Magnetic characterization of noninteracting, randomly oriented, nanometer-scale ferrimagnetic particles

Changqian Cao, Lanxiang Tian, Qingsong Liu, Weifeng Liu, Guanjun Chen, and Yongxin Pan

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[1] Studying the magnetic properties of ultrafine nanometer-scale ferrimagnetic particles (<10 nm) is vital to our understanding of superparamagnetism and its applications to environmental magnetism, biogeomagnetism, iron biomineralization, and biomedical technology. However, magnetic properties of the ultrafine nanometer-sized ferrimagnetic grains are very poorly constrained because of ambiguities caused by particle magnetostatic interactions and unknown size distributions. To resolve these problems, we synthesized magnetoferritins using the recombinant human H chain ferritin (HFn). These ferrimagnetic HFn were further purified through size exclusion chromatography to obtain monodispersed ferrimagnetic HFn. Transmission electron microscopy revealed that the purified ferrimagnetic HFn are monodispersed and each consists of an iron oxide core (magnetite or maghemite) with an average core diameter of 3.9 ± 1.1 nm imbedded in an intact protein shell. The R value of the Wohlfarth-Cisowski test measured at 5 K is 0.5, indicating no magnetostatic interactions. The saturation isothermal remanent magnetization acquired at 5 K decreased rapidly with increasing temperature with a median unblocking temperature of 8.2 K. The preexponential frequency factor $f_0$ determined by AC susceptibility is $(9.2 ± 7.9) \times 10^{10}$ Hz. The extrapolated $M_{s}/M_i$ and $B_{cr}/B_i$ at 0 K are 0.5 and 1.12, respectively, suggesting that the ferrimagnetic HFn cores are dominated by uniaxial anisotropy. The calculated effective magnetic anisotropy energy constant $K_{eff} = 1.2 \times 10^5 \text{ J/m}^3$, which is larger than previously reported values for bulk magnetite and/or maghemite or magnetoferritin and is attributed to the effect of surface anisotropy. These data provide useful insights into superparamagnetism as well as biomineralization of ultrafine ferrimagnetic particles.


1. Introduction

[2] Superparamagnetic (SP) particles of magnetite (Fe$_3$O$_4$) or maghemite ($\gamma$-Fe$_2$O$_3$) are of great interest in the fields of rock magnetism, environmental magnetism, and magnetic materials science as well as for medical and industrial applications [Moskowitz et al., 1997; Winklhofer et al., 1997; Wong et al., 1998; Hanesch and Petersen, 1999; Nolan et al., 1999; Egli, 2009; Galindo-Gonzalez et al., 2009]. For example, ultrafine-grained magnetite and/or maghemite are significant sources of magnetic susceptibility enhancement in soils and paleosols [Zhou et al., 1990; Maher, 1998; Banerjee, 2006]. Nanometer-scale magnetite particles (2–5 nm) in clusters contained in the upper beak skin of homing pigeons are assumed to form part of a biological magnetic field receptor [Winklhofer et al., 2001; Tian et al., 2007]. Furthermore, nanometer-scale magnetic particles are able to pass biological barriers that are usually inaccessible to larger particles; thus, they can be developed into novel diagnostic and therapeutic tools [Uchida et al., 2006, 2009].

[3] Néel theory [Néel, 1949] is the foundation for the quantitative interpretation of superparamagnetic grains and serves as the starting point for any modern applications to rock magnetism and paleomagnetism. The theory of thermoremanence by Néel [1949] links superparamagnetism and thermoremanence as thermally activated and blocked state, respectively, of single-domain magnetic particles [Dunlop and Özdemir, 1997; Banerjee, 2006]. However, classic Néel theory does not always perfectly describe the superparamagnetic behavior of natural and synthetic samples.

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because of the effects of magnetostatic interactions between poorly dispersed grains [Maher, 1988; Muxworthy, 2001; Muxworthy et al., 2003; Blanco-Mantecón and O’Grady, 2006]. Therefore, a population of noninteracting, superparamagnetic grains with a well-defined size distribution would help workers refine existing theories of thermal activation and superparamagnetism.

Ferritin is an iron storage protein that is ubiquitously distributed throughout the microbial, plant, and animal kingdoms. It is composed of a spherical cagelike protein shell with an outer (inner) diameter of 12–13 nm (8 nm) and a solid iron oxide or iron oxy-hydroxide core [Arosio et al., 2009]. The magnetoferritin was first successfully reconstituted with magnetic iron oxide (magnetite or maghemite) by using demetalized horse spleen ferritin (apoferritin) as a constrained reaction vessel under slow oxidation and relative high temperature (60°C–65°C) and pH (8.5) [Meldrum et al., 1992; Wong et al., 1998]. The artificial magnetoferritin potentially provides an ideal material for studying superparamagnetism. Moskowitz et al. [1997] carried out very detailed low-temperature magnetization and Mössbauer spectroscopy analyses on SP maghemite particles in reconstituted magnetoferritin. They determined the preexponential frequency factor \( f_0 \approx 10^9 \) Hz for the SP sample. However, their results could be biased by particle aggregation, as revealed by their transmission electron microscopy (TEM) images and the Henkel plot (Figures 1 and 7 in their paper). Therefore, to properly characterize SP particles, studies on magnetoferritin samples with narrow grain-size distribution and without magnetic interactions are needed.

In this study, we successfully synthesized nanometer-scale ferrimagnetic iron oxide (magnetite or maghemite) in the protein cavity using genetically engineered human H chain ferritin (HFn). These ferrimagnetic HFns were further purified to obtain monodispersed ferrimagnetic HFn. Systematic low-temperature magnetic measurements were conducted on the noninteracting ferrimagnetic HFn sample to understand the ultrafine superparamagnetism. In particular, we focused on determinations of the preexponential frequency factor \( f_0 \), the effective energy barrier constant \( K_{eff} \), and the surface anisotropy per unit of surface area \( K_s \) for the ferrimagnetic HFn.

2. Materials and Methods

The recombinant plasmid pET12b-HFn containing the sequence coding the human H chain ferritin was transformed into Escherichia coli strain Rosetta. The protein expression and purification were carried out according to the procedure of Santambrogio et al. [2000]. The purity of the protein was examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis and the protein concentration was determined by BCA Protein Assay Kit (Pierce) with bovine serum albumin as standard. The ferrimagnetic HFn
was synthesized according to the method of Uchida et al. [2006] with some modifications. All solutions were carefully deoxygenated with argon and transferred into an anaerobic chamber (COY Laboratory Products Inc.). The solution of 50 mL 100 mM NaCl with HFn (0.25 mg/mL) was added to the reaction vessel. The temperature of the vessel was kept at 65°C through an electric heating mantle and the pH was stabilized at 8.5 using 50 mM NaOH with a pH stat titrator (842 Titrand, Metrohm). Fe(II) (12.5 mM (NH₄)₂Fe(SO₄)₂·6H₂O) was added in a rate of 100 Fe/(protein min) using a dosing device (800 Dosino) connected with 842 Titrand. Simultaneously, stoichiometric equivalents (1.3 H₂O₂:Fe²⁺) of freshly prepared H₂O₂ (4.17 mM) were added as an oxidant, consistent with the following reaction. After adding theoretical 2300 Fe/(protein min) using a dosing device (800 Dosino) connected with 842 Titrand, the reaction continued another 5 min before finishing. Finally, 200 μL of 300 mM sodium citrate was added to chelate any free iron. The magnetite-forming reaction can be expressed as

\[ 3\text{Fe}^{2+} + \text{H}_2\text{O}_2 + 2\text{H}_2\text{O} \rightarrow \text{Fe}_3\text{O}_4 + 6\text{H}^+ \]

To get rid of the aggregated ferrimagnetic HFn, the synthesized ferrimagnetic HFn was further purified through two steps (1) centrifuging the synthesized ferrimagnetic HFn sample for 20 min at 10,000 g to remove aggregated clusters and (2) passing the sample through size exclusion chromatography (Sepharose 6B, GE Healthcare). Size exclusion chromatography is a useful method for fractionating proteins, water-soluble polymers, and water-based ferrofluid based on size difference [Nunes and Yu, 1987]. The method is usually achieved with a column that consists of a hollow tube tightly packed with small porous polymer beads designed to have pores of different sizes. When the ferrimagnetic HFn particles were loaded into the size exclusion column, the aggregates of ferrimagnetic HFn were unable to pass through the pore spaces because of their larger size. They would elute as different bands to achieve successful separation of monodispersed ferrimagnetic HFn. Finally, the magnetic HFn particles were loaded into the size exclusion tube tightly packed with small porous polymer beads designed to have pores of different sizes. When the ferrimagnetic HFn was further purified through two steps of (1) centrifuging and (2) passing the sample through size exclusion chromatography (Sepharose 6B, GE Healthcare). The size distribution of ferrimagnetic HFn cores, fitted with an intact protein cage. The outer diameter of the protein cage is ~12 nm. No crystal is observed outside of the protein cage. The size distribution of ferrimagnetic HFn cores, fitted into lognormal distribution function, have uniform grain size with a mean diameter of 3.9 nm and a standard deviation of 1.1 Å, which are characteristic of the (311), (400), (440), and (642) family of lattice planes for magnetite (and/or maghemite), respectively. Nanoparticles of magnetite and of maghemite of space group Fd3m cannot be distinguished by their electron diffraction patterns.

### 3. Results

#### 3.1. Transmission Electron Microscopy Analysis

[9] The bright-field TEM images of negative stained ferrimagnetic HFn (Figure 1a) show that those particles are monodispersed and each particle has an electron-dense core with an intact protein cage. The outer diameter of the protein cage is ~12 nm. No crystal is observed outside of the protein cage. The size distribution of ferrimagnetic HFn cores, fitted into lognormal distribution function, have uniform grain size with a mean diameter of 3.9 nm and a standard deviation of 1.1 Å, which are characteristic of the (311), (400), (440), and (642) family of lattice planes for magnetite (and/or maghemite), respectively. Nanoparticles of magnetite and of maghemite of space group Fd3m cannot be distinguished by their electron diffraction patterns.

#### 3.2. Magnetostatic Interactions

[10] For an assembly of randomly oriented noninteracting single domain (SD) particles with uniaxial anisotropy, \( I_r(H) \) and \( I_d(H) \) follow the Wohlfarth equation: 

\[
I_r(H) = 1 - 2I_f(H) \quad \text{[Wohlfarth, 1958]}
\]

Figure 2a shows the IRM acquisition curves [normalized to the maximum saturation isothermal remanent magnetization (SIRM)] and the DC demagnetization curves [rescaled as \( 0.5(1 + I_d(H)/\text{SIRM}) \)] [Cisowski, 1981] measured at 5 K. The R value of the Wohlfarth-Cisowski test for the measured sample is 0.5, indicating a lack of magnetic interactions. The median destructive field is 35.7 mT, which is consistent with the \( B_{cl} \) (35.5 mT). Figure 2b is the Henkel plot [Henkel, 1964] of the ferrimagnetic HFn, which displays a linear trend (\( r^2 = 0.99 \)). Again, it demonstrates that the ferrimagnetic HFn sample has nearly no magnetostatic interactions.

#### 3.3. Low-Field Magnetization Curves

[11] Figure 3a shows the temperature dependence of low-field (1.5 mT) magnetization between 5 and 60 K after the ZFC and FC treatments. The pattern is generally similar to the observation of Moskowitz et al. [1997] except for the much lower unblocking temperature for our samples. In the low field, the ZFC curve is proportional to the initial DC susceptibility. As temperature increases, the susceptibility increases as SD particles are progressively unblocked. The susceptibility peaks at 9.2 K. In contrast, the FC curve decays continuously with increasing temperature, because the magnetization was already aligned with the field direction during cooling through the blocking temperature [Moskowitz et al., 1997]. For the measured sample, its ZFC and FC curves merge at about 50 K, which corresponds to the maximum unblocking temperature. The differences

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between the FC and ZFC curves equal the thermal remanent magnetization \cite{Liu et al., 2004}. Above the maximum unblocking temperature (50 K), the reciprocal susceptibility \((1/\chi_i)\) plotted as a function of temperature can be fitted to the Curie-Weiss law (Figure 3b),

\[
\chi_i = C / (T - \theta),
\]

where \(C\) is the Curie constant and \(\theta\) is an ordering temperature \cite{Gittleman et al., 1974; Pedersen et al., 1994}. The reciprocal susceptibility plotted as a function of temperature above 50 K (Figure 3b) shows a good linear trend, which defines \(C = 6.3\) and \(\theta = 0.36\) K.

### 3.4. Demagnetization of Saturation Isothermal Remanent Magnetization

\cite{12} The thermal demagnetization curves of SIRM acquired at 5 K show a rapid decrease of remanence with increasing temperature (Figure 4). Above 15 K, the remanence decreases to less than zero, and as temperature increases to 300 K, the remanence trends asymptotically approach zero (see inset in Figure 4). The negative values above 15 K could be caused by the presence of negative residual fields in the MPMS-5XL, which is unavoidable especially after the acquisition of the SIRM at 5 K, or because of a ferrimagnetic and an antiferromagnetic phase coupling within ferritin cores. The 2.5 T ZFC and FC curves show very similar trends and are nearly superimposed. These data display classical SP behavior of ferrimagnetic particles: as temperature increases, the SD particles are unblocked into SP particles. The median unblocking temperature (the temperature where half of the remanence decayed) in the FC curve is \(T_{\text{bm}} = 8.2\) K. The remanence of ZFC and FC decreased to zero at 15 K, which means all the particles are unblocked into SP particles.

### 3.5. Hysteresis Loops

\cite{13} The hysteresis loops at different temperatures between 2 and 15 K are measured in fields of \(\pm 3\) T (Figure 5). The

![Figure 2](image1.png)  
**Figure 2.** Remanence data of ferrimagnetic HFn measured at 5 K. (a) Normalized IRM acquisition and DC demagnetization (DCD). The DCD curve was rescaled as \(0.5(1 + \text{IRM}(-H))/\text{SIRM}\). (b) Henkel plot, \(I_r(H)\) versus \(I_d(H)\), normalized by SIRM. The straight solid line is the theoretical Wohlfarth equation for noninteracting systems.

![Figure 3](image2.png)  
**Figure 3.** (a) Low-field (1.5 mT) susceptibility as a function of temperature measured after zero-field-cooling (ZFC) and field-cooling (FC) treatments. (b) Reciprocal susceptibility \((1/\chi_i)\) plotted as a function of temperature from the ZFC curve. The solid line is the best fit to equation (1) in the text.
value of the ratio of saturation remanence to saturation magnetization \( (M_r/M_s) \) at 2 K is 0.4, and the extrapolated value at 0 K is nearly 0.5, which indicates that the magnetization in the ferrimagnetic HFe is dominated by uniaxial anisotropy [Day et al., 1977]. The coercivity \( (B_c) \) of ferrimagnetic HFe at 2 K is 40.2 mT, which drops to 1.0 mT as temperature increases to 10 K. At 15 K, there is hardly any measurable coercivity. The remanence coercivity \( (B_{c\text{r}}) \) also decreases as temperature increases, from 60.6 mT at 2 K to 14.0 mT at 10 K. Owing to the SD unblocked into SP particles with increasing temperature, the hysteresis loops become more and more constricted (wasp-waisted) and hysteresis disappears at 15 K, which shows typical SP unblocking behavior [Tauxe et al., 1996].

Figure 6 shows the modified Day plot [Dunlop, 2002] for hysteresis parameters of the ferrimagnetic HFe measured at different temperatures (2–7 K) below its unblocking temperature (8.2 K). The ferrimagnetic HFe falls well within the SD + SP region. The decrease of \( M_r/M_s \) and the increase of \( B_{c\text{r}}/B_c \) with elevated temperatures indicate the proportion of SP particles increases as the SD particles are unblocked accordingly. The extrapolated value of \( B_{c\text{r}}/B_c \) corresponding to \( M_r/M_s = 0.5 \) is 1.12, which is slightly larger than the theoretical predicted value of 1.09 for randomly oriented uniaxial SD particles [Wohlfarth, 1958; Gittleman et al., 1974].

### 3.5. AC Susceptibility

Figure 7a is the in-phase \( (\chi') \) susceptibility of the ferrimagnetic HFe measured between 5 and 200 K. The curves show peaks in the range 12–16 K. The peak values of \( \chi' \) decrease and the peak temperatures \( (T_{\text{max}}) \) increase with increasing frequencies (see inset in Figure 7a). Clearly, the \( \chi' \) displays frequency dependence below 50 K, whereas above 50 K, \( \chi' \) shows nearly no frequency dependence. For a noninteracting single domain particle with uniaxial anisotropy, the thermal relaxation process follows the Néel-Arrhenius expression [Néel, 1949],

\[
\tau = 1/f_0 \exp(E_a/kT),
\]
where \( \tau \) is the relaxation time of the magnetic moment of the particles, \( f_0 \) is the preexponential frequency factor, \( E_a \) is the average effective energy barrier to be overcome for the magnetic moment of a grain to switch direction, \( k \) is Boltzmann’s constant, and \( T \) is the temperature. For uniaxial anisotropy particles, \( E_a = K_{\text{eff}}V \), where \( K_{\text{eff}} \) is the effective magnetic anisotropy energy constant, and \( V \) is the particle volume [Gittleman et al., 1974; Kim et al., 2001]. The plot of \( \ln \tau \) versus \( 1/T_{\text{max}} \) (Néel-Arrhenius plot; see Figure 7b) shows a nice linear trend, which means the ferrimagnetic HFe are noninteracting particles and follow the Néel-Arrhenius expression. Thus, according to equation (2) and the Néel-Arrhenius plot (Figure 7b), the average value of \( E_a \) is \( 3.9 \times 10^{-21} \) J. The effective magnetic anisotropy energy constant \( K_{\text{eff}} \) is estimated to be about \( 1.2 \times 10^6 \) J/m\(^3\) (1.2 × 10\(^6\) erg/cm\(^3\)) and \( f_0 = 9.2 \times 10^{10} \) Hz from the in-phase susceptibility data of the ferrimagnetic HFe.

The plot of \( T_{\text{max}} \) of out-of-phase \( (\chi'') \) susceptibility of the ferrimagnetic HFe (Figure 7c) is nearly independent of the frequency. The \( \chi'' \) data approach zero when the temperature is increased above 50 K, which indicates that the sample is completely unblocked at higher temperature at the frequencies being measured and all particles in the sample behave superparamagnetically.

### 3.7. Determination of Median Blocking Temperature

The blocking temperature is the temperature at which the relaxation time is equal to the time scale of experimental measurement. For a typical DC remanence measurement with \( t = 100 \) s and \( H \to 0 \), setting \( \tau = t \) gives the following expression:

\[
T_{\text{bm}} = E_a/k \ln(f_0t),
\]
where \( T_{\text{bm}} \) is the median blocking temperature corresponding to the median volume of the sample; the average effective energy barrier \( E_a = K_{\text{eff}}V = 3.9 \times 10^{-21} \) J and the preexponential frequency factor \( f_0 = 9.2 \times 10^{10} \) Hz were calculated from AC susceptibility data of ferrimagnetic HFe (see section 3.6). Therefore, we get \( T_{\text{bm}} = 9.4 \) K, which is very consistent with the susceptibility peak around 9.2 K (Figure 3).

However, the value of \( T_{\text{bm}} \) determined from the FC SIRM decay curve (Figure 4) is 8.2 K, which deviates from the calculated \( T_{\text{bm}} \) value and susceptibility peak temperature. The difference could be interpreted by effects from the size distribution. Theoretically, the \( T_{\text{bm}} \) determined from the decay of high-field saturation remanence (Figure 4) is less affected by size distribution and can be taken as true \( T_{\text{bm}} \); the peak temperature of low field ZFC equals \( \beta T_{\text{bm}} \), where the value of \( \beta \) depends on the particle size distribution. Jiang and Mørup [1997] simulated \( \beta \) values for different lognormal volume distributions of ferrimagnetic particle systems. They found the \( \beta \) value increased from 1 to 2 as the standard deviation \( \sigma \) increased from 0.1 to 1.3. From our sample, we can get a factor of \( \beta = 1.1 \).
3.8. Surface Anisotropy

As the particle size decreases, the fraction of atoms lying at the surface with lower atomic coordination increases. This results in the increase of site-specific surface energy, usually taken as a local uniaxial anisotropy normal to the surface. The surface anisotropy will lengthen the characteristic switching time of the particle magnetization and increase the effective energy barrier [Gilmore et al., 2005; Mazo-Zuluaga et al., 2008; Pérez et al., 2008]. For a spherical magnetite particle with diameter of $D$, the effective energy barrier $B_{eff}$ can be expressed as:

$$B_{eff} = B_s + B_s'$

where $B_s$ is the surface anisotropy and $B_s'$ is the effective uniaxial anisotropy.

*Figure 5.* Hysteresis loops of the ferrimagnetic HFn measured at 2, 5, 7, 10, 15, and 300 K.
magnetic anisotropy energy per volume unit follows [Pérez et al., 2008]:

\[ K_{\text{eff}} = K_v + 6K_s/D, \]

where \( K_{\text{eff}} \) is the effective magnetic anisotropy energy per volume unit, \( K_v \) is the core anisotropy energy per volume unit, and \( K_s \) is the surface anisotropy per unit of surface area. Assuming the magnetic mineral phase of ferrimagnetic HFn is magnetite, and the particle shape is spherical with no shape anisotropy, \( K_v = K_v = 2 \times 10^5 \text{ J/m}^3 (2 \times 10^5 \text{ erg/cm}^3) \) [Gilmore et al., 2005], then the value of \( K_s \) extracted from equation (4) for \( D = 3.9 \text{ nm} \) is \( 6.5 \times 10^2 \text{ erg/cm}^2 \). The comparison of values of \( K_{\text{eff}}, K_v \), and \( f_0 \) for the ferrimagnetic HFn in this study and other particles is listed in Table 1.

4. Discussion

[20] Synthesized magnetoferritin is susceptible to protein-protein aggregation, which causes broadening size distribution, increasing magnetostatic interactions, and blocking temperature [Moskowitz et al., 1997; Wong et al., 1998; Southern et al., 2007]. In this study, we obtained monodispersed ferrimagnetic HFn as shown by the TEM photographs, with intact protein cages and iron oxide cores (mean diameter of \( 3.9 \pm 1.1 \text{ nm} \); Figure 1). It indicates that the centrifugation and size exclusion chromatography procedures removed aggregated magnetoferritins. Results of the IRM acquisition and demagnetization curves, hysteresis loops, and the \( d \) spacings indicate that the cores of the synthesized ferrimagnetic HFn in the present study are low-coercivity ferrimagnetic minerals, magnetite or maghemite. However, it is difficult to determine the exact core composition at this stage, because (1) magnetite and maghemite have similar \( d \) spacings and (2) no measurable Verwey transition may be attributable to the thermal unblocking of SP magnetite or maghemite. The Mössbauer spectroscopy help but it requires large quantities of samples, which are unfortunately difficult to produce in the present study.

[21] The crossing point of IRM and DC demagnetization for the ferrimagnetic HFn at 5 K in this study is 0.5 (Figure 2a), which indicates the ferrimagnetic HFn samples have nearly no magnetostatic interactions. This finding was further supported by the linearity of the Henkel plot (Figure 2b) and the Néel-Arrhenius plot (Figure 7b). It should be noted that the ferrimagnetic HFn in this study is used after centrifugation and size exclusion chromatography treatments. Our ferrimagnetic HFn sample is monodispersed and magnetically noninteracting with narrow size distribution. Therefore, it provides us a good opportunity to estimate the \( f_0, K_{\text{eff}} \), surface anisotropy, hysteretic parameters, and other related magnetic behavior for the sample.

[22] The preexponential frequency factor \( f_0 \) plays a crucial role in determining the relaxation time obtained from Néel-Arrhenius expression and theoretically predicting AC magnetic, DC magnetic, magnetic resonance, and Mössbauer spectroscopy phenomena [Dickson et al., 1993; Tronc et al., 1995; Moskowitz et al., 1997; Egli, 2009]. However, the physically meaningful values of \( f_0 \) are hardly obtained, mainly because the magnetostatic interactions between particles are indeed difficult to avoid and they can influence superparamagnetic relaxation [D’Amico et al., 1995]. The \( f_0 \) determined for the ferrimagnetic HFn in this study is about \( 9.2 \times 10^{10} \text{ Hz} \), which agrees with the reported value of \( 10^{11} \text{ Hz} \) for quasi-isolated maghemite particles with very weak interactions [Dormann et al., 1999]. However, the value of \( f_0 \) in this experiment is higher than \( f_0 \approx 10^9 \) determined by Moskowitz et al. [1997]. This may be caused by different samples used in two experiments. The sample used in this earlier study was maghemite cores encapsulated in horse spleen ferritin with some interactions. Also, the \( f_0 \) of the ferrimagnetic HFn in this study is smaller than the \( f_0 \) of native horse spleen ferritin \( (10^{12}–10^{13} \text{ Hz}) \) [Guertin et al., 2007]. We argue that this difference in the values of \( f_0 \) may be caused by the different types of magnetic spin ordering and anisotropy of the core in these two types of ferritins: ferrimagnetic in ferrimagnetic HFn and antiferro-magnetic in native horse spleen ferritin. In addition, because the value of \( f_0 \) in this study was obtained by fitting to data between 12 and 16 K (Figure 7b) and extrapolating to 0 K, a potential error might be introduced by the extrapolation. Analyzing by a linear regression, we estimated the standard error for \( f_0 \) at 0 K to be \( 7.9 \times 10^{10} \text{ Hz} \).

[23] The calculated value of the effective magnetic anisotropy energy constant \( K_{\text{eff}} \approx 1.2 \times 10^7 \text{ J/m}^3 \) is significantly larger than that of bulk magnetite \( (1.34 \times 10^4 \text{ or } 2 \times 10^4 \text{ J/m}^3) \) [Dunlop and Özdemir, 1997; Gilmore et al., 2005] and bulk maghemite \( (4.7 \times 10^4 \text{ J/m}^3) \) [Birks, 1950]. Because the ferrimagnetic HFn are nearly spherical with weak shape anisotropy and no interaction, we attribute this larger \( K_{\text{eff}} \) to surface anisotropy. The surface anisotropy per unit of surface area \( K_s \) determined for ferrimagnetic HFn is \( 6.5 \times 10^4 \text{ erg/cm}^2 \), which is larger than the value of other magnetoferritin \( (2 \times 10^4 \text{ erg/cm}^2) \) [Gilmore et al., 2005]. This may also be caused by different size distributions of these two samples: \( 3.9 \text{ nm} \) for ferrimagnetic HFn and \( 7 \text{ nm} \) for previous magnetoferritin.
When the size of magnetite and maghemite get to the nanometer scale, the surface properties cannot be neglected [Kachkachi et al., 2000; Kim and Shima, 2007]. For example, the anisotropic energy of noninteracting or interacting maghemite of 3–10 nm is governed by surface anisotropy (6 × 10\(^{-2}\) erg/cm\(^2\)) [Tronc et al., 2000]. Iglesias and Labarta [2004] simulated surface anisotropy at 0 K and found that loops became elongated with a much higher closure field, increasing of coercive field and reducing the high field susceptibility. They explained that the surface anisotropy over exchange interactions created surface disordered states which became more difficult to reverse by the magnetic field. The observed hysteresis loop for the ferrimagnetic HFn at 2 K is elongated and closed at nearly 1500 mT, which supports the findings of Iglesias and Labarta [2004].

For nanometer-sized particles, the particle surface produces positive uniaxial anisotropy [Gazeau et al., 1998].

**Table 1.** Comparison of the Calculated Values of \(K_{\text{eff}}\), \(K_s\), and \(f_0\) for the Ferrimagnetic HFn Used in This Study and Previous Reported Values for Magnetoferritin

<table>
<thead>
<tr>
<th></th>
<th>Ferrimagnetic HFn</th>
<th>Magnetoferritin</th>
<th>Bulk magnetite</th>
<th>Bulk maghemite(^a)</th>
<th>Horse spleen ferritin(^b)</th>
</tr>
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<tbody>
<tr>
<td>(K_{\text{eff}}) (J/m(^3))</td>
<td>1.2 × 10(^5)</td>
<td>2.4 × 10(^{+4}) – 3.63 × 10(^{+4})</td>
<td>2 × 10(^4) – 1.35 × 10(^4)</td>
<td>4.7 × 10(^1)</td>
<td>2.5 × 10(^4)</td>
</tr>
<tr>
<td>(K_s) (erg/cm(^2))</td>
<td>6.5 × 10(^{-2})</td>
<td>2 × 10(^{-2})</td>
<td>1 × 10(^0)</td>
<td>1 × 10(^0)</td>
<td>1 × 10(^{12}) – 10(^{13})</td>
</tr>
<tr>
<td>(f_0) (Hz)</td>
<td>(9.2 ± 7.9) × 10(^{10})</td>
<td>(\times)</td>
<td>(\times)</td>
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\(^a\)Birks [1950].
\(^b\)Guertin et al. [2007].
\(^c\)Moskowitz et al. [1997].
\(^d\)Gilmore et al. [2005].
\(^e\)Dunlop and Özdemir [1997].

**Figure 7.** (a) In-phase susceptibility (\(\chi'\)) of the ferrimagnetic HFn; (b) Néel-Arrhenius plot, ln\(\tau\) versus \(1/T_{\text{max}}\) (the solid line is the best fit to the Néel-Arrhenius equation); and (c) out-of-phase susceptibility (\(\chi''\)) of the ferrimagnetic HFn.
Recent models by Labaye et al. [2002] confirmed that the surface anisotropy could compete with bulk magnetocrystalline anisotropy to form a “hedgehog”-type spin structure where all the spins were radially oriented, either outward or inward toward the center of the nanosphere. In this study, the extrapolated ratio $M_{c}/M_{s}$ is 0.5 at 0 K and is consistent with the study of Li et al. [2009], which supports that the ferrimagnetic HFn sample is dominated by uniaxial anisotropy. The extrapolated value of $B_{c}/B_{s}$ corresponding to $M_{c}/M_{s} = 0.5$ is 1.12, which is slightly larger than the theoretical predicted value of 1.09 for randomly oriented uniaxial SD particles (Figure 6) [Wohlfarth, 1958]. There are several factors that may lead to increased values of $B_{c}/B_{s}$, such as magnetostatic interactions, shape anisotropy, and surface effects [Wohlfarth, 1955; Moskowitz et al., 1997]. The shape anisotropy and magnetostatic interaction for our ferrimagnetic HFn sample can be excluded; thus, the large surface anisotropy may cause increasing values of $B_{c}/B_{s}$.

5. Conclusions

[26] We characterized the well-dispersed, noninteracting, randomly oriented nanometer-scale ferrimagnetic HFn particles using both TEM and low-temperature magnetic analyses. This experimental data allowed us to better integrate Néel theory with direct observations of superparamagnetic assemblies, which are widely distributed in geological samples and biological systems. Based on the preceding discussion, our conclusions are as follows:

[27] 1. The synthesized ferrimagnetic HFn after purification have intact protein shells with iron oxides encapsulated. The SAED, IRM, and hysteresis analyses indicate that the core of the ferrimagnetic HFn is magnetite/maghemite. The ferrimagnetic HFn cores have an average size of $3.9 \pm 1.1$ nm.

[28] 2. The ferrimagnetic HFn have nearly no magnetostatic interactions because of their intact protein shells. The observed median blocking temperature (8.2 K) is very consistent with our simulated results.

[29] 3. The value of preexponential factor $f_{0}$ in the Néel-Arrhenius equation can be obtained from the AC susceptibility data of the ferrimagnetic HFn. The value obtained is $(9.2 \pm 7.9) \times 10^{10}$ Hz.

[30] 4. The extrapolated value of $M_{c}/M_{s}$ is nearly 0.5 and $B_{c}/B_{s}$ is 1.12 at 0 K, which suggests the ferrimagnetic HFn is dominated by uniaxial anisotropy. Owing to the surface anisotropy, the effective magnetic anisotropy energy constant $K_{\text{eff}}$ determined for ferrimagnetic HFn is $1.2 \times 10^{5}$ J/m$^{3}$, which is larger than that of bulk magnetite, bulk maghemite, and magnetoferritin.

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