Morphology of nanomagnetite crystals: Implications for formation conditions

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ABSTRACT

A series of controlled abiotic syntheses of magnetite nanocrystals were carried out to explore the possibility of using morphological criteria, crystal size distributions, and shape ratio as tools for identifying nanocrystals that would be specifically produced by magnetotactic bacteria. High-quality magnetite crystals synthesized with various controlled total iron concentrations were shown to have cubo-octahedral shapes and sizes varying from 4 to 24 nm. The mean particle size of the population was found to be 10.5 ± 0.7 nm and no significant effect of the total iron concentration on the particle size was observed. Systematical analyses of size and morphology also allowed for the determination of crystal size and shape ratio distributions. Crystal sizes were observed to follow log-normal distributions. Shape factors are bounded by one, with maxima between 0.80 to 1.00. Their distributions are asymmetric, with a cut off toward the high values. Crystal morphologies and shape factors appear not to be a powerful diagnostic tool for the differentiation of abiotic vs. biotic particles. However, crystal size distributions of abiotic crystals are significantly different from those of biotic populations. Indeed, opposite asymmetry of the size distributions from biogenic and non biogenic crystals was observed, with cut off toward larger sizes for biogenic nanocrystals and with cut off toward smaller sizes for abiotic nanocrystals. This therefore constitutes a potential diagnostic tool for deciphering magnetite origin.

INTRODUCTION

Under present Earth surface conditions, nanoparticles of biotic magnetite can be intracellularly formed in the magmatosmes of magnetotactic bacteria (MTB) (Blakemore 1975). Thus, magnetite nanoparticles found in carbonate globules of the ALH 84001 meteorite—recognized in 1994 as Martian meteorite (Mittlefehldt 1994)—have been related to possible extraterrestrial life (McKay et al. 1996). Mineralogical and crystallographic criteria, particularly morphology of nanocrystals, have been used to suggest that a fraction of nanoparticles of magnetite found in the ALH 84001 meteorite are Martian magnetofossils. Six independent criteria were proposed to define a biosignature (Thomas-Keprta et al. 2000). These criteria were used to evaluate the origin of magnetites extracted from ALH 84001 carbonate globules. Within these criteria, chemical purity is the only one which cannot be considered as strictly mineralogical. The five others—single domain size and restricted anisotropic width/length ratio, crystallographic perfection, magnetite chains, unusual crystal morphology, and crystallographic direction of elongation of magnetite crystals—can be called mineralogical criteria because they are related either to the size, habit, or crystallographic properties of the crystals. Extensive characterization of ALH 84001 magnetite nanoparticles and magnetite crystals from MTB have been done (Clemett et al. 2002; Friedmann et al. 2001; Gibson Jr. et al. 2001; Taylor et al. 2001; Thomas-Keprta et al. 2001) to prove the biotic origin of the magnetite crystals found in the meteorite. Devouard et al. (1998) had previously characterized magnetite crystals from MTB and from a simple inorganic process. A group of other authors also studied magnetite formation by MTB or by other processes and magnetite crystals in ALH 84001 (Barber and Scott 2002; Bradley et al. 1996, 1997; Brearley 2003; Buseck et al. 2001; Golden et al. 2001; Kopp and Humayun 2003; McSween and Harvey 1998; Saxton et al. 1998; Treiman et al. 2002; Treiman and Romanek 1998). The conclusions arrived at by these latter studies differ radically from previous ones. The first group (Clemett et al. 2002; Friedmann et al. 2001; Gibson Jr. et al. 2001; Taylor et al. 2001; Thomas-Keprta et al. 2000, 2001) suggest that given the complexity of the ALH 84001 meteorite, the simplest mechanism that could explain the formation of a fraction of their magnetite nanoparticles sample should be the MTB biomineralization whereas the second group (Barber and Scott 2002; Bradley et al. 1996, 1997; Brearley 2003; Buseck et al. 2001; Devouard et
al. 1998; Golden et al. 2001; McSween and Harvey 1998; Saxton et al. 1998; Treiman et al. 2002; Treiman and Románek 1998) concluded that the magnetite nanocrystals found in ALH 84001 were not necessarily formed via a biotic process or perhaps were formed via a biotic process on Earth (Kopp and Humayun 2003). Other criteria, such as magnetism (Weiss et al. 2000), isotopes (Greenwood et al. 2003, 1997; Leshin et al. 1998; Saxton et al. 1998; Valley et al. 1997), crystal size distributions (Arató and Pósfai 2003), or polycyclic aromatic hydrocarbon (Frankel and Busek 2000) have been proposed and studied. The straightforward approach would be to systematically compare the properties of abiotically synthesized magnetites with biogenic ones (Devouard et al. 1998; Thomas-Keprta et al. 2000). A difficulty often encountered in abiotic syntheses, however, is the lack of control on thermodynamic conditions which leads to large crystal-size distributions and morphologies.

In this study, inorganic magnetites were synthesized under controlled chemical conditions and High Resolution Transmission Electron Microscopy (HRTEM) analysis was performed to determine precise mineralogical characteristics [crystal size distribution (CSD), shape factors, crystallographic perfection, crystal morphology, and crystallographic direction of elongation of magnetite crystals] of the precipitated particles.

**MATERIALS AND METHODS**

**Synthesis of magnetite**

Magnetite precipitation experiments were carried out by co-precipitation of ferrous and ferric ions in aqueous solution following a previously developed methodology (Faivre et al. 2004) given by:

\[
2 \text{Fe}^{3+} + \text{Fe}^{2+} + 4 \text{H}_2\text{O} \rightarrow \text{Fe}_3\text{O}_4 + 8 \text{H}^+ (1)
\]

Fifty milliliters of a solution (pH 1.25) containing dissolved FeCl₃ and FeCl₂, in the stoichiometric ratio of the solid [Fe(III)/Fe(II) = 2] were added to 500 mL of sodium nitrate NaNO₃ solution at a rate of 0.5 mL per minute using an automatic pump (Metrohm 776 Dosimat). The ionic strength of the medium was fixed at \( f = 0.1 \text{ mol/L} \) by the sodium nitrate solution. The precipitation cell was held at a constant temperature of 25 ± 0.5 °C. All solutions were previously boiled and carefully deoxygenated with nitrogen, which was kept continuously bubbling during the experiment to avoid Fe(II) oxidation. The pH was kept constant at pH = 10.5 ± 0.05 by addition of a 0.1 mol/L sodium hydroxide solution using a commercial pHstat (Metrohm 718 STAT Titriso, calibration with Schott buffer solutions: pH 7.0 and 10.0 at 25 °C). The pH was monitored with a pHmeter, integrated in the pHstat, with a Metrohm 6.0203.100, 3 mol/L KCl) combined electrode. Calibrations were done using Schott buffer solutions: pH 7.0 and 10.0 at 25 °C. The total iron concentration (hereafter abbreviated TIC) in the initial iron-bearing solution was varied in the experiments to explore the effect of this parameter on the structural properties of the nanoparticles.

**Analysis**

Nanocrystals were characterized by HRTEM using a JEOL 2010 F electron microscope operating at 200 kV, coupled with a EDS chemical analyzer (PGT-PRISM 2000 with IMIX software, probe size: 1 nm, time of analysis: 300 s). The TEM samples were prepared by deposition of a drop of magnetite nanoparticles precipitation solution onto a copper mesh grid covered with a carbon film. After 10 s, the drop was removed and the grid was allowed to dry. The nature of the synthesized particles was checked using electron diffraction diagrams. HRTEM image analysis was performed to determine the size and shape factor of the crystals according to a previously described method (Devouard et al. 1998). This technique was shown to have negligible effect on size distribution. The shape determination of nanocrystals is however not an easy task (Busek et al. 2001). The main problem is due to the fact that the crystals are viewed in projection. It is therefore not possible to distinguish between crystal faces and crystal edges. For semi-conductors, HRTEM image calculation techniques have been used to validate a model of the exact three-dimensional shape of a particle deduced from HRTEM images (Ricoleau et al. 1999). However, this method requires perfect orientation of the crystal with respect to the electron beam. This requirement is not easily achievable, especially when the particles are not isolated as in our case (Fig. 1). We have used a method based on the observation of about 100 particles for which the crystallographic orientation was known. Owing to the smallness of the particles, precise orientation was difficult, so exploitable HRTEM images were obtained only for particles fortuitously well-oriented with respect to the electron beam. For each studied particle, the observation zone axis was determined from the 2-D Fast Fourier Transform (FFT) of the image (Fig. 2). Indexing of the diffraction spots was used to identify the crystal faces. For the example, the particle in Figure 2 was observed along the [011] zone axis and exhibits (111) faces. For this particle, the habit is unambiguously a cubic-octahedron with slightly expressed (100) faces. The different possible morphologies compatible with Fd3m symmetry have been tested taking into account the crystal orientation deduced from the FFT indexation. For this particle, no other possibility has been found to interpret the observed shape. Knowing the magnetite crystal symmetry group and making the hypothesis of cubic-octahedral crystals, it was possible to “reconstruct” the projection of the assumed crystal morphologies with respect to the orientation deduced from FFT analysis (Fig. 3).

**RESULTS**

Typical images of crystals are shown in Figures 1, 2, and 3. The nature of the observed mineral phases as a function of the TIC was studied by Faivre et al. (2004). These analyses showed that for low total iron concentrations (<30 × 10⁻³ mol/L), goethite particles (α-FeOOH) coexist with poorly crystallized iron oxides and hydroxides and with magnetite. For total iron concentrations exceeding 30 × 10⁻³ mol/L, magnetite is the only phase observed (Table 1). Electron Energy Loss Spectroscopy (EELS) was used to demonstrate that the particles studied are magnetite, not maghemite (Faivre et al. 2004).

**Sizes and shape factors**

To quantify the size and shape distributions of the different inorganic magnetite samples, a statistical analysis of crystals sizes was performed. Statistical analysis of sizes and shapes was done using approximately 500 particles (about 100 particles for TIC of 0.009, 0.018, 0.06, 0.12, and 0.18 M). Magnetite crystals from the population are distributed over a narrow range and follow a log-normal distribution (Fig. 4a). This seems to be typical of inorganically produced populations (Devouard et al. 1998). This CSD type is somewhat harder to observe for single samples, especially for the TIC of 0.18 M (Fig. 4f). The general type of CSD is log-normal for each of the sample (Fig. 4b-e).

The mean particle sizes are 10.3 ± 0.7, 10.7 ± 0.7, 10.1 ± 0.6, 10.1 ± 0.6, and 11.1 ± 0.8 nm respectively for TIC of 0.009, 0.018, 0.06, 0.12, and 0.18 M and at a confidence level of 10% (Fig. 5). This indicates that the TIC has no significant effect on the particle size. The mean particle size of the population is 10.5 ± 0.7 nm. Therefore, magnetite particles are mostly in the superparamagnetic size domain.

The shape factors (also called width/length ratio) of the magnetite crystals in all of the samples have shape factors bounded by one, with maxima between 0.80 to 1.00 (Fig. 6). The distributions

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<th>Table 1. Phases observed as a function of TIC</th>
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<td>TIC (mol/L)</td>
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<td>Phases observed</td>
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Note: Phases observed were goethite (Go), poorly crystallized iron oxides or hydroxides (Io), and magnetite (Mt)
Figure 1. TEM image of magnetite crystals used for statistical analysis: (a) low magnification and (b) details from the TIC 0.06 sample.

Figure 2. Typical image of magnetite crystals (TIC = 0.06 sample). As explained in the methodology, the Fast Fourier Transformed (FTT) image corresponds to electron diffraction of the crystals and allows the determination of the zone axis of the particle. Based on these properties and the cubo-octahedron hypothesis, the simulated crystal shows good agreement with the magnetite crystal shown.
FIGURE 3. Examples of comparison between nanomagnetite morphologies observed in HRTEM and the cubo-octahedron model. For each case, the orientation of the model is done with respect to the orientation deduced from FFT of the corresponding HRTEM image. (scale bar = 5 nm)
are asymmetric, with a cut-off toward the high values. The TIC 0.12 M experiment was an exception, with frequencies continuously increasing (maximum particle density for the 0.95 to 1.00 shape factor range). Shape-factor values close to 1 indicate that the crystals are hardly elongated, whereas values close to 0 indicate that crystals are very elongated (the so called needle-shaped crystals). Since the shape-factor values of our population are mostly in the 0.8 to 1 range, the octahedral crystals that we obtained (see next paragraph for the morphology of the crystals) are not very elongated.

Crystallographic perfection and morphologies

The high-resolution transmission electron microscope investigation of nanocrystals formed via inorganic synthesis at constant chemical affinity indicates that they are essentially free of internal defects (Figs. 1, 2, and 3). As shown in Figures 1b, 2, and 3, the cubo-octahedral shape has been found to be most common among the inorganically synthesized particles. Other simulated morphologies clearly differ from observed morphologies as seen in HRTEM images. This morphology, however, tends to be a simpler octahedral habit since the \{100\}
faces are hardly developed. It is interesting to note that the cubo-octahedral morphology is also the most common morphology reported for the most studied magnetotactic bacteria, i.e., strain MS-1 (Baeuerlein 2000, 2003; Bazylnski and Frankel 2003; Konhauser 1998; Schüler and Frankel 1999).

DISCUSSION

Mean particle sizes of about 10 nm, barely elongated nanocrystals, and log-normal CSDs were observed in all experiments. Synthetic magnetite crystals prepared in this study are not necessarily representative of all types of abiotic magnetite, natural or synthetic, but they present a useful starting point for comparison with biogenic crystals. These magnetite crystals were formed under controlled chemical affinity conditions, which facilitated narrow size distributions and which might be relevant for comparison with biogenic magnetites, which are believed to grow in controlled environments.

Magnetite crystals produced by Magnetospirillum magnetotacticum (MS-1) have sizes (~30 nm) (Devouard et al. 1998) of the same order of magnitude as the abiotically formed crystals in this study (~10 nm). However, the great majority of biogenic crystals fall in the single magnetic size domain whereas the abiotic ones are all superparamagnetic. Larger crystals have been formed with the same abiotic precipitation method used in this study (Vayssières et al. 1998). Larger crystals have been formed with the same abiotic precipitation method used in this study (Vayssières et al. 1998), by changing ionic strength conditions, but all were in the superparamagnetic domain. Larger abiotic particles have been reported (Cornell and Schwertmann 1996, 2003; Devouard et al. 1998) but they are formed with less controlled chemical affinity and thus belong to large crystal-size distributions. Mean particle size might therefore not be a useful parameter to constrain the formation conditions of magnetite crystals.

The nanocrystals formed via this abiotic synthesis are essentially free of internal defects (Figs. 1, 2, and 3) with cubo-octahedral shapes based on combinations of the isometric forms {111} and {100}. This has also been observed for biomagnetite crystals (Devouard et al. 1998; Thomas-Keprta et al. 2000). This indicates that a simple aqueous inorganic processes can lead to the precipitation of high-quality crystals with well-defined morphologies, such as those observed in biologically controlled mineralization (BCM) (Baeuerlein 2000, 2003; Bazylnski and Frankel 2003; Kirschvink and Hagadorn 2000; Roh et al. 2003; Schüler and Frankel 1999). This means that there is no need for biological intervention to control the shape and crystalline order of the magnetite crystal. Solution properties are sufficient to control the morphology and crystalline order of crystals since inorganic precipitation can produce well arranged crystals with a habit similar to those observed in BCM.

Shape factors, also called width/length ratio, have been used by several authors to discuss biogenicity. Thomas-Keprta et al. (2000) observed that prismatic magnetite particles have highly restricted W/L ratio around 0.675 and 0.725 for MV-1 and ALH84001 respectively. These ratios are similar those observed for MV-1 particles (Devouard et al. 1998), but significantly smaller than those observed here and also for MS-1 particles (Devouard et al. 1998), which have W/L ratios between 0.80 and 0.95. It appears that different bacterial strains may produce crystals with different shape factors and that the shape factor distributions measured in the abiotic syntheses of this study match some of the biogenic distributions. Therefore, assigning a formation condition based on morphology or shape factor considerations remains insufficient.

Three different strains of bacteria have been shown to produce magnetite crystals that exhibited asymmetric CSDs, with sharp limits toward larger sizes (Devouard et al. 1998). The abiotically produced particles synthesized by Devouard et al. (1998) also exhibited an asymmetric CSD, but with a limit toward smaller sizes. A broader CSD distribution was found for abiotically produced particles than for the biogenic ones. This broadening might be explained by the fact that supersaturation conditions were not constant during precipitation, therefore leading to different particle sizes (Lasaga 1998). Our results show that abiotic crystals formed via processes in which the chemical affinity is controlled also exhibit an asymmetric and narrow CSD similar to biogenic particles, but with a limit toward smaller sizes. The opposite asymmetry of the CSDs from biogenic and non biogenic syntheses thus remains the sole potential criterion for distinguishing magnetite populations from biogenic or abiotic origins. As stated by Arató and Pósfai (2003) and Devouard et al. (1998), the statistical analysis of size distributions might provide a robust criteria for distinguishing between intracellular biogenic and other crystals (extracellular and non biogenic particles). However, in samples that would contain both bacterially and inorganically produced crystal populations the distinction of biogenic crystals would become ambiguous (Arató and Pósfai 2003).

In a previous study (Faivre et al. 2004), the precipitation mechanism in such magnetite formation experiments was attributed to a constant nucleation rate producing crystals of nearly identical sizes, the slight asymmetry being explained by limited growth of older crystals. CSDs (Fig. 4) were obtained from crystals that were extracted from the solution directly after precipitation and are therefore not subject to any ripening. Kile et al. (2000) associated log-normal CSDs with decaying rate of nucleation accompanied by surface controlled growth. Our constant nucleation, far from equilibrium conditions, when
compared to their results, could be associated with Ostwald ripening (Ostwald 1891, 1897) and would in this case lead to CSDs similar to those observed for biogenic magnetite particles. However, as far as we know, no Ostwald ripened size distribution has ever been observed in inorganic magnetite crystals. This might be explained by the fact that the interfacial tension could be lowered sufficiently to allow the formation of dimensionally stable particles (Vayssières et al. 1998). Therefore, experimental results show that CSDs from inorganic and biogenic populations have opposite asymmetry. It would be interesting to study CSDs from aged or ripened inorganically produced populations of magnetite.

CSDs appear to be a powerful diagnostic tool for magnetite origin. Naturally, the use of a single criterion may lead to erroneous conclusions. Therefore, mineralogical biogenic criteria should be coupled to others, such as magnetic or isotopic criteria. Further studies, using an abiotic environment resembling the biogenic one, would be of great interest to better understand the biomineralization process and therefore help to better define criteria of biogenicity.

**Figure 6.** Shape factor distribution for the total population (a) and for samples from the TIC 0.009 (b), 0.018 (c), 0.06 (d), 0.12 (e), and 0.18 M (f) experiments respectively.
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