRO instrument emitting  $\text{CoK}\alpha$  radiation was employed in the XRD analyses. Phase composition of the samples was evaluated using an X'Pert HighScorePlus software package (PANanalytical®) and the JCPDS PDF-4 database.

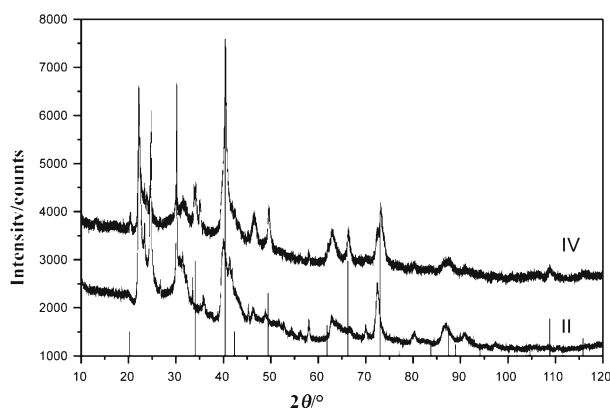
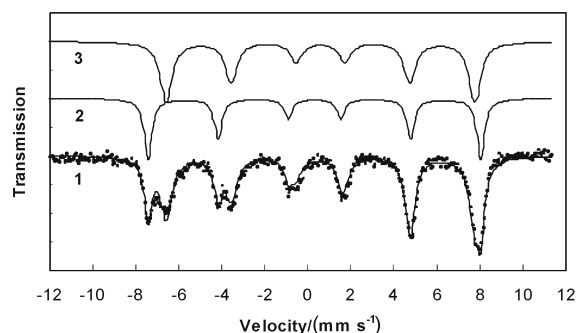
TEM images of the nanocomposites were obtained using a JEM 2010 microscope operating at 200 kV. Sample for the TEM analysis was prepared by placing a drop of ethanol suspension onto a carbon-coated copper grid and allowing it to dry.

$^{57}\text{Fe}$  Mössbauer spectra were measured using a standard transmission method in a constant acceleration mode with a  $^{57}\text{Co}(\text{Rh})$  radioactive source. The spectra were collected at temperatures of 50 K and 300 K using a closed He-cycle cryogenic system. The values of the isomer shift are related to  $\alpha\text{-Fe}$  at room temperature. For computer processing of the spectra, a NORMOS package was used yielding the values of the relative spectrum area  $A$  and the values of the hyperfine parameters including hyperfine magnetic field  $B_{\text{HF}}$ , isomer shift  $\delta$ , quadrupole splitting  $\Delta$ , and quadrupole shift  $\varepsilon$ .

The applicability of the synthesized composite as an MRI contrast agent has been tested on MR 1.5T ES EXCITE ECHOSPEED FIXED (GE MEDICAL SYSTEMS). The samples for the MRI were prepared by suspending the composite in an isoosmotic solution.

The X-ray patterns of samples II and IV demonstrate that diffraction lines corresponding to magnetite appear after the synthesis procedure (Fig. 1).

Although the amount of iron in the original natural bentonite (sample I) is very low, its Mössbauer spectrum recorded at room temperature (RT) consists of an  $\text{Fe}^{2+}$  doublet ( $\delta_{\text{Fe}} = 1.22 \text{ mm s}^{-1}$ ,  $\Delta E_{\text{Q}} = 2.69 \text{ mm s}^{-1}$ ) and an  $\text{Fe}^{3+}$  doublet ( $\delta_{\text{Fe}} = 0.30 \text{ mm s}^{-1}$ ,  $\Delta E_{\text{Q}} = 0.60 \text{ mm s}^{-1}$ ). The Mössbauer spectrum of sample V confirmed that the described synthesis procedure leads to pure magnetite. The spectrum consists of two sextets, one sextet with  $\delta_{\text{Fe}} = 0.59 \text{ mm s}^{-1}$ ,  $B_{\text{HF}} = 44.5 \text{ T}$  corresponding to octahedrally coordinated

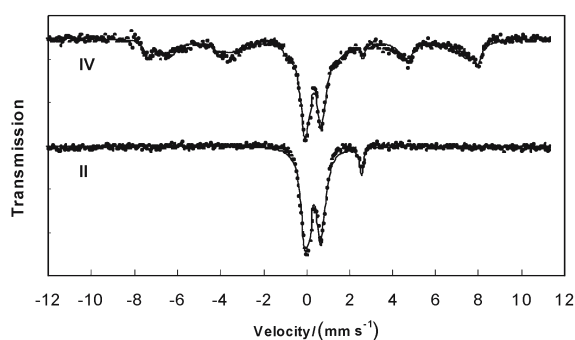
**Fig. 1.** X-Ray diffraction patterns of samples II and IV: vertical lines show the position of magnetite diffraction lines.**Fig. 2.** RT Mössbauer spectrum of sample V: experimental data (1), fitted subspectra of tetrahedrally coordinated  $\text{Fe}^{3+}$  (2) and octahedrally coordinated  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  (3).

$\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  (B site) and the second sextet with  $\delta_{\text{Fe}} = 0.31 \text{ mm s}^{-1}$  and  $B_{\text{HF}} = 47.9 \text{ T}$  corresponding to tetrahedrally coordinated  $\text{Fe}^{3+}$  (A site) (Fig. 2). The ratio of the relative areas of the sextets corresponding to A and B sites is close to the theoretical value of 1 : 2. This fact verifies that the synthesized Fe species represent magnetite [7]. The lower values of  $B_{\text{HF}}$  in comparison to  $B_{\text{HF}}$  for bulk magnetite ( $B_{\text{HF}} = 49 \text{ T}$  and  $B_{\text{HF}} = 46 \text{ T}$  for the A and B sites, respectively) are connected with different particle sizes. The value of isomer shift ( $\delta_{\text{Fe}} = 0.59 \text{ mm s}^{-1}$ ) of the B sextet indicates a mixture of divalent and trivalent iron.

RT Mössbauer spectrum (Fig. 3, Table 2) of sample IV as well as that of composite III (its spectrum is not displayed in the figure), consists of two sextets, typical for magnetically ordered magnetite and two doublets belonging to superparamagnetic magnetite and  $\text{Fe}^{2+}$  ions, respectively. Hyperfine parameters of synthesized composites are summarized in Table 2. The ratio of the relative areas of the sextets corresponding to A and B sites is close to the value of 1 : 4. This value differs from the ratio of 1 : 2 for a stoichiometric magnetite, which means that the magnetite

**Table 2.** Hyperfine Parameters of RT Mössbauer Spectra of Synthesized Composites

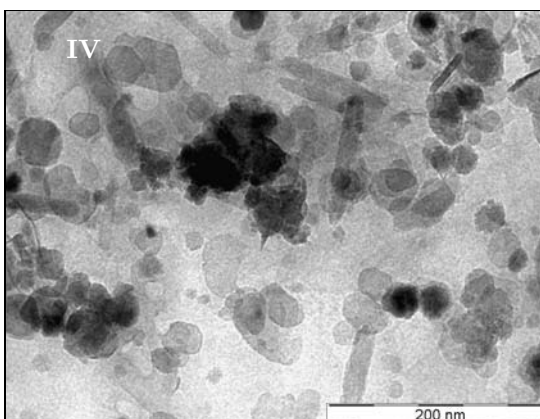
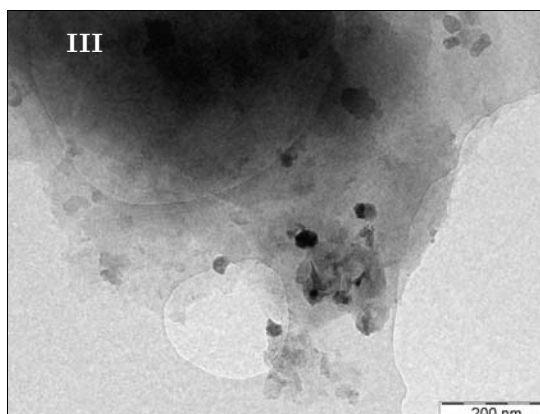
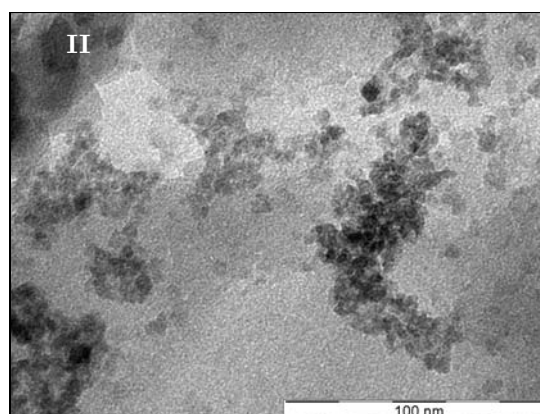
Sample	$\delta_{\text{Fe}}/(\text{mm s}^{-1})$	$\Delta E_{\text{Q}}/(\text{mm s}^{-1})$	$B_{\text{HF}}/\text{T}$	RA/%	Assignment
II	0.31	0.73	–	88.0	Superparamagnetic magnetite
	1.34	2.47	–	12.0	Rest of $\text{Fe}(\text{OH})_2$
III	0.33	0.76	–	52.1	Superparamagnetic magnetite
	1.30	2.57	–	11.8	Rest of $\text{Fe}(\text{OH})_2$
	0.29	–	45.7	6.6	Site A
	0.56	–	42.1	29.5	Site B
IV	0.31	0.78	–	40.4	Superparamagnetic magnetite
	1.34	2.52	–	4.1	Rest of $\text{Fe}(\text{OH})_2$
	0.31	–	47.6	11.6	Site A
	0.51	–	43.1	43.9	Site B

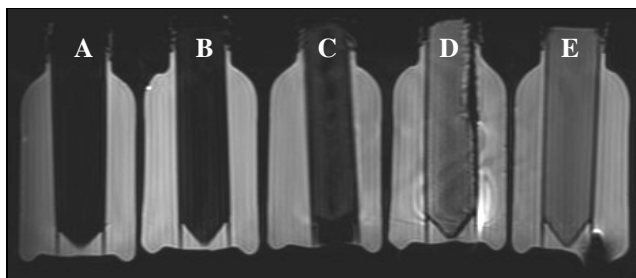
**Fig. 3.** Experimental RT Mössbauer spectra of samples II and IV.

has a low structure ordering. The low magnetic ordering, which arises from a low structure ordering is confirmed by the decreased values of  $B_{\text{HF}}$ . It is noticeable that the fractions of magnetically ordered magnetite and superparamagnetic magnetite change depending on the bentonite/iron mass ratio in the composite. RT Mössbauer spectrum of sample II comprises two doublets, thus, the composite does not contain magnetically ordered magnetite (Fig. 3, Table 2).

In the Mössbauer spectra, the doublet with  $\delta_{\text{Fe}} = 1.30\text{--}1.34 \text{ mm s}^{-1}$  and  $\Delta = 2.47\text{--}2.57$  represents  $\text{Fe}^{2+}$  ions that could be attributed to an interlayer space ions of the mineral or to non-transformed rest of  $\text{Fe}(\text{OH})_2$ . The parameters of the observed doublet are not consistent with the parameters of interlayer  $\text{Fe}^{2+}$  ions [8]. Moreover, a new sample was prepared to confirm that the above-mentioned doublet corresponds to  $\text{Fe}(\text{OH})_2$ . The sample was prepared by mixing the suspension of bentonite with solutions of  $\text{FeSO}_4$ ,  $\text{KNO}_3$ , and  $\text{KOH}$ . Its color was dark green and indicated the presence of  $\text{Fe}(\text{OH})_2$ . The Mössbauer spectrum of this sample contained a doublet with the same hyperfine parameters as those found in the spectra of samples II–IV.

TEM pictures of samples III and IV (Fig. 4) show that the mineral particles contain a lot of iron oxide particles with the mean diameter of about 20 nm. In sample III, single particles are separated and dis-

**Fig. 4.** TEM pictures of samples II–IV.



**Fig. 5.** MR images of A – siloxane spheres with  $\text{Fe}_2\text{O}_3$  and  $\text{Fe}_3\text{O}_4$  core, B – nanocomposite magnetite–bentonite, C – magnetite + bentonite mixture, D – pure magnetite, E – pure bentonite.

tributed on the whole surface area. In sample IV, bigger aggregates of particles are formed because the iron/bentonite mass ratio is higher. In the case of sample II, the diameter of the iron oxide particles is less than 10 nm.

The composite was tested as a possible gastrointestinal contrast agent for MRI [1]. For this purpose, the image of commercial product containing 300 nm aggregates of  $\text{Fe}_2\text{O}_3$  and  $\text{Fe}_3\text{O}_4$  superparamagnetic particles coated with siloxane was compared with that of the magnetite–bentonite particles. The test on phantoms (suspension of contrast agent in isoosmotic solution) is demonstrated in Fig. 5. A full reduction of the MRI signal intensity was observed for sample

II (Fig. 5B) as well as for the commercial product (Fig. 5A), when the total amount of iron was the same in both agents. A mixture of magnetite and bentonite (Fig. 5C) reduced the MRI signal intensity less than the magnetite–bentonite composite but more than pure magnetite and bentonite (Fig. 5D and 5E, respectively).

*Acknowledgements.* Financial support from the Ministry of Education, Youth, and Sports of the Czech Republic under the Research Project 1M0512 is gratefully acknowledged.

## REFERENCES

1. Giovagnoni, A., Fabbri, A., and Maccioni, F., *Abdom. Imaging* 27, 367 (2002).
2. D'Arienzo, A., Scaglione, G., Vicinanza, G., Manguso, F., Bennato, R., Belfiore, G., Imbriaco, M., and Mazzacca, G., *Am. J. Gastroenterol.* 95, 720 (2000).
3. Jung, C. W., *Magn. Reson. Imaging* 13, 675 (1995).
4. Boraschi, P., Braccini, G., Giloni, R., Cartei, F., and Perri, G., *Magn. Reson. Imaging* 22, 1085 (2004).
5. Nesse, W. D., *Introduction to Mineralogy*. Oxford University Press, New York, 2000.
6. Bruce, I. J., Taylor, J., Todd, M., Davies, M. J., Borioni, E., Sangregorio, C., and Sen, T., *J. Magn. Mater.* 284, 145 (2004).
7. Hermanek, M., Zboril, R., Mashlan, M., Machala, L., and Schneeweiss, O., *J. Mater. Chem.* 16, 1273 (2006).
8. Charlet, L. and Tournassat, C., *Aquat. Geochem.* 11, 115 (2005).