Temperature dependence of electron magnetic resonance spectra of iron oxide nanoparticles mineralized in *Listeria innocua* protein cages

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Electron magnetic resonance (EMR) spectroscopy was used to determine the magnetic properties of maghemite (γ -Fe₂O₃) nanoparticles formed within size-constraining *Listeria innocua* (LDps)- (DNA-binding protein from starved cells) protein cages that have an inner diameter of 5 nm. Variable-temperature X-band EMR spectra exhibited broad asymmetric resonances with a superimposed narrow peak at a gyromagnetic factor of $g \approx 2$. The resonance structure, which depends on both superparamagnetic fluctuations and inhomogeneous broadening, changes dramatically as a function of temperature, and the overall linewidth becomes narrower with increasing temperature. Here, we compare two different models to simulate temperature-dependent lineshape trends. The temperature dependence for both models is derived from a Langevin behavior of the linewidth resulting from “anisotropy melting.” The first uses either a truncated log-normal distribution of particle sizes or a bi-modal distribution and then a Landau-Lifshitz lineshape to describe the nanoparticle resonances. The essential feature of this model is that small particles have narrow linewidths and account for the $g \approx 2$ feature with a constant resonance field, whereas larger particles have broad linewidths and undergo a shift in resonance field. The second model assumes uniform particles with a diameter around 4 nm and a random distribution of uniaxial anisotropy axes. This model uses a more precise calculation of the linewidth due to superparamagnetic fluctuations and a random distribution of anisotropies. Sharp features in the spectrum near $g \approx 2$ are qualitatively predicted at high temperatures. Both models can account for many features of the observed spectra, although each has deficiencies. The first model leads to a nonphysical increase in magnetic moment as the temperature is increased if a log normal distribution of particles sizes is used. Introducing a bi-modal distribution of particle sizes resolves the unphysical increase in moment with temperature. The second model predicts low-temperature spectra that differ significantly from the observed spectra. The anisotropy energy density K_1 , determined by fitting the temperature-dependent linewidths, was ~ 50 kJ/m³, which is considerably larger than that of bulk maghemite. The work presented here indicates that the magnetic properties of these size-constrained nanoparticles and more generally metal oxide nanoparticles with diameters $d < 5$ nm are complex and that currently existing models are not sufficient for determining their magnetic resonance signatures. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4757964>]

I. INTRODUCTION

Magnetic nanoparticles have attracted substantial interest in nanoscience with applications in technologies such as biological and chemical sensing,¹ molecular magnetic resonance imaging,² remote-controlled drug delivery,³ and cellular targeting.⁴ Magnetic properties of nanostructures are

highly dependent on their size, shape, composition, and topology. The controlled synthesis of nanostructures with a particular size, shape, and morphology thus remains a central goal. Bio-mimetic mineralization within protein cages is an attractive approach for nanoparticle synthesis, because particles are limited to the cage inner diameter.^{5,6} Bio-mimetic mineralization has facilitated much interest in material synthesis based on molecular interactions between organic super-molecular assemblies and inorganic minerals.⁷ Protein cages provide a scaffold for preparing different metal

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compositions having unique magnetic characteristics.^{8–10} Moreover, the protein shell surrounding the nanoparticles presents a uniform spatial array of amino-acid side-chains for genetic modification,^{4,11} further synthetic processing such as covalently attaching relevant functional groups,^{12,13} or a combination thereof.¹⁴ Characterization of metal oxides mineralized within protein cages is essential to understand, design, and fabricate tailored magnetic properties for utilization in bio-nanotechnologies.

Transition-metal oxide nanoparticles exhibit properties, including superparamagnetic relaxation and a large fraction of surface spins, different from bulk magnetism and molecular paramagnetism. Superparamagnetic properties are measured by use of established methods such as AC susceptibility, Mössbauer spectroscopy, magnetometry, neutron scattering, and electron magnetic resonance (EMR) spectroscopy.^{15–17} The term EMR is used instead of electron paramagnetic resonance or ferromagnetic resonance to emphasize that superparamagnetic particles fall between these two well-understood regimes. Previous efforts have been recently reviewed to provide a unified view of the magnetic properties of nanoparticles by EMR spectroscopy with quantum and classical approaches.¹⁸ The interface between the quantum and classical regimes remains a fruitful area of research to understand the magnetic properties of molecular nanomagnets and magnetic nanoparticles and to observe the possible coexistence of classical and quantum phenomena.¹⁹

An increasing number of investigations use EMR spectroscopy to determine magnetic moment distributions and anisotropic energies of nanoparticles. Several routes have emerged to address lineshape trends in EMR spectra: in particular, lineshape analysis to determine magnetic moment distributions^{20–22} and resonant field shifts to describe the anisotropy energies^{23,24} and surface quantum effects.^{19,25,26} Often, bulk values for the magnetization and magnetocrystalline anisotropy are used as fixed parameters to assess EMR temperature-dependent magnetic-moment distributions.²¹ There has long been speculation as to whether the bulk magnetization values are appropriate to describe nanoparticle magnetic properties.²⁷ For example, as the particle size decreases, reduced bonding leads to a loss in spin exchange sufficient to induce surface spin disorder, resulting in surface anisotropy that contributes significantly to the bulk volumetric magnetocrystalline anisotropy.²⁸ Current evidence supports the need for a comprehensive model to describe the magnetic properties in this size regime.¹⁸

This investigation presents variable-temperature EMR spectra of size-constrained γ -Fe₂O₃ iron oxide nanoparticles. The spectra were simulated by use of existing models in an attempt to characterize the nature of the magnetic anisotropy, calculate the magnitude of the anisotropy energy K_1 , determine the particle magnetization as a function of temperature, and estimate the particle size distribution. *Listeria innocua* (LDps)-(DNA-binding protein from starved cells), which is a spherical protein with an inner diameter of 5 nm, was used as a size-constraining template to synthesize iron-oxide nanoparticles. Nanoparticles formed within protein cages provide an opportunity to place a size limit on the largest nanoparticles. The cage-limited maximum particle size was used as a

fixed parameter in spectral simulations. The concept of size-limited nanoparticles is in contrast to most nanoparticle systems where controlling the upper size without a template presents a difficult synthesis challenge.

Previous magnetometry measurements of these particles indicate that the particles are not simple superparamagnetic particles, but rather the magnetic system seems to be composed of superparamagnetic cores and a roughly comparable moment contained in non-saturated paramagnetic species.²⁹ Zero-field cooled (ZFC)/field-cooled (FC) magnetization data further show a sharp bifurcation of the ZFC/FC curves, confirming that there is an abrupt cut-off in the particle size. The blocking temperature of the superparamagnetic cores, determined from the ZFC/FC data, is ~ 4 K, which yielded an anisotropy energy of $K_1 = 40$ kJ/m³. Since the measured anisotropy energy is much larger than the cubic bulk anisotropy for γ -Fe₂O₃, we will assume in this paper that the anisotropy is uniaxial, due to surface magnetic properties.³⁰ The low blocking temperature and the sharp bifurcation indicate that this nanoparticle system is among the smallest and most uniform of all nanostructures studied, with the exception of molecular nanomagnets.

Theoretical interpretation of EMR variable-temperature spectra in small metal-oxide nanoparticles remains an intractable problem that requires an emendation of the current leading models. We compare and contrast two theories (static and kinetic) to simulate the variable-temperature EMR data of iron oxide nanoparticles in a size-constraining vessel. Both models describe the temperature dependence as derived from a Langevin behavior of the saturated linewidth ensuing from “anisotropy melting.” The first is a formalism developed by Berger *et al.* with an added constraint that imposes a volume limit.¹⁵ This model uses a distribution of particle sizes and a Landau-Lifshitz lineshape to describe the individual nanoparticle resonances. The essential feature of this model is that small particles have narrow linewidths and a constant resonant field at $g \approx 2$. Large particles have a broad linewidth and shift in resonant frequency as a function of temperature. The second model, developed by Raikher and Stepanov, uses a Fokker-Planck formalism to average over both thermal fluctuations and the particle distribution of anisotropy axes.³¹ For this model, we assume uniform particles with a diameter around 4 nm and a random uniaxial anisotropy. A sharp feature near $g \approx 2$ is predicted at higher temperatures, but there are significant deviations from the experimental data.

Prominent two-component lineshapes are often observed in typical EMR spectra of metal oxide nanoparticles with diameters $d < 15$ nm.^{20–22,25,32,33} Both models investigated here can account for a number of observed spectral features, but each has deficiencies. The first model leads to a nonphysical increase in μ_p as the temperature is increased if a standard log-normal distribution of particle sizes is assumed. Prior multi-frequency EMR experiments showed that the particle magnetization values, obtained with the Berger formalism, were larger than bulk magnetization values for 5 nm LDps particles.¹⁵ The second model, which more accurately accounts for the particle fluctuations and ensemble broadening, predicts low-temperature spectra that are quite different from the observed lineshape trends. Our work indicates that

these size-constrained nanoparticle systems and, more generally, metal oxide nanoparticles with diameters $d < 5$ nm have complex magnetic properties and that previous theoretical efforts have difficulty modeling metal oxide magnetic resonance signatures across a wide temperature range.

II. EXPERIMENTAL

Iron-oxide nanoparticles were synthesized by use of LDps spherical protein cages as a mineralization nanotemplate with a nominal inner diameter of 5 nm. Transmission electron microscopy (TEM) confirmed the encapsulation of the iron oxide nanoparticles within the protein cages that have an average diameter of 4.1 ± 1.1 nm.³⁴ Details of the mineralization and characterization have been described elsewhere for iron oxide nanoparticles mineralized within LDps.³⁴ X-band (~ 9.2 GHz) continuous-wave EMR spectra were recorded on a commercial spectrometer for linearly polarized microwave excitation and field modulation; hence, the EMR spectra $I(B_a)$ are proportional to the derivative of the imaginary part of the magnetic susceptibility $I(B_a) \propto \frac{d}{dB_a}(\frac{\chi''_+ + \chi''_-}{2})$, where χ''_+ and χ''_- are, respectively, the imaginary parts of the magnetic susceptibility for right- and left-circularly polarized excitations. Temperature was controlled by means of a commercial cryostat. The particles were in a frozen water solution with a concentration of approximately 0.3 mg/ml or 1.3 μ M of protein cages. The samples were cooled in zero magnetic field, and the spectra were recorded with 10 mW power and 1 mT modulation amplitude at a frequency of 100 kHz. The temperatures did not exceed the melting point of the water solution, which if present would add additional motional effects to the spectra.

III. EMR THEORY FOR NANOPARTICLES

Yulikov *et al.* describe the two main approaches for calculating superparamagnetic spectra: the kinetic and static methods.³⁵ The static model, formulated by Berger *et al.*,^{20,22} is more phenomenological and generally describes the lineshape trends as a function of temperature^{21,36} and frequency.¹⁵ The kinetic method, as discussed by Raikher and Stepanov,³⁷ calculates the moment-distribution function in the presence of both superparamagnetic fluctuations and ensemble broadening due to a distribution in anisotropy axes.^{31,37} Other models include an improved static model to describe the decrease in the magnetic anisotropy as temperature increases³⁵ and a theory for surface quantum effects.²⁵ Here, we first use the Berger formalism to describe the EMR lineshape trends and magnetic properties of iron oxide nanoparticles. While quality fits to the data can be made, there are concerns about the physical interpretation of the parameters resulting from the fits. Next, we simulate the data by means of the Raikher-Stepanov model and obtain qualitative agreement with the experimental data at 225 K but substantial deviation at lower temperatures.

A. Berger model

This model assumes an ensemble of non-interacting single-domain particles of moment μ_p , volume V , with (for the

case considered here) randomly oriented anisotropy axes. For each nanoparticle, the magnetization dynamics are described by the Landau-Lifshitz (LL) equation of motion²²

$$\frac{d}{dt}\vec{M} = \gamma \cdot \vec{M} \times \vec{B}_{\text{eff}} - \frac{\alpha\gamma}{M_s} \cdot \vec{M} \times (\vec{M} \times \vec{B}_{\text{eff}}), \quad (1)$$

where $\vec{M} = \vec{\mu}_p/V$ is the particle magnetization, B_{eff} is the effective field, which is the sum of the applied static, microwave, and anisotropy fields, γ is the gyromagnetic ratio, α is the damping parameter, and M_s is the saturation magnetization. Reference 21 includes a discussion of several other phenomenological equations of motion; however, we will focus only on the LL equation, because it provided the best description of a single magnetic nanoparticle. If the applied static field is sufficient to saturate the magnetization, Eq. (1) leads to the normalized absorption lineshape, which is proportional to the imaginary part of the magnetic susceptibility²²

$$\chi''_{LL\pm}(B, B_0, \Delta_B) = \frac{1}{\pi} \frac{B_0^2 \Delta_B}{(B \mp B_0)^2 B_0^2 + B^2 \Delta_B^2}, \quad (2)$$

where $\pm$ refers to right- and left-circular polarization, B_0 is the resonant field, and a linewidth parameter Δ_B is the half-width at half-amplitude of the resonance. For a single particle obeying LL dynamics this homogenous linewidth is related to the damping parameter by $\Delta_B = \alpha B_0$. For a low damping rate and hence narrow linewidth, $B_0 \gg \Delta_B$, the lineshape simplifies to a Lorentzian lineshape with no shift in resonant frequency, whereas the resonant field shifts slightly to lower field values for broad resonance linewidths. This is in contradistinction to the Landau-Lifshitz-Gilbert formalism which has an explicit coupling of the resonance frequency to the damping causing a large decrease in resonance frequency for large damping values ($\alpha > 0.5$). Given the large inhomogeneous broadening, the details of the individual line shape are not of importance.

The absorption intensity I in an applied magnetic field B_a is the sum of all particle resonances weighted by the particle volume amplitude²²

$$I(B_a) = \sum_i^{\text{vmax}} F_i[B_a, B_0, \Delta_B(V)] \times f_v, \quad (3)$$

where F_i is the angle-dependent individual lineshape for the i th nanoparticle with a volume V_i , B_0 is the ensemble resonant field, f_v is the volume distribution function, and Δ_{B_i} is the individual linewidth. The main assumption in this model is that the resonance lineshape averaged over all particles with a volume V_i are given by the LL susceptibility. This assumption is not in general true, as discussed in the Sec. III B, if the inhomogeneous broadening is much greater than the homogenous broadening. The absorption intensity is then given by

$$I(B_a) = \frac{d}{dB_a} \int_V \chi''_{LL} \times f_v \cdot dV, \quad (4)$$

where χ''_{LL} is related to the imaginary part of the susceptibility.

A decrease in the linewidth Δ_B is assumed for decreasing particle size due to more effective thermal averaging of the anisotropy in small particles. Spectral narrowing can be taken into account by introducing a size- and temperature-dependent linewidth by the expression²¹

$$\begin{aligned}\Delta_B &= \Delta_0 \cdot G(y) \cdot L_1(\xi), \\ L(\xi) &= \coth \xi - \frac{1}{\xi}, \\ G(y) &= \frac{1 - 3y^{-1} \coth y + 3y^{-2}}{\coth y - y^{-1}},\end{aligned}\quad (5)$$

where $\xi = \frac{M_s V B_a}{k_B T}$, $y = \frac{K_1 V_s}{k_B T}$. In Eq. (5), $L(\xi)$ is the Langevin function and $G(y)$ is called the superparamagnetic averaging factor for uniaxial anisotropy.^{30,38} Δ_0 is the saturation linewidth at 0 K, and V_s is a reference volume taken as the greatest volume in the statistical ensemble. Figure 1 is a plot of the predicted linewidth versus particle diameter for two different temperatures, 10 K and 225 K. The correctness of this functional form is debated in the literature, although all models qualitatively agree on the linewidth narrowing due to thermal fluctuations averaging the anisotropy over phase space.

The distribution of particle sizes and their accompanying magnetic properties lead to characteristic features in their EMR spectra. In characterizing the response of an ensemble of particles, we first assume a truncated log-normal volume distribution f_v of the magnetic moments μ_p . The distribution is modeled by the volume mode V_{mode} , logarithmic variance σ , a minimum volume V_{min} , and maximum volume V_{max} value, which is limited to the inner diameter of the protein cage. A bi-modal volume distribution function was also used to describe the EMR spectra, and we highlight the advantages when a two-component distribution function is used in the nanoparticle EMR lineshape simulations.

FIG. 1. EMR linewidth as a function of volume, which compares the Berger and RS models at 10 K and 225 K.

B. Raikher-Stepanov model

The Raikher-Stepanov (RS) model also assumes single-domain particles whose dynamics obey the LL equation of motion. The model is based on solutions to the Fokker-Planck equation and calculates the orientational distribution function in the presence of both thermal fluctuations and a distribution of easy axes. The dynamic susceptibility is then calculated from the equation of motion of the average nonequilibrium magnetization. For the case of particles with uniaxial anisotropy with a uniform distribution of anisotropy axes, analytic expressions for the complex susceptibility (Eq. (6) and coefficients are derived from Ref. 31) are obtained

$$\chi = \frac{n * \mu_o \mu_p^2}{V k_B T} \left\{ \frac{B}{D} + \left[\frac{2A - B}{2C - D} - \frac{B}{D} \right] \frac{\arctg[3D/(2C - D)]^{1/2}}{[3D/(2C - D)]^{1/2}} \right\}, \quad (6)$$

where the complex coefficients are given by

$$\begin{aligned}A &= 1 - \left(\frac{1}{\xi} + \frac{i}{\alpha} \right) L_1, \\ B &= \frac{2K_1 V}{3k_B T} \left[\frac{3}{\xi^2} - \left(\frac{1}{\xi} + \frac{i}{\alpha} \right) \frac{dL_2}{d\xi} \right], \\ C &= 2i\omega\tau + \left(\frac{\xi}{L_1} - 1 \right) - \frac{i}{\alpha} \xi, \\ D &= \frac{2K_1 V}{3k_B T} \frac{1}{L_1^2} \left(3 - \xi \frac{d}{d\xi} - 3 \frac{i}{\alpha} L_1 \right) L_2.\end{aligned}$$

Here, $\xi = \mu_p B_a / k_B T$, the characteristic orientational diffusion period of the magnetic moment is given by $\tau = \mu / 2\alpha\gamma_e k_B T$, and L_1 and L_2 are defined as averages of the first and second Legendre polynomials over the unit sphere

$$\begin{aligned}L_1(\xi) &= \langle \cos\theta \rangle = \frac{d}{d\xi} \left(\ln(Z_0(\xi)) \right), \\ L_2(\xi) &= \left\langle \frac{\cos^2\theta - 1}{2} \right\rangle = \left(\frac{3}{2} \frac{1}{Z_0(\xi)} \frac{d^2}{d\xi^2} Z_0(\xi) \right) - \frac{1}{2},\end{aligned}$$

where the partition function Z_0 is

$$Z_0(\xi) = \frac{2\pi}{\xi} (e^\xi - e^{-\xi}).$$

As shown in Ref. 31, these equations predict, for the imaginary part of the susceptibility, a broad asymmetric “powder pattern” at low temperatures, due to the distribution of anisotropy axes (an example powder pattern is shown in Figure 6(a)). There are more particles with easy axes perpendicular to the field and moment direction, which for positive K_1 , have lower resonant frequencies. For this case, there is more spectral weight at high field values (low frequencies). When K_1 is negative, the resonant frequencies of particles with easy axes perpendicular to the field direction have higher resonant frequencies, hence there is more spectral weight at low

FIG. 2. Variable temperature X-band (9.2 GHz) EMR spectra of encapsulated iron oxide (γ - Fe_2O_3) nanoparticles in *Listeria innocua* Dps protein cage. Experimental data (red) and simulations (black) are shown for the temperature range of 10 K–225 K.

fields. Thus, the resonant field shift is determined by the sign of the anisotropy constant.

For small particles or at higher temperatures, as discussed above, thermal fluctuations induce a melting of the anisotropy that leads to the narrowing of the linewidths as temperature increases. The RS calculation indicates that there is a thermal-fluctuation-induced reduction of the anisotropy constant such that $K_{1\text{eff}} = K_1 L_2(\xi)/L_1(\xi)$ and a

concomitant reduction in the inhomogeneous linewidth given approximately by $\delta B_u = 3K_{1\text{eff}}(\xi_0)/M_s$, where $\xi_0 = M_s V \omega / \gamma k_B T$ represents ξ evaluated at the resonance field and $\omega = 2\pi\nu$. The decrease in linewidth due to anisotropy melting is qualitatively similar to, but quantitatively different from, the Berger model. The RS model further predicts that as ξ decreases below ~ 1 , the reduction of the inhomogeneous linewidth gives way to an increase in linewidth, due to fluctuation broadening. In this limit, the particle's moment rapidly fluctuates from one orbit to another, and these rapid fluctuations dominate the linewidth. This competition results in a minimum linewidth for $\xi \cong 1$ (see Figure 1). The Berger model does not account for fluctuation broadening and assumes a monotonic reduction of the linewidth as ξ decreases.

IV. RESULTS

The variable-temperature (10 K–225 K) X-band EMR spectra of maghemite (γ - Fe_2O_3) LDps protein cages are shown in Figure 2. The key features of the EMR data are a broad linewidth characteristic of single-domain superparamagnetic nanoparticle spectra superimposed on a narrow component. The two spectral components, as determined by empirical fits to two Gaussians without using any physical model, clearly have different temperature-dependent trends (see Figures 3(a) and 3(b)). The broad asymmetric component narrows with increasing temperature (from 280 mT to 110 mT) and undergoes a concomitant shift in resonance frequency to high fields (from 240 to 320 mT), as seen in the curves labeled *Broad*. The narrow feature diminishes in amplitude and undergoes minimal broadening as the temperature decreases, while the resonance field ($g \cong 2.0328 \pm 0.0036$) remains unchanged, as seen in the curves labeled *Narrow*. LDps EMR spectra are consistent with previous observations of the two-component lineshape trends as a function of temperature in metal-oxide nanoparticles.^{21,25}

FIG. 3. Experimental and simulated (a) shift of the resonant field and (b) linewidths for the two *spectral* components as a function of temperature. The experimental data for *Broad* and *Narrow* points were fit empirically to two Gaussian functions to obtain the linewidth values. The *Simulated* points result from use of the Berger model to fit the experimental spectra.

FIG. 4. (a) Simulated Langevin functions used to generate the X-band EMR lineshape fits of *Listeria innocua* Dps iron oxide nanoparticles. (b) An illustration of the log-normal ($\sigma = 0.6 \pm 0.1$) and two-component weighted distribution functions. The inset is simulated (black) and experimental (red) spectra at 50 K based on a two-component distribution function.

The Berger model (Eqs. (1)–(5)) was used to calculate the variable-temperature EMR spectra and can describe the lineshape trends for both spectral components of LDps iron oxide nanoparticles.²² The linewidths and resonant field shifts for both broad and narrow components are well-described with an ensemble of Landau-Lifshitz lineshape functions (Eq. (4)). The apparent resonant field shifts to lower fields for large linewidths at low temperatures but remains constant for linewidths much smaller than the resonant field ($B_0 \gg \Delta_B$). The fitting parameters for a truncated log-normal distribution, derived from minimizing the sum of squares, were $V_{\text{mode}} = 65.45 \text{ nm}^3$ ($d = 5 \text{ nm}$), with a volume distribution of $\sigma = 0.6 \pm 0.1$ and a minimum volume of 0.37 nm^3 ($d = 0.89 \text{ nm}$). The adjustable parameters for each temperature were M_s and Δ_T , while the maximum diameter was constant at the inner dimensions of the cage.

The spectral parameters and constraints that are used to fit the experimental data determine the overall simulated Langevin function temperature trend (see Figure 4(a)). The magnetization M_s and temperature T parameters change the curvature of the simulated Langevin functions, which describe the evolution of the line broadening in the EMR variable-temperature spectra. The saturating linewidth decreases as temperature increases, consistent with anisotropy melting. The experimental linewidth trends are modeled by assuming a volume-dependent Langevin function; however, the physical correctness of this functional form is questionable.³³ Large particles at the asymptote of the Langevin function, $\xi > 1$, have the same saturated linewidth and, therefore, are spectrally insensitive to size variations. Small particles or narrow linewidths are more sensitive to the linear region of the Langevin function, $\xi < 1$, and are more diagnostic of the smaller volume ensemble distribution.¹⁵

The temperature dependence of the magnetization M_s parameter results from imposing a size constraint (protein cage) (see Figure 5(a)). The values are similar to bulk magnetism

(370 kA/m) from 10 K to 60 K and increase drastically above 60 K to an unrealistic value of $\sim 9100 \text{ kA/m}$ at 225 K. A magnetic moment that increases to such high values as a function of temperature is a nonphysical result. The nonphysical result of an increasing magnetic moment can be rectified by abandoning a log-normal distribution function and making use of a two-component distribution function (see Figure 4(b)). The two-component function is essentially two discrete volumes (linewidths) instead of a continuous distribution of particle sizes. Figure 4(b) inset shows a spectral fit at 50 K with a two-component distribution function. The fitting quality is similar to the log-normal distribution data across the entire temperature range. Assuming bulk magnetization, the spectra were fit to a diameter $d = 4.0 \text{ nm}$ with a weight of $88\% \pm 4\%$. The narrow component fit resulted in a diameter $d = 1.9 \pm 0.3 \text{ nm}$ with $12\% \pm 4\%$ weighting. With the present data, we cannot determine whether the narrow spectrum is due to a small superparamagnetic magnetic moment ($d < 2 \text{ nm}$) or is from surface spins on a superparamagnetic core. We caution against the assertion that the compared integrated areas imply a small population of the narrow component, because we do not know the origin of the line broadening for the narrow component.

The mechanism that leads to a decrease in the saturating linewidths (Δ_T) as a function of temperature has been described by the superparamagnetic averaging factor (Eq. (5)) for uniaxial anisotropy.^{30,38} Here, we neglected the temperature dependence of K_1 , and the saturating linewidth Δ_T was fit to the equation $\Delta_T = \Delta_0 G(K_1 V_s / k_B T)$ with $V_s = 65.4 \text{ nm}^3$ (see Figure 5(b)). The saturation linewidth varied from 240 mT to 90 mT in the corresponding temperature range of 10 K–225 K. The anisotropy energy K_1 was 52 kJ/m^3 , which is considerably higher than the cubic anisotropy energy of -4.7 kJ/m^3 , and suggests that other anisotropic effects are present, such as surface anisotropy. Surface anisotropy may contribute to the volumetric magnetocrystalline anisotropies,

FIG. 5. (a) Temperature dependence of the magnetization of a 5 nm encapsulated nanoparticle determined from fits to the Berger model. With a truncated log-normal volume distribution, departure from bulk magnetization values occurs around 60 K with the moment continuing to nonphysical values at 225 K. A two-component distribution function showed no departure from bulk magnetization values. (b). Temperature dependence of the saturating linewidth parameter (Δ_T) and the theoretical fit using the superparamagnetic averaging factor (Eq. (5)). The magnetocrystalline energy, with a reference volume of 65.4 nm^3 , is approximately 52 kJ/m^3 . RS simulation by use of parameters described in the text with $\alpha = 0.01$.

which become more pronounced in samples with relatively small nanoparticles ($d < 10 \text{ nm}$).¹⁷

RS Model linewidth simulations for the temperature range are shown in Figure 5(b), and the predicted absorption spectra at three temperatures are shown in Fig. 6(a). The parameters used in the calculations were $\mu_p = 1200 \mu_B$, $M_s = 330 \text{ kA/m}$, $d = 4.0 \text{ nm}$, $\alpha = 0.01$, $K_1 = 40 \text{ kJ/m}^3$, $\nu = 9.37 \text{ GHz}$. The resonance absorption linewidth is given by $\delta B \cong \frac{3K_1}{M_s B_r}$ at low temperatures. The derivative of the susceptibility, which corresponds to the measured EMR spectra, is shown in Figs. 6(b) and 6(c). The derivative highlights the onset of resonance at low field and a second peak occurring near $g \approx 2$. The calculated spectra at 225 K qualitatively replicate the measured spectra in that there is a sharp feature at $g \approx 2$ and spectral weight extending to low fields. However, the low-temperature spectra show considerable deviation

without accurately describing the variable-temperature lineshape trends of the two-component experimental spectra. Including a second small component into the RS model will provide a $g = 2$ feature at lower temperatures; however, the detailed lineshape cannot be explained without allowing for a distribution in particle sizes or anisotropy energies.

V. DISCUSSION

The EMR frequency and temperature spectral analysis remains under considerable debate for determining nanoparticle size distributions and magnetic properties.¹⁸ In particular, the theoretical treatment needs improvement to accurately describe the spectral trends for the two-component lineshape structure of the EMR spectra in metal oxide nanoparticles.¹⁵ We have previously reported the multi-frequency spectral evolution of three different-sized protein cages and suggested that the narrow component in the spectra is most likely due to small nanoparticles, in accordance with the Berger model. The Landau-Lifshitz lineshape function follows the correct shift in the resonant field as a function of linewidth, with larger linewidth peaks being shifted to lower fields. However, the use of the Landau-Lifshitz lineshape, which gives homogeneous broadening, to predict shifts in the resonance field is questionable since the broadening is due to inhomogeneous effects. The correlation between the observed line width and resonant frequency is most likely due to the temperature dependence of the anisotropy energy.

The physical constraint of volume limits can be considered a special treatment of the Berger model. Koseoglu *et al.* evaluated trends in M_s and K_1 as a function of nanoparticle size, assuming bulk magnetization values.³⁶ In non-constraining simulations, the “largest” particles are an important modeling parameter that result in the entire nanoparticle ensemble being modeled as having bulk magnetization properties.^{21,36} Here, the size distribution and magnetic properties were modeled by limiting the largest particle size to the inner diameter of the protein cage and

FIG. 6. The temperature dependence of the susceptibility predicted for the RS model (top). Comparison of the derivative of the susceptibility for RS and experimental data at 225 K (middle) and 30 K (bottom).

parametrically adjusted M_s and Δ_T to simulate the experimental data. The truncation of particle size provides insight into a specific case of the static model and treatment of the magnetic resonance data for nanoparticles of $d < 5$ nm. The maximum volume dependence is intrinsic to the Berger formalism and underscores the importance of “size limits” to understand EMR spectral results in metal-oxide nanoparticle systems. A nonphysical increase in the magnetic moment with increasing temperature was observed by applying size constraints with a log-normal distribution of particles in the Berger model (see Figure 5(a)).

A nonphysical temperature-dependent magnetic moment μ_p has been notably observed in nanoparticles by use of magnetometry,³⁹ where moments increase with increasing temperature. We previously have shown that the temperature-dependent magnetic moment in magnetometry may be rectified by abandoning a particle log-normal distribution of sizes and model the particles with a core moment and surface spins.²⁹ The analogous *ad hoc* assumption can also be applied to the EMR spectra, where the data were modeled with a two-component distribution function. A bimodal volume distribution gives similar fitting results as the lognormal distribution of particle volumes (see Figure 4(b)), without the nonphysical increase in magnetic moment across the entire temperature range (see Figure 5(a)). However, the physical interpretation of the narrow component is yet debatable, because it clearly follows temperature trends different from the broad component or paramagnetic iron spectra. As a result, the true origin for the narrow component is unknown; i.e., either a small population of nanoparticles at ~ 2 nm or collectively from surface spins.

The narrow component has been attributed to smaller nanoparticles^{32,40} or is perhaps due to surface spins in which the smallest particles of < 100 nm³ have a high surface-to-volume ratio.^{25,26} Nanoparticles consisting of a few unit cells may require the spin Hamiltonian formalism to give an accurate description of the EMR lineshapes, similar to the theoretical treatment molecular nanomagnets.¹⁸ Such nanoparticle structures would most likely have a resonant field that is invariant with respect to the angular dependence of the applied magnetic field, with minimal resonant influence from a weak magnetic core. Li *et al.* performed EMR on LDps iron oxide nanoparticles and showed no angle-dependence on the resonance position for the narrow component. The results suggested that the narrow component is due to a population of paramagnetic ion nucleation clusters.³⁰ For the broad component, Li *et al.* found evidence of uniaxial anisotropy by measuring the angle dependence and calculated an anisotropy value of $K_1 = 42.7$ kJ/m³ at 76 K.³⁰ They showed that anisotropy energy density increases with decreasing particle size, supporting the importance of surface anisotropy. Our results on the same system are comparable from variable temperature EMR spectra with $K_1 = 52$ kJ/m³ and magnetometry with $K_1 = 40$ kJ/m³. Therefore, small nanoparticles may have considerable surface effects that contribute to the increase in anisotropy energy.

The model of Raikher and Stepanov uses a more precise calculation of the linewidth that includes a spectral competition between superparamagnetic fluctuations (homogeneous

broadening) and a random distribution of anisotropies as a function of temperature (inhomogeneous broadening). As temperature rises, the RS model predicts that the orientational fluctuations weaken the inhomogeneous broadening arising from a distribution of anisotropy axes and strengthen the homogenous-superparamagnetic broadening. A powder-pattern spectrum is calculated at low temperatures with the asymmetry determined by the sign of the anisotropy energy (see Figure 6). As temperature increases, the spectrum shows qualitative agreement with the two-component spectra and eventually collapses into a single Lorentzian lineshape. However, a single Gaussian lineshape is experimentally observed for large nanoparticles and at high magnetic fields.^{15,33} The Landau-Lifshitz lineshape function also predicts a Lorentzian lineshape at higher temperatures and larger particles.

In contrast to paramagnetic broadening as temperature increases, superparamagnetic anisotropies undergo motional averaging due to thermal fluctuations of magnetic moments.²¹ Motional averaging requires the thermal fluctuation period to be faster than the resonant period, which is *not* the case in this temperature range, based on integration of the stochastic Langevin equation.⁴¹ Therefore, we prefer the analogy of “anisotropy melting” presented by Raikher and Stepanov for describing spectral narrowing in superparamagnetic particles EMR spectra.³¹ The spectral behavior of the narrow component displays temperature-dependent trends that are characteristic of neither paramagnetic iron, iron-sulfur clusters,⁴² nor superparamagnetic nanoparticles.⁴³ The narrow component may require a quantum-mechanical approach to fully clarify the line-broadening mechanisms and temperature trend. If the underlying physics between the narrow and broad components are different, nanoparticle systems at the quantum and classical interface may need a combination of theories to account for lineshape trends in nanoparticle EMR spectra.^{18,19,25} Therefore, the use of other methods, such as the quantum approach suggested by Noginova *et al.*, can be used for modeling the two-component lineshape EMR spectra of small metal oxide nanoparticles.²⁵ Moreover, our results also show a small but discernable signal at half the resonant position ($B_0/2$), which has been attributed to both quantum¹⁹ and classical⁴⁴ effects in nanoparticle systems. A giant-spin model may offer a more fundamental analysis and comprehension of the complex lineshape behavior of superparamagnetism in magnetic nanoparticles.²⁵

VI. CONCLUSION

Magnetic properties of iron oxide nanoparticles formed within *Listeria innocua* protein cages have been investigated by variable-temperature X-band EMR spectroscopy. These nanoparticles present an ideal case study to test existing theoretical models given their small size < 5 nm and existence of an upper size constraint. Static models, such as the Berger model, can fit the EMR temperature spectra; however, the information obtained from the simulations must be carefully evaluated, and other measurement techniques are needed to restrict the variability in the parameters when the experimental data are fitted. In the Berger model, the magnetization M_s

increased with increasing temperature with a log-normal distribution of particles and with size limits imposed by the protein cage. A two-component analysis resolves the problem of a nonphysical increasing magnetic moment, yet the origin of the narrow component remains uncertain. The temperature dependence of the saturating linewidth was used to determine the anisotropy energy. The RS model predicts the temperature dependence of the line width of the large superparamagnetic component; however, the predicted complex asymmetric line shape is not observed. We believe a comprehensive model is needed to describe simultaneously EMR spectral trends and magnetometry data that conform to consistent magnetic properties for both methodologies. To this end, accurately modeling the magnetic properties of nanoparticle systems with EMR may be used for quality control to develop finely tuned nanoparticles for multiple applications.

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