Doctoral Thesis

Magnetic Properties of Iron-oxide Nanoparticles and Methods for their Characterization

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MAGNETIC PROPERTIES OF IRON-OXIDE NANOPARTICLES AND METHODS FOR THEIR CHARACTERIZATION

A thesis submitted to attain the degree of

DOCTOR OF SCIENCES OF ETH ZURICH

(Dr. sc. ETH Zurich)

presented by

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TO

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ABSTRACT

Iron-oxide nanoparticles, also known as magnetic nanoparticles, are a key component in the field of nano- and biotechnology, which has become a multi-billion dollar industry. Iron oxides include magnetite, its partially oxidized form maghemite, and the completely oxidized form of hematite. These materials appeal to scientists because of their tunable magnetic properties at the nanoscale. They exhibit great potential for a wide range of applications, such as magnetic data storage devices, sensors, catalytic materials, wastewater treatment adsorbents, pigments, coatings, magnetic resonance imaging, bio-separation, drug delivery, hyperthermia, and many more. Each application uniquely demands specific characteristics for achieving high quality performances in their respective fields. Hence a detailed magnetic characterization of the magnetic nanoparticles not only at the bulk scale but also at individual constituents/components is extremely important, because these can influence the overall magnetic behaviour and thereby the technical performances.

This dissertation aims at developing methods and protocols that aid in characterizing the system of magnetic nanoparticles. The information on magnetic properties can then be used to evaluate the purity of chemical composition, the effective particles size, and/or the degree of interaction among the particles. The study uses mostly biological and biomimetic magnetite nanoparticles, which were prepared under the EU project ‘Biomimetic and Biomineralized Magnetic Nanoparticles for Magnetic Resonance Imaging’ (Bio2MaN4MRI).

A set of magnetic and non-magnetic methods has been used at low and/or room temperature to address several questions important for the characterization of magnetic nanoparticles. These methods include hysteresis loops, acquisition of backfield isothermal remanent magnetization (dc-IRM), first order reversal curves (FORC), ac-susceptibility as a function of temperature and frequency, transmission electron microscopy, and X-ray diffraction. The questions, that were addressed, are: i) how to estimate the relative fraction of smaller, i.e., superparamagnetic (SP) particles, and larger i.e., single domain (SD) particles in a mixture; ii) how useful is the Day-Dunlop plot in estimating SP content in a particle system of magnetite; iii) what magnetic methods are best in characterizing purity of chemical composition; and iv) how well do self-assembled magnetite particles displays magnetic anisotropy.

FORC diagrams are known to provide information on the coercivity spectrum arising from ferromagnetic (s.l.) minerals in a material, and the spectrum of interaction field arising from the particle system. They can also be used to obtain a first-order estimate on the amount of SP particle sizes by deconvolving the reversible and the irreversible magnetization. The SP content of well-controlled biogenic and synthetic magnetite nanoparticles was estimated and a good agreement was found between this semi-quantitative estimate of SP particles and the concentration obtained from TEM images. It was also demonstrated that the Day-Dunlop plot is not particularly suitable in estimating the concentration of SP particles in a mixture of SP and SD magnetite. The results from this study should inspire having a closer look at the
theory that underlies this plot.

FORC diagrams are also shown to be very sensitive in detecting the first stage of surface chemical alteration of magnetite to hematite through the appearance of what is known as a 135° ridge. As the degree of oxidation continues, this feature disappears as the hematite shell increases with respect to the magnetite core. These results demonstrate that the 135° ridge is not simply an artifact of data processing but arises from weak interaction between two phases with non-overlapping coercivities.

In a study of self-assembled single crystals of magnetite, it has been shown that it is not possible to reach a strong degree of alignment of the easy axis of magnetization within the plane of deposition. The difference in magnetic properties, when considering these within the plane of deposition compared to normal to the plane of deposition, come close to following theoretical predictions that have been established in micromagnetic studies. Torque magnetometry is also shown to be a sensitive method for illustrating the role of shape versus magnetocrystalline anisotropy in aligned magnetosomes.

In the final chapter of the thesis the methods and information that were acquired in the previous studies are used in practical applications. The first example deals with the magnetic characterization of the biogenic and biomimetic magnetic nanoparticles that were developed under the EU-project. Feedback on the magnetic properties aided in improving techniques in the synthesis of biomimetic magnetite to produce reproducible nanoparticles with more constrained size distribution and composition. The methods were also used to understand how biomedical image (MRI and MPI) performance can be improved by tailoring the magnetic properties of the nanoparticles. A second application, determined which magnetic parameters are best for predicting performance of iron oxide nanoparticles for MRI and/or MPI. It was verified that initial susceptibility is the best criterion for judging performance. However, also the bifurcation temperature, i.e., the temperature at which the first SP particle starts to behave as a blocked SD particle, is related to image performance, reflecting the significance of the largest particles size close to the SP and SD boundary.

The results from the individual studies in the thesis should be of interest to anyone synthesizing iron oxide nanoparticles, with regards to characterization of magnetic performance or tuning the particles for desired magnetic behaviour. For example relative sensitivity of image contrast for MRI and MPI can easily be assessed using the initial magnetization curve and the bifurcation temperature. If optimal magnetic performance is obtained then the amount of particles needed for an application can be reduced in general. This is an important factor if the particles are being used for magnetic targeting of drug delivery or hyperthermia treatment. This work also has relevance for the paleomagnetic and rock magnetic communities, because an improved understanding on the magnetic behaviour of well-characterized magnetic system aids in characterization of less well constrained systems.
ZUSAMMENFASSUNG


Verschiedene magnetische und nicht-magnetische Methoden wurden bei Tief- und/oder Raumtemperatur eingesetzt, um wichtige Fragen betreffend Charakterisierung von magnetischen Nanopartikel beantworten zu können. Diese Methoden beinhalten Hysterese-Schlaufen, Rückfeld-isothermische remanente Magnetisierung (DC-IRM), Umkehrkurven erster Ordnung (FORC), AC-Suszeptibilität in Abhängigkeit von Temperatur und Frequenz, Transmissionselektronenmikroskopie, und Röntgenstrahldiffraction.

Folgende Fragen sind zu beantworten: i) wie kann der relative Anteil von kleineren, d.h., super-paramagnetischen Partikel (SP), und grösseren, d.h., Einzeldomänen-Partikel (SD), in einer Mischung bestimmt werden; ii) wie nützlich ist die Day-Dunlop Darstellung zur Bestimmung des SP Anteils in einem Partikelsystem von Magnetit; iii) welche magnetischen Methoden sind am besten geeignet die Reinheit der chemischen Zusammensetzung zu bestimmen; und iv) wie stark zeigen selbstorganisierende Magnetitpartikel eine magnetische Anisotropie.

Partikel in einer Mischung von SP und SD Magnetit. Die Resultate der vorliegenden Studie sollten dazu animieren die zugrunde liegende Theorie dieses Diagramms zu überprüfen. Es wurde auch gezeigt, dass FORC Diagramme äusserst sensitiv sind, um die erste Stufe der chemischen Veränderung der Oberfläche bei der Umwandlung von Magnetit in Hämatit sichtbar zu machen, durch das Auftreten der sogenannten „135° ridge.“ Mit weiter steigendem Grad der Oxidationsschicht verschwindet dieses Merkmal wieder. Diese Resultate demonstrieren, dass die „135° ridge“ nicht einfach ein Artefakt der Datenverarbeitung ist, sondern durch schwache Wechselwirkung zwischen zwei Phasen von nicht überlappenden Koerzitivfeldstärken verursacht wird.


Im Schlusskapitel der Dissertation werden die Methoden und Resultate aus den oben beschriebenen Studien zur Lösung von praktischen Problemen angewandt. Das erste Problem befasst sich mit der Charakterisierung von biogenen und biomimetischen magnetischen Nanopartikel, welche innerhalb des EU-Projekts hergestellt wurden. Der Feedback von Informationen über die magnetischen Eigenschaften hat dazu beigetragen die Technik in der Synthese von biomimetischem Magnetit zu verbessern und Nanopartikel mit homogener Grösse und Zusammensetzung zu produzieren. Die in dieser Dissertation etablierten Methoden wurden auch dazu benutzt, um zu verstehen wie die Ergebnisse von biomedizinischer Bildverarbeitung (MRI und MPI) verbessert werden können, durch gezielt anpassen der magnetischen Eigenschaften der Nanopartikel. In einer zweiten Anwendung wurde bestimmt, welche magnetischen Parameter am besten sind, um die Eignung von Eisenoxid Nanopartikel für MRI oder MPI zu beurteilen. Es wurde verifiziert, dass die Anfangssuszeptibilität der beste Indikator ist, um die Eignung zu beurteilen. Auch hat die Bifurkationstemperatur, d.h., die Temperatur wo die ersten SP Partikel beginnen zu blockieren, einen Einfluss auf die Bildqualität, was die Bedeutung der grössten Partikel nahe der SP/SD Grenze unterstreicht.

CHAPTER 1

Introduction

Relative sizes of naturally occurring items. The nanometer length scale is a thousand times smaller than bacteria and ten million times smaller than the width of a human hair.
1.1 NANOPARTICLES

The International Organization for Standardization (ISO) in cooperation with the European Committee for Standardization (CEN) has defined the usage of the term *nano* as

1. **Nanoscale** (or *nano range*): size range from approximately 1 nm to 100 nm.
2. **Nano-object**: material that is confined in one, two, or three dimensions at the nanoscale. This includes nanoparticles (all three dimensions in the nanoscale), nanofibres (two dimensions in the nanoscale) and nanoplates (one dimension in the nanoscale). Nanofibres are further divided into nanotubes (hollow nanofibre), nanorods (solid nanofibre) and nanowire (electrically conducting or semiconducting nanofibre).

In this thesis a broad definition for ‘nanoparticles’ is used in which at least one dimension should be on a nanoscale.

1.1.1 CURRENT STATE OF THE ART

Nanoparticle research is currently an area of intense scientific and technological interest. This is reflected by an eight-fold increase in the number of new publications covering all aspects of nanoparticles (NP) over the past 10 years (Fig. 1.1).

![Fig. 1.1: a) Number of new scientific publications on MNPs over the past 10 years (Source: Web of Science, in January 2015).](Image)

Research includes publications that involves novel methods for the synthesis of NP, distinct characterization techniques applied to them, and their use in many nano- and biotechnological applications [e.g., Dhanabal and Gurunathan, 2015; Hao et al., 2010; Ludwig et al., 2012; Majewski and Thierry, 2007; Mathukumaran and Philip, 2015; Riaz et al., 2014; Yan et al., 2015]. Miniaturization and size-dependent properties such as quantum confinement in semiconductor particles, surface plasmon resonance in some metal particles and
superparamagnetism (SP) in magnetic materials, has steered the explosion in NP research. Furthermore, NP represents a bridge between bulk materials and atomic or molecular structures. Many of the unique properties of NP are attributed to their high surface-to-volume ratios when compared to the bulk part with same compositions.

1.1.2 MAGNETIC NANOPARTICLES

Magnetic nanoparticles (MNP) are a class of engineered NP that can be manipulated by an external magnetic field. Two crucial features, which are responsible for their novel magnetic properties, are finite-size effects and surface effects [Lu et al., 2007]. Single domain (SD) and SP limits are the most prevalent studied finite-size effect. A SD particle is a uniformly magnetized particle with all atomic spins statistically aligned in the same direction; they possess a magnetic remanence and coercive force. A superparamagnet is defined as having large magnetic moments where thermal energy is larger than anisotropic energy. SP particles are characterized by the absence of both remanence and coercivity. As the MNP size decreases, the number of surface atoms becomes greater, making surface and interface effects significant. Surface anisotropy and core-surface exchange anisotropy are consequences from surface effects. MNP are grouped into three major groups namely; diamagnetic, paramagnetic, and ferromagnetic. A short description of these magnetic classes is presented in chapter 2.1.2, but can also be found in standard textbooks on the physics of magnetism or magnetic mineralogy [Coey, 2010; Cornell and Schwertmann, 2003; Dunlop and Özdemir, 1997]. MNP consists of cations, e.g., Fe, Ni, Co, Cr, and their oxides, such as magnetite (Fe₃O₄), maghemite (γ-Fe₂O₃), hematite (α-Fe₂O₃), cobalt ferrite (Fe₂CoO₄), and chromium di-oxide (CrO₂).

The MNP that are investigated in this thesis are limited to iron oxides: magnetite, maghemite and hematite.

1.1.2.1 MAGNETITE

Magnetite has an inverse spinel structure with oxygen forming a face-centered cubic crystal system (Fig. 1.2a). Its structural formula is Fe³⁺[Fe³⁺Fe²⁺]O₄, where Fe²⁺ and Fe³⁺ are found on the octahedral sublattice, while the tetrahedral lattice is filled with only Fe³⁺. The net magnetic moment of magnetite is due to Fe²⁺ on the octahedral sublattice. Magnetically, magnetite is ferrimagnetic, and has a Curie temperature (T_C) of 853 K; above this temperature it exhibits paramagnetism. Pure stoichiometric magnetite has a saturation magnetization (M_s) of 480 kA/m or 92 Am²/kg. Pure multidomain (MD) magnetite or SD magnetite with magnetocrystalline anisotropy exhibits a Verwey transition (T_V) around 120 K [Verwey and Haayman, 1941].
1.1.2.2 MAGHEMITE

Maghemite has structural formula $\text{Fe}^{3+}[\text{Fe}^{3+}\text{Fe}^{3+}_{2/3} \square_{1/3}]\text{O}_4$. It is the end member of a solid solution series with magnetite. With progressive oxidation the end member has two thirds of the original $\text{Fe}^{2+}$ oxidized to $\text{Fe}^{3+}$ with the simultaneous removal of one third of the original $\text{Fe}^{2+}$ from the octahedral site. This removal occurs by diffusion producing vacancies (□) in the spinel structure where a $\text{Fe}^{2+}$ cation had previously resided. The saturation magnetization for maghemite is 380 kA/m or $\approx 80 \text{ Am}^2/\text{kg}$. It is metastable at high temperature, which makes determination of its Curie temperature difficult to assess.

1.1.2.3 HEMATITE

Hematite $\alpha-[\text{Fe}^{3+}]_2\text{O}_3$ is the most stable form of iron-oxide with hexagonal close pack structure (Fig. 1.2b). It exhibits antiferromagnetism with a Néel temperature ($T_N$) of 948 K; above this temperature it is paramagnetic. $M_S$ for hematite is $\approx 2.5 \text{ kA/m}$ or 0.4 $\text{ Am}^2/\text{kg}$. The low magnetization is due to canted basal plane and defect. It has a diagnostic transition temperature, called the Morin transition ($T_M$) around 250 K, at which the magnetic moments rotate from the basal plane and align along the c-axis ([Morin, 1950a]. Below $T_M$, hematite is a perfect antiferromagnet. The exact $T_M$ is a function of particle size and induced internal stress ([Kündig et al., 1966; Muench et al., 1985]).

Fig 1.2: a) Crystal structure of magnetite [Source: http://jolisfukyu.tokai-sc.jaea.go.jp/fukyu/tayu/ACT05E/05/0503.htm] and, b) crystal structure of hematite above and below the Morin transition temperature, after Fuller (1987).

1.1.3 POTENTIAL APPLICATIONS OF MAGNETIC NANOPARTICLES

MNP are attractive for many applications because of their tunable properties. This allows them to be tailored for specific applications (Fig. 1.3). Each potential application requires distinct characteristics from MNP. For example: data storage, which is one of the economically most important application, needs MNP with stable and switchable magnetic...
states. Biomedical applications are also becoming economically important. An example of one use of MNP is in hyperthermia treatment, which is employed for cancer therapy, particularly tumors. This method is based on the principle that by applying an AC field the MNP generates heat on the order of 42°C - 46°C, which destroy the cancer cells \[Berry and Curtis, 2003; Mornet et al., 2004\]. In practice biomedical applications require SP particles that are non-toxic and stable at room temperature, which is a major challenge in their synthesis \[e.g., Bangs, 1996; Gupta and Gupta, 2005; Lu et al., 2007\]. MNP are also being used in various environmental applications. For example, when functionalized to attract a pollutant it can be introduced into contaminated sites and then the pollutant can be removed by magnetic extraction technique \[e.g., Elliott and Zhang, 2001; Masciangioli and Zhang, 2003\].

![Fig. 1.3: Examples of high performance capabilities of MNP in various fields of application.](image)

### 1.1.4 CHALLENGES IN THE FIELD OF MNP

Key to any application using MNP is their magnetic response, and this requires a complete characterization of their magnetic behavior. This is essential as the magnetic properties of MNP will depend on chemical composition, mean particle size, size-distribution, shape, anisotropy, defects in the crystal lattice, functionalization, and interaction with matrix and neighboring particles. These factors are very hard to control in practice, therefore it is not uncommon that the magnetic properties of nano-materials of the same type can be markedly different. This provides a major challenge towards their reproducibility on an industrial scale. Other few specific but vital challenges are outlined in below.

#### 1.1.4.1 EFFECTIVE MAGNETIC PARTICLE SIZE

Particle size is determined generally using electron microscopic or X-ray diffraction techniques and this defines their physical particle size. In contrast magnetic properties are
used to define the effective magnetic particle size. Ideally physical size should be the same as effective magnetic particle size, but particle interaction, aggregation or, agglomeration, can cause a considerable deviation between the two sizes. Figure 1.4 shows an example from uncoated synthetic magnetite particles prepared by co-precipitation of Fe (II) and Fe (III) salts. The sample has a mean physical particle size of about 15 nm as determined by high resolution transmission electron microscopy. The effective magnetic particle size, however, must be at least partly ≥ 25 nm, because some particles are magnetically blocked, as seen from the spread in the coercivity (for coercivity cf. Chapter 2.1.2) distribution represented along the horizontal axis, that extends to 60 mT (Fig. 1.4c).

![Image](image_url)

*Fig. 1.4: a) Transmission electron microscopic (TEM) image, b) physical size distribution obtained from TEM analysis and; c) FORC distribution for synthetic magnetite nanoparticles.*

1.1.4.2 SURFACE EFFECTS

As the diameter of MNP decreases to less than 10 nm, surface plays an increasingly significant role in governing the magnetic properties of a material [Estrader et al., 2015]. An example of the effect of size on magnetic properties is shown in Figure 1.5. The magnetic moment of magnetite will normally saturate by a maximum field of 300 mT. However, the hysteresis loop for magnetite with diameter under 10 nm is not saturated by 1 T. This could be due to surface oxidation of the particle to hematite or it can also be an effect from surface spins.

1.1.4.3 STOICHIOMETRY

High values of $M_S$ are desirable for many bio-medical applications. Here stoichiometry plays a vital role because it governs $M_S$ of the particles. Stoichiometry can be affected by oxidation or defects. For example $M_S$ for pure stoichiometric magnetic is 480 kA/m, but as the non-stoichiometry increases $M_S$ decreases. Magnetite often loses its stoichiometry either during synthesis or after storing for some period.
Fig. 1.5: Magnetic hysteresis loop for a synthetic magnetite nanoparticles with diameter below 10 nm.

1.1.5 SCOPE OF THE THESIS

1.1.5.1 MOTIVATION OF THE WORK

The research in this dissertation was conducted under the EU project Biomimetic and Biomineralized Magnetic Nanoparticles for Magnetic Resonance Imaging (Bio2MaN4MRI). The project focused on nano-biotechnology research to develop biomimetic methods in the synthesis of MNP for applications in magnetic resonance imaging (MRI). The ETH-Zürich was responsible for assessing magnetic properties of the cultured biogenic and synthesized MNP. My particular interest was on developing protocols or methods for the magnetic characterization of iron-oxides with respect to their effective magnetic particle size and stoichiometry. These protocols should be of interest to any group who are synthesizing MNP, so that they can optimize their methods to obtain particles with tailored magnetic behavior. This work also has relevance to the paleomagnetic and rock magnetic community. In geologic material one often has a mixture of compositions, grain size, degree of stoichiometry, and magnetic interaction. Synthesized materials are more limited in these terms. Therefore a good understanding of synthetic materials helps in our understanding the factors that have the greatest influence on magnetic properties in natural materials.

1.1.5.2 ORGANIZATION OF THESIS

This dissertation consists of seven chapters. The proceeding chapter 2 presents the theoretical background and the methods adopted for analyzing MNP.

Chapter 3 examines the discrimination of magnetic domain states or sizes of MNP. It is comprised of three sections. The first section presents a semi-quantitative method to establish relative percentage fraction of SP and SD in an assemblage of MNP. The second section is an experimental study that evaluates the usefulness of theoretical predictions for SP-SD mixtures
on a modified Day-plot, which is widely used in the paleomagnetic community for defining magnetic domain states and sizes [Day et al., 1977; Dunlop, 2002a]. The third section is a part of a large study titled “The magnetic signature of nanoscale magnetite mesocrystals” [Reichel et al., submitted to ACS Nano]. Mesocrystals are crystals that are assembled from smaller sub-units. The study demonstrates the effectiveness of using magnetic methods to evaluate the effective magnetic particle size in contrast to the physical particle size.

Chapter 4 deals with partial-chemical alteration of magnetite NP and concerns with identifying the first occurrence of surface oxidation on magnetite and/or maghemite MNP with the aid of first order reversal curves (FORC) [Kumari et al., 2014; Muxworthy et al., 2005; Pike et al., 1999; 2001; Roberts et al., 2000]. FORC analysis has shown a feature, known as a 135° ridge, which is often found when a material has two magnetic phases with strongly contrasting coercivities. This study provides insight to the conditions that are required for the manifestation of a 135° ridge on a FORC diagram.

Chapter 5 focuses on MNP that were obtained from magnetotactic bacteria (MTB). It describes methods used for the preparation of magnetic field-directed self-assembled biological magnetite nanoparticles and their characterization. Low and high-field anisotropy of magnetic susceptibility results are applied to determine the effectiveness of the particles alignment in biologic samples and to understand the role of shape anisotropy, crystalline anisotropy and remanence in the process of alignment in these biological samples.

Chapter 6 is dedicated to the application of MNP for magnetic resonance or particle resonance imaging, and consists of two sections. The first section presents an overview on the magnetic characterization of magnetite NP that were produced under the EU-Bio2Man4MRI project. The study also compares overall contrast performances between biological and synthetic, and small vs. large magnetite nanoparticles from the prospective for applications in MRI. The second section examines which magnetic properties are important for judging the quality of MNP for magnetic resonance and magnetic particle imagining.

The final chapter, Chapter 7 summarizes the main findings in this thesis and presents an outlook for further work.
Magnetic field produced by electric current in a solenoid coil and that of a bar magnet
(Source: http://hyperphysics.phy-astr.gsu.edu/hbase/magnetic/elemag.html)
2.1 FUNDAMENTAL CONCEPTS ON MAGNETISM

This chapter presents a short description on fundamentals of magnetism and the relevant theory applied in the thesis. More complete information can be found in several books e.g., Fundamentals and Frontiers [Dunlop and Özdemir, 1997]; Magnetism and Magnetic Materials [Coey, 2010]; Introduction to Magnetic Minerals [Cullity and Graham, 2005]; and Paleomagnetism: Magnetic Domain to Geologic Terranes [Butler, 1992]. A brief description of the instrumentations and methods that were employed for the characterization of the iron oxide MNP is also provided in this chapter.

2.1.1 DEFINITIONS AND UNITS

A microscopic current arising from the orbital motion and intrinsic spin of the electrons generate a magnetic field. This field is characterized by the magnetic induction $B$, and is measured in Tesla T, i.e., newton per ampere meter (N/Am). In practice however a magnetic field quantity, known as the magnetic field strength $H$, is used. It describes the strength of magnetic field inside a magnetic material and is expressed in ampere per meter (A/m). The two magnetic fields are related to each other through:

$$B = \mu_0 (H + M)$$  \hspace{1cm} (2.1)

where $\mu_0$, is the permeability of free space, a constant with a magnitude of $4\pi \times 10^{-7}$ N A$^{-2}$; $M$, the intensity of magnetization or simply the magnetization, which is the density of magnetic dipole moments $m$ in a volume $V$ (Equation 2.2). $M$ and $m$ are measured in A/m and Am$^2$ respectively.

$$M = m / V$$  \hspace{1cm} (2.2)

Another commonly used expression for an isotropic material with the intensity of magnetization parallel to the applied field $H$ is given by equation 2.3. Where, the proportionality constant $\chi$, is known as magnetic susceptibility. It measures the ease with which a material can be magnetized; it is dimensionless.

$$M = \chi H$$  \hspace{1cm} (2.3)

Most commercial instruments that measure magnetic properties are still based on cgs units. The system of magnetic units and its conversion from SI to cgs system is given in Table T2.1. This table summarizes the SI and cgs system of units along with their conversion factors for selected basic magnetic quantities, which will be used in later chapters. In the thesis I use the SI system of units and define the magnetic field by the magnetic field strength $H$. 
Table T2.1: Magnetic parameters and their units.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>SI</th>
<th>cgs</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic induction, (B)</td>
<td>T</td>
<td>Gauss, (G)</td>
<td>(1 \ G = 10^{-4} \ T)</td>
</tr>
<tr>
<td>Magnetic field, (H)</td>
<td>A/m</td>
<td>Oersted, (Oe)</td>
<td>(1 \ Oe = 79.6 \text{ kA/m})</td>
</tr>
<tr>
<td>Magnetization, (M)</td>
<td>A/m</td>
<td>emu/cm(^3) (G)</td>
<td>(1 \ G = 10^3 \text{ A/m})</td>
</tr>
<tr>
<td>Magnetic moment, (m)</td>
<td>Am(^2)</td>
<td>emu</td>
<td>(1 \text{ emu} = 10^{-3} \text{ Am}^2)</td>
</tr>
<tr>
<td>Magnetic susceptibility, (\chi)</td>
<td>Dimensionless</td>
<td></td>
<td>(\chi \text{ (cgs)} = 4\pi\chi \text{ (SI)})</td>
</tr>
</tbody>
</table>

2.1.2 CLASSIFICATION OF MAGNETIC MATERIALS

As stated above magnetism arises from the orbital and spin moment of electrons; whereby the moment from protons is negligible. All materials exhibit magnetism and can be broadly classified into three categories based on the nature of their magnetic susceptibility.

2.1.2.1 DIAMAGNETISM

Every material with orbiting electrons has a diamagnetic component. Diamagnetism arises from the net magnetic moment from the precession of electron orbits. A purely diamagnetic material has all of its electrons coupled such that it possesses only a net orbital moment in an applied field, in which the magnetic moments are aligned antiparallel with respect to the applied field (Fig. 2.1a). The classical theory of diamagnetism was first proposed by Paul Langevin in 1905 based on the Larmor precession of electrons. According to it, the diamagnetic susceptibility \(\chi_{\text{dia}}\), is small, negative, reversible and temperature independent (Fig. 2.1b and 2.1c). Graphite is the most diamagnetic material with \(\chi_{\text{dia}}\) in between \((80-200)\times10^{-6}\) in SI units.

![Fig 2.1: Schematic diagram for a diamagnetic material showing, a) the alignment of net magnetic moments with respect to the applied field \(H\), b) magnetization as a function of applied field, and c) magnetization as a function of temperature \(T\). (after Moskowitz, 1991) result](#)

2.1.2.2 PARAMAGNETISM

Paramagnetism is a statistical phenomenon in which the resultant magnetic moment aligns
with the direction of applied magnetic field (Fig. 2.2a). It originates from non-interacting, unpaired electron spins. The magnetization from a paramagnetic material is governed by a competitive effect between the thermal energy that randomize the spins and the magnetic field that bias spins in its direction. The induced magnetization is described by Langevin theory. Paramagnetic susceptibility $\chi_{\text{par}}$ is small, positive, and reversible. Further $\chi_{\text{par}}$ is approximately inversely proportional to the temperature, $T$ (Fig. 2.2b and 2.2c). At very low temperature the magnetic moments may become blocked; this is known as the paramagnetic Curie temperature, $\Theta$. Olivine and siderite are the examples of paramagnetic minerals with $\chi_{\text{par}}$ around $10^{-3}$ (SI).

![Schematic diagram for a paramagnetic material](image)

Fig 2.2: Schematic diagram for a paramagnetic material, showing a) the alignment of magnetic dipole moments for zero and positive field strength, b) variation in magnetization with respect to $H$ and c) $T$. (after Moskowitz, 1991)

### 2.1.2.3 FERROMAGNETISM

The term ferromagnetism (s.l.) describes materials with permanent magnetization i.e., a net magnetic moment in the absence of the applied field. Ferromagnetism is derived from the uncompensated spins that are interacting. Based on the nature of the coupling interaction within the material, ferromagnetism (s.l.) can be divided into different types. True ferromagnetism (s.s.) arises from the direct electron exchange between the neighboring atoms that are sufficiently close together. It is a quantum-mechanical effect that aligns statistically all magnetic moment parallel to the applied field (Fig. 2.3a) and is encountered only in metals, e.g., Fe, Ni and Co. The other variations in ferromagnetism are due to the superexchange coupling between the neighbouring atoms that leads to the formation of sublattices within the material. This interaction occurs through the electron shell of an intermediate anion, e.g., oxygen ions in oxide components. Antiferromagnetism is a condition in which the spin moments of the adjacent sublattices are equal but opposite in direction of magnetization (Fig. 2.3b). An example of such material is ilmenite ($\text{FeTiO}_2$). When a sublattice has defects, then the magnetizations from the adjacent sublattices are no longer exactly equal and opposite. This gives a net magnetization in the direction perpendicular to the lattice average direction. Materials with such characteristics exhibit parasitic ferromagnetism or in case of spin misalignment, a spin-canted antiferromagnetism (Fig. 2.3c)
and 2.3d). Hematite and goethite are the minerals that show this behavior. When the two sublattices have different magnetizations values due to the differences in the number of cations that accounts for their magnetization, ferrimagnetism occurs (Fig. 2.3e). Magnetite and maghemite are common ferrimagnetic materials.

**FERROMAGNETISM (s.l.) AND TEMPERATURE**

The magnetization of a ferromagnetic material is temperature dependent. With an increase in temperature the distance between the contiguous orbitals increases, leading to a decrease and eventually a complete loss of exchange interaction or magnetization. This temperature, at which the magnetization reduces to zero and reverts to paramagnetism, is defined as Curie temperature ($T_C$) in ferro- and ferrimagnetic materials and Néel temperature in antiferromagnetic materials (Fig. 2.4a).

Certain materials undergo phase transitions at specific temperature below $T_C$ or $T_N$. For example the cubic structure of magnetite changes to monoclinic at around 120 K and is called the Verwey transition [Verwey and Haayman, 1941] (Fig. 2.4b). Around 250 K the lattice spins in hematite switch from within the basal plane to the c-axis, where the lattice moments are exactly antiparallel, thus perfectly antiferromagnetic. This is known as Morin transition (Fig. 2.4b) [Morin, 1950a]. The exact temperature and whether $T_V$ or $T_M$ is seen is dependent on particle size, chemical stoichiometry, lattice defects and internal stress induced [e.g., Dézsi et al., 2008; Goya et al., 2003; Kündig et al., 1966; Muench et al., 1985; Ye et al., 2007].
Because both the $T_C$ and $T_N$ and the transition temperatures ($T_V$ and $T_M$) are material specific, they are used as a diagnostic for their presence.

**Fig 2.4:** a) Magnetization decay of a ferromagnetic mineral with increasing temperature ($T$). b) Verwey transition and Morin transition at $T$ below $T_C$ and $T_N$ respectively.

**FERROMAGNETISM (s.l.) AND APPLIED FIELD**

The magnetic response of a ferromagnetic material in an applied field is expressed by a magnetic hysteresis, i.e., a closed loop showing the lagging of magnetization behind the changing external field through a complete cycle (Fig. 2.5).

**Fig 2.5:** Schematic diagram of a magnetic hysteresis loop.
The magnetic hysteresis loop, enables the interpretation of the bulk magnetism using four basic parameters namely; saturation magnetization $M_S$, the maximum magnetization which does not increase with increasing magnetic field $H$; saturation remanent magnetization $M_{RS}$, magnetization remaining at zero field; coercivity $H_C$, magnetic field needed to apply to remove the induced magnetization; and remanent coercivity $H_{CR}$, magnetic field applied to remove remanent magnetization $M_{RS}$.

**FERROMAGNETISM (s.l.) AND MAGNETIC DOMAINS**

The classical theory of magnetization for ferromagnetism was first postulated by Weiss in 1907. He hypothesized the presence of magnetic domains, i.e., the regions in ferromagnetic materials where all the magnetic moments are aligned. Depending on the grain size and shape of a ferromagnetic material, the magnetic behavior can be distinguished as multi-domain (MD), pseudo-domain (PSD), single-domain (SD) and superparamagnetic (SP) (Fig. 2.6).

![Diagram of magnetic domain state as a function of particle size](http://www.irm.umn.edu/hg2m/hg2m_d/hg2m_d.html)

The MD state occurs in relatively large grains that are characterized by the presence of more than domains, each separated by a domain wall. The division of domains occurs for energy purposes. In the absence of field the net magnetization of these grains is ideally zero and thus do not carry a stable magnetization. Smaller grains possessing homogenous magnetization with only one magnetic domain and no domain walls are referred as SD. They carry a stable remanent magnetization. The response of a SD grain to a progressively increasing field is illustrated in Figure 2.5. There is yet another class of domain state PSD,
between MD and SD, which corresponds to a mixed characteristic from MD and SD states. These PSD grains are large enough to contain domains, but magnetization is not balanced out completely because the domain walls are fixed. Therefore, their magnetization can be stable and the particles behave magnetically more like SD grains. With further decrease in size, i.e., < ca. 20-30 nm, magnetic grains once more loses its stable magnetization. This state is called SP and is due to the randomizing effect from thermal energy. The physics of SP is described by Néel-relaxation [Néel, 1947].

MAGNETIC ANISOTROPY

The magnetization process is influenced by the symmetry of the lattice structure of a ferromagnetic material. This causes the directional variability in magnetization as a function of the direction of the magnetizing field. It is related to the crystal structure or shape of the grain and is termed as magnetic anisotropy.

![Fig 2.7: a) Magnetic moment in response to H along [100] and [111] crystallographic axis of magnetite, and b) external magnetic field lines and the demagnetizing field (Hd) for an ellipsoidal shaped magnetic material (Redrawn from http://www.irm.umn.edu/hg2m/hg2m_c/hg2m_c.html).](image)

Depending upon the crystallographic orientation of the material, different field strengths are required to reach the Ms (Fig. 2.7a). This intrinsic property of a material is known as magnetocrystalline anisotropy and is attributed to the spin-orbit coupling. In addition, shape anisotropy arises in materials with strong Ms, and is due to a non-spherical material that has a preferred, i.e., easy, axis of magnetization (Fig. 2.7b). The shape leads to a difference in the strength of the demagnetizing field. This varies in its strengths along different axis and is proportional to the dimension of the particle. The third type of anisotropy, called stress
anisotropy, is due the mechanical stress and involves both the magnetoelastic and the magnetostrictive effects.

The directional variability of magnetic susceptibility in a material is called the anisotropy of magnetic susceptibility (AMS). For an anisotropic material the susceptibility equation 2.3 still holds, but in this case $\chi$ can be described mathematically by a second-order symmetric tensor [Hrouda, 1982]. Geometrically it can be expressed as an ellipsoid with three principal axes $\chi_1 \geq \chi_2 \geq \chi_3$. The shape and the degree of anisotropy is quantified by using, magnetic lineation, ($L = \chi_1 / \chi_2$), magnetic foliation, ($F = \chi_2 / \chi_3$), and the degree of anisotropy, ($P = \chi_1 / \chi_3$).

AMS can be classified in two ways, depending upon the strength of applied magnetic field. The first is low-field anisotropy of magnetic susceptibility, (LF-AMS), where the magnetic field is $\leq 700 \text{ A/m}$ and involves combined contributions from all diamagnetic, paramagnetic and ferromagnetic material. However the presence of a strong ferromagnetic material will generally overwhelm the other contributions. The second method is high-field anisotropy of magnetic susceptibility, (HF-AMS), where the apply fields is sufficient to saturate the ferromagnetic component. HF-AMS is used to identify the anisotropic carriers i.e., magnetite, hematite or paramagnetic material. It allows the separation of individual contributions from paramagnetic, diamagnetic and ferromagnetic components [Martin-Hernandez and Hirt, 2001; 2004; Schmidt et al., 2007].
2.2 INSTRUMENTATION AND METHODS

2.2.1 MAGNETIC METHODS

The following provides a short description of the instrumentation that were used to make the majority of magnetic measurements in this dissertation. These were made at the Laboratory of Natural Magnetism, ETH-Zurich.

2.2.2 MICROMAG INSTRUMENT

VIBRATING SAMPLE MAGNETOMETER (VSM)

A Princeton Measurement Corporation (PCM), MicroMag VSM, model 3900, was used to define the elementary magnetic properties. Its principle of operation is based on Faraday’s Law, whereby a magnetic material is vibrated in an applied field. The rate of change of induced magnetization will be proportional to the induced voltage in the sensing coils. It can measure magnetic moments in the range 50 nAm$^2$ to 10 mAm$^2$ as a full scale measurement with a standard deviation of 0.5 nAm$^2$ at room temperature with 1 s averaging time.

The following experiments have been performed to characterize the magnetic properties of the NP using this VSM:
- hysteresis loops to obtain saturation magnetization ($M_S$) and coercivity ($H_C$) of the bulk material and to estimate for the domain states, magnetic minerals and their purity;
- backfield isothermal remanent magnetization (IRM) to attain remanent coercivity ($H_{CR}$) of the bulk material and to interpret the chemical purity of the NP;
- first-order reversal curves (FORC) analysis for peak coercivity ($H_{CP}$), coercivity distribution ($H_c$), grain size spectra and interaction field ($H_U$);
- hysteresis loops, dc-IRM and moment as a function of rotational angle to quantify the degree of anisotropy in magnetically aligned particles;
- low temperature hysteresis, IRM, FORC, field-cooled (FC), zero-field-cooled (ZFC) measurements to aid in interpretation of grain size spectra, interaction and compositional purity; and
- zero-field cooled and field-cooled magnetization to estimate the blocking and bifurcation temperature and the size distributions.

ALTERNATING FIELD GRADIENT MAGNETOMETER (AGM)

A PCM MicroMag Alternating Gradient Magnetometer (AGM), was used for samples with weaker magnetic signal as it has a full scale magnetic moment range from 1 nAm$^2$ up to 5 mAm$^2$ with 10 pAm$^2$ sensitivity. The measurement principal of AGM is similar to that of
VSM, but in this case the sample is fixed and the uniform magnetic field alternates [Flanders, 1988].

Both the MicroMag instruments have an accuracy of 2% versus calibration with a standard reference material Yttrium iron garnet, (YIG) sphere from National institutes of standards and technology, (NIST).

2.2.2.1 TORQUE MAGNETOMETER

A home-built torque magnetometer was used to measure HF-AMS [Bergmuller et al., 1994b]. It is based on the principle of the torque experienced by a material with anisotropic susceptibility, when placed in a homogeneous external magnetic field. This torque tries to align sample’s easy axis of magnetization along the external field. The torque magnetometer measures the deviatoric susceptibility tensor with a sensitivity of $5 \times 10^{-9}$ SI.

The anisotropy of the aligned magnetosomes (cf. Chapter 5) was determined in the three orthogonal planes using torque magnetometer by applying fields between 500 mT to 1000 mT and rotating with a 15° increment.

2.2.2.2 AGICO MKF 1A SUSCEPTIBILITY BRIDGE

An AGICO MKF-1A Kappabridge susceptibility meter was employed to determine LF-AMS. It is based on the principle of Wheatstone-bridge and measures susceptibility with a sensitivity on the order of $10^{-8}$ SI [Pokorný et al., 2011]. The instrument also is outfitted with a cryostat, which allows measurement of susceptibility as a function of temperature between 77 to 290 K. Directional anisotropy in the case of aligned magnetosomes (cf. Chapter 5) and the susceptibility as a function of low temperature for MNP (cf. Chapter 6) in order to observe a Verwey or Morin transition, were measured using the Kappabridge.

2.2.3 NON-MAGNETIC METHODS

2.2.3.1 ELECTRON MICROSCOPY

Electron microscopic techniques were performed at two laboratories to obtain the structural, morphological, and size information. Mainly the measurements were carried out by Prof. M. Pósfai, using Philips JEOL 3010 transmission electron microscopes, at the Department of Earth and Environmental Sciences, University of Pannonia. Low temperature scanning electron microscopy, (SEM) measurements were performed on self-assembled magnetosomes using field emission-SEM (Gemini 1530 equipped with a cryo-stage), at the Scientific Center for Optical and Electron Microscopy (ScopeM) ETH-Zurich.
Characterization of Particle Size: Magnetic Domain State

Magnetite nanoparticles with varying mean particle size.
(Source: http://nanonet-nm.org/2013/02/21/nanotechnology-and-shale-gas-production/)

This chapter consists of three individual studies that are concerned with characterizing the particle size or magnetic domain state of magnetite nanoparticles.
3.1 DISTINGUISHING MAGNETIC PARTICLE SIZE OF IRON OXIDE NANOPARTICLES WITH FIRST ORDER REVERSAL CURVE (FORC)

Monika Kumari, Marc Widdrat, Éva Tompa, Rene Uebe, Dirk Schüler, Mihály Pósfai, Damien Faivre and Ann M Hirt; Published in Journal of Applied Physics; 2014; doi: 10.1063/1.4896481

3.1.1 INTRODUCTION

Magnetic iron oxide nanoparticles have attracted immense interest due to various technological applications that exploit their magnetic properties. Recent years have witnessed unprecedented growth in the number of studies on the synthesis or application of magnetic nanoparticles (MNP) [e.g., Hao et al., 2010; Hong et al.; Lu et al., 2007; Majewski and Thierry, 2007; Riaz et al., 2014]. Potential application may require different properties of the MNP, however, their monodispersibility with controlled size, stability, composition, magnetic properties and functionality are critical for any application. Often transmission electron microscopy (TEM) and X-ray diffraction (XRD) are used to study the morphology and the size distribution of MNP. It is important to note that the principle size distribution does not necessarily correspond to the effective magnetic particle size. Most commonly, magnetic behavior is described by magnetic hysteresis loops. Hysteresis loops reflect the bulk magnetic properties of all the particles in a sample. However, hysteresis loops do not fully differentiate between mineral composition, size distributions, domain state or magnetic interactions. Similar loops can be obtained from particles with different properties, and thus lead to ambiguous interpretation. First order reversal curves (FORC) [e.g., Carvallo et al., 2006; Muxworthy et al., 2005; Pike et al., 1999; 2001; Roberts et al., 2000] provide an efficient way to achieve a detailed magnetic characterization of MNP in terms of composition, domain state and particle interaction. Establishing FORC diagrams for a wide range of well-characterized MNP is essential in interpreting their magnetic behavior [Dobrotă and Stancu, 2013; Giri et al., 2013; Hirt et al., 2014].

This study presents FORC diagrams for different MNP characterized by TEM or XRD. It demonstrates the effectiveness of using the reversible and irreversible components of magnetization from FORC data set to obtain a semi-quantitative measure of the superparamagnetic (SP) and magnetically blocked particles. The threshold diameter, $d_{th}$, for the SP and single domain (SD), i.e., magnetically ordered, magnetite nanoparticles depend on the measurement time used for the FORC acquisition. With a measurement time of 100 ms, $d_{th}$ is 25 nm. Furthermore, we demonstrate by masking the dominant part on the FORC response aids in revealing the existence of secondary/minor magnetic contributions, thus
helping in a more accurate characterization of the magnetic properties.

### 3.1.2 METHODS AND SAMPLES

#### 3.1.2.1 SAMPLES

We examined three sets of MNP. The first set comprises of magnetosomes that were extracted from four distinct strains of the magnetotactic bacterium Magnetospirillum gryphiswaldense. Each magnetosome consists of isometric magnetite nanoparticle surrounded by a lipid membrane. Magnetosomes were extracted from strains ΔF3D (BT23.6), ΔFe4 (BT28.7), ΔA11 (BT32) [Lohsse et al., 2011], and Δmms48 (BT41) [Lohsse et al., 2014], and each sample contained magnetosomes either isolated or in chains. The second set consists of chemically synthesized magnetite nanoparticles prepared by co-precipitation of iron (II) and (III) salts [Baumgartner et al., 2013]. These uncoated particles are designated as SX14, SX17, SX33 and SX36, where the first letter stands for synthetic, second corresponds to the measurement type for size distribution, i.e., XRD, followed by the mean size of the particles (in nm). The third sample set is a mixture, obtained by adding ferrofluid (FF) from Chemicell GmbH, FluidMAG-D with a mean hydrodynamic diameter equal to 45 nm and an iron-core diameter (d) of 10 nm, to a small fraction of magnetotactic bacteria (MTB) (≤5%) that contain SD magnetite in chains.

#### 3.1.2.2 TRANSMISSION ELECTRON MICROSCOPY

For TEM measurements the magnetosomes and the synthesized magnetite nanoparticles were directly deposited onto carbon-coated copper grids. Measurements were carried out using a JEOL 3010 microscope, operated at 300 kV and a Philips CM20 transmission electron microscope operated at 200 kV accelerating voltage. Bright-field, amplitude-contrast images, high-resolution transmission electron microscope (HRTEM) images and selected-area electron diffraction (SAED) patterns were obtained to study the sizes, shapes and structures of the crystals. Particle sizes were measured on digitized images using ImageJ software.

#### 3.1.2.3 X-RAY DIFFRACTION MEASUREMENTS

Synthetic samples were dried under atmospheric conditions on a Kapton thin film. X-ray diffraction measurements were performed in transmission with a 100 µm beam of the wavelength λ ≈ 0.82656 Å at the µ-Spot beam line, BESSY II, Berlin. The size was determined by fitting the (311) peak by Scherrer analysis with a pseudo-Voigt function and instrumental broadening correction [Fischer et al., 2011].

#### 3.1.2.4 MAGNETIC MEASUREMENTS

Samples for FORC measurements were dried to obtain in powder form, and later
immobilized by placing in gently pressed gelcaps. FORC were performed on Princeton Measurements Corporation, Vibrating Sample Magnetometer (VSM; Micro-Mag Model 3900) at room temperature. Each FORC measurement begins with first saturating the sample by a positive applied field of 1T, followed by tracing 140 magnetization curves. A field increment of 2.3 mT and an average measurement time of 100 ms were used. A FORC diagram is a contour plot of the FORC distribution with $H_C$ on the horizontal axis and $H_U$ on the vertical axis. The FORC data were transformed into a FORC diagram using the Winklhofer MATLAB code [Winklhofer and Zimanyi, 2006]. All FORC diagrams are processed with smoothing factor (SF) of 2. In a FORC diagram, the horizontal axis denoting the coercive field ($H_C$) is sensitive to the composition and grain size, while the vertical axis ($H_U$) provides information on magnetic interaction between particles [Roberts et al., 2000].

3.1.3 RESULTS AND DISCUSSION

3.1.3.1 BIOGENIC NANOPARTICLES: MAGNETOSOMES

In all samples smaller magnetosomes were randomly scattered on TEM grids, whereas larger magnetosomes (>25 nm) showed a preference to form chains. The mean size of the magnetite nanoparticles in the individual samples within this set varied from 23.6 nm to 41.0 nm (Fig. 3.1.1). All samples can be divided into fractions of magnetite nanocrystals, with diameter, $d > 25$ nm and a fraction $\leq 25$ nm. BT23.6 consists mostly of nanoparticles with diameter $d \leq 25$ nm whereas BT41 contains principally magnetite with $d > 25$ nm (Fig. 3.1.1). The samples BT28.7 and BT32 lie between these two end members.

![Fig. 3.1.1: a) Bright-field transmission electron microscope (TEM) images (top) and; b) corresponding particle size distributions (bottom) as measured from TEM micrographs, of the extracted magnetosomes. Sample names are indicated on the top of the TEM images. Red line separates the particles with sizes below and above 25 nm in diameter (d).](image)
The FORC distribution of BT23.6 is located near the origin of the plot, i.e., where $H_U$ and $H_C$ are zero. This distribution is characteristic for the presence of mostly SP particles whose magnetization relaxes on the order of 100 ms. The FORC distribution is displaced slightly above the $H_U = 0$ axis, indicating the absence of magnetic interactions among the particles [Pike et al., 2001; Roberts et al., 2000].

An increase in the contribution from SD particles is seen by the increase in the spread along $H_C$ axis. Hence, BT41, which contains mainly SD particles, has the largest $H_C$ spread (Fig. 3.1.2a). Further FORC analysis allows deconvolution for reversible and irreversible components [Winklhofer et al., 2008]. The reversible part has a contribution from SP particles and the reversal magnetization of SD particles, while the irreversible component arises from the magnetically blocked particles. When the magnetization of magnetite is dominated by shape anisotropy, the reversible contribution for non-interacting SD particles is 50%. We used the peak susceptibility, i.e., $\partial M/\partial H$, to obtain a semi-quantitative estimation of the SP fraction, assuming non-interacting particles with shape anisotropy. Figure 3.1.2b clearly indicates that each sample is a mixture of SP and SD particles. BT23.6 is predominantly made up of SP particles with a very small contribution from blocked particles. The relative proportion of SP particles is 44% (Table T3.1.1.).

Fig. 3.1.2: a) FORC diagrams with smoothing factor SF (top); and b) irreversible (solid-red)/reversible (dashed-blue) components to the magnetization (bottom), for isolated magnetosomes. Sample names indicated on the top of the FORC diagrams.
With increasing average particle size, there is a progressive increase in irreversible susceptibility. BT28.7 has 35% SP particles, BT32 has 23% and BT41 has 18%. The amount of SP particles is slightly higher than what is predicted from the TEM images in all samples except BT23.6. This discrepancy is discussed below.

### 3.1.3.2 CHEMICALLY SYNTHESIZED UNCOATED NANOPARTICLES

All samples contained magnetite nanoparticles. They vary in their size distribution and mean particle size. For example, SX14 and SX17 have $d < 25$ nm and show a low spread in particle size distribution (Fig. 3.1.3). Sample SX33 exhibits uniformly distributed aggregates of magnetite over the grid, with a mean size of 33 nm, but size spreads from 10 nm to 100 nm. Larger particles are aggregates of small-sized crystals. Sample XS36 comprises essentially small particles (~15 nm); with some larger aggregates composed of randomly or similarly oriented nanocrystals.

![Image of TEM images of synthetic uncoated magnetite nanoparticle](image)

**Fig. 3.1.3:** Bright-field TEM images of synthetic uncoated magnetite nanoparticle, a) SX14; and b) SX17. Particle size distributions as measured from TEM micrographs using Image J software for c) SX14; and d) SX17. Red line separates the particles with sizes below and above 25 nm in d.

The FORC distribution for SX14 is centered at the origin with an offset along $H_U$, signifying the presence of SP particles (Fig. 3.1.4a); however, the spread along $H_C$ and $H_U$ is larger compared to BT23.6, suggesting a significant amount of larger particles [Roberts et al., 2000]. The FORC diagram for SX17 exhibits spread in $H_C$ up to 35 mT while $H_U$ is ± 40 mT. Again the spread in both $H_C$ and $H_U$ suggests a broader particle size distribution compared to that obtained in TEM analysis. The FORC distribution of SX33 shows a broad distribution along
the $H_U$ axis, indicating the highest degree of particle interaction in the sample group. Sample SX36 shows a FORC distribution that is slightly displaced away from the origin along $H_C$ indicating the dominance of magnetically ordered particles over unblocked particles. In case of highly aggregated particles magnetic properties reflect the effective magnetic particle size.

Separation of the FORC distribution into its reversible and irreversible components indicates that all synthetic samples consist of a mixture of SP and SD particles, in varying proportions (Fig. 3.1.4b). Sample SX14 shows the highest amount of SP particles, similar to BT23.6 (Table T3.1.1). While 18% of the magnetization of SX36 is carried by SP particles, SX17 has approximately 34% contributions from un-blocked particles.

![FORC diagrams](image)

Fig. 3.1.4: Synthetic uncoated magnetite nanoparticles, a) FORC diagrams with smoothing factor $SF$; and b) blocked (solid-red)/un-blocked (dashed-blue) components to the magnetization. Sample names indicated on the top of the FORC diagrams.

### 3.1.3.3 MIXTURE OF SP AND SD MAGNETITE

Figure 3.1.5a - 3.1.5b shows the FORC distributions for the individual ferrofluid sample of SP magnetite particles and the chains of SD magnetite, taken from magnetotactic bacteria, respectively, whereas Figure 3.1.5c shows the FORC diagram for their mixture. Based on the value of magnetic moment acquired at 1 T for the SD fraction and the mixture, we estimate that $\leq 5\%$ of the SD + SP mixture is SD magnetite. The FORC distribution for the mixed system is characterized by peak coercivity at the origin of the diagram with an upward offset
along $H_U$. This is characteristic for SP magnetite and resembles the FORC distribution from the individual ferrofluid sample. There is a secondary feature in the FORC distribution with a very weak contribution close to the noise level that is observed in the coercivity range from 10 mT and 20 mT. The derivative of induced magnetization shows that the reversible component is dominant, but there is also a contribution from the magnetically blocked magnetite in the mixture. Truncating the dominant SP contribution, i.e. masking the initial coercivity from zero to 5 mT on the FORC diagram, leaves an elongated distribution with a peak coercivity $\approx 14$ mT (Fig. 3.1.5b). The truncated FORC distribution is in agreement with FORC diagrams that have been measured on pure samples of magnetotactic bacteria with magnetite in chains (Fig. 3.1.5b) [Egli et al., 2010]. This example demonstrates that although the SD component is only $\leq 5\%$, it is possible to extract a secondary contribution from the binary mixture of magnetite by masking dominant coercivity contributions to the FORC distribution. The method may also find application in the detection of a high coercive material such as hematite in presence of soft magnetic material.

![FORC diagram](image)

Fig. 3.1.5: FORC diagram of a) SP ferrofluid (FF); b) SD magnetotactic bacteria (MTB); c) mixture of SP (FF) and SD (MTB) particles (inset shows the ratio of superparamagnetic versus blocked particles with respect to the reversal field); and d) FORC diagram showing the presence of the SD component, obtained after masking the dominant superparamagnetic component for coercivity ranging from 0 mT to 5 mT.

3.1.3.4 COMPARISON OF THE EFFECTIVE MAGNETIC PARTICLE SIZE WITH PHYSICAL PARTICLE SIZE

In the biogenic samples, for which the particle-size distribution is known, we find a good agreement between the estimated amounts of the SP particles in all samples except BT23.6. The SP content is underestimated in this sample with the smallest grain size. BT23.6 does not contain chains of magnetite particles. We performed low temperature hysteresis measurements that showed at 30K the magnetization ratio is 0.56 (Fig. 3.1.6). Not all particles are blocked but an increase in magnetization ratio suggests that the magnetocrystalline
anisotropy is playing a role. If we assume that magnetocrystalline anisotropy dominates then only approximately 13% of the reversible component arises from the SD particles depending upon if the easy axes is 111. In this case the SP fraction in BT23.6 would be 76%. Therefore, if both magnetocrystalline and shape anisotropy are important, the amount of SP particles would be between 44% and 76%. In all of the analyzed samples the proportion of SP particles, which is estimated from FORC analysis, is higher than the estimates from TEM image analysis and X-ray diffraction. One possible reason for this discrepancy may be that the SP contribution is derived from the magnetization curve. The magnetic susceptibility of SP particles of magnetite is higher than that of SD particles [Dunlop and Özdemir, 1997], because SP grains are more efficient in aligning in an applied magnetic field. For this reason the total susceptibility will be biased towards the SP particle sizes, whereas TEM and X-ray diffraction will measure physical particle size. In addition if particles show magnetic interaction or aggregate, then they behave magnetically as a larger particle [Hirt et al., 2014].

Table T3.1.1: Comparison of SP component estimated form TEM and X-ray diffraction compared to the semi-quantitative measure of SP from FORC measurements. *size obtained from XRD measurements, Diameter, $d$, in nm, N.A = not available

<table>
<thead>
<tr>
<th>Sample name</th>
<th>% Fraction of SP particles (TEM)</th>
<th>% Fraction of SP particles (FORC)</th>
<th>Average particle size (TEM / XRD*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT23.6</td>
<td>61</td>
<td>44</td>
<td>23.6</td>
</tr>
<tr>
<td>BT28.7</td>
<td>33</td>
<td>35</td>
<td>28.7</td>
</tr>
<tr>
<td>BT32</td>
<td>19</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td>BT41</td>
<td>14</td>
<td>18</td>
<td>41</td>
</tr>
<tr>
<td>SX14</td>
<td>N.A</td>
<td>44</td>
<td>14*</td>
</tr>
<tr>
<td>SX17</td>
<td>N.A</td>
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<td>17*</td>
</tr>
<tr>
<td>SX33</td>
<td>N.A</td>
<td>27</td>
<td>33*</td>
</tr>
<tr>
<td>SX36</td>
<td>N.A</td>
<td>18</td>
<td>36*</td>
</tr>
</tbody>
</table>

In summary it is a question as to what information is needed for an application when assessing particle size, i.e. physical size or effective magnetic size. Isolation of the reversible and irreversible components to the magnetization of the FORC distribution provides information on the relative percentage of particles that are unblocked or blocked over the time scale of the measurement. This information is important in understanding the magnetic behavior of a material. TEM and XRD on the other hand provide information on the physical particle size. It must be noted, however, that this method is only valid for mixtures of SP+SD particles, and not applicable if larger particle sizes are present.
CONCLUSIONS

This study demonstrates how FORC analysis can be used to characterize a nanoparticle system. We have shown that magnetite nanoparticles formed by bio-mineralization have a low degree of magnetic interaction due to the presence of a membrane that encloses individual particles. This membrane also helps to prevent oxidation and agglomeration of the individual particles. The synthetic particles do not have any coating. Therefore, these have a tendency to aggregate, to interact magnetically, or both, which affects the effective magnetic grain size. The increase in aggregation or magnetic interaction is seen by a spread in coercivity spectrum, such that the aggregates appear as larger particles. This can lead to a difference in interpreting between the physical size distribution as found in TEM and X-ray diffraction, and the effective magnetic size distribution as obtained from FORC analysis.

The second feature that can be exploited is in using the derivative of the induced magnetization for the blocked and unblocked component has a potential for obtaining a semi-quantitative measure of the percentage of SP particles versus magnetically blocked particles (Table T3.1.1). This information is important for characterizing the magnetic behavior in a nanoparticle system. Theoretically the limit for the domain size can be varied, by changing the measurement time, i.e., by increasing the averaging time for a measurement the boundary between SP and SD behavior will be shifted to larger particle size. Table T3.1.1 shows a good correspondence between the physical grain sizes with the magnetic grain size for the nearly idealized system of magnetosomes. However, magnetic particle size shows a higher contribution from the smaller SP particles than the physical size defined from TEM due to the enhanced contribution of SP susceptibility to the magnetization. Furthermore we demonstrated how masking the dominant part on the FORC diagram reveals the existence of secondary/minor magnetic contributions and thus helps in a more accurate interpretation of the magnetic properties.
3.2 EXPERIMENTAL MIXTURES OF SUPERPARAMAGNETIC AND SINGLE DOMAIN MAGNETITE WITH RESPECT TO DAY-DUNLOP PLOTS

Monika Kumari, Rene Uebe, Éva Tompa, Wolfram Lorenz, Fredrik Ahrentorp, Christian Jonasson
Christer Johansson, Mihály Pósfai, Dirk Schüler and Ann M Hirt; Published in Geochemistry, Geophysics, Geosystems Journal; 2015; doi: 10.1002/2015GC005744

3.2.1 INTRODUCTION

The grain size of ferromagnetic (s.l) minerals plays an important role in paleomagnetism when one needs to understand the stability of magnetization in rocks and sediments. It is also of great interest in environmental magnetic studies, where grain size can reflect provenance, and will be influenced by geochemical processes. Many methods have been proposed to obtain the grain size distribution of magnetic minerals. These include microscopic methods, e.g., transmission electron microscopy, scanning electron microscopy, and methods that exploit magnetic properties that are sensitive to grain size [Carter-Stiglitz et al., 2001; Day et al., 1977; Dunlop, 2002a; b; Heslop and Roberts, 2012; Jackson et al., 1990; Kumari et al., 2014; Tauxe et al., 1996; von Dobeneck, 1996]. In practice, the Day plot has established itself as one of the most common tools to define domain states and grain sizes for magnetite and more recently for greigite [Roberts et al., 2011].

The Day plot combines theoretically derived parameters with empirical results, which were obtained from titanomagnetite with varying grain size fractions. It represents the ratio of remanent coercivity ($H_{CR}$) to coercivity ($H_C$) plotted as a function of the ratio of remanent magnetization ($M_{RS}$) to saturation magnetization ($M_S$). The Day plot is categorized into three sections that indicate single domain (SD), pseudo-single domain (PSD), or multi-domain (MD) behavior. Later Dunlop [2002a] modified the original Day plot by suggesting new boundary limits for the three domain states. By assuming non-interacting magnetite SD grains the boundaries in domain state were set at $M_{RS}/M_S \geq 0.5$ and $1 \leq H_{CR}/H_C \leq 2$; the limits are $0.02 \leq M_{RS}/M_S \leq 0.5$ and $2 \leq H_{CR}/H_C \leq 5$ for PSD grains; and $M_{RS}/M_S \leq 0.02$ and $H_{CR}/H_C \geq 5$ for MD grains. He also incorporated mixing lines for grain size distributions that include SD-PSD and SD-MD mixtures, and calculated a range of mixing lines for superparamagnetic (SP) and SD mixtures depending upon the size of the SP grains [Dunlop, 2002a]. These theoretical curves for SD-SP mixtures are derived under the following approximations: a) the two end members consist of stoichiometrically pure magnetite, whereby the ratio of magnetization shows linear additivity with respect to the end members; b) the SD magnetite consists of identical, uniaxial and non-interacting grains, in which induced magnetization is linear.
between $-M_{RS}$ and $H_C$ and backfield isothermal remanent magnetization is linear between $M_{RS}$ and $-H_{CR}$; and c) the SP magnetite is also made up of identical and non-interacting grains whose magnetization curve is governed by a Langevin function that is linear to the applied magnetic field, $H$, and saturates at lower values of $H$.

A large number of paleomagnetic studies that employ a Day-Dunlop plot find often that the samples fall into the PSD size range [e.g., Chiara et al., 2014; Cinku et al., 2013; Guzmán et al., 2011; Hao et al., 2012; Kapper et al., 2014; Li et al., 2014; Stantson et al., 2011; Tianshui et al., 2012]. One possible reason could be that the samples consist of mixtures of grain sizes. Few studies have investigated how mixtures of magnetite with known particle sizes match with predictions of SD-MD, SD-PSD and SD-SP mixtures on a Day-Dunlop plot [Dunlop, 2002a; b; Dunlop and Carter-Stiglitz, 2006; Lanci and Kent, 2003; Lees, 1997]. Dunlop and Carter-Stiglitz [2006] evaluated one mixture that consisted of SD magnetite obtained from magnetotactic bacteria for the SD end member and ferrofluid with an average magnetite diameter of 10 nm for the SP end member. Their SD-SP mixing curve falls below and/or to the left of the expected theoretical mixing trend for 10 nm SP particles. The authors attributed this difference to interactions, which will affect the effective particle volume if aggregation occurs, thereby reducing the initial SP susceptibility. The authors did not, however, experimentally determine if particle interaction occurs.

This study re-examines Day-Dunlop plots for SD-SP mixtures of well characterized end members. The first case repeats the experiment of Dunlop and Carter-Stiglitz [2006] with a mixture of bacterial SD magnetite and ferrofluid. The second case considers a mixture of SD and SP magnetite, which are both of bacterial origin. The results are compared to the earlier study of Dunlop and Carter-Stiglitz [2006] and sources of discrepancies with theoretical curves are discussed in relationship to the applicability of the plot in predicting the percentage of SP particles and their sizes in a SD-SP mixture.

### 3.2.2 METHODS AND SAMPLES

#### 3.2.2.1 METHODS

**TRANSMISSION ELECTRON MICROSCOPY**

Transmission electron microscopy (TEM) analyses of the end members, i.e, SD-bacteria, SP-ferrofluid and SP-bacteria, were made at three different places. The SD-bacteria were imaged at the Scientific Center for Optical and Electron Microscopy (ScopeM) at ETH-Zurich using a FEI Morgagni 268, operated at 100 kV. The ferrofluid was analyzed at the Research Institute for Technical Physics and Materials Sciences in Budapest, Hungary using a JEOL 3010 microscope, operated at 300 kV, and TEM micrographs of SP-bacteria were obtained at...
LMU Munich using a FEI Tecnai F20 transmission electron microscope at an accelerating voltage of 200 kV.

**MAGNETIC MEASUREMENTS**

Induced magnetization, acquisition of an isothermal remanent magnetization in backfield (DC-IRM) and first-order reversal curves (FORC) [Carvallo et al., 2006; Muxworthy et al., 2004; Pike et al., 1999; 2001; Roberts et al., 2000] were performed on a vibrating sample magnetometer (VSM, model 3900) from Princeton Measurement Corporation (PCM). Hysteresis loops were measured between ± 1 T with a 100 ms averaging time, using a varying field increment: a 0.5 mT field increment was used between ± 20 mT, a 1 mT increment in the range |20| to |100| mT, and 10 mT increment in higher fields. The DC-IRM curve was acquired at room temperature by saturating SD-bacteria in a field of + 1 T, and then remagnetizing the sample in the opposite direction, to obtain $H_{CR}$. Measurements were made with a 0.01 T increment and 100 ms averaging time. Because it was not possible to normalize the magnetic moment by the Fe content, we give results in terms of the total magnetic moment.

The FORC protocol for the SD-bacteria plus SP-ferrofluid series consists of 180 curves with a field increment of 1.3 mT. However, a set of 240 curves for the mixtures of SD-bacteria and SP-bacteria were made with a field increment of 0.61 mT to increase the signal-to-noise ratio, due to the weak magnetic properties of the samples. FORC data were processed using the M.Winklhofer MATLAB code [Winklhofer and Zimanyi, 2006] with smoothing factor, SF = 2, unless otherwise given. The first point artifact is removed. In a FORC diagram, the horizontal axis denotes the distribution of coercive field ($H_C$), which is sensitive to composition and grain size of the ferromagnetic (s.l.) phases, while the vertical axis ($H_U$) provides information on magnetic interaction between the particles. The full width at half maxima $H_{C1/2}$ and $H_{U1/2}$ were used to characterize interactions in these samples.

Frequency-dependent susceptibility (AC susceptibility) in a large frequency range at room temperature was measured with two AC susceptometer instruments, the DynoMag system from Acreo Swedish ICT AB (frequency range 1 Hz – 500 kHz) and a prototype high frequency (HF) AC susceptometer (frequency range 20 kHz to 10 MHz) at Acreo Swedish ICT AB. Measurements were all carried out at 298 K in the frequency range from 1 Hz to 10 MHz [R M Ferguson et al., 2013]. The field amplitude is 0.5 mT for the DynoMag system and 30 µT for the HF system. Both AC susceptometer systems are calibrated against a Dy₂O₃ sample (paramagnetic substance) over the entire frequency range with respect to signal response amplitude and phase. The AC susceptibility is given in volume susceptibility (SI units). The sample volume is used to normalize the measured AC magnetic moments. Since
the samples are liquids it is easier to use the sample volume and report the volume susceptibility. It is simple to convert the volume susceptibility to mass susceptibility by divide the volume susceptibility by the density of the samples (close to 1000 kg/m$^3$, carrier liquid is water) to get the mass susceptibility in m$^3$/kg. The sample volumes are 0.2 mL.

An AC susceptibility model is based on the Debye expression, which is integrated over a size distribution (log-normal function) for the Brownian relaxation part and a Cole-Cole expression for the Neel relaxation [Öisjöen et al., 2010]. This AC susceptibility model was fitted to the AC susceptometry data to determine the particle size distribution and type of magnetic relaxation (Brownian and Néel relaxation or a combination) of the magnetic nanoparticle systems [Öisjöen et al., 2010]. Neel relaxation is the internal relaxation process in single domain nanocrystals and is dependent for instance on the energy barrier, i.e., the product of the magnetic anisotropy and the volume of the single domain nanocrystal. Brownian relaxation is the stochastic relaxation process, which occurs when particles are placed in a liquid; it is dependent on the viscosity of the liquid and the hydrodynamic volume of the particle [Krishnan, 2010]. The different relaxation process for a nanoparticle system in a carrier liquid can be determined by studying in which frequency range the relaxation features occurs, i.e., changes in the in-phase and out-of-phase components of the AC susceptibility. Generally magnetic nanoparticles dispersed in a carrier liquid that exhibit Brownian relaxation the relaxation is seen below 100 kHz while the Néel relaxation is observed above 100 kHz [Krishnan, 2010].

Frequency-dependent susceptibility was also measured as a function of temperature on a Quantum Design Physical Properties Measurement System (PPMS-14T) at the Institute of Solid State Physics, ETH Zürich in seven frequencies between 10 Hz and 10 kHz, using a 5 K measurement interval between 5 K and 300 K.

In order to characterize magnetostatic interaction in SD-bacteria, IRM acquisition and alternating field (AF) demagnetization curves were performed using a 2G Enterprises SQUID rock magnetometer from (Model 755), kept inside a magnetically shielded room. The sample was first subjected to AF demagnetization along all three axes and subsequently given a magnetization in a known direction, using an ASC Impulse Magnetizer (Model IM-10-30). The magnetization acquired was then measured as a function of applied magnetic field strength. Finally, the sample was step-wise demagnetized in the direction in which the remanence was acquired.
3.2.2.2 SAMPLE DESCRIPTION

END MEMBERS

The end-members that were used for the two SD-SP mixing series consist of: a) *Magnetospirillum gryphiswaldense* strain MSR-1, as the source of SD-magnetite for both series; b) ferrofluid, FluidMAG-D, (Article number: 4101-1; 4101-5), which was purchased from Chemicell GmbH with a mean hydrodynamic diameter of 45 nm and magnetite nanocrystal size of 10 nm as one SP end member; and c) *Magnetospirillum gryphiswaldense* strain ΔA12, whose mean particle size is reported to be 18.4 ± 6.0 nm [Lohsse et al., 2011], as the SP end member in the second series. Note that the minor variations in cultivation conditions and growth phases can lead to smaller or larger average size. The individual magnetite nanoparticles within MSR-1 and ΔA12 bacterium are surrounded by a lipid bilayer that protects the magnetite from oxidation and clustering. The synthetic SP particles were coated with starch that provides steric repulsion between the particles to prevent oxidation and aggregation.

SD-SP MIXING SERIES

Two different samples were prepared from the MSR-1 sample. Both were dried in a small capillary tube and are referred to as SD-bacteria. They served as the initial material for both mixing series; a) SD-bacteria plus SP-ferrofluid, and b) SD-bacteria plus SP-bacteria. A small amount of the SP sample was added incrementally to the fixed amount of SD-bacteria. Measurements were performed on dried and immobilized samples after each addition.

3.2.3 RESULTS

3.2.3.1 TEM RESULTS

TEM analysis was done for the end members. The SD-bacteria contain chains of euhedral magnetite crystals with an average size of 35 ± 10 nm, but with a distribution in particle sizes between ca. 10 nm to 60 nm (Fig. 3.2.1a). The magnetite particles from ferrofluid formed a continuous film on the TEM grid and were embedded in an amorphous material that resulted in a poor TEM image contrast. This made it difficult to measure the particle size distribution due to aggregation. A mean iron-core size was determined from 24 individual particles and gave an average size of 6 nm (Fig. 3.2.1b). The diameter of particle aggregates, however, is approximately 10-12 nm. The ΔA12 bacteria contain isolated magnetite crystals that are dispersed and have an average size of 14 ± 6 nm (Fig. 3.2.1c). This size falls within the range reported for the ΔA12 strain by Lohsse et al. [2011]; however there are also a few larger magnetosomes of single domain size in individual bacterium cells.
Fig. 3.2.1: Bright-field transmission electron microscopic images of: a) SD-bacteria; b) SP-ferrofluid; and c) SP-bacteria. Inset shows the corresponding particle size distributions as measured from TEM micrographs.

3.2.3.2 MAGNETIC RESULTS

END MEMBER MAGNETIC PROPERTIES

Figure 3.2.2 shows the hysteresis loops, IRM acquisition and FORC diagram of the SD-bacteria at room temperature. The hysteresis loops are open with $H_C = 11.3$ mT (Fig. 3.2.2a) and the dc-IRM curve is saturated at fields of approximately 100 mT (Fig. 3.2.2b). $M_{RS}/M_S$ and $H_{CR}/H_C$ from the hysteresis loops are 0.45 and 1.3, respectively. These values are consistent with those reported for magnetosomes in intact chains [Denham et al., 1980; Fischer et al., 2008; Moskowitz et al., 1993]. They deviate slightly, however, from the ideal values for non-interacting SD magnetite with shape anisotropy reported by Day et al. [1977], i.e., $M_{RS}/M_S = 0.5$ and $H_{CR}/H_C = 1.5$. This is attributed to the presence of a minor contribution from nascent SP particles at the ends of the chains [Pan et al., 2005]. The FORC diagram shows a characteristic elongated coercivity distribution with a peak around 13.5 mT and $H_{U1/2} = 3.5$ mT (Fig. 3.2.2c). The low value of $H_{U1/2}$ indicates negligible or no interaction among the individual magnetite nanoparticles. The FORC diagram also displays a pronounced negative region in the lower $H_U$ region close to $H_C = 0$ indicative of non-interacting SD grains [Newell, 2005]. The FORC diagram also displays a pronounced negative region in the lower $H_U$ region close to $H_C = 0$ indicative of non-interacting SD grains [Newell, 2005]. The FORC diagram also displays a pronounced negative region in the lower $H_U$ region close to $H_C = 0$ indicative of non-interacting SD grains [Newell, 2005]. The FORC diagram also displays a pronounced negative region in the lower $H_U$ region close to $H_C = 0$ indicative of non-interacting SD grains [Newell, 2005]. The FORC diagram also displays a pronounced negative region in the lower $H_U$ region close to $H_C = 0$ indicative of non-interacting SD grains [Newell, 2005]. The FORC diagram also displays a pronounced negative region in the lower $H_U$ region close to $H_C = 0$ indicative of non-interacting SD grains [Newell, 2005]. The reversible and irreversible components for the SD-bacteria show that some particles are relaxing over the measurement scale of 100 ms, in which the fraction of SP particles is estimated to be $\approx 16 \%$ of the total magnetic response [Kumari et al., 2014].

The hysteresis loops for both SP samples are closed, thus having a negligible magnetization ratio (Fig. 3.2.2d). The magnetic moment of the SP bacteria is very weak, which accounts for the noisier measurements. Their FORC distributions are centered at the origin of the diagram with a positive shift along the axis of the interaction field (Fig. 3.2.2e and 3.2.2f).
Fig. 3.2.2: a) Hysteresis loops; b) dc-IRM; and c) FORC distribution with an inset showing reversible and irreversible magnetization, for SD-bacteria. Note: $m_s = 10\mu A m^2$ for SD-bacteria; d) Hysteresis loops for SP samples; and FORC distributions with an inset showing reversible and irreversible magnetization, for e) SP-ferrofluid and f) SP-bacteria.
The AC susceptibility (in-phase $\chi'$ and out-of-phase $\chi''$), measured as a function of excitation frequency, for the FluidMAG-D sample from Chemicell GmbH is shown in Figure 3.2.3. Because the measurements were made on a liquid sample both Brownian relaxation and Néel relaxation are important. The peak in $\chi''$ at about 400 Hz is due to the Brownian relaxation and the gradually decrease in $\chi'$ and the non-zero value in $\chi''$ at frequencies above about 10 kHz is due to the AC susceptibility contribution from the Néel relaxation. The experimental data was fitted to an AC susceptibility multi-core particle model [Öisjöen et al., 2010] as described above. Since this model is a superposition of both the Brownian and the Néel relaxation, the amount that each relaxation contributes to the total AC susceptibility at low frequencies can be determined. Brownian relaxation was estimated to be 72% of the total signal while Néel relaxation was 28%. A mean value of the magnetite nanocrystal size can be estimated from the Néel relaxation time determined from the used model for data fitting and using the Néel relaxation time expression (assuming low fields and negligible magnetic interactions):

$$\tau = \tau_0 \exp(K_a V/kT)$$

where $\tau$ is the Néel relaxation time; $\tau_0$ is the attempt time, $K_a$ is the magnetic anisotropy constant, $V$ is volume, $k$ is Boltzmann’s constant and $T$ is temperature. From the result of the data fitting, we get, $\tau = 1.2 \times 10^{-6}$ s; and assuming $K_a = 2 \times 10^4 - 4 \times 10^4$ J/m$^3$ and $\tau_0 = 10^{-9} - 10^{-10}$ s, (typical values of $K_a$ and $\tau_0$ for magnetite nanocrystals at room temperature), the average value of the magnetite nanocrystal diameter is in the range of 11-15 nm for a spherical volume.

Fig. 3.2.3: $\chi'$ and $\chi''$ components of the AC susceptibility as a function of frequency at room temperature. Dotted lines show the fit to the AC susceptibility model.
SD-SP MIXING SERIES

Mixture of SD-Bacteria and SP-Ferrofluid

\( M_{RS} \), which is obtained from the hysteresis or FORC measurements, can be used to control whether remanent magnetization changed with the incremental addition of the SP sample. The value stays relatively constant with the incremental addition of SP ferrofluid, which demonstrates that the properties of the SD end member are not changing during the addition of the SP component (Fig. 3.2.4a). The difference between the values of \( H_{CR} \) obtained from the FORC and dc-IRM measurements reflects the different modes of magnetic field control between the two measurement methods, and the smaller measurement interval used in the FORC measurement. This difference becomes important with increasing content of SP material (Fig. 3.2.4b). Hence the magnetic parameters, i.e. \( M_S \), \( M_{RS} \), \( H_C \) and \( H_{CR} \), for both SD-SP mixing series were obtained from the FORC measurements. The values of \( M_{RS} \) and \( H_{CR} \) have a standard deviation of ± 0.36 \( \mu \)Am\(^2\) and ± 0.37 mT about their mean values, respectively. The standard deviation is well within the error estimation limit, which is 1.5 \( \mu \)Am\(^2\) for \( M_{RS} \) and 2 mT for \( H_{CR} \). No systematic variation is observed in either parameter.

Fig. 3.2.4: Effect of successive addition of SP-ferrofluid in SD-bacteria + SP-ferrofluid series on a) \( M_{RS} \) and b) \( H_{CR} \). Red represents the data obtained from hysteresis loops/dc-IRM and blue corresponds to data points from FORC analysis. Note: ‘0’ represents the initial SD-bacteria sample, i.e., without addition of ferrofluid.
Figure 3.2.5a shows the magnetization and coercivity ratios on a Day plot obtained for a series of mixtures of SD-bacteria and SP-ferrofluid, as a function of increasing concentration of the SP fraction. Similar to the result of Dunlop and Carter-Stiglitz [2006], the values lie to the left of the theoretical curve for 10 nm. Our results show a good agreement, however, for a mixing line with 6 nm up to 54.7 % addition by volume of ferrofluid. Above this the remanence ratio is higher than expected, and this deviation increases with increasing concentration. Figure 3.2.6a, 3.2.6c and 3.2.6e shows the FORC distribution and the cross-sectional coercivity spectrum for the initial sample (0% SP-ferrofluid), an intermediate member of the series (64% SP-ferrofluid), and the final sample in the mixing series (94.2% of ferrofluid). It clearly demonstrates; 1) a shift in the peak FORC distribution function towards the origin of the FORC diagram, 2) an increase in the reversible peak of the induced magnetization, with a constant magnitude of the irreversible component, and 3) a constant magnitude of $H_{C1/2}$ of the high coercivity (SD) (Table T3.2.1) with incremental addition of SP sample to the SD-bacteria (Fig. 3.2.6b, 3.2.6d and 3.2.6f).

![Day-Dunlop plot](image)

**Fig. 3.2.5:** Day-Dunlop plot for mixtures of a) SD-bacteria plus SP-ferrofluid, and b) SD-bacteria plus SP-bacteria. Red curve with solid points represents experimental data, while black curve with solid points are theoretically-predicted mixing curves. Note: triangles are for mixing series with SP-ferrofluid and diamonds for mixing series with SP-bacteria. Open diamond represents the measurements from non-dipolar sample volume, and hence the dotted line is connecting this measurement point. Blue curves are the mixing curves of SD plus SP mixtures for different concentrations of SP content. Concentration of the SP particles for the points on the mixing lines can be found in Table T3.2.1 and T3.2.2.
Table T3.2.1. Magnetic parameters for the SD-bacteria plus SP-ferrofluid mixing series.

$\mu_o H_c$—coercive force; $\mu_o H_{CR}$—remanent coercivity; $m_{RS}$—remanent saturation moment; $m_S$—saturation moment, $\text{Area}$—area of normalized hysteresis loop; $\mu_o H_{1/2 \text{ high}}$—full width at half maxima of coercivity peak from FORC distribution of SD contribution, $\mu_o H_{1/2 \text{ low}}$—full width at half maxima of coercivity peak from FORC distribution of SP contribution.

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<th>$\mu_o H_{CR}$ (mT)</th>
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<th>$m_{RS}$ (μAm$^2$)</th>
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Table T3.2.2. Magnetic parameters for the SD-bacteria plus SP-bacteria mixing series.

$\mu_o H_c$—coercive force; $\mu_o H_{CR}$—remanent coercivity; $m_{RS}$—remanent saturation moment; $m_S$—saturation moment, $\text{Area}$—area of normalized hysteresis loop; $\mu_o H_{1/2 \text{ high}}$—full width at half maxima of coercivity peak from FORC distribution of SD contribution, $\mu_o H_{1/2 \text{ low}}$—full width at half maxima of coercivity peak from FORC distribution of SP contribution.

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Fig. 3.2.6: FORC distribution with an inset showing reversible and irreversible magnetization in the left column and coercivity distribution taken at $\mu_0 H_a = 0$ in the right column; a) and b) (100%) SD-bacteria end member, c) and d) SD-bacteria plus 64% SP-ferrofluid mixture, and e) and f) SD-bacteria + 94.2% SP-ferrofluid mixture. The cross-sectional coercivity distribution is obtained by a profile along the horizontal axis $H_C$ through the FORC distribution function.
Mixtures of SD-Bacteria and SP-Bacteria

The magnetization and coercivity ratios for the mixing series of SD-bacteria and SP-bacteria also fall below the theoretical mixing curve for SP grain size \( \geq 14\) nm on the Day-Dunlop plot (Fig. 3.2.5b). Due to its weak magnetic signal, larger amounts of the SP bacteria had to be added to the sample, which gave a larger sample volume. For this reason the last experimental point in this series no longer approximates a dipole signal. Similar to the ferrofluid mixing series we also observe; 1) a shift in the peak FORC distribution function towards the origin of the FORC diagram (Fig. 3.2.7a, 3.2.7c and 3.2.7e), 2) an increase in the magnitude of the reversible peak and constant magnitude of irreversible peak of the induced magnetization (insets from Fig. 3.2.7a, 3.2.7c and 3.2.7e), and 3) an enhanced peak near the origin but a constant \( H_{C1/2} \) for high coercive phase at \( \approx 14\) mT as a function of incremental addition of SP-bacteria to the SD-bacteria (Fig. 3.2.7b, 3.2.7d and 3.2.7f) (Table T3.2.2).

3.2.4 DISCUSSION

3.2.4.1 ROLE OF MAGNETIC INTERACTIONS

The mixing series of SD bacteria with SP ferrofluid shows a good agreement with the theoretical mixing assuming particles with 6 nm diameter at low concentration but a deviation occurs with higher ferrofluid concentration. The curvature in the mixing line has also been seen by Dunlop and Carter-Stiglitz [2006], who attributed the sigmoidal shape to a change that occurs in the shape of the magnetization curve when the SP concentration exceeds 50%. A 6 nm particle size agrees with what was found on TEM images from the few isolated particles, but does not agree with the bulk magnetic behavior, for which the AC susceptibility suggests particles with 11-15 nm diameter. Aggregation of smaller particles could account for the difference. Dormann et al. [1999] also observed the effect of interparticle interaction in ferrofluids with higher concentration.

The mixing series of SD bacteria and SP bacteria follow a trend that lies below the theoretical mixing line for \( \sim 14\) nm particles on the Day-Dunlop plot. The observed discrepancy could be accounted for by smaller SP particle size, which does not appear to be likely, or may be due to the presence of magnetic interaction, a skewed SP particle size or shape distribution, chemical purity of the samples, or a combination of these. In the following we perform a set of experiments to test these different hypotheses for the observed discrepancy in the two sets of data.

\( H_{C1/2} \), for the SD (large coercivity) and SP (low coercivity) are relatively constant with a standard deviation = \( \pm 0.38 \) and 0.34 mT, and no systematic variations, again as expected.
Fig. 3.2.7: FORC distribution with an inset showing reversible and irreversible magnetization in the left column and coercivity distribution taken at $\mu_0H_u = 0$ in the right column; a) and b) SD-bacteria end member, c) and d) SD-bacteria plus 25.4% SP-bacteria mixture, and e) and f) SD-bacteria plus 32% SP-ferrofluid mixture.

from the hysteresis parameter. This suggests that SD-bacteria are essentially not influenced by the concentration of the SP-ferrofluid upon addition (Table T3.2.1). Similar is the case for
SD-bacteria plus SP-bacteria mixing series except that in this case $H_{C1/2}$, for the large coercivity exhibits a larger standard deviation of ± 0.75 probably due to the low signal to noise ratio. The low coercivity phase however, has $H_{C1/2}$, relatively constant with and 0.06 mT (Table T3.2.2).

We also apply a test, proposed by Stacey and Banerjee [1974], for examining the stability of the mixing series at each step. Their test suggests that for concentrations > 54.7 %, the SP-ferrofluid mixing series is unstable. For the SD-bacteria plus SP-bacteria mixing series, however, all measurements, except the last in the series, are stable.

### 3.2.4.2 REMANENT MAGNETIZATION CURVES

We first test for interaction in the SD bacteria end member by using R-value of the Wohlfarth- Cisowski test [Cisowski, 1981]. In an ideal case of non-interacting SD particles; the IRM acquisition and AF-demagnetization of IRM curves are symmetric with respect to each other, and their crossing point, R, is at 0.5. Figure 3.2.8 shows the results for the SD bacteria end member, which has $R = 0.45$. This indicates that the SD-bacteria have negligible or no interactions. The small fraction of SP particles at the ends of chains would be enough to depress R slightly [Li et al., 2012].

![Fig. 3.2.8: Wohlfarth-Cisowski test on SD-bacteria.](image)

### 3.2.4.3 AC SUSCEPTIBILITY VERSUS TEMPERATURE

Magnetic interaction in the SP end members was assessed using AC susceptibility as a function of temperature. The AC susceptibility separated into its in-phase ($\chi'$) and out-of-phase ($\chi''$) parts is shown in Figure 3.2.9. Both end members show a broad blocking temperature spectrum that covers practically the entire temperature range.
Néel [1949] demonstrated that in the case of a broad distribution in blocking temperature the $\chi''$ component of AC susceptibility is proportional to the frequency dependence of the $\chi'$ susceptibility. This relationship can be used to verify the origin of thermal relaxation, and has been named the $\frac{\pi}{2}$ law (Equation 3.2.2) [Egli, 2009; Hrouda et al., 2013]:

$$\chi'' = -\frac{\pi}{2} \frac{\chi'(L) - \chi'(H)}{d \ln(f(L) - f(H))}$$  \hspace{1cm} (3.2.2)

where $\chi'(L)$ is the in-phase susceptibility at low frequency, $\chi'(H)$ is the in-phase susceptibility at high frequency, $f(L)$ and $f(H)$ are the low and high frequencies respectively. The $\frac{\pi}{2}$ law was tested at frequencies $f(H) = 10,000$ Hz and $f(L) = 100$ Hz. The blocking behavior of a fraction of the SP-ferrofluid follows the $\frac{\pi}{2}$ law, but is violated at lower temperature (Fig. 3.2.9b). Therefore another phenomenon is responsible for $\chi''$ below ca. 100 K. SP-bacteria, on the other hand, display a good agreement with the $\frac{\pi}{2}$ law (Fig. 3.2.9d), which indicates that $\chi''$ is purely due to thermally activated non-interacting SP grains [Shcherbakov and Fabian, 2005; Worm and Jackson, 1999].

![Fig. 3.2.9: AC susceptibility as a function of temperature for SP-ferrofluid, a) In-phase, b) out of phase; and for SP-bacteria, c) In-phase and d) out of phase. Insets to b and d show corresponding logarithm of relaxation time versus inverse of blocking temperature as a function of frequency. Note: Dashed black curve is obtained using equation (3.2.2) for frequencies 10,000Hz and 100 Hz.](image-url)
The temperature, at which $\chi''$ has a peak value, i.e., the average blocking temperature ($T_B$) of the particle system, can also be examined as a function of frequency. Because $\chi''$ is comparatively unresponsive to temperature-related phenomena that are not associated with SP behavior, the change in $T_B$ should follow Néel-Brown theory (Equation 3.2.1). We observe that $T_B$ increases with increasing frequency in both samples (Fig. 3.2.9), and this relationship is shown in the insets to Figure 3.2.9 for both the SP-samples. The linear fit of the data gives $\tau_o$, which should range between $10^{-8}$ to $10^{-11}$ s for a non-interacting SP assembly [Dormann et al., 1999; Ghasemi, 2012]. The $\tau_o$ is $3.2\times10^{-21}$ s for the SP-ferrofluid, which has no physical meaning because it is a few orders of magnitude below the empirically defined range. This suggests that Equation (3.2.1) is inappropriate to describe the system of ferrofluid, and the inconsistency can be explained by moderate interactions among the magnetite particles in ferrofluid [Muxworthy, 2001]. In the case of SP-bacteria $\tau_o$ is $1.1\times10^{-10}$ s, which is well within the empirically accepted range, thereby further supporting the presence of non-interacting magnetite particles.

3.2.4.4 DETERMINATION OF MEAN PARTICLE SIZE AND SIZE DISTRIBUTION USING LANGEVIN THEORY

The Langevin function can be applied to magnetization curves of the SP samples to estimate the mean effective magnetic particle size and their size distribution by approximating that magnetic interaction and magnetic anisotropy effects has negligible influence on the magnetization. Assuming a log-normal distribution of particle size we model initial magnetization curves to compare the effective magnetic size with the physical size as depicted from TEM analysis. The simulated curves for initial magnetization are in good agreement with that obtained using experimental data for SP-ferrofluid (Fig. 3.2.10a) and the SP-bacteria (Fig. 3.2.10b). The SP-ferrofluid has a calculated average core-diameter of $11.7 \pm 1.5$ nm (inset to Fig. 3.2.10a). This value is close to the value provided by the manufacturer but also the average size obtained from the model fitting of the AC susceptibility data over the frequency range of 1 Hz to 10 MHz. Therefore measurements on the bulk ferrofluid sample suggest that the effective particle size is larger than what was determined from isolated particles in TEM analysis. SP-bacteria have a calculated mean diameter of $13.4 \pm 1.5$ nm and agree with the TEM analysis (inset to Fig. 3.2.10a). The magnetosomes of SP-bacteria of this mutant strain are likely to follow a normal size distribution. The SP-bacteria used in this study, however, showed a closer fit by assuming a log normal distribution of particle sizes. Assuming either a normal size distribution or log normal size distribution does not produce a significant difference in the mean size estimation.
3.2.4.5 THEORETICAL DETERMINATION OF MIXING SERIES USING END-MEMBERS

An alternative approach to compare experimental results with the theoretical curves of Dunlop [2002a] is to assume a linear mixing model based on the magnetic properties of the end members. This approach uses the following equations to evaluate magnetization and coercivity ratios for mixtures:

\[
\frac{M_{RS}}{M_S} = f_{SD}\left(\frac{M_{RS}}{M_S}\right)_{SD}
\]  
(3.2.3)

where, \(f_{SD}\) corresponds to the volume fraction of SD, and \(\left(\frac{M_{RS}}{M_S}\right)_{SD} = 0.46\) for SD-bacteria and

\[
\frac{H_{CR}}{H_C} = \left(\frac{H_{CR}}{H_C}\right)_{SD}
\]  
(3.2.4)

with, \(\left(\frac{H_{CR}}{H_C}\right)_{SD} = 14.2\) mT, the remanent coercivity for the SD-bacteria. \(H_C\) represents the coercivity of the mixtures. It corresponds to a balance point field, and was determined from the magnetization curves of the end-members of the two series. For SD-bacteria we use the magnetization curve between \(M = -M_{RS}\) and \(H = H_C\), and for the two SP samples we use the initial magnetization to obtain a balance point field corresponding to different volume fraction of the SD component. The balance point field is defined as the field at which the magnetization of the SD component is equal in magnitude, but in opposite direction, with respect to the magnetization of the SP component. Experimental and theoretical data for the mixing series of SD-bacteria and SP-ferrofluid show a good agreement for low SP concentrations and again for the final samples (Fig. 3.2.5a). There is a departure when the SP concentrations are 60% or higher, this could be explained by interactions within the SP-ferrofluid, as suggested by various magnetic methods described above. Therefore at low concentrations there may be less interaction between the SP particles, but this increases with

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**Fig. 3.2.10:** Initial magnetization as a function of applied field at room temperature for, a) SP-ferrofluid, and b) SP-bacteria. Solid circles represent the experimental data points and open squares are from simulation using Langevin theory applied to SP state. Corresponding insets show the probability as a function of particle diameter.
higher concentrations. This would cause a deviation to higher coercivity ratios. It should be noted, however, that even the sample with the highest SP-ferrofluid concentration lies below the theoretical 10 nm curve on the Day-Dunlop plot. Figure 3.2.5b displays experimental data and the theoretical fit for the mixtures of SD-bacteria and SP-bacteria. The experimental data match theoretical prediction trends based on the end members. It is important to note, however, that the curve lies below what one would expect for 14 nm SP particles.

3.2.5 CONCLUSIONS

Both experimental and theoretically predicted data of the two SD-SP mixing series show inconsistency with the SD-SP curves on the Day plot as predicted by Dunlop [2002a] for their respective grain sizes. The mixtures of SD-bacteria with SP-ferrofluids follow the predicted mixing trends on the Day-Dunlop plot up to 54.7% addition of ferrofluid by volume assuming the TEM defined particle size of 6 nm. AC-susceptibility and initial magnetization curves suggest moderate magnetic interactions in the ferrofluid. Modeling of Langevin behavior and the Néel behavior in an AC susceptibility model applied to experimental data indicates a larger particle core size (≈11 nm), which may reflect the size of aggregates produced from magnetic interactions or agglomeration of smaller grains. Therefore the aggregated particles are still SP, but their core diameter increase. This leads to a deviation of the mixing curve toward larger particle size.

The experimental mixing line for the mixtures of SD-bacteria and SP-ferrofluid follows the theoretically predicted line, but suggests lower coercivity and magnetization ratios than what would be expected from the mixing lines of Dunlop [2002a]. In both series adjustment of particle size and shape distribution in the theory may bring experimental results closer to the theoretical SD-SP mixing lines of Dunlop [2002a].

However, these results demonstrate that caution should be used when trying to determine the percentage of SP particles and their size from the SD-SP mixing curves on Day-Dunlop plots. Such mixtures may lie in the PSD field on the plot and be indistinguishable from mixtures of SD and PSD grains or SD and MD grains. Tests for SP contributions in a rock or sediments would help eliminate this as an option when interpreting hysteresis data on the Day-Dunlop plot. Therefore without any corroborative information, a simple comparison of magnetization and coercivity ratios with mixing curves on the Day-Dunlop plot may lead to an ambiguous interpretation of particle size.
3.3 PHYSICAL PARTICLE SIZE VERSUS MAGNETIC PARTICLE SIZE: A STUDY ON MESOCRYSTALS

3.3.1 INTRODUCTION

Mesocrystals are single crystals that are formed from the ordered assembly of smaller units. X-ray diffraction and high-resolution transmission electron microscopy (HRTEM) show that the structure is consistent throughout the mesocrystal. An open question is whether the magnetic properties of the mesocrystal reflect the entire particle or the smaller units. Reufer et al. [2011], showed that the magnetic properties of synthetic spindles of hematite with a long axis of 150–300 nm and a short axis of 29–70 nm, displayed either single domain behavior or superparamagnetic (SP) behavior, depending on the synthesis method. The particles that showed SP behavior were more porous, such that the smaller units were isolated enough so as not to magnetically interact, although the crystal structure indicated that all units were similarly aligned. Frandsen et al. [2005] also observed similar behavior in an aggregate of hematite nanoparticles.

The effective magnetic size of nanoparticles is of great importance in their application. In this study the magnetic properties of synthetic magnetite mesocrystals is investigated. We are specifically interested in whether the magnetic properties represent the mesocrystal or the smaller units, which are assembled to form the larger unit. The results from this study are part of a more complete study: The magnetic signature of nanoscale magnetite mesocrystals, by V. Reichel et al. [2015] which has been submitted to ‘ACS Nano’ (Appendix A).

3.3.2 METHODS AND SAMPLES

3.3.2.1 SAMPLES

The samples, which were evaluated in this study, are mesocrystals of magnetite that are self-assemble from smaller units and the supernatant, in which the smaller units were synthesized. These particle synthesis is described in detail in Baumgartner et al., [2014]. Magnetite mesocrystals were precipitated using a computer-controlled titration set up (Appendix A). Briefly, the iron solution was added drop wise over one hour to a 10 ml solution of polyarginine at a concentration of 0.1 mg/ml. These simple settings lead to the formation of these unique particles with monodispersity even higher than that observed in magnetosomes of magnetotactic bacteria, typically used as reference for monodispersity [Thomas-Keprta et al., 2000]

3.3.2.2 TRANSMISSION ELECTRON MICROSCOPY

Transmission electron microscopy (TEM) was used for studying the sizes, and
compositions of the particles of individual nanocrystals within the particles. High-resolution TEM (HRTEM) images were obtained at the Department of Earth and Environmental Sciences, University of Pannonia, using Philips JEOL 3010 transmission electron microscopes operated at 300-kV accelerating voltage. Images were recorded using a Gatan Orius CCD camera mounted on JEOL 3010. Particle sizes were measured on digitized images using ImageJ software.

3.3.2.3 MAGNETIC MEASUREMENTS

The samples were magnetically characterized using hysteresis loop, backfield isothermal remanent magnetization (IRM) and first order reversal curves (FORC). All measurements were made on a Princeton Measurements Corporation vibrating sample magnetometer (Model 3900). Hysteresis loops were made by cycling the field between ± 1T with an average time of 300 ms. FORCs were measured by first saturating the sample in a positive applied field of 1 T. A series of 140 first-order magnetization curves originating from the major hysteresis loop were determined with a field increment of 2.3 mT and an average measurement time of 100 ms or 5 s. The FORC data were transformed into a FORC diagram using the Winklhofer MATLAB code [Winklhofer and Zimanyi, 2006].

3.3.3 RESULTS

3.3.3.1 PHYSICAL PARTICLE SIZE

The micrograph shows that the nanoparticles are typically organized in chains (Fig. 3.3.1a). It is clearly visible that amorphous material, presumably the polyarginine, surrounds the crystals. The particles are mainly monodisperse as confirmed by the size distribution (Fig. 3.3.1b). The mean particle size is 38.9 ± 3.7 nm.

3.3.3.2 MAGNETIC PARTICLE SIZE

The magnetite mesocrystals show a thin hysteresis loop, with coercivity, $H_C = 4.2$ mT, and the ratio of saturation remanent magnetization ($M_{RS}$) to saturation magnetization ($M_S$) is 0.29 (Fig. 3.3.2a). The hysteresis loop is saturated by 200 mT after removing the high field contribution arising from a paramagnetic contribution (Fig. 3.3.2a inset). Acquisition of a backfield IRM curve shows that the magnetization is saturated by ≈ 150 mT. These results indicate that the synthesized magnetite is pure and shows no significant degree of oxidation to hematite (Fig. 3.3.2b). The FORC distribution for both averaging times shows essentially a spread along the coercivity axis from zero to approximately 20 mT and the presence of small closed contours in the vicinity of the origin (Fig. 3.3.3a and 3.3.3b). The fact that the distribution is very close to origin supports the presence of smaller SP particles. If the coercivity is masked between 0 to 30 mT, there is clear evidence for an increase in coercivity.
spectrum up to 45 mT with 5 s averaging time (insets in Fig. 3.3.3a and 3.3.3b). The longer averaging time allows the very fine SP particles to relax so that they do not contribute as much to the total magnetization, allowing the blocked part to have a stronger contribution. This can be seen when the FORC measurement is decomposed into its reversible and irreversible components (Fig. 3.3.3c). The irreversible component is stronger in the FORC measured with a 5 s averaging time. It should be noted, however, that the SP contribution does not change significantly; ca. 25.6% and 24.3% of the magnetic behavior of the samples can be attributed to SP particles for 100 ms and 5 s averaging time, respectively [Kumari et al., 2014].

Fig. 3.3.1: a) HRTEM image of mesocrystalline magnetite nanoparticles; b) Size distribution of magnetite nanoparticles.

Fig. 3.3.2: a) Hysteresis loop with an inset showing the saturation attained at high field; and b) backfield IRM for the mesocrystalline magnetite nanochains.
Another approach that strongly confirms the presence of SP particles in mesocrystals is the gradual increase in coercivity from 4.2 mT at 300K to 13.5 mT at 30K (Fig. 3.3.4a). It is interesting to note that the supernatant shows a closed loop at 300 K, which reflects that only SP particles are present. The magnetization curve opens gradually with decreasing temperature and resembles the mesocrystal sample at 30 K (Fig. 3.3.4a and 3.3.3b). These results suggest that some of the SP units within the mesocrystals are sufficiently isolated from one another by polyarginine so that they retain their reversible magnetic behavior.

Fig. 3.3.4: Hysteresis loops as a function of temperature from 300 K to 30 K for the mesocrystalline magnetite nanochains; and b) supernatant. Inset showing the hysteresis loop at low fields.
3.3.4 DISCUSSION

HRTEM show that mesocrystals consists of magnetite nanoparticles greater than 30 nm, and no smaller crystals are present in the sample. The crystal structure is the same throughout the mesocrystal, all units are similarly aligned (Appendix A, Fig. 1). Magnetic characterization of these crystals, on the other hand, indicates the crystals behave magnetically as mixtures of SP and stable SD particles, in which the stable SD behavior dominates. Therefore interaction between the units is large enough to act as a SD crystal. Other units, however, are sufficiently isolated by residual polyarginine to display SP behavior.

3.3.5 CONCLUSIONS

The work demonstrates observed divergence between the effective magnetic particle size estimated from magnetic measurements and the physical particle size accounted for by TEM analysis. It further illustrates the importance of detailed magnetic measurements to analyze the effective size-distribution of nanoparticles in mesocrystals or any nanoparticle assemblage in general. This information is crucial in evaluating the applicability of nanoparticles in their foreseen application.
First Order Reversal Curves:

Is $135^\circ$ Ridge an Artifact?

*First order reversal curve distributions with $135^\circ$ ridge in the region where $H_U < 0$*
4.1 135° RIDGE ON THE LOWER HALF OF THE FIRST ORDER REVERSAL CURVE DIAGRAM: AN ARTIFACT?

Monika Kumari, Marc Widdrat, Izabela Bobowska, Damien Faivre, Ann Hirt (in progress)

4.1.1 INTRODUCTION

There has been an explosion in the number of studies that report on the synthesis or application of magnetic nanoparticles (MNP) in the past decade [e.g., Hao et al., 2010; Laurent et al., 2008; Lee et al., 2008; Lu et al., 2007; Majewski and Thierry, 2007; Mascolo et al., 2013; Rümenapp et al., 2012]. The magnetic properties of a nanoparticle system are key to the effectiveness of the particles in application. Most studies, however, only report on the saturation magnetization, and coercive force, but do not investigate if the MNP are chemically pure, or if particle interaction occurs. First order reversal curve (FORC) analysis is a powerful technique for characterizing magnetic minerals, their domain state or particle size, and the extent of interactions between particles [e.g., Carvallo et al., 2006; Muxworthy et al., 2005; Pike et al., 1999; Roberts et al., 2000]. The method has been shown to be useful in analysing particle size distributions, chemical alterations and degree of interactions in nanoparticle systems [Dobrotă and Stancu, 2013; Hirt et al., 2014]. Although FORC diagrams are ideal to examine these points, one feature that appears on FORC diagrams, a 135° ridge with a series of alternating patches of positive and negative intensity in the FORC density function ($\rho$) that starts at the maximum of the FORC distribution, requires further investigation to understand its origin [e.g., Acton et al., 2007; Carvallo et al., 2003; Davies et al., 2005; Panagiotopoulos, 2011; Rong et al., 2011].

The 135° ridge was first reported by Carvallo et al. [2003] in FORC diagrams, which were generated from micromagnetic models based on isolated or assemblages of single-domain (SD) grains. A 135° ridge of positive and negative peaks in FORC density ($\rho$) was found in arrays of interacting elongated particles. Davies et al. [2005] observed pairings of negative and positive peaks in the FORC distribution for doped perovskite cobaltite La$_{1-x}$Sr$_x$CoO$_3$ when the Sr concentration was $\geq 0.30$. They attributed the feature to coalescence of ferromagnetic clusters, having a long-range order that leads to a decrease or increase in reversal field dependent susceptibility as a function of the domain states to the applied field. Acton et al. [2007], found a 135° ridge in the FORC distribution measured on Bjurböle chondrules. Here they suggest that the ridge originates from interaction between two or more magnetic components with highly contrasting coercivity. Rong et al. [2011] saw a similar ridge in their study on a SmCo/$\alpha$-Fe nanocomposite, where they attributed the feature to magnetostatic interaction between the magnetically low and high coercivity phases.
Panagiotopoulos [2011] produced similar ridge-like feature using a Preisach approach and defined a region of interaction, I, on the FORC diagram, where \( \rho \) is essentially zero. In this region weak interaction between two phases of different coercivities and non-overlapping distributions produces a strong dependence of magnetization on the applied field through the lower coercivity phase and on reversal field through the high coercivity phase. This constellation results in a non-zero \( \rho \) and thereby a ridge with positive/negative features. On the other hand, some studies have attributed the feature to an artifact of processing the data [Chen et al., 2007; Egli, 2013].

We have observed a 135° ridge on the FORC diagrams of some biomimetic magnetite nanoparticles. One example is for a sample M1 (sample 107 in Chapter 6.1) that has a saturation magnetization, \( M_S = 53.6 \text{ Am}^2/\text{kg} \). At room temperature (RT) it displays a thin hysteresis loop with a coercivity, \( H_C = 10.3 \text{ mT} \), and a remanent coercivity, \( H_{CR} = 19.7 \text{ mT} \). Its dc-IRM acquisition curve is not saturated in fields \( \leq 1000 \text{ mT} \). This suggests the presence of an additional high coercivity phase, probably due to oxidation of magnetite to hematite. The FORC diagram shows alternating positive and negative patches of the FORC distribution function, which define the 135° ridge (Fig. 4.1a). At low temperature, i.e., at 90 K and 50 K, the hysteresis loop becomes wider, with \( H_C = 16.4 \text{ mT} \) and 18.1 mT, respectively. The corresponding dc-IRM curve displays \( H_{CR} = 28.5 \text{ mT} \) at 90 K and 30.9 mT at 50 K. The FORC diagram continues to display a 135° ridge (Fig. 4.1b and 4.1c), and the two phases has become more prominent. This is particularly well developed in the FORC diagram at 50 K, with a third large density patch displaced downward by ca. 10 mT, between the density patches arising from the other two phases; this arises from interaction between the high and low coercivity phases.

Because this feature was found on FORC diagrams in other biomimetic samples that were characterized under the BIO2Man4MRI project, we got interested in gaining a better understanding of the conditions under which the 135° ridge is expressed on the diagram. For this purpose we consider firstly mixtures of two iron-oxides with contrasting coercivities, i.e., magnetite/maghemite and hematite, and secondly biomimetic Fe-oxide nanoparticles that have undergone varying degree of surface oxidation. We further investigate if this feature can be removed by reducing the samples to its un-oxidized state.

### 4.1.2 SAMPLES AND METHODS

#### 4.1.2.1 SAMPLES

We examine four sets of samples, whereby the first set consists of pure synthetic spindle shaped hematite H and pure magnetite M samples. The hematite sample was synthesized...
using the method described in Reufer et al. [2010]. The long axis of the spindles is around 300 nm and short axis is about 50 nm. The magnetite sample was synthesized under the Bio2Man4MRI project (sample 238, Appendix B, section: 2.1). M has a relatively high $M_S$ of 70.6 Am$^2$/kg, which indicates that it is a relatively pure magnetite, and a bimodal coercivity distribution, which suggests a mixture of superparamagnetic (SP) and SD particles sizes. These two samples are then mixed by adding ca. 0.3 mg of M to 31 mg of H to give a sample HM. Samples of biomimetic Fe-oxide nanoparticles have been prepared by co-precipitation of Fe(II) and Fe(III) salts, at different pH and temperatures, to obtain magnetite nanoparticles with distinct mean diameters (Appendix B, section: 2.1). We focus on three sets of these particles. Group I consists of M2 (sample 241 in Chapter 6.1), which initially did not show a 135° ridge on the FORC diagram, but developed a 135° ridge after four months of storage in the refrigerator. Group II, comprised of M3 (sample 125 in Chapter 6.1), exhibits no initial ridge. It was heated first in air and subsequently in argon gas, to explore the effect that the change in oxidation state has on the FORC distribution of the initial M3 sample. And Group III, containing M4, had a ridge initially, and was later further oxidized by heating.

**Fig. 4.1:** Top: FORC diagrams with smoothing factor SF for M1 at a) RT, b) 90 K, and b) 50 K. Bottom: cross-sectional coercivity distribution obtained through FORC analysis for M1 at d) RT, e) 90 K and f) 50 K.
4.1.2.2 METHODS

The physical particle size, morphology and compositions of all initial samples of biomimetic Fe-oxide nanoparticles were obtained using high resolution transmission electron microscopy (TEM) and selected area diffraction. The JEOL 3010 and Philips CM20 were operated with 300 and 200 kV accelerating voltages, respectively.

All magnetic measurements were carried out using a Princeton Measurements Corporation, Vibrating Sample Magnetometer (VSM; Micro-Mag Model 3900). Hysteresis was measured using a multiple segment-sampling rate to have a higher density of measurements in low field. All hysteresis loops were measured with a 100 ms averaging time for each segment, and a maximum field of 1 T. Acquisition of isothermal remanent magnetization (IRM) was obtained by applying a 1 T field in one directions, and then incrementally applying a backfield in the opposite direction. These dc-IRM curves were used to check for the presence of hematite, because remanent methods are more sensitive to the presence of a high coercivity phase compared to methods that measure induced magnetization.

FORC measurements were made by first saturating the sample with a positive applied field of 1 T and then ramping down the field to a reversal field (H_R), followed by measuring magnetization (M) as the field (H) increases from reversal field back to positive saturation. A series of 140 FORC with a field spacing of 2.3 mT is used for the analysis. FORC data are transformed into FORC diagrams using M.Winklhofer MATLAB code [Winklhofer and Zimanyi, 2006]. The FORC distribution is determined by The FORC distribution is obtained from a mixed second order derivation:

\[
\rho (H_R, H) = - \frac{\partial M(H_R, H)}{\partial H_R \partial H} \quad (4.1)
\]

The coordinate system can be changed from (H_R, H) to (H_C, H_U), where H_C describes the coercivity, H_C = (H – H_R)/2, and H_U the interaction field, H_U = (H + H_R)/2. The FORC diagram depicts the FORC distribution with H_C on the horizontal axis and H_U on the vertical axis. All FORC diagrams are processed with smoothing factor (SF) = 2, unless otherwise stated. Samples were oxidized by heating in a Memmert oven in air. Samples were reduced partially or fully by heating from room temperature to 700ºC in an inert atmosphere of argon using an Agico MFK1-FA susceptibility bridge outfitted with an oven unit.

4.1.3 RESULTS

4.1.3.1 TRANSMISSION ELECTRON MICROSCOPY

TEM analysis indicates that M2 is pure magnetite with an average particle size of ca. 15
nm. It should be noted that although most particles fall in superparamagnetic particles sizes, some particles have a diameter up to 30 nm. M3, consists of compact aggregates of pure magnetite with a spread in particle size from 10-90 nm, and a mean particle size of 33 nm. M4 is also made up of uniformly distributed aggregates of magnetite, with a mean particle diameter of 36 nm.

4.1.3.2 MAGNETIC PROPERTIES

GENERAL GROUP: SAMPLES H, M AND HM

Sample HM, comprised of M and H, was designed to test the idea postulated in Acton et al. (2007) that two phases with contrasting coercivity produce the 135° ridge. The magnetic properties of the starting materials were first characterized. The hysteresis loop for M shows a coercivity (Hc) 13.1 mT, which is in the range for pure magnetite Peters and Dekkers [2003] (Fig. 4.2a). The sample’s hysteresis loop is close to saturation but the high-field magnetization may reflect paramagnetic contributions from salts or very fine particles [Khurshid et al., 2012; Thapa et al., 2004]. Acquisition of a backfield IRM shows that the remanent magnetization has a coercivity of remanence, $H_{CR} = 20.3$ mT, and the IRM is saturated by $\approx 220$ mT, which is also compatible with pure magnetite (Fig. 4.2b). The FORC diagram for M shows a peak in $H_C$ at $\approx 16$ mT but the coercivity distribution extends from 0 to approximately 60 mT, which reflects the broad particle size distribution (Fig. 4.3a). The spread along the $H_U$ axis suggests that there is moderate interaction between particles.

![Fig. 4.2: a) Hysteresis loops and, b) dc-IRM curves; for M, H and HM.](image)

The hysteresis loop for the hematite sample shows a mean $H_C$ of 21.5 mT and the loop is not saturated in the maximum applied field (Fig. 4.2a). The dc-IRM curve also shows that the
sample is only approaching saturation of the remanent magnetization in the maximum field and $H_{CR} = 62.6$ mT (Fig. 4.2b). Although the peak coercivity is relatively low, the FORC analysis shows a broad coercivity distribution and a significant part of the magnetization above 80 mT (Fig. 4.3b).

Although the mixed sample HM, is predominantly hematite, the magnetite fraction dominates the magnetic properties of the sample. The bulk $H_C$ is 16.7 mT, which is close to that of the pure magnetite sample, but the IRM backfield curve shows a contribution of the higher coercivity phase to the remanent magnetization (Fig. 4.2a and 4.2b). The FORC distribution is also similar to M, but shows a broader coercivity distribution from the contribution of H (Fig. 4.3c). Although there is more inherent noise in the FORC distribution, there is no indication for a 135° ridge.

**Fig. 4.3:** FORC diagrams with smoothing factor SF, of a) M, b) H, and c) HM. The cross-sectional coercivity distribution obtained through FORC analysis d) M, e) H, and f) HM.

**GROUP I: SAMPLE M2**

The biomimetic sample M2, has a $M_S = 72.6$ Am$^2$/kg, which is indicative of stoichiometric magnetite. The initial sample has a thin open hysteresis loop with $H_C = 10.1$ mT, and the dc-
IRM curve saturated in fields < 300 mT (Fig. 4.4a and 4.4b). The FORC distribution for the initial M2 sample consists of closed contours near the origin with a peak distribution around 13.3 mT. The coercivity distribution extends up to 60 mT, and there is no 135° ridge (Fig. 4.5a). The sample was then re-measured after four months of storage in a refrigerator. The hysteresis loop remains essentially the same (Fig. 4.4a). HCR also remains about the same when compared to the initial sample. The dc-IRM curve of the re-measured sample, however, is no longer saturated, which indicates a slight amount of oxidation. A considerable effect is seen in the FORC distribution, which now has a prominent 135° ridge. The peak coercivity is slightly displaced downward from the origin, indicative of interaction between the two phases. The horizontal cross-sectional coercivity spectrum of the re-measured M2, extends up to ≈ 80 mT. This supports initial oxidation of magnetite to hematite.

**GROUP II: SAMPLE M3**

M3 shows a slightly open hysteresis loop with $H_C = 10.2$ mT, and the dc-IRM curve is saturated by $\approx 220$ mT with $H_{CR} = 18.1$ mT (Fig. 4.6a and 4.6b), which suggest pure magnetite. The initial FORC measurement has peak coercivity at $\approx 12.7$ mT, and no 135° ridge (Fig. 4.7a). The sample was later heated in an oven for 72 hours at a constant temperature of 130°C. The hysteresis loop is similar to the initial loop but $H_C$ increases to 13.3 mT (Fig. 4.6a). The IRM acquisition curve is no longer saturated in the maximum applied field of 1 T (Fig. 4.6b), and has $H_{CR} = 22.5$ mT. The FORC diagram shows a broadening of the coercivity distribution with bimodal distribution, a lower coercivity at $\approx 5.98$ mT and the higher coercivity at $\approx 16.7$ mT (Fig. 4.7b). A 135° ridge is dominantly displayed, extending form the higher coercivity peak.

---

Fig. 4.4: a) Hysteresis loops, and b) dc-IRM curves of M2, measured at initial state (June 2013), and after 4 months of initial measurements (November 2013).
Fig. 4.5: FORC diagrams with smoothing factor SF, of M2, a) initial stage, and b) after 4 months of storage in a refrigerator. Cross-sectional coercivity distribution obtained through FORC analysis of M2, c) initial stage, and d) after four months of storage.

Fig. 4.6: a) The magnetic hysteresis loops and, b) dc-IRM curves of M3 at room temperature.
The oxidized M3 sample was subsequently heated in an inert atmosphere of Ar until 700ºC holding the peak temperature for 20 minutes, which did not result in a major change in the hysteresis properties or IRM acquisition curve. There is a slight decrease in $H_C$, but $H_{CR}$ remains almost the same. A major difference is observed in the FORC diagram which no longer displays a 135º ridge, although the high coercivity phases remains (Fig. 4.7c). This is reflected in the coercivity distribution which is slightly broader than the initial measurement but with a similar peak coercivity.

![FORC diagrams](image)

**Fig. 4.7**: Top: FORC diagrams with smoothing factor SF, of M3, a) initial stage, b) after heating for 72 hours at 130º C in air, and c) after heating to 700º C in Ar atmosphere. Bottom: the cross-sectional coercivity distribution obtained through FORC analysis corresponding to the above FORC diagrams.

**GROUP III: SAMPLE M4**

The synthetic magnetite nanoparticles comprising sample M4 have $H_C = 8.8$ mT and $H_{CR} = 16.8$ mT in its initial state (Fig. 4.8a and 4.8b). The dc-IRM curve is saturated at $\approx 220$ mT. The FORC distribution displays the presence of a prominent 135º ridge with alternative positive and negative FORC distribution function (Fig. 4.9a). The peak coercivity is about 4.3
mT and the main FORC distribution is shifted downward with respect to the origin by 3 mT. This suggests for an interactions among the two phases that were present in the sample. The presence of two phases is further evident from the coercivity profile which exhibits two peaks. M4 was then heated up to 600°C for 15 minutes at the maximum step with 5°C interval, in air using AGICO MKF-1A Kappabridge susceptibility meter. This created more hematite in M4, as evident from the hysteresis loop that remains open up until 1 T and the dc-IRM curve that is not saturated till 1 T (Fig. 4.8a). Heating also had a significant effect on the $H_C$ and $H_{CR}$ that increased up to 19.7 mT and 37.2 mT respectively (Fig. 4.8b). The FORC diagram of the heated M4 no longer exhibits the original 135° ridge (Fig. 4.9b), but has two distinct peak coercivity with the first at 1.1 mT and a second around 30 mT. The lower coercivity peak can be attributed to SP grains that are being created during the heating. The horizontal cross-sectional coercivity spectrum extends above 80 mT and illustrates the creation of a high coercive material, consistent with hematite.

![Fig. 4.8: a) The magnetic hysteresis loops and, b) IRM acquisition curves of M4 at room temperature.](image)

### DISCUSSION

4.1.4 The experimental results demonstrate the following features for the manifestation of the 135° ridge on a FORC distribution: i) a mixture of two separate phases with contrasting coercivities will not produce a 135° ridge, ii) partially oxidized magnetite to hematite can exhibit the ridge on its FORC diagram, iii) it is possible to generate the ridge by changing the oxidation state of the initial sample, and iv) beyond a certain threshold of oxidation the sample will no longer display the ridge feature. In the first case, the bulk magnetite properties of HM are dominated by magnetite, even though it makes up approximately 1% of the total sample mass. This is because magnetite has a much higher saturation magnetization, compared to hematite. Similar results were also seen by [Frank and Nowaczyk, 2008], who
demonstrated that magnetite completely dominates the hysteresis properties of artificial mixtures of hematite and magnetite up to 2 volume %. It should be noted that the bulk $H_C$ of $H$ is relatively low, compared what would be expected for the single-domain spindle size. Although the spindles show a continuous lattice structure, there is high enough porosity within, such that the crystal has areas that are isolated enough to show superparamagnetic behavior. This leads to a lowering of the bulk coercivity.

![FORC diagrams](image)

**Fig. 4.9:** FORC diagrams with smoothing factor SF, of M4, a) initial stage, and b) after heating up to 600°C in air. Insets represent the cross-sectional coercivity distribution obtained through FORC analysis.

The 135° ridge was found in the case where the dc-IRM acquisition curve shows clear evidence for hematite, but only with a small degree of surface oxidation. In this case, cooling to lower temperature often leads to a FORC distribution with two clearly contrasting coercivities, and a third density patch, which lies between the density patches of the two phases, but offset by a negative interaction field. This suggests that the higher coercivity hematite contains very fine particles sizes so that part of the hematite phase behaves superparamagnetically. With cooling all the hematite particles become magnetically ordered and the coercivity contract and interaction is more evident (Fig. 4.1). Surface oxidation can
either result from storage [Widdrat et al., 2014] or through a certain amount of heating as seen in samples M2 and M3. More persistent heating, however, also leads to the creation of high coercivity hematite, but not to a ridge feature in the FORC diagram.

The above results can be explained by the theoretical findings of Panagiotopoulos [2011], who has modeled two-phases with non-overlapping coercivity distributions using a simple Preisach approach. Under the conditions outlined below it is possible to generate a 135° ridge, consisting of alternating patches of positive and negative density, in the case where the magnetization depends both on the field \( H \) and the reverse field \( H_R \), i.e., there is irreversible change. For this reason Panagiotopoulos [2011] describes this region on the FORC diagram by “I”. Although the flux density may be zero or negligible in this region, there is a strong dependence of \( M(H_R, H) \) on \( H \) in the region of the low coercivity phase and on \( H_R \) in the region of the high coercivity phases. In the presence of weak interaction, \( M(H_R, H) \) is no longer simply \( M_{low}(H) + M_{high}(H_R) \), but must take into account the effective fields so that:

\[
M(H_R, H) = M_{low}(H_{low}) + M_{high}(H_{R,high})
\] (4.2)

assuming the low coercivity phase is negatively saturated, i.e., \( M_{low}(H_R) = -1 \) and \( H_{R,high} = H_R - \gamma_{high,low} \), where \( \gamma_{high,low} \) is the total interaction field experience by the high coercivity hysterons when the low coercivity is saturated (note: \( \gamma_{low,high} \) would be the opposite case). Therefore

\[
M(H_R, H) = M_{low}(H_{low} + \gamma_{low,high} M_{high}(H_R - \gamma_{high,low})) + M_{high}(H_R - \gamma_{high,low})
\] (4.3)

This means that FORC no longer vanishes in the first term of (4.3), and explains why the arguments made by Egli [2013] that the ridge is an artefact, do not hold. The positive and negative density patches result because:

\[
\frac{\partial^2 M(H_{low})}{\partial H \partial H_R} = \gamma_{low,high} \frac{\partial^2 M(H_{low})}{\partial H_{low}^2} \frac{dM_{high}}{\partial H_R} \] (4.4)

It can be seen that the expression of this twin feature will be proportional to the interaction constant. An important point is that the interaction must be weak, i.e., \( \gamma_{low,high} \) or \( \gamma_{high,low} \) are small compared to the coercivity difference between the high and low coercivity phases, so that there is no overlap in the direct switching field.

Therefore initial oxidation of the magnetite nanoparticles leads to a hematite shell in which these conditions are met and the ridge is apparent. The strength of the ridge may increase at lower temperature because there is less overlapping of the coercivities of the two phases, when all hematite particles are magnetically ordered. As the amount of hematite increases...
with further oxidation, then there is no longer a weak interaction between the two phases. This may also explain why the ridge is not found in the first case with a simple mixing of magnetite and hematite.

### 4.1.5 CONCLUSIONS

A 135° ridge has been reported in FORC diagrams, which results from a non-vanishing contribution in a region where the FORC distribution should be zero or negligible. For this reason the feature has been considered to be an artifact. On the other hand it is only found in materials that contain both a high and low coercivity phases. It has been shown that this feature can arise from weak interaction between the two phases from Preisach theory. The experimental results in this study demonstrate that a 135° ridge, with alternate patches of positive and negative intensity in the FORC density function ($\rho$), on the lower half of the FORC diagram, is not an artifact, but arises when there is weak interaction between the low coercivity magnetite and high coercivity hematite. If the amount of the high coercivity hematite increases, however, there is a direct overlap in the switching fields, and the feature is no longer found.
Self-assembly is the process where the constituents of an ensemble come together in certain pattern either through interactions or external force.
5.1 ASSESSING SELF-ASSEMBLY OF BIOGENIC MAGNETITE NANOPARTICLES

Monika Kumari, Stephan Handschin, Rene Uebe, Dirk Schüler, and Ann M Hirt; in preparation for Geophysical Journal International

5.1.1 INTRODUCTION

Structural organization of magnetic nanoparticles (MNP) is of fundamental and technical importance, by virtue of magnetic anisotropy that can be exploited for data-storage, permanent magnets, optical switches, sensors, and biomedical applications [e.g., Hoinville et al., 2003; J. Lee; et al., 2007; Nie et al., 2010; Parekh et al., 2006; Sun, 2006]. Several studies have been focused on self-assembly of MNP that involves their preparation, characterization and application [e.g., Sahoo et al., 2004; Sun et al., 2000; Tracy and Crawford, 2013; Wu et al., 2005; Xu et al., 2010]. Further research has also been conducted on the magnetic anisotropy of self-assembled magnetite crystals present inside the magnetotactic bacteria [e.g., Alphandéry et al., 2009; Li et al., 2012; Li et al., 2013]. These studies have applicability in: i) bioscience for understanding the mechanism of bio-mineralization inside magnetotactic bacteria; ii) paleomagnetism to aid in the identification of magnetofossils; iii) physics and material science for the construction of controlled anisotropic materials.

The magnetic coercivity of an anisotropic material is highly dependent on the applied field with respect to the easy axis of magnetization. For MNP that are aligned in a chain, the coercivity will have its maximum value when the field is applied parallel to the long axis of the chain, and should be zero when the field is applied perpendicular to the chain (Fig. 5.1a) Previous studies on aligned magnetosomes inside magnetotactic bacteria, however, have shown that magnetic hysteresis loops are open i.e., a non-zero coercive force ($H_C$), when the applied field $H$, is normal to the aligned direction [Alphandéry et al., 2009; Li et al., 2013] (Fig. 5.1b). This is attributed to the predominance of dipolar interaction between the particles over the alignment of easy axes. Micromagnetic modeling of biological samples with magnetosomes in chains demonstrated an increase in $H_C$ when the sample is rotating away from the easy axis i.e. from 0° to ≈ 50° along the chain axis, followed by a decrease, from 55° to 90°. Experimental observations, however, found only a decrease in $H_C$ when rotating from 0° to 90° (Fig. 5.1b).

In this study dispersed magnetosomes were self-assembled by an external magnetic field. The spatial organisation of the magnetosomes was analysed with a cryogenic scanning electron microscope (cryo-SEM) and the efficiency of alignment was assessed with magnetic
measurements that includes hysteresis and isothermal remanent magnetization (IRM) analysis of the oriented and unoriented samples. These results are compared with the micromagnetic modeling of Li et al. [2013], in order to explain the variation in $H_C$ as a function of rotation angle $\phi$, where $\phi$ is the angle between $H$ and the direction of aligned magnetosome. We also examine low-field and high-field anisotropy of magnetic susceptibility (AMS), of the aligned magnetosomes. The potential of high-field torque measurements is demonstrated for evaluating magnetic contributions that arises from shape anisotropy, crystalline anisotropy and remanance in a self-assembled structure.

Fig 5.1: a) Theoretical magnetic hysteresis loops as a function of angle (0°-black; 45°-blue; and 90°-red) between the applied field and the direction of alignment, b) variation of $H_C$ as a function of applied field with respect to the long axis of the magnetosome chain. Solid circles represents $H_C$ from micromagnetic modeling and open circles are results for bacteria, taken from Li et al. [2013].

5.1.2 METHODS AND SAMPLES

5.1.2.1 SAMPLES

Magnetosomes of magnetotactict bacterium Magnetospirillum gryphiswaldense, MSR-1 wild type strain were used in this study. Each magnetosome consists of isometric magnetite nanoparticle with a mean particle size of 33.3 ± 6.6 nm (Fig. 5.2). They are enclosed by a lipid bilayer that prevents them from oxidation and agglomeration. The extracted magnetosomes suspended in a 10 mM Hepes buffer plus 1 mM EDTA, were repeatedly washed and suspended using double distilled water prior to their alignment, to remove all residual materials.

5.1.2.2 ALIGNMENT PROCEDURE

A Si-wafer of 4 by 4 mm was used as a substrate. They were cleaned using first with acetone, then propanol, and finally water in an ultra-sonic bath. Afterwards, they were dried
and treated with a negative glow discharge for one minute to enhance the dispersion property. The suspension of magnetosomes was then centrifuged and consecutively dropped on the substrate with a micro-liter pipette. The substrate was placed at the center of the self-designed adjustable magnetic unit (Fig. 5.3). By evaporation of the solvent in air, the magnetosomes were deposited on Si-wafer in the presence of 300 mT field. The individual magnetosomes align their easy axis in the direction of applied field and formed chains; this is the oriented sample (OS). A reference sample (RS) was prepared under similar conditions in the absence of a field (magnets removed). It should be noted, however, that the magnetosomes were in the Earth’s magnetic field. After the cryo-SEM images, both samples were immobilized by a carbon coating of about 20 nm. This was done to ensure complete immobilization of the particles during handling, transportation and magnetic measurements.

![Fig 5.2: Magnetosome size distribution.](image)

![Fig 5.3: Designed device to apply magnetic field during solvent evaporation.](image)
5.1.2.3 SCANNING ELECTRON MICROSCOPY (SEM)

Cryo-SEM measurements were carried out using field emission-SEM, Gemini 1530 equipped with a cryo-stage, at the Scientific Center for Optical and Electron Microscopy, ETH-Zurich. Both the OS and the RS were transferred onto the cryo-stage under high vacuum. They were then coated at with 2 nm tungsten, (W), at an elevation angle of 45° and 2 nm by varying elevation angle from 0°-90° and then from 90°-0°. Finally the W-coated samples were transferred to the cryo-SEM under cryo atmosphere for imaging.

5.1.2.4 MAGNETIC MEASUREMENTS

Hysteresis loops and the acquisition of backfield IRM were measured as a function of H and φ, at room temperature on Princeton Measurements Corporation, Vibrating Sample Magnetometer (VSM; Micro-Mag Model 3900). Samples were manually rotated with a 10° increment, denoted by the angle φ, with respect to an axis either normal to the plane of the sample or within the plane of the sample, using the rotating stage on the VSM. Centering of the sample between the pick-up coils of the magnetometer was controlled manually before each measurement. The hysteresis loops were measured between ± 1 T with a 100 ms averaging time. This provided information on saturation magnetization (Ms), remanent magnetization (MRS), and Hc. Remanent coercivity (HCR), was obtained from IRM curves measured in maximum field strength of 1 T with a 0.5 mT field increment.

Magnetic susceptibility is a second order symmetric tensor that can be characterized geometrically by an ellipsoid with principle axis $\chi_1 \geq \chi_2 \geq \chi_3$. The shape of the anisotropy can be described in terms of its elongation or degree of lineation, where $L = \chi_1 / \chi_2$, and the degree of flattening or foliation by $F = \chi_2 / \chi_3$. Low-field AMS was measured on an AGICO MKF-1a susceptibility bridge with an applied field and a frequency of 200 A/m and 976 Hz respectively. A statistical analysis regarding the significance of the anisotropy is given in the measurement program [Jelinek, 1981]. Based on the 15 measurement position the degree of anisotropy can be tested with an F-test. AMS in high-field was determine on a torque magnetometer [Bergmuller et al., 1994a]. Torque measurements were conducted in three mutually perpendicular planes with an angular increment of 30° in six fields between 1000 mT and 1500 mT at room temperature. The torque magnetometer measures the deviatoric susceptibility tensor, while low-field susceptibility bridge determines the full susceptibility tensor.

5.1.3 RESULTS

5.1.3.1 SEM RESULTS

Low temperature SEM indicates a uniform distribution of magnetosomes in all directions
with a few short chains in RS (Fig. 5.4a). These short chains are either original, i.e., the extraction from bacterium MSR 1 left some magnetosomes in chains, or self-assembled due to the Earth’s magnetic field, which was sufficient for aligning. The application of H during solvent evaporation in OS prompts uniformly dispersed magnetosomes to statistically arrange themselves into short chains along the field direction (Fig. 5.4b).

5.1.3.2 HYSTERESIS PROPERTIES

Figure 5.5 shows the hysteresis loops measured as a function of rotational angle $\phi$ for two cases. In the first case the samples were rotated with the field H applied in the plane of alignment, i.e., in-plane (Fig. 5.5a). In the second case the sample was rotated such that the applied H varies from parallel to the plane of alignment, to normal to this plane, i.e., in-plane to out-of-plane (Fig. 5.5b). Saturation is reached by 200 mT in the first case for both OS and RS independent of $\phi$, therefore, the hysteresis loops are only shown to 100 mT for both cases. RS shows the same loop independent of $\phi$, and this holds for both cases, the in-plane rotation and the in- to out-of-plane rotation (Fig. 5.5). $M_{\text{RS}}/M_{\text{S}} = 0.45$, which is close to what one would expect for randomly oriented, single domain magnetite. The slightly lower value may be related to smaller superparamagnetic particles [Pan et al., 2005] (Fig. 5.2).

![Fig 5.4: Micrographs of magnetosomes on Si-wafer a) in the absence of external magnetic field (H), b) in the presence of H.](image)

There is a larger variation in the hysteresis property for the second case (cf. Fig. 5.5 insets). When H is parallel to the aligned chain, $M_{\text{RS}}/M_{\text{S}}$ has its highest value of 0.48, which decreases to 0.29 for Case 1, and = 0.03 for Case 2. $H_{\text{CR}}$ and $H_{\text{CR}}/H_{\text{C}}$, however, show an increase between 0° and 90° (Fig. 5.6). $H_{\text{C}}$ exhibits monotonic decrease when rotating in the plane from 0° to 90° as seen in the Figure 5.6b and 5.7b. Although there is an initial increase in $H_{\text{C}}$ from 0° to 60° when measuring from in-plane to out-of-plane, at higher angles from 60° up to 90° a decrease is observed (Fig. 5.6b and 5.7a).

Using the convention of Alphandéry et al. [2009] the change in the hysteresis parameters
for OS can be described by $\Delta M_{RS}/M_S$ and $\Delta H_C$, where

$$\Delta M_{RS}/M_S = [(M_{RS}/M_S)_{\text{par}} - (M_{RS}/M_S)_{\text{per}}]/(M_{RS}/M_S)_{\text{par}}, \text{ and}$$

$$\Delta H_C = [(H_{CR}/H_C)_{\text{par}} - (H_{CR}/H_C)_{\text{per}}]/(H_{CR}/H_C)_{\text{par}}$$

The subscripts par and per are used for H parallel and perpendicular to the direction of alignment, respectively. For case 1, $\Delta M_{RS}/M_S$ shows a 40% difference when rotating from parallel to perpendicular to the chain alignment, whereas $\Delta H_C$ shows a 16% difference. A larger difference is found for case 2, in which $\Delta M_{RS}/M_S = 94\%$ and $\Delta H_C = 52\%$. The low values of anisotropic measures are attributed to the inhomogeneous deposition and partial merge of magnetosomes on the Si-wafer.

![Fig 5.5: Magnetic hysteresis loops as a function of $\phi$, for OS and RS; a) in-plane to in-plane rotation; and b) in-plane to out-of-plane rotation. Blue curve represents RS for all $\phi = 0^\circ$ to $90^\circ$.](image)
Fig 5.6: Magnetic parameters for OS (solid diamonds with dashed lines) and RS (black dashed line) as a function of \( \phi \), for in-plane rotation (blue) and in-plane to out-of-plane rotation (red). a) Ratio of magnetization; b) coercivity; c-d) remanent coercivity; and e-f) ratio of coercivities. Note: Dashed black line represents RS.
5.1.3.3 ANISOTROPY OF MAGNETIC SUSCEPTIBILITY

The results from low-field AMS measurements are summarized in Table T5.1. Both OS and RS pass the test of anisotropy in the plane in which the magnetosomes lie. The degree of lineation is 3% for RS and 14% for OS. There is a strong anisotropy when comparing the in- to out-of-plane anisotropy, i.e., the magnetic foliation.

Table T5.1: Anisotropic measurement parameters and F-test for 95% significance for low-field AMS. F12 indicates the in-plane anisotropy and F23 the in- to out-of-plane anisotropy F-test values.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Lineation</th>
<th>Foliation</th>
<th>F12</th>
<th>F23</th>
</tr>
</thead>
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<td>5.57</td>
<td>219.7</td>
<td>7105.4</td>
</tr>
<tr>
<td>RS</td>
<td>1.03</td>
<td>5.61</td>
<td>9.9</td>
<td>7491.5</td>
</tr>
</tbody>
</table>

Figure 5.8a shows torque as a function of angle for OS at 600 mT applied field. There is a very clear 2θ signal for planes 1 and 2, where the sample is rotated from in-plane to out-of-plane, which illustrates the strong uniaxial anisotropy related to the magnetization lying in one plane. No torque is found when the field is applied parallel and perpendicular to the aligned magnetosomes. The third plane, which shows in-plane rotation, has a very weak torque signal in comparison. The amplitude of the 2θ signal as a function of field is relatively constant for the three planes, which demonstrates that the torque arises from the saturated magnetite particles (Figure 5.8 b).

To increase the resolution of magnetic anisotropy in the third plane, torque measurements
were repeated using a longer averaging time to measure the torque response (25 ms vs. 4 ms) and higher saturating fields. Figure 5.9 shows the Fourier components of the torque signal obtained for the third plane at a field of 1300 mT using Fourier analysis. The Fourier analysis shows contributions from the even coefficients reflecting the magnetic anisotropy, where the 4θ component is larger than the 2θ component.

**Fig 5.8:** a) Angular variation of torque signal in three mutually perpendicular planes, for OS at 600 mT; and b) amplitude of the 2θ signal as a function of field square.

**Fig 5.9:** a) Example of the dominant Fourier components of the torque signal for an applied field of 1300 mT, where anisotropy components, 2θ (black) and 4θ (red) are shown by solid lines and original torque signal by the dotted line.
5.1.4 DISCUSSION

Although RS shows a weak alignment of magnetosomes the shape of the hysteresis loop is the same when measured as a function of φ either in the plane of the magnetosomes (Case 1) or in- to out-of-plane (Case 2). Therefore the sample can be considered to represent a random distribution of the easy axes of magnetization. The low-field AMS, however, shows that there is a weak alignment of the magnetosomes in the plane of deposition, with a lineation of 3%. This suggests that the Earth’s magnetic field is strong enough to cause a weak alignment. The low-field AMS shows that the sample OS has a stronger lineation on the order of 14%. The change in hysteresis parameters as a function of φ for the aligned magnetosomes for Case 1, follow a similar change that was also observed by [Li et al., 2013]. \( M_{RS}/M_S \) has a maximum value of 0.5, compare to 0.8 as observed by Li et al. [2013] for the intact bacteria, which suggests that shape anisotropy dominates over the magnetocrystalline anisotropy in the aligned magnetosomes. The variation in \( H_C \) with reference to the in-plane to out-of-plane measurements is consistent with the micromagnetic models for aligned magnetotactic bacteria with magnetosomes in chains [Li et al., 2013]. The authors attributed this to an incoherent mode in a model of a chain of spheres when the field angle is close to 0º and coherent mode when the angle is closer to 90º. From 90º to 180º the samples exhibit a mirror angular relationship compared to the case with φ decreasing from 90º to 0º. Variations from 180º to 360º are similar to those from 0º to 180º (Fig. 5.5).

The high-field AMS shows that the anisotropy in the plane of the deposited bacteria has a strong biaxial component, which arises because the magnetosomes are arranged in chains have two easy axis of magnetization. The 4θ term suggests for the effect coming from crystalline anisotropy, i.e., [111] as the easy-axis of magnetization. The 2θ term in the in-plane probably arises from the fact that we are not rotating about the [100] axis, of the magnetosomes, which would only result in a pure 4 component to the torque, as also observed by Martín-Hernández et al. [2006], who analysed high-field torque on single crystal of magnetite.

The results from this study indicate that it is not easy to attain a perfect alignment of the easy axes of single crystals, which was also observed by Li et al. [2013] and Alpandéry et al., [2009]. Although \( M_{RS}/M_S \) suggests that shape anisotropy dominates, the significant 4θ component demonstrates that there is a biaxial anisotropy in the plane of deposition. Rotating the sample from in-plane to out-of-plane, however, approaches micromagnetic model predictions for the hysteresis parameters. This is an important factor to consider in the design of anisotropic materials for applications. As a next step it would be interesting to repeat this experiment using particles that already have anisotropy, such as ellipsoidal or rod-shaped particles.
5.1.5 CONCLUSIONS

We have prepared the magnetic-field directed self-assembly of biologic magnetite nanoparticles and shown that the RS still shows some magnetosomes aligned in the earth’s field, although much weaker than which are aligned with external magnets. This has implications for paleomagnetic studies and suggests that the Earth’s field can lead to formation of chains. OS showed preferred arrangement of magnetosomes along the field direction. The various magnetic properties for OS and RS were studied. Larger anisotropy is seen when rotating to out-of-plane compared to the in-plane rotation. Experimental results suggest that the change in $H_C$ as a function of $\varphi$ is directly related to the strength of anisotropy, greater the anisotropy more close we are towards the micromagnetic model proposed by Li et al [2013]. For the first time low and high field AMS results were shown for demonstrating the effectiveness of the particles alignment in magnetosomes. It opens opportunity to exploit the torque measurements for demonstrating magnetic contributions e.g. shape anisotropy, crystalline anisotropy and remanence that may play a role in the process of alignment. Further field-directed self-assembly can be anticipated as a viable alternative to lithography-mediated patterning for device purposes or to sedimentologists for interpreting the fossils containing ruptured magnetotactic bacteria.
Iron-oxide nanoparticles in Biomedical Imaging

Enhanced image contrast by magnetic nanoparticles.

(Source: https://ispub.com/IJRA/14/1/14083)
6.1 NEGATIVE CONTRAST AGENTS FOR MRI: BIOLOGICAL VS. SYNTHETIC AND SMALL VS. LARGE IRON-OXIDE NANOPARTICLES

6.1.1 INTRODUCTION

Magnetic nanoparticles (MNP) are receiving considerable attention in the development of novel bio-technological and nano-technological applications [e.g., Bogart et al., 2014; Curtis and Wilkinson, 2001; Gazeau et al., 1997; Mørup et al., 2011; Pankhurst et al., 2003]. Among them magnetite/maghemite nanoparticles are promising due to their low toxicity and magnetic properties that are exploitable for diagnosis and therapies [e.g., Fahlvik et al., 1993; Gupta and Gupta, 2005; Landfester and Ramirez, 2003; Sjögren et al., 1997; Weissleder et al., 1989]. Magnetic resonance imaging (MRI) is one such example of an image modality used for creating diagnostic images of various parts of a human body.

MRI image contrast is affected by different parameters that are characteristic of the target tissues and contrast materials, e.g., proton density, which is present in the form of water and macromolecules (proteins, fats) in the tissue, relaxation times \( T_1 \) and \( T_2 \), which are the response of the protons to the radio frequency, and the flow of the arterial blood that contribute to the loss in signal intensity [Deene, 2015]. The relaxation time \( T_1 \) and \( T_2 \) can be modulated by using positive or negative contrast agents. A paramagnetic contrast agent constitutes a positive contrast agent and has a predominant effect on \( T_1 \) shortening. Ferromagnetic contrast agents on the other hand are negative contrast agents influencing \( T_2 \). The relaxivity \( R_1 = 1/ T_1 \) and \( R_2 = 1/ T_2 \), which is the inverse of relaxation time, is used to expresses the degree of image contrast. The contrast of a \( T_2 \)-weighted image increases with increasing \( R_2 \), where \( R_2 \) is indirectly associated with the magnetic response of the contrast agent. Magnetic properties of ferromagnetic particles are governed by their composition, shape, size and crystal anisotropy (cf. Chapter 2).

Studies have been made that suggest enhanced image contrast is obtained for larger iron-oxide nanoparticle [Ahmad et al., 2012], but no systematic study has been made. No biogenic magnetite based contrast agents are commercially available at present, although magnetosomes have been shown to exhibit high ratio of \( R_2/ R_1 \) compared to commercially available contrast agents for MRI [Lisy et al., 2007]. Hence this study, which was conducted under the EU project ‘Bio2Man4MRI’ describes the magnetic characterization of cultured biogenic and synthetic biomimetic, magnetite nanoparticles in relation to image contrast for MRI. The EU-Bio2Man4MRI was a three year project based on nano-biotechnology. The conclusion, presented in this section is a condensed overview of large interdisciplinary study...
from biological, chemical, materials, medical and physical science groups. Theoretical models were also developed to explore physical parameters controlling better performance of ‘biologic with synthetic’ and ‘small with large’ magnetite nanoparticles as a negative contrast agent for applications and commercialization in MRI.

A complete overview that considers different aspects of the particle usages, e.g., their size, morphology, crystal structure, magnetics, biocompatibility, stability and contrast performances, is given in a joint publication (Appendix B). We are specifically interested in comparing smaller particles, i.e., superparamagnetic (SP) with larger particles, i.e. single domain (SD), and synthetic with biogenic magnetite nanoparticles. The following is a detailed report on the magnetic characterization of all samples evaluated under Bio2Man4MRI project.

### 6.1.2 SAMPLES AND METHODS

#### 6.1.2.1 SAMPLES

During the project period we received 44 synthetic and 19 biogenic magnetite nanoparticles from the partner groups headed by Damien Faire in Max Planck Institute of Colloids and Interfaces, Potsdam, Germany and Prof. Dirk Schüler in the Department of Biology, LMU Munich, Germany respectively. Prof. Dirk Schüler has recently moved to Department of Microbiology of University Bayreuth, Germany. Detailed sample information is given in the Table 6.1.1.

Table T6.1.1. Sample information. †Include magnetotactic bacteria (BC), magnetosomes (MS) and partly disaggregated magnetosomes (SMS) for each.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Number of samples</th>
<th>Received on</th>
<th>Sample names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic</td>
<td>07</td>
<td>Dec. 2011</td>
<td>99, 102 – 107</td>
</tr>
<tr>
<td>Synthetic</td>
<td>10</td>
<td>Apr. 2012</td>
<td>121, 123 – 125, 127, 132, 135 – 137,139</td>
</tr>
<tr>
<td>Biogenic</td>
<td>02</td>
<td>Jun. 2012</td>
<td>LMU-08(BC), LMU-08(MS)</td>
</tr>
<tr>
<td>Synthetic</td>
<td>05</td>
<td>Sep. 2012</td>
<td>158 – 162</td>
</tr>
<tr>
<td>Biogenic</td>
<td>09</td>
<td>Jan. 2013</td>
<td>†LMU-08, LMU-09, LMU-11</td>
</tr>
<tr>
<td>Synthetic</td>
<td>05</td>
<td>May 2013</td>
<td>238, 240 – 2434</td>
</tr>
<tr>
<td>Biogenic</td>
<td>03</td>
<td>Jul. 2013</td>
<td>LMU-22 – LMU-24 (only BC)</td>
</tr>
<tr>
<td>Biogenic</td>
<td>05</td>
<td>Feb. 2014</td>
<td>LMU-28 – LMU-32 (only MS)</td>
</tr>
<tr>
<td>Synthetic</td>
<td>07</td>
<td>Mar. 2014</td>
<td>M1 - M7</td>
</tr>
<tr>
<td>Synthetic</td>
<td>02</td>
<td>Jul. 2014</td>
<td>stabilized 243, Resovist®</td>
</tr>
</tbody>
</table>
6.1.2.2 METHODS

All magnetic measurements were carried out using a Princeton Measurements Corporation, Vibrating Sample Magnetometer (VSM; Micro-Mag Model 3900) at room temperature. Hysteresis loops were measured under a maximum applied field of 1 T with 100 ms averaging time and variable measurement spacing. The backfield isothermal remanent magnetization curves were measured up to a maximum field of 1 T. FORC measurements were made by first saturating the sample with a positive applied field of 1 T and then ramping down the field to a reversal field \( (H_R) \), followed by measuring magnetization as the field increases from reversal field back to positive saturation. A series of 140 FORC is used unless noted otherwise for the analysis. FORC data are transformed into FORC diagrams using M.Winklhofer MATLAB code [Winklhofer and Zimanyi, 2006]. The coercivity spectrum which is the reflection of individual constituents in the sample is given by the horizontal axis \( H_C \). The vertical axis \( H_U \), known as interaction axis, however, provides information on interactions between the particles.

6.1.3 RESULTS

6.1.3.1 INDUCED MAGNETIZATION

Saturation magnetization \( (M_S) \), is the best indicator for the chemical purity of the synthesized magnetite nanoparticles. The theoretical value of \( M_S \) for stoichiometric magnetite is 92 Am\(^2\)/kg, but non-stoichiometry, surface oxidation, structural defects, magnetic interactions between particles, or very small particles size can reduce \( M_S \). Fourteen synthetic samples have \( M_S \) (Fig.6.1.1) lower than empirically chosen value of 62 Am\(^2\)/kg [cf. Peters and Dekkers, 2003], suggesting that: i) the composition is not pure; ii) there is significant magnetic interaction between the uncoated particles; and iii) there is a significant proportion of particles < 10 nm [Thapa et al., 2004]. The \( M_S \), of sample 125 is > 92 Am2/kg, the theoretical limit, which is attributed to the presence of iron traces from the synthesis. For similar reasons samples 121, 123, 124, 127 and, 135 exhibit slightly higher values for \( M_S \).

The \( M_S \) of biogenic samples could not be determined, as their exact iron content, which is needed for the normalization, is not known. Saturation remanent magnetization \( (M_{RS}) \) is not only influenced by composition, but also by particle size. The magnetization ratio \( (M_{RS}/M_S) \) for randomly oriented, non-interacting magnetite, dominated by a magnetostatic anisotropy and crystalline anisotropy, are approximately 0.5 and 0.8 respectively. The magnetization ratios for biogenic samples varied from 0.43 to almost zero, depending upon their nature (e.g., chains vs. individual magnetosomes) and/or size (Fig. 6.1.2). LMU-11-BC has nearly zero magnetization ratio, owing to the size of the magnetosomes, which is less than 25 nm. For
size $\leq 25$ nm in diameter the magnetite nanoparticles are in the SP regime, at room temperature with 100 ms measurement time.

Fig. 6.1.1: Saturation magnetization ($M_s$) of synthetic magnetic nanoparticles.

Fig. 6.1.2: Magnetization ratio of biological magnetic nanoparticles.
6.1.3.2 BACKFIELD ISOTHERMAL REMANENT MAGNETIZATION CURVES

DC-IRM curves were analyzed to check for the oxidation. Backfield IRM analysis at room temperature and low temperature (30/40/50K; depending upon the stability of the temperature and the strength of the measurement signal) shows three types of acquisition curves. The first type refers to the one that saturates at or below 300 mT. The second type curve is unsaturated with increasing magnetic moment indicating the presence of high coercive material, i.e., hematite, while the third type is again unsaturated but with decreasing magnetic moment that suggests thermal relaxation during the measurement of the sample due to superparamagnetic (SP) particles (Table T6.1.2 and Table T6.1.3). Thirteen samples out of 42, showed a clear indication for the presence of high coercive phase, most likely the hematite. In addition to this, 6 more samples may have hematite as suggested by the presence of 135° ridge on the lower half of their FORC diagrams at temperatures ≤ room temperature.

Table T6.1.2. Summary for the chemical alteration of synthetic magnetite/maghemite. * The magnetic moment decreases between 300 mT and 1000 mT. # The FORC diagram shows 135° ridge only at temperatures < 300 K. Note: RT is room temperature and LT is < RT.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>IRM curve (RT)</th>
<th>IRM curve (LT)</th>
<th>135° Ridge on FORC diagram (RT/&lt; 290 K)</th>
<th>Chemical alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>102</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No/Yes *</td>
<td>Probable</td>
</tr>
<tr>
<td>103</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>104</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>105</td>
<td>Saturated</td>
<td>Not saturated</td>
<td>No/Yes *</td>
<td>Yes</td>
</tr>
<tr>
<td>106</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>107</td>
<td>Not saturated</td>
<td>Not saturated</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>121</td>
<td>Saturated</td>
<td>Not saturated</td>
<td>No/No</td>
<td>Yes</td>
</tr>
<tr>
<td>123</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>124</td>
<td>Saturated</td>
<td>Not saturated</td>
<td>Yes/No</td>
<td>Yes</td>
</tr>
<tr>
<td>125</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>127</td>
<td>Saturated</td>
<td>Not saturated</td>
<td>No/Yes *</td>
<td>Yes</td>
</tr>
<tr>
<td>132</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>136</td>
<td>Saturated</td>
<td>Saturated</td>
<td>No/Yes *</td>
<td>Probable</td>
</tr>
<tr>
<td>137</td>
<td>Saturated</td>
<td>Saturated</td>
<td>Yes/Yes *</td>
<td>Probable</td>
</tr>
<tr>
<td>139</td>
<td>Not saturated</td>
<td>Saturated</td>
<td>No/Yes *</td>
<td>Yes</td>
</tr>
<tr>
<td>158</td>
<td>Saturated</td>
<td>-</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
The dc-IRM curves from most biogenic samples are saturated for fields < 300 mT, thereby indicating the presence of only a low coercive phase (Table T6.3.3). The LMU-29-MS, LMU-30-MS and LMU-31-MS, however exhibits dc-IRM curve that are not saturated by 300 mT. This suggests for two distinct chemical compositions one attributed to magnetite/maghemite, and the other probably to hematite. Important to note is that none of the saturated loops from the biogenic samples have shown the clear presence of 135° ridge on their FORC diagrams. LMU-32-MS showed hints for the ridge presence in their FORC distribution.

6.1.3.3 COERCIVITY

The coercivity (Hc) is strongly influenced by not only the particle size distribution but also the chemical purity. Magnetic hysteresis loop represents an average behavior of the sample; whereas FORC diagrams provides detailed information about the coercivity spectrum from
the particles within a sample (H_C). FORC analysis exhibits two distinct types of FORC diagram. First type refers to unimodal meaning FORC with single peak coercivity (H_{CP}), representing the presence of only one phase material and the bimodal FORC displaying two H_{CP}, implying the presence of a two phase material. Based on this, 20 out of 42 synthetic samples are found to have bimodal FORC implying either the presence of two distinct coercive materials, or a bimodal size distribution. In the case of the unimodal FORC distribution, the H_C of all the samples (except 176 and 177), as determined from hysteresis loops, is lower than the corresponding H_{CP} obtained through FORC analysis. In case of bimodal FORC distribution, however H_C lies between the two H_{CP} (Fig. 6.1.3). Because these particles should be spherical, larger values of H_C may provide a hint for chemical oxidation.

Table T6.1.2. Summary for the chemical alteration of biogenic magnetite/maghemite. * The magnetic moment decreases between 300 mT and 1000 mT.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>IRM curve (RT)</th>
<th>135° Ridge on FORC diagram</th>
<th>Chemical alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMU-04-BC</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-04-MS</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-08-BC</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-08-MS</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-08-SMS</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-09-BC</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-09-MS</td>
<td>saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-09-SMS</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-11-BC</td>
<td>-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-11-MS</td>
<td>-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-11-SMS</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-22-BC</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-23-BC</td>
<td>Saturated</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-24-BC</td>
<td>-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-28-MS</td>
<td>-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LMU-29-MS</td>
<td>Not saturated</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>LMU-30-MS</td>
<td>Not saturated</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>LMU-31-MS</td>
<td>Not saturated</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>LMU-32-MS</td>
<td>Not saturated#</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Similar analysis on biogenic samples revealed six samples with bimodal FORC distributions, and 12 with unimodal FORC distributions (Fig. 6.1.4). The presences of chains
Fig. 6.1.3: Coercivity distribution for synthetic magnetite nanoparticles.

Fig. 6.1.4: Coercivity distribution for biological magnetite nanoparticles.

of magnetosomes, in biogenic cells are reflected by a large spread of $H_C$, up to 80 mT. LMU-23-BC has the highest spread in HC, thereby indicating longer magnetosomal chains. In principle magnetosomes samples should have low values for HC, as they are not in chains. In general, however, the magnetosome samples exhibits a greater spread in HC, which could be
accounted for by: i) the presence of large SD size, ii) chemical oxidation to hematite, or iii) the presences of still some chains.

6.1.3.4 MAGNETIC INTERACTIONS

The magnetic field interaction ($H_U$), obtained from the FORC analysis provides a quantitative measure for the intensity of magnetic interactions between the particles. The local field interaction between the similar particles is described by the spread in $H_U$ for SD particles, the higher the spread in $H_U$, the larger the amount of the magnetic interaction between the magnetic particles. A positive upward shift of the peak FORC distribution function at or near zero $H_C$, implies the absence of magnetic interactions from assembly of SP particles. A mean field interaction can be defined from the FORC distribution function that lies above or below the $H_C$-axis. FORC analysis supports for a considerable dipolar interaction between the particles in all synthetic samples, due to the broad spread in interaction field ($H_U$) (Fig. 6.1.5). This is not unexpected, because the particles are not coated and are likely to agglomerate. Biogenic samples exhibit $H_U \leq 10$ mT, except LMU-09-MS, LMU-29-MS, LMU-31-MS and LMU-32-MS (Fig. 6.1.6). This indicates for no or negligible interaction between magnetosomes. LMU-09-MS, LMU-29-MS, LMU-31-MS and LMU-32-MS, however have a spread in $H_U$ above 10 mT that indicates for moderate interactions. Mean field interaction is either negligible or completely absent in all samples.

![Graph showing estimation of local interaction field ($H_U$), and mean interaction field for synthetic magnetite nanoparticles.](image)

Fig. 6.1.5: Estimation of local interaction field ($H_U$), and mean interaction field for synthetic magnetite nanoparticles.
6.1.3.5 STABILIZED 243

For MRI applications, particles should not show magnetic interaction, because this affects the effective magnetic particle size. After synthesis and magnetic characterization, the NanoPET GmbH partner from the project treated the particles to make them colloidal stable. Sample 243 was stabilized with L-3,4 Dihydroxyphenylalanin (DOPA) which also act as a coating to break down magnetic interaction between the particles. The stabilization lowers the magnetization ratio, from 0.09 to 0.01 and coercivity ratio, from 2.46 to nearly zero. The FORC distribution, for the stabilized 243 is concentrated at the origin, as would be expected for purely SP particles, and the magnetization is largely reversible when compared to initial 243 (Fig. 6.1.7). These results indicate that the stabilization of synthetic 243 with DOPA is successful in breaking down particle interaction, so that their magnetic properties are solely SP.

6.1.4 DISCUSSION

The initial phase of the project Bio2Man4MRI has produced synthetic particles with lower values in $M_s$, but also wide variability partly due to non-stoichiometry and magnetic interaction. Adulteration by pure iron traces was also a problem. As the project progressed, a significant improvement was seen in producing magnetic nanoparticles, with more stable and reproducible magnetic properties. For example, the last set of synthetic magnetite...
nanoparticles had a limited size distribution and a reasonably high $M_S$. The biological samples also had a narrower particle size distribution in the later samples. We have demonstrated that stabilization of the synthetic, inorganic particles prevented aggregation, so that small particles are almost exclusively SP in their magnetic properties.

![Fig. 6.1.7: FORC distributions with smoothing factor SF for a) Initial synthetic 243, and b) stabilized 243 magnetite nanoparticles.](image)

### 6.1.5 CONCLUSIONS

A comprehensive multidisciplinary study was performed on 14 best samples to examine their efficiency, as a contrast agent in MRI. The image contrast in MRI signal has an indirect relationship with the magnetism of the particles; hence it is difficult to interpret image contrast in direct relation to the particle magnetism. In the complete study it can be shown that the higher the magnetization and the coercivity ratio, the greater the relaxivity ratio is for MRI (Appendix B). The study also demonstrates that both synthetic and biologic samples exhibit enhanced relaxation properties, compared to the known MRI contrast agent Resovist®. In our samples we demonstrated that the larger the core diameter, the higher relaxivity $R_2$ is. The $R_1$ relaxivity is low and is almost independent to the particle size. Thus the $R_2/ R_1$ relaxivity values of these particles are highly improved. The large synthetic particles have insufficient colloidal stability hence only the small particles were investigated. These particles offer enhanced contrast properties with respect to Resovist® but reduced contrast compared to the biological magnetosomes. No enhanced toxicity was found for larger particles but biological particles have higher toxicity. Therefore, combining size effect, colloidal stability and toxicity, improvements in the stabilization of the synthetic particles, in particular the particles with large core diameter have potential for providing particles with enhanced relaxation properties without problems associated with toxicity.
6.2 OPTIMIZATION OF MAGNETIC PROPERTIES OF IMMOBILIZED NANOPARTICLES FOR MAGNETIC RESONANCE AND PARTICLE IMAGING

Monika Kumari, Alexander Kraupner, and Ann M Hirt; in preparation for Nanoscale

6.2.1 INTRODUCTION

Iron oxide nanoparticles have attracted enormous interest in biomedical imaging i.e., magnetic resonance imaging (MRI) and magnetic particle imaging (MPI). The tuning of iron oxide nanoparticles as a contrast agent for MRI can enhance the visibility of images by shortening $T_2$ relaxation time. Superparamagnetic (SP) particles contribute to a decrease in $T_2$ relaxation time with a simultaneous increase in relaxivity $R_2 (1/T_2)$ by introducing a large magnetic field inhomogeneity in the target region, which aggravates rapid dephasing of neighboring protons [Brix et al., 2008; Chavhan et al., 2009]. SP nanoparticles are particles whose magnetization relaxes on the time scale of measurement [Dunlop and Özdemir, 1997]. For example, using a measurement time of 100 ms shows that iron-oxide particles with a magnetic single-core-diameter, $d$, $\leq$ 25 nm are SP at 300 K. Magnetite particles with $d$, between 25 nm to 80 nm, however are homogeneously magnetized, i.e., single domain (SD), which means that their magnetization is blocked and cannot reverse on 100 ms time scale. SD particles are characterized by a remanent magnetization and coercivity. From a magnetic perspective MRI demands SP particles exhibiting high magnetic moment, and no net magnetization in the absence of a magnetic field, $H$ [Gundersen et al., 1990; Hendrick and Mark, 1993]. Iron oxide nanoparticles are also gaining attention as tracers for MPI, a new modality that will be used for clinical imaging. Ideally, MPI requires nanoparticles with broad harmonic spectra and a steep initial magnetization curve, which saturates in fields $> 20$ mT [Ferguson et al., 2013]. The particle harmonic spectrum of a sample is influenced by the behavior of magnetic relaxation, i.e., Néel and Brownian relaxations. There are numerous studies underway to synthesis nanoparticles to produce MNP with improved contrast properties for MRI, or tracer material for MPI [e.g., Ferguson et al., 2011; Huh et al., 2005; Jun et al., 2008; J-H. Lee et al., 2007; Löwa et al., 2015; Nasongkla et al., 2006; Seo et al., 2006; Zhao et al., 2001]. At present “Gold-standard” Resovist® is being used as the standard for comparing the MPI signal strength in terms of harmonic spectra [Gehrke et al., 2013; Löwa et al., 2015; Ludwig et al., 2012; Ludwig et al., 2013]. Since Resovist® is no longer commercially available; efforts focus presently on synthesizing and testing new materials that will yield similar if not better, image quality. FeraSpin™ R, manufactured by nanoPET Pharma GmbH (Berlin), is one example of such a product.
FeraSpin™ R consists of elementary crystallites of iron-oxide with \(d \approx 5-7\) nm. Some of these crystallites aggregate to form particles with a larger diameter, such that there is a broader distribution due to these multi-cores. To better understand the physical properties that contribute to the suitability of these magnetic nanoparticles (MNP) for MRI and MPI, a series of different multi-core size fractions obtained from FeraSpin™ R namely, FeraSpin™ XS, FeraSpin™ M and FeraSpin™ XL were investigated. Fractionation yields particles with identical chemical composition but a narrower multi-core size distribution, when compared to FeraSpin™ R. FeraSpin™ R has a mean hydrodynamic diameter of 60 nm and the FeraSpin™ XS, FeraSpin™ M and FeraSpin™ XL have mean hydrodynamic diameters of 15, 35 and 55 nm, respectively, as determined by dynamic light scattering (Fig. 6.2.1a). It should be noted that hydrodynamic diameter represents the size of a multi-core particle in a solution, and not that of the magnetic core. Figure 6.2.1b illustrates the differences among the hydrodynamic diameter; magnetic single-core-diameter and multi-core diameter. Several studies have been made on FeraSpin™ R and the FeraSpin™ series in relation to their performance in MPI [e.g., Gehrke et al., 2013; Gehrke et al., 2012; Ludwig et al., 2012; Ludwig et al., 2013; Yoshida et al., 2013]. This is the first study that performs a more detailed magnetic characterization on immobilized FeraSpin™ R, FeraSpin™ series and Resovist®. Information on the static magnetic properties is essential, particularly for MPI applications that demands fixation of the MNP to cells and tissues. Further this information helps to distinguish the dependence of magnetic properties on particle size, and how this contributes to final performance for MRI and MPI. An important question is whether the magnetic
properties reflect the size of the elementary crystallites that constitutes a multi-core or their aggregates. Here, aggregation can either be due to clustering of the elementary crystallites of a multi-core or clustering of the multi-core units. Either of the case will change the magnetic core-diameter from 5 or 7 nm; hence the effective magnetic core-diameter. When coating is enough to breakdown the magnetic interactions, effective magnetic core-diameter should be the same as the core-diameter, which is smaller than the hydrodynamic diameter. Note that in the following results and discussion are concerned with the effective magnetic core size, because this is what controls the magnetic properties.

In this study magnetic methods are used to characterize the magnetic properties of the particles as a whole, which will be important in their performance. The temperature dependence of low-field susceptibility is used to assess the chemical purity of the magnetite samples. First order reversal curves (FORC) [Newell, 2005; Pike et al., 2001; Roberts et al., 2000] are used to define particle effective magnetic core-size distribution, fraction of SP and SD particles in a mixture [Kumari et al., 2014], interactions among the individual crystallites [R Egli et al., 2010; Pike et al., 1999], and the compositional purity [Carter-Stiglitz et al., 2001; Hirt et al., 2014; Muxworthy et al., 2005]. We further test for interaction using FORC at 50 K and temperature dependent AC-susceptibility. Interaction plays a role in modulating the effective magnetic particle size, the particle anisotropy and their uniform dispersion. This is important because these factors regulate image contrast. The magnetic properties are finally studied with respect to their performances in MRI and MPI. The results from this study are used to demonstrate the role of chemical composition, effective magnetic particle size and interactions on image performances. These findings should help manufacturers and end-users in tailoring the magnetic properties so that they lead to enhanced imaging.

6.2.2 METHODS

All magnetic measurements were performed on dried samples immobilized in a sealed capillary tube. Samples were dried under ambient conditions in the fume hood by solvent evaporation. Low field susceptibility was measured on an AGICO KLY2 susceptibility bridge using liquid nitrogen in field strength of 0.25 mT. Induced magnetization as a function of applied field, FORC analysis and ZFC-FC measurements were performed on a Princeton Measurement Corporation (PCM), vibrating sample magnetometer (VSM, model 3900) at the Laboratory of Natural Magnetism, ETH-Zurich. Multiple segment hysteresis loops were measured with a field of ± 1T and 100 ms averaging time. A series of 140 FORC were made using 1.2 mT field increments and finally the data were processed with Winklhofer MATLAB code [Winklhofer and Zimanyi, 2006]. The first-point artifact is corrected by this routine. Low temperature measurements were achieved by the installation of cryostat on VSM. For the ZFC-FC measurements demagnetized samples were initially cooled from 300 K to 20 K in
absence of H, and then induced magnetization was measured as a function of temperature with a weak field of 10 mT at every 2 K. Similarly FC data were obtained by cooling the sample from RT to 20 K in a 1 T applied field. The field was then removed and a field of 10 mT was applied during warming. AC-susceptibility was measured as a function of temperature on a Quantum Design Physical Properties Measurement System (PPMS-14T) at the Institute of Metal Research, ETH Zurich in five frequencies: 100, 300, 1000, 3000 and 10,000 Hz. Measurements were made between 10 K and 300 K with an interval of 5 K.

6.2.3 RESULTS

6.2.3.1 MAGNETIC RESULTS

LOW-FIELD SUSCEPTIBILITY

Monitoring low-field susceptibility shows that there is an abrupt loss at about 265 K in all samples (Fig. 6.2.2). This susceptibility loss is characteristic for Morin transition in hematite, $T_M$ [Morin, 1950b; Morrish, 1994]. The change in susceptibility for FeraSpin™ XS is not as apparent as in other samples, which may be due to its suppression by very fine sized grains and high internal stress induced in fine particles [Kündig et al., 1966; Muench et al., 1985].

![Low-field susceptibility as a function of temperature for all samples.](image)

Fig. 6.2.2: Low-field susceptibility as a function of temperature for all samples.

INDUCED MAGNETIZATION

The room temperature (RT) magnetic hysteresis loops are closed virtually with zero remanence for FeraSpin™ R, its multi-core size fractions (FeraSpin™ Series), and Resovist® (Fig. 6.2.3a), signifying a predominance of SP particles. The magnetization curves of each sample shows a steep initial increase, but no sample reaches saturation in a field of 1 T. This
is due to the presence of hematite [Rochette et al., 2005], although ultra-fine particles [Khurshid et al., 2012] may also prevent saturation.

Fig. 6.2.3: a) Magnetization curves for all samples with magnetization normalized to the maximum magnetization, a) at 300 K, and b) at 30 K.

At 30 K all samples have an open hysteresis loop (Fig. 6.2.3b), indicating some portion of the particles undergo magnetic ordering at low temperature. FeraSpin™ XL with largest hydrodynamic diameter has a coercivity, $H_C$, of 11.7 mT. FeraSpin™ XS, the smallest multi-core fraction has a very thin magnetic loop at 30 K with $H_C = 1.1$ mT. This suggests that an even lower temperature would be needed to freeze the magnetic moment of all particles in FeraSpin™ XS. FeraSpin™ M, FeraSpin™ R, and Resovist® have a $H_C$ of 6.2 mT. The observed difference in the magnetic hysteresis loop at 30 K for all samples suggests aggregation among the elementary crystallites, because coating may not be perfect so that interaction persists between cryastallites. This would lead to differences in mean effective magnetic particle size.

**FIRST ORDER REVERSAL CURVES (FORC)**

FORC distributions for FeraSpin™ R, FeraSpin™ series and Resovist® at room temperature are located close to the origin of the FORC diagram (Fig. 6.2.4). All FORC diagrams show a low spread on the coercivity axis and a positive shift with respect to zero interaction field. This indicates for the presence of essentially all “non-interacting” SP particles [Pike et al., 2001; Roberts et al., 2000]. FeraSpin™ XL shows the least spread along the interaction axis, $H_U$, while, FeraSpin™ XS has the largest. The FeraSpin™ series shows an increase in the positive shift in the peak interaction field with decreasing average particle size (Fig. 6.2.4). Resovist® has broadest coercivity profile extending up to 8 mT, suggesting
larger fractions of particles with $d \geq 25$ nm compared to the FeraSpin$^\text{TM}$ R. The elementary crystallite size in Resovist$^\text{®}$ is also on the order of 5-7 nm, therefore the higher coercivity show that there is an aggregation of elementary crystallites [Ferguson et al., 2012; Gehrke et al., 2012; Yoshida et al., 2012].

Fig. 6.2.4: FORC distributions for FeraSpin$^\text{TM}$ R, its fractions and the Resovist$^\text{®}$. Material name and the smoothing factor SF used are indicated at the top corresponding to each diagram.

Fig. 6.2.5 displays FORC distributions of FeraSpin$^\text{TM}$ R, FeraSpin$^\text{TM}$ M and FeraSpin$^\text{TM}$ XL at 50 K. As temperature decreases the magnetic moments of the SP particles starts to order, i.e., become like SD. The difference observed in the individual FORC diagrams at 50 K indicates aggregation among the elementary crystallites of 5-7 nm, which reflects the mean effective magnetic core-size for each sample. FeraSpin$^\text{TM}$ R (Fig. 6.2.5a) has a bimodal FORC distribution, i.e., two distinct peaks in its coercivity distribution at about 2 mT and 11 mT.
The peak coercivity at 2 mT is typical for very small magnetic particles that still are not completely ordered, while, the higher coercivity comes from the particles that have undergone magnetic ordering. The fact that the coercivity profile persists until approximately 70 mT, may reflect ordered hematite. The small spread along interaction axis accounts for little interaction among particles in FeraSpin™ R.

![Image](image.png)

Fig. 6.2.5: FORC distributions at 50 K for a) FeraSpin™ R, and its fractions, b) FeraSpin™ M, and c) FeraSpin™ XL (top). Corresponding coercivity spectrums of d) FeraSpin™ R, e) FeraSpin™ M, and f) FeraSpin™ XL (bottom).

FeraSpin™ M has a unimodal FORC distribution at 50 K with a spread in coercivity up to 50 mT spread along interaction from -20 mT to 40 mT (Fig. 6.2.5b and 6.2.5e). This suggests that there may be more interaction among the multi-cores that results in a larger effective magnetic particle-size. FeraSpin™ XL shows a unimodal FORC distribution with a plateau in peak coercivity from 7 mT to 13 mT (Fig. 6.2.5c and 6.2.5f). The coercivity distribution extends to higher fields, which probably arises from the hematite. The FORC distribution is more contained at the origin, which suggests that the concentration of magnetic particles that are ordered is higher, compared to the other samples. It also has a narrower spread at higher
fields along $H_U$, which suggests little to no interaction of the multi-cores.

A semi-quantitative estimation for relative fractions of SP and SD particles in FeraSpin$^\text{TM}$ R, FeraSpin$^\text{TM}$ M and FeraSpin$^\text{TM}$ XL at 50 K was obtained by deconvolving the reversible and irreversible components of induced magnetization from the FORC data, assuming magnetite nanoparticles have uniaxial shape anisotropy [Kumari et al., 2014] (Table. T6.2.1). FeraSpin$^\text{TM}$ XL contains 75% of SD particles, while FeraSpin$^\text{TM}$ R and FeraSpin$^\text{TM}$ M both contain around 65-70% SD particles at 50 K.

Table T 6.2.1. Semi-quantitative estimation of SP and SD fraction with the corresponding $T_S$ and $T_B$.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>% fraction of SP particles (50 K)</th>
<th>% fraction of SD particles (50 K)</th>
<th>$T_S$ (K)</th>
<th>$T_B$ (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeraSpin$^\text{TM}$ XS</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>31</td>
</tr>
<tr>
<td>FeraSpin$^\text{TM}$ M</td>
<td>31</td>
<td>69</td>
<td>154</td>
<td>140</td>
</tr>
<tr>
<td>FeraSpin$^\text{TM}$ XL</td>
<td>25</td>
<td>75</td>
<td>ca. 300</td>
<td>208</td>
</tr>
<tr>
<td>FeraSpin$^\text{TM}$ R</td>
<td>34</td>
<td>66</td>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td>Resovist$^\circledR$</td>
<td>-</td>
<td>-</td>
<td>220</td>
<td>92</td>
</tr>
</tbody>
</table>

ZERO-FIELD-COOLED AND FIELD-COOLED MAGNETIZATION

The temperature dependence of magnetization in zero-field-cooled (ZFC) and field-cooled (FC) curves shows that all samples exhibit superparamagnetism, in which the average blocking temperature, $T_B$, can be defined from the peak temperature in the ZFC curve (Fig. 6.2.6). The temperature at which the first magnetic particles start to block is defined at the bifurcation point; $T_S$, on the ZFC and FC curves. $T_B$ and $T_S$ of each sample are summarized in Table. T6.2.1. All samples show a wide blocking spectrum except for FeraSpin$^\text{TM}$ XS, due to its narrow particle size distribution. FeraSpin$^\text{TM}$ XL has $T_S$ around RT, confirming the presence of larger particles that are blocked. Resovist$^\circledR$ and FeraSpin$^\text{TM}$ R have similar values for $T_S$, although $T_B$ for Resovist$^\circledR$ is smaller, indicating that the particle size distribution is skewed to smaller particles. From $T_B$ it can be seen that FeraSpin$^\text{TM}$ XL contains the largest particle size, whereby the average size decreases for FeraSpin$^\text{TM}$ M and FeraSpin$^\text{TM}$ R, followed by Resovist$^\circledR$ and FeraSpin$^\text{TM}$ XS. It should be noted that FeraSpin$^\text{TM}$ R has a broadest plateau at it $T_B$ that reflects a broad range of particle sizes, although the average effective magnetic particle size is nearly equal to that of Resovist$^\circledR$. This explains the large deviation observed in the average effective size of the Resovist$^\circledR$ when compared to the FeraSpin$^\text{TM}$ R on the basis of ZFC-FC curves.
Fig. 6.2.6: Temperature dependent ZFC (red)-FC (blue) magnetization curves for FeraSpin™ R, its fractions and Resovist®.
AC susceptibility is used to determine the origin of thermal relaxation and magnetic interaction by decomposing it into its in-phase ($\chi'$) and out-of-phase ($\chi''$) susceptibility (Fig. 6.2.7). For all samples, except for FeraSpin™ XS, $\chi''$ shows a good agreement with Néel relaxation, also known as the $\frac{\pi}{2}$-Law [Shcherbakov and Fabian, 2005; Worm and Jackson, 1999] (Fig. 6.2.7). This suggests thermal relaxation is solely due to the SP components in FeraSpin™ R, FeraSpin™ M, FeraSpin™ XL and Resovist®, FeraSpin™ XS, on the other hand, does not follow Néel relaxation, which suggests a significant effect arising from dipolar interaction or alternatively surface effects, e.g., uncoupled surface spins, from the ultra-fine particles. Each sample exhibits a unique $\chi''$ susceptibility spectrum, where the temperature of peak $\chi''$ also indicates the average particle size of the fractions. It can be seen that the peak temperature in $\chi''$, decreases from FeraSpin™ XL to FeraSpin™ M to FeraSpin™ XS. The absence of a true peak and the weak frequency dependency over the measured temperature range in $\chi'$ in FeraSpin™ XL, indicates that the average $T_B$ is close to room temperature. FeraSpin™ R and Resovist® carry two $T_B$, one at 40 K representing contribution from fine particles and the second around 300 K indicative of larger particles. Because FeraSpin™ XS and FeraSpin™ M displayed $\chi''$ susceptibility characteristic for a unimodal size distribution, the Néel-Brown equation was used to evaluate the pre-factor $\tau_o$. In this case $\tau_o = 9.9 \times 10^{-132}$ s for FeraSpin™ XS. This has no physical meaning, and is interpreted as reflecting interaction in the particle system. FeraSpin™ M has $\tau_o = 1.1 \times 10^{-14}$ s, which is slightly high for the empirically defined range of $10^{-8}$ to $10^{-11}$ s [Dormann et al., 1999; Ghasemi, 2012]. Therefore interaction may also be important in this sample.

6.2.3.2 IMAGING PERFORMANCES

The efficiency of image contrast in MRI is a function of spin-lattice relaxivity ($R_1$ or $1/T_1$) and spin-spin relaxivity ($R_2$ or $1/T_2$). SP iron-oxide nanoparticles indirectly cause a rise in the image contrast by introducing significant changes in $R_2$ and moderate to no changes in $R_1$. $T_2$-weighted image performance is expressed in terms of $R_1$, $R_2$ and $R_2/R_1$ (Table T6.2.2). FeraSpin™ XL has the highest negative contrast property in MRI ($R_2/R_1$) and the fraction FeraSpin™ XS the least.

MPI performance is based on the richness of the harmonic spectrum of the sample, i.e., high spectral amplitude and large harmonic number. The relative ranking of MPI signal strength is based on the harmonic spectrum given in the literature [Gehrke et al., 2012; Ludwig et al., 2013] for FeraSpin™ fractions and Resovist® (Table T6.2.2). The MPI performance of FeraSpin™ R and Resovist® is comparable due to their similar harmonic spectra [Gehrke et al., 2013; Ludwig et al., 2012]. The largest particle size fraction has the best performance in
suspension, but the FeraSpin\textsuperscript{TM} M particle size is best when immobilized. In both the cases FeraSpin\textsuperscript{TM} XS shows the least suitability for MPI.

Fig. 6.2.7: AC-susceptibility as a function of temperature at five frequencies 100 Hz (grey triangles), 300 Hz (purple squares), 1000 Hz (orange circles), 3 KHz (red triangles), and 10 KHz (blue diamonds). Néel relaxation was tested for the differences in $\chi''$ with $f(H) = 10$ KHz and $f(L) = 100$ Hz and (dashed black line) for all FeraSpin\textsuperscript{TM} samples. NOTE: Due to the large spread in data points at 100 Hz for Resovist\textsuperscript{®}, the Néel relaxation was calculated with $f(H) = 10$ KHz and $f(L) = 3$ KHz.
Table 6.2.2. Efficiency of image performance in MRI and MPI. MPI performance is ranked from 1 (best) to 4 (least). Note: MRI measurements were made at 1.4T @ 300 K.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>R₁</th>
<th>R₂</th>
<th>R₂/R₁</th>
<th>MPI (Suspension)</th>
<th>MPI (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeraSpin™ XS</td>
<td>13</td>
<td>49</td>
<td>3.8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>FeraSpin™ M</td>
<td>9.9</td>
<td>117</td>
<td>11.2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>FeraSpin™ XL</td>
<td>7.9</td>
<td>270</td>
<td>34.2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>FeraSpin™ R</td>
<td>10</td>
<td>185</td>
<td>18.5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Resovist®</td>
<td>9.3</td>
<td>174</td>
<td>18.7</td>
<td>2</td>
<td>NA</td>
</tr>
</tbody>
</table>

6.2.4 DISCUSSION

The hysteresis curves of each sample in this study are identical for the measurements, on wet and dried samples. This indicates that immobilization of these samples through solvent evaporation has no effect on their chemical and magnetic properties. All samples investigated here are SP materials at room temperature, despite the difference in the amount of magnetite aggregation between the elementary crystallites. They are, however, no longer chemically pure magnetite or even the mixture of magnetite or maghemite, but have undergone some degree of further surface oxidation to hematite, as seen from the clear Morin transition. Although surface oxidation will reduce the effective magnetite core, based on the average blocking temperature, it is clear that FeraSpin™ XL has the largest average effective magnetic particles size, followed by FeraSpin™ M, FeraSpin™ R and Resovist®, which have a similar average effective magnetic size, and FeraSpin™ XS as the smallest particles. A clear relationship can also be seen in the lack of magnetic saturation, and T_B (Fig. 6.2.3, Table T6.2.1). FeraSpin™ XS, for example, has the smallest T_B, but also the largest contribution of the high coercivity hematite as seen in the magnetization curves. FeraSpin™ XL, on the other hand has the least contribution from hematite and the largest T_B. AC susceptibility also reflects the difference in average effective magnetic particle size for different multi-core fractions, and also shows that FeraSpin™ R and Resovist® have a significant population of particles with two mean effective magnetic particle size distribution.

FORC analysis at 50 K, which is below T_B for all samples except FeraSpin™ XS, shows that FeraSpin™ R and FeraSpin™ XL have limited interaction between their multi-cores, whereas FeraSpin™ M has more particle interaction. This was further verified from the predicted \( \tau_o \) for FeraSpin™ M, although the amount of interaction is not severe, because \( \chi'' \) conforms to Néel relaxation (Fig. 6.2.7). FeraSpin™ XS does not follow predicted Néel behavior for SP relaxation as shown in Figure 6.2.7a; therefore, it also suffers from
interaction and/or surface effects. In summary, each sample varies in the amount of deviation from perfect linear behavior of the imaging signal due to differences in the strength of interactions between the particles.

Comparing the performance in MRI with the magnetic results we found that the relaxivity ratio displays a linear dependency on the slope of the magnetization curve, which is normalized by maximum moment for fields from 0 to 20 mT (Fig. 6.2.8a), and the bifurcation temperature $T_S$ (Fig. 6.2.8b). Therefore MRI performance is controlled on a first order by the particle size distribution of the magnetic nanoparticles. Deviation from the linear relationship, however, will arise from particle anisotropy, inter-particle interactions and agglomeration, and changes in composition. Ideally particles should have a high susceptibility, which has been noted in other studies [e.g., Ferguson et al., 2013; Ludwig et al., 2012; Ludwig et al., 2013]. In practice this means that the particles should be slightly under the boundary between SP and SD particle size, because these have the highest susceptibility [Dearing et al., 1996]. We demonstrate that the same criteria hold for MPI performance of particles in suspension. Interesting is that the harmonic spectrum was broader for the fixed FeraSpin™ M fraction compared to the fixed FeraSpin™ XL. Here we speculate that fixing affected the magnetic behavior by suppressing the Brownian motion. Hence those particles that are close to being magnetically ordered at RT are only governed by the Néel relaxation.

![Fig. 6.2.8: Relaxivity ratio as a function of a) the initial slope of the moment normalized magnetization curve, up to 20 mT, and b) average blocking temperature. In both cases the probability value that is used for testing statistical hypothesis, is $p < 0.05$, suggesting $> 95 \%$ significance of the relationship between the variables in the linear regression model of the data set. R-squared is a statistical measure of how close the data are to the fitted regression line (black line). Note: Diamond represents the experimental data; grey for FeraSpin™ XS, red for FeraSpin™ M, orange for FeraSpin™ R, blue for FeraSpin™ XL, and purple for Resovist®.](image)

From a synthesis point of view, it should be noted that larger particles have a further
advantage in that their surface area is a much smaller part of their volume compared to small particles. Therefore surface oxidation and surface spins have less of an effect on the bulk magnetic properties. Depending on whether particles will be in a solution or fixed, it is important to find the optimal size for an application. It is also important to note that the magnetic hysteresis and the FORC diagrams were obtained with a measurement time 100 ms for which nanoparticles up to ≈ 25 nm falls under SP regime. In present MPI system measurements are performed with average time of 20 μs. The reduction in measurement time from 100 ms to 20 μs will affect the magnetic relaxation dynamics due to the decrease in the SP limit from 25 nm to ≈ 20 nm. This in turn will contribute to deviation from a perfect linear relation of the image signal to the slope of the magnetization curve and the T_B. Further it may also account for the observed discrepancy for FeraSpin™ M when fixed.

6.2.5 CONCLUSIONS

Our findings show that all samples are magnetite with some degree of aggregation and hematite surface oxidation. Both affect only the effective magnetic particle size, as seen from T_B. MRI performance can be assessed on a first order from the magnetization curve and the T_S. It has a direct relationship to the bulk particle size of the SP particles the larger the particle size within SP range the better the performance. This holds because magnetite particles on the SP-SD boundary have the highest susceptibility and can easily align in a field. The same holds for MPI performance on SP particles in suspension. Fixing the particles, however, shows that particles close to magnetic ordering did not perform as well. This result suggests that fixation may lead to a state in which the particles undergo magnetic ordering and therefore cannot respond as easily to an applied field. More study with a broader examination of different magnetic particle would aid in verifying linear relation of imaging strength. In turn this would allow for an easy and quick method in quantifying or assessing the efficiency of any new materials for in imaging.
CHAPTER 7  Summary and Outlook
7.1 SUMMARY AND OUTLOOK

7.1.1 SUMMARY

Magnetic nanoparticles of iron oxides are being used in a wide variety of applications in sensor industrial and medical applications. The ultimate performance is dependent on how well their physical properties are tailored for their specific use. Important for any application is to know the compositional purity of the material and the effective magnetic particle size distribution. This effective magnetic particle size can be different from the physical particles size. For example, magnetic interaction between aggregates of particles can lead to a larger effective magnetic particle size, whereas surface oxidation of a magnetite or maghemite nanoparticle to hematite will reduce the core size. This work in this dissertation is concerned with establishing methods, which will aid in the characterization of iron oxide nanoparticles.

First-order reversal curves (FORC) have been shown to provide information on the coercivity distribution and particles interaction in geological applications. It is also a powerful method in the application of magnetic nanoparticles, because these systems are generally more constrained than natural materials. Much information can be extracted from the measurements, such as the reversible and irreversible contributions to the magnetization of the material. Although the reversible component will be affected by the reversible part of a magnetically ordered particle, it will also reflect the reversible part arising from particles that are not magnetically ordered or blocked. Under certain assumptions it is possible to make a first-order estimate of the amount of superparamagnetic (SP) particles in a system as shown in Chapter 3.1.1, where we found a good agreement between particle size analyses made by transmission electron microscopy (TEM). This is useful for groups synthesizing particles because it can indicate if interactions, i.e., aggregation, are occurring. The methods in the thesis were also useful in showing whether the magnetic properties of “large” magnetic particles that are composed of subunits, reflect the original small units are the aggregated whole. Hysteresis properties are also used to characterize magnetic particle size. An empirical plot, the Day-Dunlop plot, is often used to delineate bulk particle size of magnetite in a material, including mixtures of single domain and SP particles. There has been little experimental verification of these mixing curves. Here we demonstrate that in a relatively well-defined particle system, measured magnetization and coercivity ratios do not follow the prediction. Although more experimental data would be needed to better understand why the theory fails, the results should motivate a closer look at this problem.

Surface oxidation is a common problem in the synthesis of magnetite nanoparticles. It is easy to detect if there is a significant amount of oxidation, however it is a question whether it
can be detected for small amounts. We have demonstrated initial surface oxidation is seen in FORC analysis with a 135° ridge. Often this is the first expression of oxidation, before detection with selected area diffraction (SAED) in TEM analysis. This early detection can be important in preventing further oxidation, which will affect the effective magnetic particle size.

There are many applications in material science that are interested in producing materials with anisotropic magnetic properties. Often synthesis involves self-assembly of individual isotropic particles. Anisotropy in the hysteresis properties, saturation remanent magnetization and coercivity are used as a measure of the degree of alignment. We have demonstrated the usefulness of torque magnetometry in self-assembled euhedral particles of magnetite.

The biomimetic and biologic samples, which evaluated in the thesis project, were produced under the EU Project, Bio2Man4MRI. The methods and results from the first part of the thesis are applied in the final chapter. I further show which magnetic properties can be used to assess their performance for magnetic resonance imaging (MRI) and magnetic particle imaging (MPI). Optimizing these magnetic properties will help in creating particles with superior contrast properties.

These results also have important applications in environmental and rock magnetic studies. Unravelling SP component in natural materials namely, soils, sediments, and rocks, plays vital role in the diagnosis of environmental conditions and processes. For example, the creation of SP magnetite grains by bacteria reflects anaerobic atmosphere [Karlin et al., 1987; Lovley et al., 1987; Maher, 1998]. While oxidation of stronger magnetic minerals (e.g., magnetite) to weakly magnetic materials (e.g., hematite), suggests oxidizing atmosphere. Information about grain size of iron oxides also has implications in archaeology, soil science and paleoclimate [Heller and Evans, 1995; Jordanova, 2001; Liu et al., 1992]. For example, processes that create new iron oxide materials or reductively dissolve existing iron-oxides, can lead to a broad particle size spectrum. In addition, understanding SP/SD magnetite content and its role in the stability of magnetization in rocks as a magnetic recorder is of great significance in paleomagnetism. It can also be indicative of changes that occur during metamorphic events, which results in remagnetization of a rock [Channell and McCabe, 1994; Jackson, 1990; Xu et al., 1998].

7.1.2 OUTLOOK

Although the results from this thesis have shown the necessity of a good characterization of the magnetic properties to guarantee performance for an application, it also opened many new questions, or highlighted uncertainties in our present understanding. Magnetic methods may
not provide answers to all questions, but when used together with other methods we can continue to improve understanding into which magnetic methods or parameters deliver the best information to predict a specific performance in an application.

One example is in the 135° ridge, as a signature from an interface between the two magnetic phases with contrasting coercivities. From my experience under the Bio2Man4MRI project, the appearance of this ridge on a FORC diagram is the very first indication for oxidation. Preliminary work with Prof. E. De Grave has suggested the potential of using Integral Low-Energy Electron Mössbauer Spectroscopy (ILEEMS) together with Mössbauer spectroscopy to determine surface oxidation. Unfortunately the method requires a concentration larger than what we normally had for each sample in the project. Although hematite could not be uniquely determined on the surface of the particles, preliminary results do indicate that there is an additional phase present on the surface. A study by Almeida et al. [2014] has shown the use of environmental transmission electron microscopy (ETEM) in combination with off-axis electron holography for detecting surface oxidation of magnetite to maghemite. Thus a comparison of ETEM with off-axis electron holography would be interesting on a sample that exhibits a 135° ridge on its FORC diagram with the one that do not show it.

Panagiotouplos [2011] developed his model for the expression of the 135° ridge using a Preisach approach. In the future micromagnetic modelling could be made so that other factors, such as dipolar interaction, microstructural character of the sample, or the nature of the reversible component of magnetization can be considered.

The magnetic anisotropy on an aligned magnetosomes by ambient evaporation has been examined through torque magnetometry. The above method of alignment however deviates from the ideal conditions for structured nanoparticles. A future study could employ single magnetosomes that are aligned in 1- and 2-dimension using lithography in the study of the degree of anisotropy that can be reached. This information from torque magnetometry could provide better insight into the magnetic anisotropy of biogenic nanoparticles.

Although the linear relationship between the MRI and MPI images with respect to the slope of the magnetization curve and the bifurcation temperature are under statistical significance obtained for five samples. It would be useful to expand these results to other particles to further validate this relationship.
APPENDIX A

THE MAGNETIC SIGNATURE OF NANOSCALE MAGNETITE MESOCRYSTALS

Victoria Reichel, András Kovács, Monika Kumari, Éva Tompa, Mihály Pósfai, Ann M. Hirt, Martial Duchamp, Rafał E. Dunin-Borkowski, and Damien Faivre

ABSTRACT

Mesocrystals are usually micrometer-sized crystallographically-ordered assemblies of nanoparticles that are indistinguishable from single crystals of the same phase by diffraction techniques. Here, we study 40 nm mesocrystals of magnetite that have been precipitated in the presence of polyarginine and are composed of ca. 10 nm building blocks in a consensus crystallographic orientation similarly as in mesocrystals of larger size. We use both bulk magnetic measurements and magnetic induction maps recorded from individual particles using off-axis electron holography to show that most of the mesocrystals exhibit single domain magnetic behavior, with larger crystals occasionally supporting more complicated magnetic states. The magnetic state of each 40 nm mesocrystal is therefore primarily determined by the size of the superstructure and not that of the constituent sub-units, in a similar fashion to its structural properties. Our results have significance for understanding both the magnetic properties and the formation pathways of nano-sized, mesocrystalline ferrimagnetic particles.
Mesocrystals, or mesoscopically-structured crystals, are typically micrometer-scale superstructures that consist of ordered assemblies of nanoparticle substructures [Colfen and Antonietti, 2005; 2008; Kim et al., 2014]. The term ‘mesocrystal’ also commonly implies a formation mechanism whereby smaller subunits assemble into a larger, organized structure [Kim et al., 2014]. Such well-organized arrays of building blocks form entities that can have the properties of single crystals. In particular, single crystalline signatures are often measured when mesocrystals are analyzed using techniques based on X-ray diffraction (XRD) or transmission electron microscopy (TEM). Mesocrystals are frequently observed in biominerals [Seto et al., 2012] and can also be obtained synthetically by precipitation either in the presence [Kim et al., 2014; Wang et al., 2006; Wang et al., 2005; Fang et al., 2008; Kulak et al., 2007; Xu et al., 2006] or in the absence [Nozawa et al., 2011; Faivre et al., 2015] of organic additives. Fundamental questions related to mesocrystals include how they are structured and whether their properties differ from those of their single crystalline counterparts. In the case of magnetic materials, it is of great interest to determine whether the magnetic properties of a mesocrystal reflect those of the global entity or those of the individual sub-units. However, a model system in the nanometer-scale size range with size-dependent magnetic properties has yet to be defined. In this work, we propose such a model system and describe its magnetic properties.

Magnetite is a ubiquitous iron oxide mineral that is found on Earth and in planetary settings, as well as in the biological world [Nozawa et al., 2011; Faivre et al., 2015; Fortin et al., 2005; Cornell and Schwertmann, 2003]. Magnetite nanoparticles have numerous industrial and technological applications [Prozorov et al., 2013; Lang et al., 2007; Duong et al., 2014; Weissleder et al., 2014]. The magnetic properties of magnetite crystals are dependent on Neel relaxation [Néel, 1949], which in turn depends on particle size, morphology and interparticle interactions, as well as on the temperature and timescale of the measurement [Muxworthy et al., 2006; 2009]. For example, for a measurement time of 100 ms at room temperature, isolated equidimensional magnetite particles with diameters of below approximately 25 nm are superparamagnetic (SP), i.e., the directions of their magnetic moments change due to thermal fluctuations on the order of the measurement time. Particles with diameters of between approximately 25 and 80 nm can usually be magnetized homogeneously and are then considered to support stable single domain (SSD), i.e., thermally blocked, magnetic states. Particles that are larger than approximately 80 nm are usually multi-domain (MD) [Dunlop, 1997]. Therefore, studies of magnetite nanoparticles that are larger than 25 nm (SSD or MD) but composed of sub-units smaller than 25 nm (SP) promise to provide answers to the question of whether the magnetic properties of a mesocrystal are controlled by the global
entity, the individual building blocks or a mixture of the two.

Several magnetite superstructures have been reported in the literature, including 30 μm microdisks composed of 20 nm building blocks (i.e., on the SP/SSD threshold) [Yao et al., 2013] and 200 nm hollow spheres consisting of 40 to 50 nm aligned nanoparticles [Guan et al., 2009], both exhibiting thermally blocked states. However, these types of particles are based on sub-units that are potentially too large to display SP behavior and the mesocrystals themselves are too large to display SSD behavior. In a study of 174 nm particles composed of misaligned 10.9 nm nanocrystals (as determined from XRD using the Scherrer formula [Ge et al., 2007]), the magnetic properties were reported to be reminiscent of SP particles, i.e., of those of the sub-units. However, to the best of our knowledge, there has been no previous report of the magnetic properties of SSD sized mesocrystals based on SP building blocks, even though magnetism is one of the most interesting physical properties of mesocrystals for providing information about interaction potentials between the individual building blocks.

Synthetic magnetite can be formed from aqueous solution at room temperature via nanoparticulate intermediates [Baumgartner et al., 2013]. While such processes can lead to the formation of SSD particles independent of the presence of any additive, to the best of our knowledge, imaging by electron microscopy has not previously revealed any mesocrystalline nature in such crystals [Baumgartner et al., 2013]. The use of additives has been reported to control the mechanism of magnetite formation and possibly the resulting nanoparticle dimensions [Prozorov et al., 2007; Spionen et al., 2013; Baumgartner et al., 2014; Arakaki et al., 2003; Lenders et al., 2015]. In particular, the addition of polyarginine leads to the formation of monodisperse particles with sizes of approximately 40 nm [Baumgartner et al., 2014], which are therefore expected to be within the SSD size range. Here, we show that such crystals exhibit typical features of mesocrystalline structures, as they diffract like single crystals but are formed from assembled sub-structures, as shown by high-resolution TEM. Magnetic characterization of these crystals indicates that they display predominantly SSD behavior, although some of the largest mesocrystals that we describe are able to support more complicated magnetic states.

RESULTS AND DISCUSSION

The nanoparticles that are reported here formed within the first hour of our typical experiments (see the Methods section below). Some of them occur in two-dimensional clusters, while others self-organize into chains when deposited on TEM grids (Figs. 1a and b). The measured size distribution of the particles indicates that they are monodisperse, with a mean diameter of 38.9 ± 3.7 nm (inset to Fig. 1b). The size distribution of the particles is similar to that of magnetosomes from magnetotactic bacteria, which are often used as a
reference for monodispersity [Thomas-Keprta et al., 2000]. Bright-field (BF) TEM images show that an amorphous material (presumably the polyarginine) surrounds the crystals and that the particles have irregular shapes (Fig. 1b).

![Fig. 1: (a) High-angle annular dark-field (HAADF) scanning TEM (STEM) image and (b) BF TEM image of magnetite mesocrystals assembled in chains. The particles display light and dark contrast in (a) and (b), respectively. The web-like feature in the background in (b) is the lacy C support film. The inset in (b) shows the particle size distribution, as measured from TEM images.](image)

The particles are, as expected, composed of Fe and O, as seen in elemental maps measured using energy-dispersive X-ray spectroscopy (EDXS) (Fig. 2). In addition to the signals from the 40 nm particles, Fe and O signals are also recorded from smaller particles that are dispersed on the TEM grid (e.g., in the boxed area to the right of the lower part of the magnetite chain in Fig. 2d). These smaller features may represent the precursor iron oxide particles that first form during the synthesis process. The presence of C in the particles was checked in EDXS maps obtained from parts of the particles that extended above holes in the lacy C support film and thus could be analyzed without the contribution of a C-containing background (Fig. S1). The inhomogeneous distribution of the C signal observed within the particles (Fig. S1b) suggests that some C is present inside them, perhaps originating from polyarginine; however, the presence of C-bearing contamination arising from interaction
between the intense and very small electron probe and the particle surface cannot be excluded.

Fig. 2: Compositional analysis of a chain of eight magnetite mesocrystals. (a) HAADF STEM image; (b) Fe, (c) O, and (d) Fe+O elemental maps obtained from EDXS spectrum images. The boxed area in (d) encloses smaller particles that are also composed of Fe and O.

Elemental maps recorded using electron energy-loss spectroscopy (EELS) also confirmed the Fe–O composition of the particles (Fig. S2). From EELS spectrum images, it is also possible to extract information about the relative thickness of the magnetite mesocrystals $t/\lambda$, where $t$ is the thickness and $\lambda$ the mean free path of the electrons, by taking the ratio of the integrated intensities of the zero-loss peak and the total EEL spectrum. According to such thickness map (Fig. S2b), the shapes of the magnetite particle sometime deviate from spherical morphologies; the particle shown in Fig. S2a is relatively flat, with a thicker part at the red spot that matches the regions producing dark contrast in the BF STEM image.

The 40 nm particles have irregular, angular outlines with reentrant angles, suggesting that they are composed of sub-units, as seen in both BF TEM and higher-magnification HAADF STEM images (Figs. 1b and 2a). High-resolution TEM (HRTEM) images also confirm that each 40 nm particle is composed of smaller sub-units, as indicated by the distinct contrast (typically bright) along their boundaries and by the presence of different HRTEM contrast in the parts that make up the entire particle and have variable thicknesses in the electron beam direction (Figs. 3 and S3). Nevertheless, the Fourier transforms of the HRTEM images of most of the particles are similar to single-crystal-like diffraction patterns. Although twinning and slight mis-orientations between different parts of the aggregates sometimes occur, the
individual sub-units of the particles are typically assembled in consensus crystallographic orientations. For example, the lower part of the particle in Fig. 3 consists of two octahedral crystallites that share the same orientation with each other, as well as with the rest of the particle. Therefore, aligned internal substructures are encountered throughout the mesocrystals and the 40 nm magnetite particles can be described as mesocrystals in the sense of the definition given above.

![HRTEM image of a mesocrystalline magnetite particle recorded with the electron beam parallel to the [110] direction of magnetite. The Fourier transform of the mesocrystal is shown in the inset and indicates that, even though the particle consists of several smaller crystallites, it is similar to the diffraction pattern of a single crystal.](image)

We used off-axis electron holography in the TEM to study the magnetic induction within and around individual magnetite mesocrystals arranged in rings and chains to determine how the mesocrystalline nature of the particles and the interactions between them influence their magnetic properties. Briefly (see the Methods section for details), off-axis electron holograms were recorded in magnetic-field-free conditions after the sample had been magnetized in opposite directions. Each hologram was processed to obtain the phase and the amplitude of the electron wave in real space [Dunin-Borkowski et al., 2004]. Subsequently, half of the sum of phase images corresponding to the two oppositely magnetized states was used to calculate the mean inner potential (MIP) contribution to the phase, which is approximately proportional to the specimen thickness, while half of the difference between the phase images was used to calculate the magnetic contribution to the phase. Contours and colors were then added to the
magnetic contribution to the phase to create magnetic induction maps, in order to facilitate the visualization and quantification of the magnetic field within and around each crystal within the field of view.

A representative magnetic induction map of a ring of six 40 nm magnetite mesocrystals, which is shown in Fig. 4a, demonstrates that each particle contains a single magnetic domain. The ring configuration of the mesocrystals constrains the magnetic field to form a flux-closed state. A similar magnetic induction map recorded from a chain of magnetite mesocrystals, which is shown in Fig. 4b, also indicates that most of the particles contain single magnetic domains, with the direction of the magnetic induction now determined by the overall direction of the chain. However, the two largest (ca. 50 nm) mesocrystals shown in Fig. 4b appear to have multi-domain states, as suggested by the changes in the directions of the contour lines and the colors within them. These two particles (at the lower end and the right of the chain) may correspond to the minimum size, for which the particles are no longer single domain. Alternatively, it is possible that the other particles are essentially single crystals, while the multi-domain particles consist of imperfectly aligned subunits. Another interesting feature of the magnetic induction maps shown in Fig. 4 is that the phase contours are more closely-spaced in the ring than in the chain, suggesting the particles in the ring have more saturated magnetic states than those in the chain, perhaps because the ring geometry results in stronger magnetostatic interactions between adjacent particles than the chain.

**Fig. 4:** Magnetic induction maps recorded in magnetic-field-free conditions using off-axis electron holography from (a) a ring and (b) a chain of magnetite mesocrystals. The colors and contours show the direction and strength of the projected in-plane magnetic flux density, respectively and are superimposed on a combination of the mean inner potential contribution to the phase and an off axis electron hologram. A color wheel is shown as an inset at the lower left corner of each image. The phase contour spacing is $2\pi/256$ radians in each image. The white arrows indicate the direction of the magnetic induction in each particle. The thin white lines show the outer edge of each mesocrystal.
As discussed above, phase information obtained using off-axis electron holography can be also used to measure both the magnetic and the mean inner potential contribution to the phase quantitatively. The magnetic phase shift across each mesocrystal in the ring is approximately 0.34 radians, which is slightly smaller than the value of 0.50 radians that would be predicted for a uniformly magnetized 40 nm sphere of magnetite. In the case of a homogeneous material, the mean inner potential contribution to the phase is proportional to the particle thickness in the electron beam direction. For a spherical, 40 nm particle of magnetite, assuming a mean inner potential of 17 V and an accelerating voltage of 300 kV, the mean inner potential at the center of the particle relative to its surroundings is predicted to be 4.4 radians. For the particles shown in Fig. 4, the measured mean inner potential contribution to the phase varies between 2.9 and 4.0 radians, suggesting that the particles have a density that is slightly lower than that of pure magnetite. We interpret this feature as a consequence of the mesocrystalline nature of the particles. As is also visible in HRTEM images, small gaps and perhaps organic material remain between the individual sub-units in each mesocrystal. As a result, both the mean inner potential and the magnetic contribution to the phase are lower than they would be for single crystals of magnetite of the same size.

We also analyzed the bulk magnetic properties of the sample. The mesocrystalline magnetite nanoparticles showed a thin hysteresis loop, with a coercivity Hc of 4.2 mT and a ratio of saturation remanent magnetization (Mrs) to saturation magnetization (Ms) of 0.29 (Fig. 5a). The hysteresis loop is saturated by 200 mT after removal the high-field slope for the paramagnetic behavior (inset to Fig. 5a). Acquisition of a backfield isothermal remanent magnetization (IRM) curve is saturated by ≈ 150 mT. These results indicate that the synthesized magnetite is pure and shows no significant degree of oxidation (Fig. 5b). The first order reversal curve (FORC) distribution shows a spread along the coercivity axis from zero to approximately 20 mT and the presence of small closed contours in the vicinity of the origin (Fig. 5c). The fact that the distribution is narrow and very close to the origin demonstrates the presence of smaller particles. A significant contribution from multi-domain particles would lead to a broader FORC distribution at the origin. Moreover, the decomposition of FORC measurements into reversible and irreversible components of induced magnetization suggests that ca. 25% of the magnetic behavior of the samples can be attributed to superparamagnetic particles (Fig. 5d) [Kumari et al., 2014]. Therefore, three quarters of the particles appear to be SSD, while one quarter show SP behavior.

The results of the FORC measurements are consistent with the TEM observations, since the latter indicate the presence of precursor particles that are only a few nm in size (e.g., in the boxed area in Fig. 2d) and are therefore superparamagnetic. In contrast, each of the larger, mesocrystalline magnetite particles contains a single magnetic domain, 1 as demonstrated by
most of the crystals in the magnetic induction maps recorded using off-axis electron holography. The individual sub-units within each mesocrystal interact magnetically, so that they can be treated as a single magnetic unity, in agreement with the fact that ordered aggregations of magnetite have been reported to exhibit higher coercivities than dispersed distributions of particles of similar size [Xuan et al., 2007; Yao et al., 2013].

Fig. 5: Bulk magnetic characterization of magnetite mesocrystals. (a) Hysteresis loops with the inset showing the saturation attained at high field; (b) backfield IRM; (c) FORC diagram with a smoothing factor of 2; (d) derivative of the magnetic moment of the reversible (blue curve) and irreversible (black curve) part of the induced magnetization of the mesocrystalline magnetite nanochains.

Each 40 nm magnetite particle can therefore be regarded as a magnetic mesocrystal, since it is composed of a number of sub-units, while its structural and magnetic properties are determined by the size of the superstructure rather than that of its components. This mesocrystalline nature led us to speculate on the possible mechanism that results in the
controlled organization of the sub-units. We performed syntheses over prolonged periods of time (up to 5 h) and did not observe particle growth (Fig. S4), showing that the mesocrystals form within the first hour and that only the number of particles increases with additional time. When magnetite was synthesized without organic additives, the first particles (primary particles) were about 2 nm in size. The size of the subunits in the mesocrystals (9 ± 3 nm) differs from that of the primary particles. Therefore, the formation of the mesocrystals does not occur by simple stabilization of primary particles by polyarginine, but is thought to happen in a three-stage process (Fig. 6), whereby the primary particles first form and then aggregate into magnetite nanoparticles with sizes of approximately 10 nm, as observed in our earlier synthesis experiments performed in the absence of additives [Baumgartner et al., 2013]. The latter size agrees well with the sizes of the sub-units observed in the mesocrystals. We speculate that the ca. 10 nm sub-units aggregate along similar crystallographic orientations via oriented attachment to form the mesocrystals, thanks to the additive, in a process that is reminiscent of what has been described for goethite [Yuwono et al., 2010]. This aggregation along similar crystallographic orientations, which is the key to the single-crystal-like structure, is in our case additionally favored by the magnetic properties of the materials in the form of exchange coupling, as has also been observed for the less magnetic iron oxide hematite [Frandsen et al., 2005; Reufer et al., 2011; Pósfai et al., 2006].

A final interesting feature of our samples is that the magnetite mesocrystals form chains and layers instead of dense, three-dimensional clusters, as is typical for inorganically-synthesized magnetite particles of similar size. We attribute this colloidal stability to the amorphous shell of organic material that surrounds each particle and likely prevents their agglomeration.

CONCLUSIONS

We have used 40 nm magnetite particles composed of 10 nm sub-units as a model system to study the relationship between the structures and magnetic properties of nanoscale mesocrystals. We show that the magnetic properties of each mesocrystals are dominated by those of the superstructure. We hypothesize a three-step process for the formation of the mesocrystals, in which the exact role of the additive remains to be clarified.

METHODS

SYNTHESIS OF MAGNETITE (Fe₃O₄) NANOPARTICLES

Magnetite nanoparticle synthesis was performed as described previously [Baumgartner et al., 2014]. Briefly, a computer-controlled titration device was used. The device consists of a titration unit (Methrom Titrino 888) containing a 5 ml cylinder, a dosing unit (Metrohm
Dosimat 805) containing a 1 ml unit and a Biotrode pH electrode. A 50 ml reaction vessel with thermostat was used, the temperature being kept constant at 25°C. In addition, the reactor was kept under a controlled nitrogen atmosphere. 10 ml of d.d. H2O was filled in the reaction vessel, to which polyarginine was directly added to reach a concentration of 0.1 mg × ml⁻¹. Iron (II) chloride tetrahydrate and iron (III) chloride hexahydrate were used in a stoichiometric ratio of magnetite (FeII / FeIII = 1/2) to prepare a 0.1 M iron solution. A 0.1 M NaOH solution was used for titration. Before use, all solutions were deoxygenated with nitrogen. The reaction was started by the addition of the iron solution (1 μl × ml⁻¹) to the reaction vessel containing the polyarginine solution under continuous stirring using a mechanical stirrer. The pH value was kept under permanent control at pH 11 by the NaOH titration unit of the Metrohm device.

TRANSMISSION ELECTRON MICROSCOPY

Conventional transmission electron microscopy (TEM) was used to study the sizes, shapes, structures and compositions of the particles, as well as the crystallographic orientations of individual nanocrystals within the particles. Low-magnification BF images, HRTEM images and selected-area electron diffraction patterns were obtained at the Institute for Technical Physics and Materials Science in Budapest using Philips CM20 and JEOL 3010 TEMs at accelerating voltages of 200 and 300 kV, respectively. Images were recorded either on image plates (on the CM20) or using a Gatan Orius charge-coupled device (CCD) camera (on the JEOL 3010). Digital Micrograph and SingleCrystal software was used for TEM data processing and interpretation. Particle sizes were measured from digitized images using ImageJ software. Advanced STEM and off-axis electron holography were performed using aberration corrected TEMs in the Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons in Forschungszentrum Julich, Germany. A probe-aberration corrected FEI Titan 60-300 TEM was used for obtaining BF and HAADF STEM images of the magnetite particles. The annular dark-field detector inner semi-angle was 69 mrad. Elemental maps of Fe, O and C were obtained from spectrum images acquired using both EDXS and EELS. EELS spectrum images were also used to produce thickness maps of the particles. For spectrum imaging, low-loss and core-loss energy-loss spectra were recorded simultaneously using a Gatan dual-EELS Enfinium spectrometer.

In order to study the magnetic properties of individual particles and their chains, off-axis electron holography experiments were performed using an image-aberration-corrected FEI Titan 60-300 TEM operated at 300 kV and a Fischione dual-axis tomography holder. Electron holograms were recorded in magnetic-field-free conditions in Lorentz 1 mode using a Gatan direct electron detection (K2-IS) camera in counting mode. This camera provides better
signal-to-noise ratio and fewer pixel artifacts than a conventional charge-coupled device (CCD) camera. In order to perform magnetization reversal experiments, the sample was tilted by ±75° and magnetized in the direction of the electron beam using the objective lens field of 1.41 T. The objective lens was then switched off, the sample tilted back to 0° in magnetic-field-free conditions and electron holograms of the resulting magnetic remanent states were acquired using a biprism voltage of 200 V. The sample was magnetized three times in opposite directions. Each magnetic induction map was created by adding phase contours to half of the difference between phase images recorded with the specimen magnetized in opposite directions. TEM images were processed used Gatan Digital Micrograph and Semper software. Further details of the approach used to perform off-axis electron holography experiments and analysis are given elsewhere [Dunin-Borkowski et al., 2004].

**BULK MAGNETIC CHARACTERIZATION**

Our earlier studies showed that during magnetite synthesis first an unknown precursor phase, possibly ferrihydrite, forms. Some of these primary precursor particles are retained in the sample even when the 40 nm magnetite particles form [Baumgartner et al., 2013]. In order to reduce the amount of the precursor particles in the material used for magnetic characterization, the samples were washed three times, with a magnet used to separate the mesocrystalline magnetic particles from the impurities after synthesis. The supernatant was removed and the pellet was resuspended in d.d. H₂O. To avoid pH changes after synthesis, d.d. H₂O was adjusted to a pH value of 11. Two TEM samples were prepared, one from the supernatant and the other from the pellet, showing that the supernatant contained precursor particles only, whereas the pellet included the larger mesocrystalline magnetite nanoparticles and some precursor particles as well. Sample preparation for magnetic measurements involved drying of the sample under ambient conditions and placing the dry powder in gently pressed gelatin gel caps. The mesocrystalline magnetite particles were magnetically characterized using hysteresis loop, backfield isothermal remanent magnetization (IRM) and first order reversal curves (FORC) using a Princeton Measurements Corporation vibrating sample magnetometer at room temperature. Hysteresis loops were measured with an average time of 300 ms. The hysteresis ratios, remanent to saturation magnetization (Mrs/Ms) and remanence coercivity to coercivity (Hcr/Hc) reflect the composition, particle size and the chemical purity of the sample. The theoretical value for the magnetization ratio of non-interacting magnetite, dominated by magnetostatic anisotropy, is 0.5. FORCs were measured by first saturating the sample by a positive applied field of 1 T. A series of 140 magnetization curves originating from the major hysteresis loop with a field increment of 2.3 mT and an average measurement time of 100 ms were obtained. The FORC data were transformed into a FORC diagram using the Winklhofer MATLAB code [Winklhofer and Zimanyi, 2006].
APPENDIX B

BIOLOGICAL VS. SYNTHETIC AND SMALL VS. LARGE MAGNETITE NANOPARTICLES AS MRI CONTRAST AGENT – A COMPREHENSIVE PHYSICAL AND THEORETICAL STUDY

Reinis Taukulis, Marc Widdrat, Monika Kumari, David Heinke, Monika Rumpler, Éva Tompa, René Uebe, Alexander Kraupner, Andrejs Cebers, Dirk Schüler, Mihály Pósfai, Ann M. Hirt, Damien Faivre, accepted in Magnetohydrodynamics.

ABSTRACT

Magnetite nanoparticles, especially superparamagnetic iron oxide nanoparticles (SPION), are established contrast agents for magnetic resonance imaging (MRI). Magnetosomes, which are magnetite nanoparticles of biological origin, have been shown to have better contrast properties than current formulations, possibly because of their larger size and high monodispersity. Here, we present an integrated study of magnetosomes and synthetic magnetite nanoparticles of varying size, hence different magnetic properties. We investigate not only the relaxation times as a measure for the contrast properties of these particles but also their cytotoxicity and demonstrate the higher contrast of the larger particles. A theoretical model is presented that enables us to simulate the $R_2/R_1$ ratio of a contrast agent and confirm that larger particles offer higher contrast. The results from this study illustrate the possibility to obtain colloidal stability of large magnetic nanoparticles for MRI applications, and serve as an impetus for a more quantitative description of the contrast effect as a function of size.
INTRODUCTION

The use of magnetic nanoparticles has developed into a major research area for bio- and nanotechnologists [Bogart et al., 2014]. Translation to clinical studies has already started for magnetite nanoparticles in imaging studies among other application, due to their low toxicity and magnetic properties [Reddy et al., 2012; Zhou et al., 2014]. Magnetic resonance imaging (MRI) is a particularly well-established diagnostic tool for obtaining images of any space in the human body. The main advantage of MRI is that it is harmless to the patient while delivering high resolution images of the body interior.

The magnetic properties of magnetite nanoparticles are dependent on their size. For equi-dimensional magnetite nanoparticles in the size range from 30 to 100 nm, the particles will be homogeneously magnetized, i.e., have one magnetic domain. This is referred to as stable single domain (SD) state and is defined by a high coercive force ($H_c$). Below 30 nm, the particles are in the superparamagnetic (SP) state. These are also SD, but the anisotropy energy is sufficiently low compared to thermal energy for flipping the magnetic moment of the particle at the timescale of observation. SP magnetite saturates quickly in fields < 150 mT and have no coercivity [Dunlop and Özdemir, 1997]. Contrast enhancement in magnetic resonance imaging (MRI) is achieved by changing the magnetic relaxation times $T_1$ and $T_2$ of the protons contained in the tissue. For MRI, two different classes of contrast agents exist: agents that influence mainly the signal in $T_2$- (i.e., negative contrast agents, reducing the signal) or $T_1$- weighted images (i.e., positive contrast agents, increasing the signal). The degree of the $T_2$ contrast effect is typically represented by the spin–spin relaxivity $R_2$ ($R_2=1/T_2$), where higher values of $R_2$ result in a larger contrast effect. Because $R_2$ is strongly related to $\mu$, the magnetic moment of the contrasting nanoparticles, which in turn is dependent on the size as seen above, it is not surprising that larger particles should provide enhanced properties as contrast agents relative to smaller ones [Ahmad et al., 2012; Xuan et al., 2009]. However, an experimental proof obtained on particles of similar batches is lacking as well as the related theoretical description relating the contrast properties to the particles size.

SPION (superparamagnetic iron oxide nanoparticles) combine a magnetic core with a coating agent that is an approved pharmaceutic for contrast agent use in MRI. Although commercial formulations are already on the market (e.g. Ferumoxtran for lymph node diagnosis, Feridex or Resovist for liver diagnosis) [Laurent et al., 2008; Reddy et al., 2012], none uses biogenic magnetite. SPION are smaller than magnetosomes and by far less monodisperse than the biological magnetite [Faivre and Schüler, 2008]. Therefore, magnetosomes potentially provides a better contrast agent for MRI. Their potential for MRI
has been recognized, because they have been shown to possess an even larger $R_2/R_1$ ratio than commercially available drugs [Lisy et al., 2007]. However, it is currently not possible to form magnetosomes in amounts required for pharmaceutical applications and their cytotoxicity remains to be fully characterized.

We have recently shown that the particle size of magnetite nanoparticles can be controlled in aqueous-based processes [Baumgartner et al., 2013b] and that particles of dimensions similar to those of bacterial magnetosomes can be obtained by this process [Baumgartner et al., 2013a]. In addition, we have also demonstrated that the dimension of the magnetosome particles can also be controlled, in this case by genetic engineering [Lohsse et al., 2011; Murat et al., 2012; Scheffel et al., 2008]. Here, we thus present an integrated study comparing the structural and magnetic properties of magnetite nanoparticles. We assess their potential as contrast agent for MRI by testing not only their contrast properties but also their biocompatibility. In particular, we test if the high contrast properties obtained for magnetosomes are only due to their size or if further properties such as monodispersity or colloidal stability are at the origin of this remarkable feature. In addition, we present a method to calculate the relaxivity of magnetic nanoparticles in MRI. To our knowledge, this is the first quantitative description of the relationship between contrast properties and particle size and confirms the results we obtained experimentally.

RESULTS AND DISCUSSION

The main findings from this study can be illustrated from the results of five biogenic and six synthetic samples (Table 1). The samples are categorized as small biogenic (SB), large biogenic (LB), small inorganic (SI) and large inorganic (LI).

STRUCTURE, SIZE AND MORPHOLOGY OF THE NANOPARTICLES

All samples consist of nanoparticles of pure magnetite, as indicated by the observed peaks in the X-ray diffractogram (Fig.1) and by diffraction maxima measured in SAED patterns (Fig. 2c and d).

SYNTHETIC SAMPLES

The size of the individual nanoparticles ranges from a few to several tens of nanometers (Table 1). The mean sizes, determined by TEM for the particles in the SI samples, are in good agreement with the sizes obtained using synchrotron X-ray diffraction. The size of larger particles (LI samples) is difficult to determine from TEM micrographs because of aggregation.

All synthetic samples are very similar in that they contain clusters of nanoparticles in
random orientations (Fig. 2a), with most particles having euhedral (octahedral or cuboctahedral) shapes and typically perfect structures (Fig. 2b), as suggested by high-resolution TEM images. SAED patterns obtained from both clusters of crystals (cf. ring patterns in Fig. 2c) and from individual nanoparticles are consistent with the structure of defect-free magnetite (Fig. 2d).

*Fig. 1:* X-ray diagram of representative bacteria (LB3 3, orange) and synthetic (LI 2, red) samples as well as reference materials.

*Fig. 2:* (a) A typical view of magnetite nanoparticles in SII. (b) HRTEM image of several nanoparticles, showing euhedral shapes and perfect crystalline structures. (c) SAED pattern obtained from a cluster of nanoparticles, and (d) a Fourier transform of the HRTEM image of particle D in (b). Both the ring pattern in (c) and the intensity maxima in (d) can be indexed according to the structure of magnetite.
Table 1: Summary of the properties of the nanoparticles. Acronyms used for a sample’s names: SB stands for Small and Biological, LB for Large and Biological, SI for Small and Inorganic, LI for Large and Inorganic, s for stabilized. dc , dh are core and hydrodynamic diameter, respectively. M/Mr and Hc/Hc are magnetic properties described in the chapter on magnetic properties; the following 6 columns depict the biocompatibility properties of the samples. Finally, the final 3 columns depicts the MRI contrast properties of the samples.

<table>
<thead>
<tr>
<th>Sample description</th>
<th>dc [nm]</th>
<th>dh [nm]</th>
<th>Stabilizing agent</th>
<th>M/Mr</th>
<th>EZ4U NIH3T3 [IGC50, mg/ml]</th>
<th>EZ4U RAW264.7 [IGC50, mg/ml]</th>
<th>EZ4U MC3T3-E1 [IGC50, mg/ml]</th>
<th>LDH NIH3T3 [IGC50, mg/ml]</th>
<th>LDH RAW264.7 [IGC50, mg/ml]</th>
<th>LDH MC3T3-E1 [IGC50, mg/ml]</th>
<th>R1 [L/mmol*s]</th>
<th>R2 [L/mmol*s]</th>
<th>R2/R1</th>
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<tr>
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<td>24</td>
<td>90</td>
<td>0.05</td>
<td>N/A</td>
<td>9.0</td>
<td>320.7</td>
<td>35.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LB1</td>
<td>41</td>
<td>143</td>
<td>0.38</td>
<td>1.5</td>
<td>8.6</td>
<td>541.5</td>
<td>63.1</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>LB2</td>
<td>38</td>
<td>84</td>
<td>0.38</td>
<td>1.3</td>
<td>0.94</td>
<td>0.05</td>
<td>0.13</td>
<td>0.82</td>
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<td>0.72</td>
<td>0.18</td>
<td>0.12</td>
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<td>0.12</td>
<td>11.0</td>
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<tr>
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<td>0.68</td>
<td>0.19</td>
<td>0.13</td>
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<td>0.43</td>
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<td></td>
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<td>0.52</td>
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<td>0.58</td>
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</table>
The size distribution of particles was measured in SI1, indicating that the maximum of the distribution is at 15 nm and extends to approximately 30 nm. LI2 and LI3 also consist of mostly small (~15 nm) magnetite nanoparticles; however, these samples also contain larger aggregates that apparently formed from randomly or similarly oriented nanocrystals.

**BIOLOGICAL SAMPLES**

In all biological samples, the magnetosomes extracted from different strains of the magnetotactic bacterium *Magnetospirillum gryphiswaldense* (Table S1), are still enveloped by the magnetosome membrane. This surface layer prevents clustering/aggregation of the particles, and results in a dispersed distribution of magnetosomes on the TEM grid. Typically, particles larger than 30 nm diameter form chains, whereas smaller particles appear randomly scattered (Fig. 3).

![Fig. 3](image)

**Fig. 3:** (a) Bright-field TEM images of typical magnetite magnetosomes from five different bacterial samples, with the sample identifiers indicated on the top (cf. Table 1). (b) Particle size distributions as measured from TEM micrographs. (c) Shape factor (width/length) distributions of particles in the same samples, with the fraction of twinned or aggregated particles indicated in each panel.

Compared to magnetosomes of the wildtype, particles of mutant strains (LB2) show a difference in both particle size and shape. Whereas wildtype particles produce a negatively-skewed size distribution, size distributions in mutant samples are lognormal-like (SB2), Gaussian (SB1 and LB3) or broad, double-peaked (LB1) (Fig. 3b). The mean size of the distributions increases from 23.6 nm (SB1) to 41 nm (LB1). Concerning the shapes of the
particles, samples LB2 and SB2 contain wild-type-like, mostly single crystalline, euhedral particles (Fig. 4a).

Fig. 4: HRTEM images of (a) a single-crystal magnetosome with a perfect structure from SB2, with the Fourier transform of the image in the lower left, indicating that the crystal is viewed along the [223] crystallographic direction, and (b) an aggregate magnetosome that consists of at least three individual crystals, from sample LB3.

The shape factor distribution is relatively narrow in these samples and resembles the curves that are typically obtained for magnetosomes from the wildtype (Fig. 3c). In the other samples, however, a large fraction of the magnetosomes are either twinned or multiply aggregated. This effect is most pronounced in the case of SB2, which contains relatively large crystals, most of which are composed of several crystallites (Fig. 4b). The aggregated nature of the magnetosomes clearly affects their shape factor distribution, because samples containing a large fraction of twinned or aggregated particles produce broad shape distributions (Fig. 3c). The TEM-based particle size and shape measurements are entirely consistent with the bulk magnetic data as shown below.

MAGNETIC PROPERTIES

Biological samples show a large variation in their magnetic properties (Table 1). SB1 has a closed hysteresis loop, which would be expected for true SP behavior (Fig. 5). The hysteresis loops of the other biological samples are open, and the ratios of the remanent saturation magnetization to saturation magnetization ($M_r/M_s$) and remanent coercivity to coercivity ($H_{cr}/H_c$) are compatible with a mixture of SD and SP particle sizes. The synthetic samples all show an open hysteresis loop (Fig. 5). The magnetization ratio is variable and suggests SD particle size with a significant SP contribution. The approach to saturation magnetization of synthetic particles is slower (low susceptibility) and requires much higher fields to reach
saturation as compared to biological particles.

Fig. 5: Normalized hysteresis loops for chemically synthetized and biologically mineralized magnetite nanoparticles with average particle size in the a) SSD and b) SP domain.

Fig. 6: FORC diagrams for biogenic and synthetic magnetite nanoparticles. Smoothing factor is 2.

First order reversal curve (FORC) analysis is a powerful technique for characterizing ferromagnetic minerals (s.l.), their domain state (SP and SD), and the extent of interactions between particles [Carvallo et al., 2006; Muxworthy et al., 2005; Pike et al., 1999; Roberts et al., 2000]. FORC distributions located near the origin, i.e. at the cross section of interaction
axis ($H_b$) and the coercivity axis ($H_a$), with an upward offset signify non-interacting particles, whereas a spread along $H_a$ signifies a distribution in particle size. A spread along $H_b$ on the FORC diagram indicates the presence of interacting SD particles. FORC diagrams for SB1, SB2, SI1, and SI2 reveal the dominance of magnetite nanoparticles that are SP (Fig. 6). The other samples contain a broader mixture of SP and SD particle sizes.

Decomposing the FORC measurements into the reversible and irreversible parts of the induced magnetization clearly shows the relative magnitudes of the SP and SD contributions (Fig. 7) [Kumari et al., 2014]. Samples LB1, LB2, LI2, LI3, and LI4 have a larger contribution of irreversible magnetization, i.e., SD particle size. LB3 has less of a contribution from SD particles, and samples SB2, SI1 and SI2 are largely SP. Only SB1 is almost purely SP in its magnetic behavior.

![FORC Diagrams](image)

**Fig. 7:** Derivative of magnetic moment of the reversible (blue curve) and irreversible (red curve) part of the induced magnetization versus reversal fields ($H_r$) for both biologic and synthetic samples.

From FORC analysis one sees that the biological SD samples exhibit a lower spread on the $H_b$ (interaction) axis, varying between ±15 mT, compared to the synthetic samples that range from -30 mT to +40 mT. This suggests a lower degree of interaction among magnetite particles from biologic origin as compared to chemically synthesized. There is also a larger spread in the coercivity distribution of the synthetic samples, e.g., it ranges from 0 mT to 40 mT for SI1 with the smallest particle size distribution, compared to SB1. It should be noted
that the larger degree of interactions in the synthetic samples may lead to a larger effective magnetic particle size in comparison to their physical size, i.e., aggregates of SP particles behave like SD size. Afterward SI1 was coated with L-3,4 Dihydroxyphenylalanin (L-DOPA) (SI1s), which inhibited particle interaction. The FORC distribution is concentrated at the origin, as would be expected for purely SP particles, and the magnetization is largely reversible, similar to what is found for SB1. Resovist shows a largely reversible magnetization similar to what is seen in SI1s and SB1. Truncating the FORC distribution to suppress the very low coercivity, however, shows that there are particles with a higher coercivity. This higher coercivity tail is not found in SB1 and SI1s.

In summary, FORC analysis demonstrates that the magnetic properties of samples SB1, SB2, SI1 and SI2 are dominated by the SP fraction in the sample. Samples LB1, LB2, LB3, LI2, LI4 and LI4 may also have SP particles, but the SSD particles dominate. Coating the synthetic particles is successful in breaking down particle interaction, so that their magnetic properties are SP.

**BIOCOMPATIBILITY**

The biocompatibility of the synthetic and biological samples was tested by different assay systems: EZ4U and LDH assay, using various cell lines: MC3T3-E1 osteoblast, NIH3T3 fibroblasts and RAW264.7 macrophages (Table 1). IGC$_{50}$ values obtained by EZ4U and LDH assays give similar results for all synthetic and biological samples within one cell line. More specifically, IGC$_{50}$ values of the synthetic particles, which were determined in the three cell lines, range from 0.15 – 0.65 mg/ml in the case of EZ4U assay and between 0.20 – 0.65 mg/ml in the case of LDH assay. These results demonstrate that IGC$_{50}$ values of the synthetic particles with sizes between 17 – 36 nm do not depend on size.

The biocompatibility of numerous types of artificially synthesized nanoparticles, e.g. chitosan, silica and zinc oxide, has been determined using various cell lines [Javid et al., 2013; Kroll et al., 2011; Peng et al., 2006; Sayes et al., 2007]. IGC$_{50}$ values of 0.1 – 0.25 mg/ml were reported for iron oxide nanoparticles [Hussain et al., 2005; Zhang et al., 2012]. The IGC$_{50}$ values from this study are not broader and only slightly higher than values reported in these other investigations and show that cytotoxicity of our synthetic inorganic iron oxide particles is comparable to synthetic particles from e.g. chitosan or zinc oxide.

The IGC$_{50}$ values of the biological particles show a larger variation than those of synthetic particles. They are, however, similar within one cell line. IGC$_{50}$ values evaluated by EZ4U and LHD assay in MC3T3-E1 and RAW264.7 cells range from 0.05 – 0.19 mg/ml and 0.07–0.43 mg/ml, respectively. In NIH3T3 cells IGC$_{50}$ values are higher than in the other two cell
lines, which cannot be explained at present. IGC\textsubscript{50} value in NIH3T3 cells, determined by EZ4U and LDH assays, ranges from 0.48–0.94 mg/ml. Thus, the applied genetic manipulation of the biological nanoparticles did have an effect on IGC\textsubscript{50} values in the tested cell lines. In general, the synthetic particles are less toxic to the cells than the biological ones, probably due to the presence of remaining bacterial cell components on the magnetosome membrane.

**COLLOIDAL STABILITY**

The biological samples were found to be colloidally stable without further stabilization. This behavior can be attributed to the magnetosome's membrane, which is sufficient to stabilize the particles in aqueous media. The synthetic particles on the other hand, specifically those exhibiting large crystal sizes and hence high magnetic attraction forces, are prone to aggregation. Thus, stabilization is essential for these nanoparticles, because a surface modification during the synthesis step is missing.

Particles can typically be colloidally stabilized using two different approaches: electrostatic or steric stabilization. Iron oxide nanoparticles for biomedical applications are typically sterically stabilized by using biocompatible dispersants, such as dextran [Gamarra et al., 2005], polyethylene glycol [Tiefenauer et al., 1996] or poly (vinyl alcohol) [Laurent et al., 2008]. These dispersants are physically adsorbed on the particle surface and prevent the particles from aggregation due to steric repulsion. Because non-ionic stabilization polymers do not lead to any colloidally stable dispersion, cationic polymers such as polyethyleneimine (PEI) and chitosan were found to be good dispersants for the synthetic particles (data not shown). PEI and chitosan are known to offer an exceptionally strong adhesion because of additional ionic interactions with the particle surface [Petri-Fink et al., 2008]. Electrostatic stabilization, in turn, can be achieved by introducing an anionic/cationic charge [Laurent et al., 2008] either due to anions/cations or due to monomers with a charged functional group; i.e. citric acid [Wagner et al., 2002]. The most promising results were obtained using L-DOPA, which is known to exhibit high affinity anchor groups for iron oxide particles that leads to highly stable dispersions [Amstad et al., 2009].

The stabilization of the synthetic particles LI1, SI1, and SI2 was successful, and the stabilized dispersion of SI2s using L-DOPA is exemplary shown in Fig. 8. The change in colloidal stability can be clearly visually observed and is also reflected by the hydrodynamic diameter, which is in the range of 100 – 125 nm after the stabilization. The stabilized particles show good colloidal stability until present, six months after treatment. Stabilization of the samples LI2, LI3 and LI4 was not successful. This can be attributed to the larger core sizes, which lead to stronger magnetic interaction (i.e., larger contribution of irreversible magnetization, cf. Figure 7), which results in irreversible aggregation.
CONTRAST PROPERTIES IN MRI

In general, paramagnetic and superparamagnetic substances lead to a shortening of the relaxation times $T_1$ (spin–lattice relaxation time) and $T_2$ (spin-spin relaxation time) of the hydrogen protons in the surrounding tissue. Iron oxide nanoparticles exhibit an especially strong shortening of $T_2$, for which they are also called $T_2$-contrast agents. The higher the shortening of $T_2$, which is equivalent to a high $R_2$ relaxivity, the higher the obtained $T_2$ weighted contrast is. Contrast agents in general are characterized by the ratio of the relaxivity $R_2/R_1$. Therefore, a high $T_2$ weighted contrast can be obtained by combining high $R_2$ values with low $R_1$ values. In order to evaluate the findings, the contrast properties of the here investigated particles were compared with the known contrast agent Resovist®.

**Fig. 8:** Sample SI2 a) before and b) after stabilization using L-DOPA. The log-normal distribution of the hydrodynamic diameter (c) of the original and stabilized particles clearly indicates the stabilization effect.

**Fig. 9:** Concentration dependent relaxation rates (a) $1/T_1$ ($R_1$) and (b) $1/T_2$ ($R_2$) determined at 0.94T @ 39°C. The biological particles are represented by the squares and synthetic particles are represented by triangles, respectively.

Figure 9 illustrates the relationship between the relaxation rates $1/T_1$ and $1/T_2$ and iron concentration. As expected, both relaxation rates increase with increasing iron concentration. The relaxation rates of all particles show good linearity in the measured concentration range,
which is an indication for non-interacting particles and colloidal stability. Colloidal stability is mandatory for the determination of the relaxivity values \( R_1 \), \( R_2 \) and \( R_2/R_1 \).

The dependency of the relaxivity on the core diameter \( d_c \) of the different particles is shown in Figure 10. Independent of the origin of the particles, biological or synthetic, the \( R_2 \) relaxivity increases roughly linearly with the core diameter \( d_c \). The increase of the \( R_2 \) relaxivity with the particle size can be explained by the fact, that larger particles exhibit stronger magnetic moments, which leads to a faster dephasing of the hydrogen protons and, therefore, to a stronger shortening of \( T_2 \). It is known that the \( T_2 \)-effect dominates for iron oxide particles over the \( T_1 \)-effect. We also observe a slight dependence of \( R_1 \) on size: a decreasing \( T_1 \) effect is obtained for increasing core diameter. Considering both the \( R_1 \) and \( R_2 \) relaxivities, the decisive \( R_2/R_1 \) ratio increases with the core diameter.

It has to be noted that in addition to the dependence of the relaxation properties on the core diameter \( d_c \), a strong dependence is also found on the hydrodynamic diameter \( d_h \) (data not shown). Therefore, only particles with similar hydrodynamic diameters can be compared directly (see Table 1).

\[ \text{Fig. 10: Size dependence (} d_c \text{) of the } R_1 \text{ relaxivity (square), } R_2 \text{ relaxivity (circle) and the ratio } R_2/R_1 \text{ (triangle). Biological particles are represented by the filled symbols and synthetic particles are represented by blank symbols, respectively.} \]

The biological particles were measured in the original state because of their sufficient colloidal stability. Compared to Resovist, the smallest biological particles SB1 (\( d_c = 24 \) nm) exhibit a 1.5-fold increase in \( R_2 \) and a 3.2-fold increase in \( R_2/R_1 \). In accordance with the observed size-dependence, the large biological particles LB2 (\( d_c = 38 \) nm) show an improved relaxation behavior with an 2.2 fold higher \( R_2 \) relaxivity and, due to the low \( R_1 \) relaxivity, even a 4.9-fold higher \( R_2/R_1 \) relaxivity.
The relaxation properties of the original synthetic particles could not be measured because of colloidal instability; therefore only the L-DOPA-stabilized particles were used for the determination of the relaxation properties. The \( R_2 \) relaxivity is increased compared to that of Resovist, but due to the overall smaller core diameters only to a limited degree. The S12s particles with the smallest core diameter (\( d_c = 18 \) nm) show a 1.4-fold increase in \( R_2 \) and a 1.8-fold increase in \( R_2/R_1 \). The observed size dependence also applies to the synthetic particles, where the sample L1Is with a larger core diameter (\( d_c = 28 \) nm) shows enhanced relaxation effects. Accordingly, L1Is provides a 2.5-fold increase of \( R_2/R_1 \) compared to Resovist.

This study is focused on the determination of the MRI contrast properties of the here investigated particles and shows the advantage of bigger particles in \( R_2 \)-weighted imaging. Besides the contrast properties, for a successful use of the particles \textit{in vivo}, aspects like blood half-life, biodistribution, clearance properties and particle surface characteristics are expected to play important roles. Especially the blood half-life of particles is crucial because for applications concerning the imaging of the blood vessel system (angiography) a long particle blood half-life is essential. In contrast to the size-dependent behavior of the contrast properties the blood half-life of particles is in general higher the smaller the particles are [Roohi et al., 2012]. In addition, the different surface coatings of the biological and synthetic particles (magnetosome membrane vs. L-DOPA coating) leads to different blood kinetics and affects the \textit{in vivo} behavior of the particles [Chouly et al., 1996]. These examples show that not only the contrast properties of the particles are decisive for a new MRI imaging agent but also aspects concerning the particle behavior \textit{in vivo} have to be taken into account.

**SIMULATION OF CONTRAST PROPERTIES**

In order to better understand factors that affect relaxivity in a particle system, a model has been developed to calculate changes in relaxivity by varying different physical parameters, such as the core and hydrodynamic diameters, the particle’s magnetization and its anisotropy, external field strength, or the ratio between “inner” and “outer” viscosities. Expressions for the longitudinal \( T_1 \) and transversal \( T_2 \) relaxation times of protons are derived from a semi-classical model, where the protons are described in the frame of quantum mechanics, and magnetic nanoparticles are treated classically as macroscopic objects. Magnetic nanoparticles create random magnetic fields acting on the protons. Their randomness originates first from the distance between the nanoparticle and the proton, which is random due to thermal diffusion of water molecules; and second due to thermal fluctuations of the magnetic moment of the particle. As a result the longitudinal and transversal relaxation times of the proton are expressed through time correlation functions of the magnetic moment of the nanoparticle and
the function \( j \) [Ayant et al., 1975], describing the thermal motion of the proton in the vicinity of the nanoparticle.

Time correlation functions of the magnetic moment are calculated using the “egg-yolk” model [Shliomis et al., 1993], which takes into account the simultaneous Brownian rotation of the particle (“egg”) and the magnetic moment (“yolk”). The model accepts three non-dimensional parameters corresponding to external field strength, anisotropy of the particle, and ratio of “inner” and “outer” viscosities of the particle. These parameters can be calculated from the physical parameters of the system as follows:

\[
\xi = \frac{\mu H}{k_B T}; \quad \sigma = \frac{KV_m}{k_B T}; \quad \epsilon = \frac{\tau_m}{\tau_B}
\]

where \( \mu \) is the magnetic moment of the particle, \( H \) the external magnetic field, \( k_B \) the Boltzmann constant, \( T \) the temperature, \( K \) the magnetic anisotropy constant of the particle, \( V_m \) the magnetic volume of the particle, and \( \tau_m \) and \( \tau_B \) the characteristic rotational diffusion times of the “yolk” and the “egg”, respectively. In the limits of a weak field and a rigid dipole, the time correlation functions may be well approximated by single exponentials. The decay times of these exponentials are in good agreement with theoretical expressions as shown in Figures 11 and 12.

\[ \text{Fig. 11: Correlation decay times in the weak field limit (} \xi = 10^{-2} \text{) of magnetic moment components parallel (blue) and perpendicular (red) to the anisotropy axis of the particle. Numerical simulation was performed with } \epsilon = 0.5 \text{ (circles) and } \epsilon = 1 \text{ (squares). Lines represent the theoretical expressions [Shliomis et al., 1993]}. \]

For an intermediate range of magnetic field strength and magnetic anisotropy, the time correlation functions should be approximated by sums of exponentials as follows:
\[
\bar{\mu}_z\bar{\mu}_0 = \mu^2 \left( C_{z,0}^2 + \sum_k C_{z,k} e^{-\tau/\tau_{z,k}} \right)
\]

\[
\bar{\mu}_x\bar{\mu}_0 = \mu_y\bar{\mu}_y = \mu^2 \sum_k C_{xy,k} e^{-\tau/\tau_{xy,k}}
\]

Numerical simulation gives correct values for the mean-square fluctuation of \(\mu_z\) namely:

\[
\sum_k C_{z,k} = 1 - \frac{L(\xi)}{\xi} - L(\xi)^2
\]

where \(L(\xi)\) is the Langevin function. Figure 13 shows two decay times of \(\bar{\mu}_z\bar{\mu}_0\) at intermediate values of anisotropy and external field. They result from contributions of the magnetic moment components parallel and perpendicular to the particle’s anisotropy axis, leading to a slow and a fast decay time, respectively.

**Fig. 12:** Correlation decay times in the rigid dipole limit \((\sigma=10^{+3})\) of magnetic moment components parallel (blue) and perpendicular (red) to the external field. Numerical simulation (circles) is compared to theoretical expressions (lines) [Raikher et al., 1994].

As a result for the longitudinal \((T_1^{-1} = R_c)\) and transversal \((T_2^{-1} = R_c)\) coefficients of the relaxivity \(R_{1,2}(c)\) (\(c\) is the molar concentration of iron in the sample), we obtained [Taukulis et al., 2012]:

\[
R_1 = A \times \left\{ 6C_{z,0}^2 i\omega_1 \tau_D + 6 \sum_k C_{z,k} \left( i\omega_1 \tau_D + \frac{\tau_D}{\tau_{z,k}} \right) + 14 \sum_k C_{xy,k} j \left( i\omega_1 \tau_D + \frac{\tau_D}{\tau_{xy,k}} \right) \right\}
\]

\[
R_2 = \frac{R_1}{2} + A \times \left\{ 4C_{z,0}^2 + 4 \sum_k C_{z,k} j \left( \frac{\tau_D}{\tau_{z,k}} \right) + 6 \sum_k C_{xy,k} j \left( \frac{\tau_D}{\tau_{xy,k}} \right) \right\}
\]
where

\[ A = \frac{16\pi\gamma I M_s^2\rho MV_m}{135DRn_{Fe}} \]

and

\[ j(z) = \text{Re}\left\{ \frac{1 + \frac{1}{4}z^{1/2}}{1 + z^{1/2} + \frac{4}{9}z + \frac{1}{9}z^{3/2}} \right\} \]

and where \( \gamma_I \) is the proton gyromagnetic ratio, \( M_s \) the saturation magnetization of the particle, \( \rho \) the density of the particle, \( M \) the molar weight of molecules that constitute the particle, \( D \) the self-diffusion coefficient of water molecules, \( R \) the radius of the particle, \( n_{Fe} \) the number of magnetic atoms in the in the formula unit of the constituting material, and \( \tau_D = R^2/D \) the characteristic diffusion time of the proton in the vicinity of the particle.

Fig. 13: Numerically computed decay times (circles) of \( \mu_{z_{\perp}} \mu_{z_{\parallel}} \) with \( \sigma = 5, \epsilon = 0.1 \). As a comparison, the theoretical decay time of the rigid dipole is shown (line). Squares represent the theoretical decay times for the magnetic moment components parallel (blue) and perpendicular (red) to the anisotropy axis of the particle [Shliomis et al., 1993].

The model described by the relations (1) and (2) was checked with existing experimental data. The relaxivity coefficient \( R_2 \) increases linearly with size of the nanoparticle according to [Vuong et al., 2012]. This result is in agreement with relation (2). It is known that the relaxivity of the nanoparticles \( R_1 \) exhibits maximum as a function of the external magnetic field strength. It flattens out with the increase of the particle size or magnetic anisotropy constant as shown in [Levy et al., 2013] by comparison of the relaxivity data for maghemite and cobalt ferrite nanoparticles. Our numerical simulation confirms this behavior, as shown in
Figure 14. The verified model was applied to the experimental data.

Fig. 14: Numerical simulation of $R_1$ with different magnetic anisotropy constants. $K = 10^3$ (blue line), $K = 5 \times 10^3$ (green line) and $K = 10^4$ (red line). Other parameters of the simulation are $\varepsilon = 0.0043$, core and hydrodynamic diameters of the particle $d_c = d_h = 20\text{nm}$, $T = 312K$, $M_s = 300\text{kA/m}$, $\eta = 6.7 \times 10^{-4}\text{Pa}\cdot\text{s}$, $M = 0.23\text{kg/mol}$, $\rho = 5.15\text{g/cm}^3$, $n_{Fe} = 3$.

Experimental data for various synthetic inorganic and biological samples are shown in Table 1. Numerical simulations were performed to compute relaxivities using the model and are shown in Table 2. The physical parameters of the particles, i.e., core diameters and field of saturation magnetization were chosen to reflect values obtained from the real particles. An exception was the hydrodynamic size of the particle that was kept close to the magnetic core size, with only a small (2.5 nm) non-magnetic layer. This reflects the ability of water molecules to enter the non-magnetic coating layer. Use of the measured hydrodynamic size leads to modeled relaxivity values that are in disagreement with experimental values.

Table 2: Results of numerical simulation. Common parameters: magnetite particles in water, anisotropy energy density $K = 10 \text{ kJ/m}^3$, $B = 0.94T$, $T = 39^\circ\text{C}$. Here $\varepsilon$ is the ratio between “inner” and “outer” viscosities of the nanoparticle.

<table>
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<tr>
<th>Sample</th>
<th>$d_c$ [nm]</th>
<th>$d_h$ [nm]</th>
<th>$M_s$ [kA/m]</th>
<th>$\varepsilon$</th>
<th>$R_1$ [L/mmol*s]</th>
<th>$R_2$ [L/mmol*s]</th>
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<td>LI1s</td>
<td>28</td>
<td>33</td>
<td>357</td>
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<tr>
<td>SI1s</td>
<td>17</td>
<td>22</td>
<td>358</td>
<td>0.0023</td>
<td>17.5</td>
<td>160</td>
<td>9.1</td>
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<tr>
<td>SI2s</td>
<td>18</td>
<td>23</td>
<td>329</td>
<td>0.0022</td>
<td>14.9</td>
<td>153</td>
<td>10.3</td>
</tr>
<tr>
<td>SB1</td>
<td>24</td>
<td>29</td>
<td>300</td>
<td>0.0024</td>
<td>11.9</td>
<td>234</td>
<td>19.7</td>
</tr>
<tr>
<td>LB1</td>
<td>41</td>
<td>46</td>
<td>300</td>
<td>0.0030</td>
<td>8.1</td>
<td>723</td>
<td>89.3</td>
</tr>
<tr>
<td>LB2</td>
<td>38</td>
<td>43</td>
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<td>0.0029</td>
<td>8.7</td>
<td>617</td>
<td>70.9</td>
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<tr>
<td>SB2</td>
<td>29</td>
<td>34</td>
<td>300</td>
<td>0.0026</td>
<td>10.8</td>
<td>349</td>
<td>32.3</td>
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To demonstrate the effect of saturation magnetization $M_s$ on relaxation parameters, various values were used in numerical simulation and the results are shown in Figure 15 - 17. Other parameters were kept the same as in Table 2 and $d_h = d_c + 5$ nm. Both $R_1$ and $R_2$ increase with $M_s$, as is expected by relations (1) and (2) but their ratio is invariant. In general, the numerical model predicts that at constant external field (0.94 T) the most important physical parameters of the particles are their magnetic core size and saturation magnetization as these parameters change the relaxivities the most. Effects from other parameters are small or vanishing.

![Figure 15: Comparison of $R_1$ measured at 0.94T (markers) with those computed numerically for various values of magnetization (lines).](image1)

![Figure 16: Comparison of $R_2$ measured at 0.94T (markers) with those computed numerically for various values of magnetization (lines).](image2)
CONCLUSIONS

This multidisciplinary study has shown that stable magnetic nanoparticles with a limited size distribution can be produced either biologically or synthetically, with particle size distributions being narrower for the biological samples. Small particles have predominantly SP behavior, whereas the magnetic properties of samples consisting of larger particles show a combination of SP and SD behavior. Stabilization of the synthetic, inorganic particles prevents aggregation, so that small particles are almost exclusively SP in their magnetic properties.

Both types of particles, the biological as well as the synthetic ones, provide enhanced relaxation properties compared to the known MRI contrast agent Resovist. A significantly larger particle core diameter leads to higher $R_2$ relaxivity. Combined with a low $R_1$ relaxivity, the $R_2/R_1$ relaxivity values of these particles are highly improved. Due to the insufficient colloidal stability of the large synthetic particles, only the small particles were investigated. These particles offer enhanced contrast properties with respect to Resovist but reduced contrast compared to the biological magnetosomes. No enhanced toxicity was found for larger particles, while higher toxicity was measured for biological particles. Therefore, combining size effect, colloidal stability and low toxicity with improvements in the stabilization of the synthetic particles, in particular of those with large core diameter, has the potential of providing particles with enhanced relaxation properties without problems associated with toxicity.
METHODS

We produced and analyzed 50 bacterial and 80 synthetic samples of magnetite nanoparticles. Procedures of the production of both biogenic and synthetic samples were continually refined in response to the results of sample characterization, in order to achieve the highest purity magnetite and the best performance of the particles as MRI contrast agents. We exemplify our findings on typical samples presented in Table 1.

MAGNETITE SYNTHESIS

Magnetite nanoparticles were synthesized with the modified co-precipitation method [Baumgartner et al., 2013a; Baumgartner et al., 2013b]. Briefly, the system was controlled by a titration set-up (Metrohm, 776 Dosimat and 719 S Titrino). The iron was added as Fe\textsuperscript{II} / Fe\textsuperscript{III}-chloride solutions (1 M, Fe\textsuperscript{II} / Fe\textsuperscript{III} = 1 / 2) at a rate of 1 µl × min\textsuperscript{-1} to a total volume of 10 ml. The pH and the temperature are kept constant during the process (pH = 9 ± 0.4 or 11 ± 0.1 with 1 M NaOH; Temperature = 25 ± 0.1 °C). All solutions were degased before using and the system was kept under nitrogen atmosphere during the synthesis.

STABILIZATION OF SYNTHETIC PARTICLES

The colloidally unstable particle dispersion was treated with an ultrasonic tip (Bandelin Sonoplus, duration = 30 s with 60 % power) to reduce the size of large particle aggregates. Afterwards, the particle dispersion was mixed 1 / 1 (v / v) with a 10 wt% polymeric solution and a saturated solution of L-DOPA, respectively. The solution was shaken for 3 h (Thermomixer comfort, Eppendorf) and finally centrifuged (Biofuge pico, Heraeus) for 5 min @ 4000 rpm. The resulting supernatant, containing the stabilized particles, was removed and analyzed in terms of particle size (DLS) and iron content.

MAGNETOSOME FORMATION, ISOLATION AND PREPARATION

For the formation of biogenic magnetite particles Magnetospirillum gryphiswaldense wildtype and mutant strains (Table S1) were grown anaerobically at 25°C in a 30 L Biostat C fermentor (B. Braun Biotech International, Melsungen, Germany) using a modified flask standard medium [Uebe et al., 2010]. Cells were harvested after 24 - 36 h by tangential flow filtration and centrifugation (9250 g, 15 min, 4°C). Pelleted cells were resuspended and washed in an ice-cold wash buffer (20 mM Heps, 5 mM EDTA, pH 7.4) twice. Subsequently, cell pellets were stored at -20°C until use. For magnetosome isolation cells were resuspended in 50 mM Heps, 1 mM EDTA, 0.1 mM PMSF, pH 7.4 (7 ml per g fresh weight) and disrupted by 3 passages through a Microfluidizer M110-L system equipped with a 100 µm HZ10 diamond interaction chamber. After removal of cell debris by centrifugation (1,000 rpm, 10 min, 4°C) the supernatant was subjected to magnetic separation as described
earlier [Lang and Schüler, 2008]. The resulting enriched magnetosome suspension was applied on a 50 % (w/w) sucrose cushion and centrifuged at 230,000 g at 4°C for 2 h. Finally, the supernatant was removed; the pelleted magnetosomes were resuspended in 10 mM Hepes, 1 mM EDTA, pH 7.4 and subsequently stored under an inert nitrogen atmosphere at 4°C in order to prevent oxidation.

**X-RAY DIFFRACTION**

The particles were analyzed as previously described [Baumgartner et al., 2013a; Fischer et al., 2011]. Briefly, the material was dried with an α-quartz standard on a Kapton thin film and measured in transmission with a 100 µm beam at a wavelength $\lambda \approx 0.82656$ Å at the μ-Spot beam line of the BESSY II synchrotron radiation facility, Berlin [Paris et al., 2007]. The particle size was determined by Scherrer analysis i.e. by fitting the (311) peak with a pseudo-Voigt function and after correcting for instrumental peak broadening.

**TRANSMISSION ELECTRON MICROSCOPY**

The nanoparticles were deposited from a suspension onto Cu transmission electron microscopy (TEM) grids covered by either lacey or continuous Formvar+carbon films. Bright-field and high-resolution (HRTEM) images, as well as selected-area electron diffraction (SAED) patterns were obtained using a JEOL 3010 transmission electron microscope, operated at 300 kV accelerating voltage and equipped with a Gatan Imaging Filter for the acquisition of energy-filtered elemental maps. Images were recorded on a CCD camera. Additionally, bright-field images and SAED patterns were also recorded on imaging plates using a Philips CM20 TEM operated at 200 kV. For the processing and interpretation of data we used Digital Micrograph and SingleCrystal software.

**MAGNETIC MEASUREMENTS**

All magnetic measurements were carried out using a Princeton Measurements Corporation, Vibrating Sample Magnetometer (VSM; Micro-Mag Model 3900) at room temperature. Hysteresis loops were measured under a maximum applied field of 1 T with 100 ms averaging time and variable measurement spacing. FORC measurements were made by first saturating the sample with a positive applied field of 1 T and then ramping down the field to a reversal field ($H_r$), followed by measuring magnetization as the field increases from reversal field back to positive saturation. A series of 140 FORC with a field spacing of 2.4 mT was used for the analysis. FORC data were transformed into FORC diagrams using M. Winklhofer MATLAB code [Winklhofer and G. T. Zimanyi, 2006]. FORC diagrams were processed with a smoothing factor of 2.
The following various cell lines were used to determine IG\textsubscript{50} values of magnetic nanoparticles (MNPs): NIH3T3 (fibroblasts), MC3T3-E1 (osteoblasts) and RAW264.7 (macrophages). These cells were cultured in Dulbecco’s modified eagle’s medium (DMEM) with 10% fetal bovine serum (FBS) and 50 µg/ml gentamycin. Cultures were performed under humidified atmosphere at 37°C and 5% CO\textsubscript{2}. For experiments cells were seeded in DMEM with 2% FBS and 50 µg/ml gentamycin at a density of 2 x 10\textsuperscript{4} cells × cm\textsuperscript{-2} in a 96-well plate. After 24 hours of cultivation the culture medium was changed and inorganic samples or biological samples were added to the cells. The IG\textsubscript{50} values were calculated from dose-response curves ranging from 0.005 to 2 mg / ml inorganic or biological samples × cm\textsuperscript{-2} according to Lee et al. by using nonlinear least-squares analysis [Lee et al., 2000]. For identifying IG\textsubscript{50} values two different biochemical assays were used. First was the EZ4U assay (colorimetric MTT assay; Biomedica, Austria), which is based on the principle that living cells reduce uncoloured tetrazolium salts into intensely coloured formazan derivates that requires functional mitochondriae. Second was the CytoTox ONE Membrane Integrity Assay (Promega), which measures lactate dehydrogenase (LDH) release that is based on the conversion of resazurin into resorufin. Because MTT and LDH assays are colorimetric assays, MNPs and BMGs were removed prior to the incubation with the assay substrates to prevent optical interference [Alexandra Kroll et al., 2009].

**MRI**

The contrast properties of the magnetic nanoparticles were determined using a Bruker mq 40 contrast agent analyzer (0.94 T @ 40 MHz). The relaxation times T\textsubscript{1} and T\textsubscript{2} in [ms] were measured at 39 °C using aqueous particles dispersions of 0.1, 0.25 and 0.5 mM iron, respectively. The relaxivity R\textsubscript{1} and R\textsubscript{2} in [L/mmol * s] were determined by applying the linear relationship of the reciprocal of the relaxation time 1 / T\textsubscript{1,2} in [s\textsuperscript{-1}] and the iron concentration c(Fe) in [mM]. Finally, the relaxivity R\textsubscript{1,2} was defined by the slope of the obtained linear equation.

**DLS**

Dynamic light scattering (DLS) was used to characterize the colloidal stable particles in terms of their size. The hydrodynamic diameter d\textsubscript{h} was measured with a Submicron Particle Sizer Model 370 from Nicomp Particle Sizing Systems.
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CURRICULUM VITAE

MONIKA KUMARI

Nationality: Indian

EDUCATION

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<td>08/2003 – 06/2005</td>
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<td>ETH Zurich, Department of Earth Sciences, Institute of Geophysics</td>
<td>Role: Lecturer for the Rock and Environmental Magnetism; (51-4107-00L), 2 hours</td>
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<td>01/2011 – 06/2011</td>
<td>Internship at GreenTEG GmbH (Spin-off), Zurich, Switzerland,</td>
<td>Role: Electro-deposition and characterization of p-type and n-type material for thermoelectric generators (TEGs)</td>
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<td>06/2009 – 03/2010</td>
<td>India, IIT Kharagpur, Department of Physics &amp; Meteorology,</td>
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PUBLICATIONS


Taukulis, R., M. Widdrat, M. Kumari, D. Heinke, M. Rumpler, É. Tompa6, R. Uebe, A. Kraupner, A. Cebers, D. Schüler, M. Pósfai, A. M. Hirt, D. Faivre (2015), Biological vs. synthetic and small vs. large magnetite nanoparticles as MRI contrast agent - a comprehensive physical and theoretical study, Magnetohydrodynamics. (Accepted).


