Mn Incorporation in Magnetosomes: New Posibilities for the Nanotechnological Applications of Biomagnetite

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INTRODUCTION.

Magnetite (Mgt), a ferrous-diferric oxide (Fe_3O_4), is a commonly-occurring mineral on Earth, usually found in all kind of natural terrestrial environments and even in extraterrestrial material (for a review, see Thomas-Keprta *et al*., 2000). Moreover, thanks to its excellent magnetic properties, it is a material widely used in a number of technological applications, ranging from quantum computing to cancer therapy.

Although it is fairly easy to make small (<10 nm) poorly crystalline and, generally, shapeless magnetic particles via chemical routes, the magnetic moment of such magnetic nanostructures is normally too small to be suitable for practical use (e.g., be manipulated by an applied magnetic field). An ideal material can be described as one with the maximal magnetic moment per particle, where the particles exhibit good crystallinity, belong to one of the highly magnetic compounds, such as Mgt or cobalt ferrite, and are close to the superparamagnetic limit in size, so the particles still remain in a monodomain state (Prozorov *et al*., 2007). These strict requirements make biomineralization one of the most successful and promising approaches, especially the particles produced by biologically controlled mineralization (BCM). In addition, biomineralization pathways offer materials with minimal harm to environment and biocompatibility, much sought in medical applications, including magnetic resonance imaging (MRI).

BCM-Mgts are the result of an exquisitely controlled process exerted by magnetotactic bacteria. Such a control over the biomineralization process result in the production of crystals that are structurally well-ordered, chemically pure, with species-specific crystal habits and narrow size distribution, which imply consistent, well defined and excellent magnetic behavior (usually single magnetic domain).

However, these well defined properties of BCM-Mgts also reduce the range of applications of those Mgts, due to the well constrain and narrow range distribution of the magnetic properties of the Mgts produced by magnetobacteria. Therefore, the main goal of this research is to study the potential incorporation of Mn in BCM-Mgts and the effect of such incorporation on the magnetic properties of BCM-Mgts. The final goal is to produce new non stoichiometric particles with novel magnetic properties.

MATERIALS AND METHODS.

Magnetosomes were obtained from cultures of *Magnetospirillum gryphiswaldense* strain MSR-1 grew microaerobically in FSM culture medium (Heyen and Schüler, 2003). Two types of samples were produced, here referred as "pure" magnetosomes and "Mn-doped" magnetosomes. The idea was to initially maintain cells in FSM culture medium to which the Fe source was removed (to avoid the formation of magnetosomes) and then, to grow these cells in FSM culture medium containing either Fe ("pure" magnetosome samples) or Fe and Mn ("Mn-doped" magnetosome samples) to allow magnetosome formation. "Pure" magnetosomes we obtained from cells of *M. gryphiswaldense* grown in FSM culture medium containing $Fe(C_6H_5O_7)$ (50 μM), added to the medium prior to inoculation. "Mn-doped" magnetosomes were obtained identically, but adding either $MnCl_2$ or $MnSO_4$ (50 μM) along with $Fe(C_6H_5O_7)$ (50 μM) to different batches (ratio Mn:Fe = 1:1).

Cells from both experiments were harvested and washed. Those samples were French pressed (18,000 lb) three times to extract the magnetosomes. They were further purified by magnetic separation, sealed anaerobically, and kept frozen.

The particles obtained were exhaustively analyzed. Analytical Electron Microscopy (AEM-HRTEM) was used to determine their morphology and chemical composition. Crystal structure was analyzed using Raman spectroscopy and magnetic properties were identified through a Verwey Transition temperature test.

RESULTS AND DISCUSSION.

After growth, cells and magnetosome Mgt crystals from "Mn-doped" magnetosome samples appeared to be normal morphologically and in size (Fig. 1).

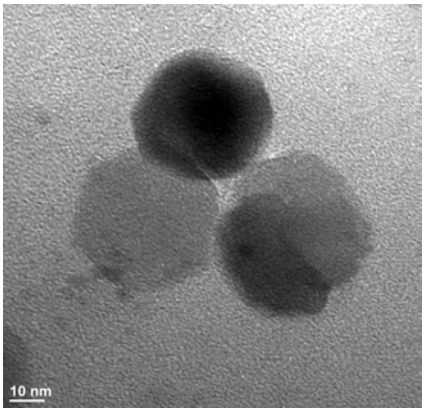

fig 1. *Magnetosome Mgt crystals*

On average, the cells grown in the presence of Mn contained a slightly smaller number of magnetosomes. Energy dispersive analyses (EDAX) showed that Mn was associated with the magnetosome Mgt crystals. From the results of localized AEM-HRTEM analysis, the Mn content was 1.04-1.14% of the total amount of metal in the magnetosome Mgt crystals.

palabras clave: Magnetita, Biomineralización, Bacterias Magnetotácticas , Nanopartículas.

key words: Magnetite, Biomineralization, Magnetotactic Bacteria, Nanoparticles.

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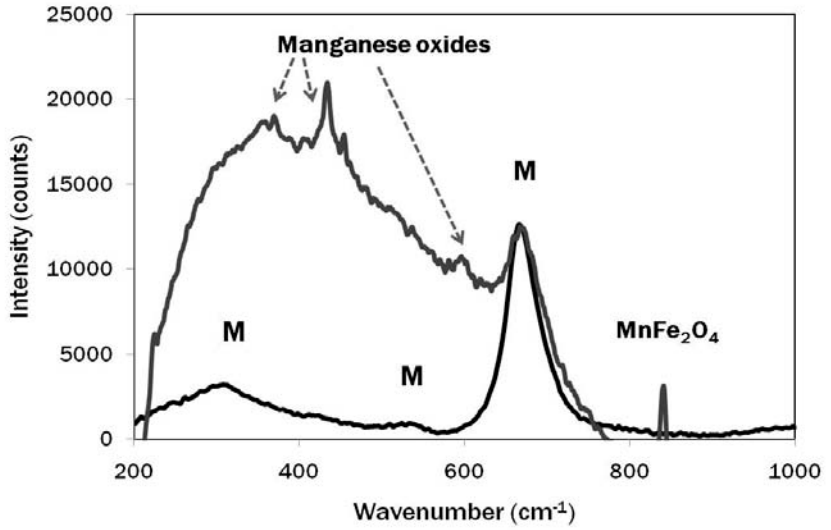

fig 2. *Raman spectra of pure magnetosomas (black line) and Mn-doped magnetosomas (grey line).Peaks of Mgt are named as M.*

Regarding crystal structures Raman spectra for pure- and Mn-doped magnetosomes are shown in Fig. 2. The pure magnetosome spectrum shows peaks at 663 cm^{-1}, 304 cm^{-1} and 533 cm^{-1}. These peaks have been identified by a number of authors as characteristics of Mgt (Rull *et al*. 2004). The peak at 663 cm^{-1} displays a narrow Lorentzian shape, indicating a well-crystallized Mgt. The Mn-doped magnetosome sample shows the strongest Mgt peak at 665 cm-1 and additional peaks at 370 cm^{-1}, 432 cm^{-1}, 452 cm^{-1}, 590 cm^{-1} and 840 cm^{-1}. The peaks at wavenumbers below 665 cm^{-1} correspond to a Mn-oxide (Julien *et al*., 2004), while the peak at 840 cm^{-1} may correspond to jacobsite. These minerals probably formed in the bulk culture medium, since they were not detected in the magnetosomes by transmission Electron Microscopy. There is a wide an intense band below 550 cm^{-1} which is due to fluorescence caused by the presence a high amount of organics (probably magnetosome membranes and cell debris) associated to the magnetosomes. Interestingly, the peak at 665 cm-1 (strongest peak for Mgt) is slightly wider in the Mn-doped magnetosome spectrum than in that of the pure magnetosome. Since the wider the peak, the less crystalline the sample is (Rull *et al*., 2004), our results indicate that the Mgt in the Mn-doped magnetosome sample is less crystalline than the Mgt formed in the pure magnetosome sample, probably due to a structural alteration caused by the incorporation of Mn in the Mgt structure.

The incorporation of Mn was also confirmed by the change in the magnetic properties of "Mn doped" Mgt compared to those of "pure" Mgt (Fig. 3). While the blocking temperature (temperature at which a material stops exchanging isotopes with external environment) remains unchanged, there is a complete suppression of the Verwey transition temperature in "Mn-doped" samples, being the Verwey temperature the one at which a material changes to behave as an electrical conductor to an isolating one. Such a change implies that Mn is incorporated into Mgt crystal structure substituting Fe ions and, therefore, causing a change in the spin aligment that affects the magnetic properties of the crystals. Such a change would not have occurred if the Mn were just adsorbed onto the crystal surface.

This is important regarding application of these Mn-doped magnetosomes, since this kind of particles are required in Manganese-enhanced magnetic resonance imaging for tracking biological activity of viable cells that can provide unique information in numerous applications (Silva and Bock, 2008).

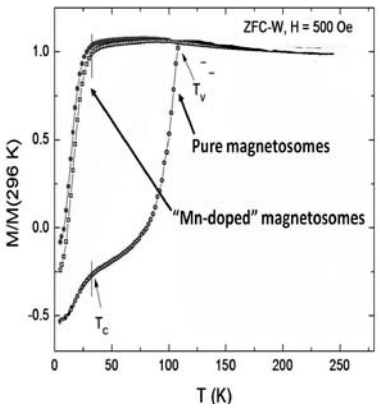

fig 3. *Magnetization (M) versus temperature (T) magnetization curves (ZFC-W measurements.*

CONCLUSIONS.

The results presented in this study confirm that when *M. gryphiswaldense* grows in a culture medium containing Mn, such a cation incorporates into the crystalline structure of the Mgt crystal in the magnetosome, causing a slight alteration in the crystalline structure of the Mgt that can be detected by Raman spectroscopy and that is confirmed trough changes in particle magnetism. This has a significant potential in tuning the bulk magnetic properties of Mgt biomineralized by magnetotactic bacteria. The ability to culture magnetotactic bacteria to produce doped Mgt nanoparticles has significant implications for medical applications such as manganese-enhanced magnetic resonance imaging.

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