How to SPION Glass Transitions

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ABSTRACT

As nanomaterials migrate into commercial products, determining their fate is an issue of increasing importance[1]. The magnetic properties of superparamagnetic iron oxide nanoparticles (SPIONs) are used in a variety of health-related applications[2-5] even as iron oxide nanoparticles are implicated in free-radical generation[6]. Methods used for characterizing SPION structure and magnetic properties[3, 7, 8] provide limited information about their solution behavior. We address this by demonstrating that electron paramagnetic resonance (EPR) can be used in conjunction with SPIONS to detect changes in solvent composition and state. Changes in the viscosity, upon addition of methanol to the water, yield narrowed linewidths within the SPION EPR spectrum. Characteristic temperature-dependent EPR variations from SPIONs in a methanol:glycerol glass suggest SPIONs reorient upon glass melting. Pre-alignment with an external magnetic field demonstrates how SPION orientation can be monitored. The method presents an intriguing avenue for observing magnetic SPIONs in solution.

Keywords: SPION, electron spin resonance

1 INTRODUCTION

The magnetic properties of iron oxide nanoparticles make them candidates for applications such as magnetic contrast agents[9], novel surface coatings[2], and drug delivery vehicles[10]. The structural and magnetic characterizations[7] of magnetic nanoparticles provide limited information about their solution behavior. Enhanced detail of the magnetic characteristics of iron-oxide nanoparticles comes from electron paramagnetic resonance (EPR) studies. In this study, SPION EPR data reveal solvent-dependent and state-dependent changes. The method represents a unique method for accessing information about the solution behavior of SPIONs

2 EXPERIMENTAL

Magnetite SPIONs: SPION preparation is detailed in reference 3. In brief, the SPIONs were prepared in a co-precipitation reaction of $FeCl_3$, $FeCl_2{\cdot}4H_2O$, with NH_4OH. From this reaction, a black precipitate was obtained which was then heated at 80 °C for 30 min, washed several times with water followed by ethanol, and finally dried in a vacuum oven at 70 °C.

Dried samples were first washed 3x with 75 µM HCl in methanol followed by sonication in 75 µM HCl in methanol for 30 minutes using a Fisher Scientific FS-30 sonicator bath. The 75 µM HCl was empirically observed to facilitate the formation of monodisperse solutions of SPIONs. The resulting solution was approximately 1.5 mg/mL magnetite and was used as the stock solution for further experiments. An aliquot of this solution was then mixed with 75 µM HCl in glycerol to yield a working sample of 0.6 mg/mL magnetite SPION in 50:50 (v:v) methanol:glycerol. The sample was then put into a 100 µL quartz capillary that was subsequently flame sealed on both ends. To form a glass, the capillary was flash cooled with liquid nitrogen and transferred to the pre-cooled EPR cavity (180 K). For the angle studies of the magnetite SPIONS, the SPIONs were pre-aligned in a magnetic field for 20 minutes using a 5 kG permanent shaped magnet.

EPR: Spectra were acquired using Bruker X-band EMX spectrometer equipped with a TE_{101} cavity and a Bruker N_2 temperature controller. The EPR experiments used 2.0 mW of microwave power over an 8000 Gauss scan width centered at 4000 Gauss using 2048 points. The modulation amplitude was 10 G, the time constant 41 msec, and the conversion time was 82 msec. The total acquisition time for the scan was 168 seconds. A 3 K increment, or decrement, was used for the variable temperature experiments between 180↔231 K. The temperature of the instrument was allowed to equilibrate for 5 minutes prior to acquisition such that the total acquisition time for a warming, or cooling, experiment was 468 seconds. The EPR spectra are shown without y-axis labeling, arbitrary units, for clarity. To obtain the angle dependence of the SPION EPR signal, a goniometer was used to rotate the sample around an axis parallel to the applied magnetic field. An increment of 20 degrees was used along with 133 seconds equilibration time, followed by micro-tuning before each acquisition. The total time for each acquisition was 300 seconds and the duration of the experiment was ~1

hour. Data were processed using the program Octave to obtain the B_{eff} and the results were graphically presented via Excel.

3 RESULTS

Magnetite SPIONs were prepared from a reductive precipitation with ammonium hydroxide[8]. The resulting nanoparticles have a log-normal size distribution with a norm diameter of 13 nm (+/- 1 nm). The inverse spinel structure of magnetite ($Fe^{3+}{}_2Fe^{2+}O_4$) SPIONs puts Fe^{3+} ions into octahedral and tetrahedral sites while the Fe^{2+} occupies the remaining octahedral site within the unit cell of the lattice (figure 1a)[11]. Anti-ferromagnetic coupling between tetrahedral iron ions (Fe^{3+}) and octahedral iron ions (Fe^{3+}) effectively pair the spins of the Fe^{3+} ions in these positions. The result leaves electron exchange between octahedral Fe^{3+}/Fe^{2+} ions across the crystal lattice to establish the SPION superparamagnetism (figure 1b). Our 13 nm nanoparticles were suspended in 75 µM HCl in water via sonication and studied between 274 and 294 K using an X-band EPR spectrometer equipped with a liquid nitrogen cryostat (figure 1c). Magnetically dilute conditions were confirmed through double-integration of the SPION's EPR spectrum as a function of SPION concentration[12]. Above 1.6 mg/mL SPION, dipole-dipole interactions between the SPIONs lead to relaxation effects that reduce the integrated spin-count and set an upper limit for the magnetically dilute solution. The EPR spectrum is typically presented as the first derivative of the microwave power absorption by the sample as a function of the swept magnetic field (8000 G). A broad resonance is centered around B_{eff}=2774 G in the magnetite SPION EPR spectrum, representative of superparamagnetic Fe^{3+}[13, 14]. There is also the presence of a shoulder at ~4500 G, possibly the result of interior defects within the SPION's lattice. A kinetic analysis of the oxidation of magnetite using HCl revealed the magnetite→maghemite(γ-Fe_2O_3) conversion occurs within a short time, hours, and is complete within months[15]. No observable changes were detected in the EPR spectrum through the course of these experiments, with samples sitting on the bench for 2 months. Based upon this observation, we conclude the oxidation state of the SPIONs is highly stable.

Previous work with iron oxide nanoparticles in a borate glass revealed the EPR spectra depends upon the nanoparticle size distribution, shape, and temperature[16-19]. The spectra from our SPIONs show some lineshape narrowing as the temperature is raised from 274→294 K; however, overall they are fairly insensitive to changes in the temperature. This result came as a surprise because previous work with maghemite (γ-Fe_2O_3) nanoparticles in borate glasses suggested significant temperature-dependent changes should be observed in our nanoparticles[17]. The origin for this temperature effect comes from the competition between thermal fluctuations and the driving force of aligning the SPION's magnetic moments parallel to the applied field[13]. Thermal motions induce deviations from an orientation parallel to the applied field that result in broadened linewidths which subsequently undergo dramatic increases as the temperature decreases[17]. Conversely, warming results in motional narrowing within the SPION EPR spectrum.

In an effort to get below the freezing point of water, the

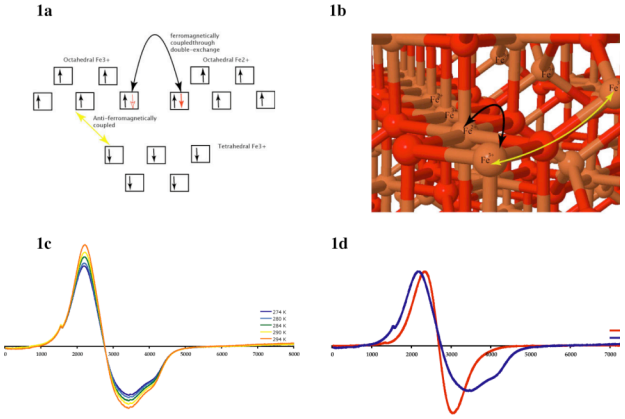

Figure 1. (a),(b) The basic spin-physics of superparamagnetism result from a double exchange mechanism. (c) The variable temperature (274→294 K), first-derivative EPR spectra from magnetite SPIONs suspended in water (~0.3 mg/mL) as function of field strength (Gauss). (d) Comparison of EPR spectra of magnetite SPIONs in water (blue) and 50:50 methanol:water (v:v, red) at 274 K. The spectra were normalized to unit peak intensity for the purpose of comparison.

SPIONs were suspended in a solution of 50:50 water:methanol. The comparison of SPIONs in water:methanol versus water (figure 1d, both at 274 K) reveals a significant sharpening of the EPR linewidth and loss of the up-field fine-structure. The viscosity of pure methanol at 274 K is roughly one-third that of water at 274 K. The narrowed EPR linewidths of the SPIONs in water:methanol appear to reflect the decreased viscosity of the water:methanol mixture; however, the resulting spectra showed limited variation as the temperature was lowered to the mixture's freezing point. Once frozen, occlusion of the SPIONs from the crystal environment and subsequent onset of inter-particle dipolar coupling could not be ruled out. As a result, another sample of SPIONs was prepared in 50:50 methanol:glycerol (v:v). This cryogenic solution forms a well-behaved glass below 195 K[20] and offered an excellent model for studying the SPION's temperature-dependent behavior. EPR spectra were recorded at 3 K increments starting at 180 K, with five minutes equilibration time between steps, to 231 K (figure 2a). Qualitative comparison with figure 1c suggests the methanol:glycerol solution mimics the viscosity of water. As the temperature increases, the B_{eff} shifts 100 G to lower field values while the linewidth narrows significantly. The

sample was then cooled in 3 K decrements, using a five minute equilibration time, back to 180 K (figure 2b). Intriguingly, the spectra display little change as the temperature decreases. Quantitative analysis of the B_{eff} shows a remarkable transition upon warming through the glass transition temperature (figure 3, red diamonds). The B_{eff} begins a transition at ~190 K that is complete by ~228 K. Of note is that the transition begins at a temperature close to the melting temperature of the methanol:glycerol

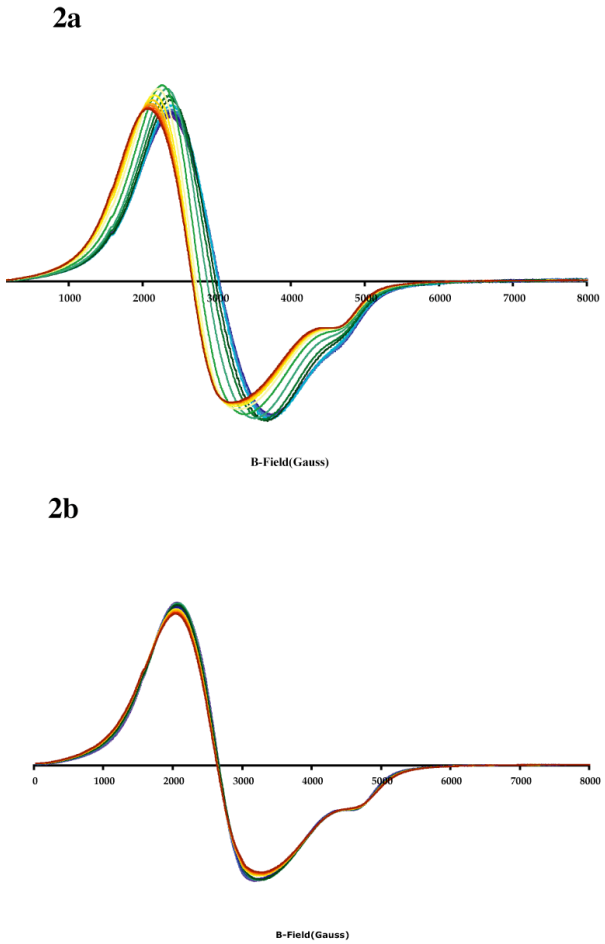

Figure 2 (a) First-derivative EPR spectra from magnetite SPIONs suspended in 50% methanol:glycerol (v:v) as the temperature is warmed from 180 → 231 K through 3 K increments or (b) cooled from 180 K(blue) to 231 (red).

glass (195 K). As the temperature rises from 195→225 K, the glass is undergoing a phase transition to a highly viscous liquid state. Above 225 K, the SPIONs are insensitive to the changes in the temperature. Remarkably, the curve is not reversible; the B_{eff} is effectively constant as the sample is cooled (figure 3, blue diamonds).

Within the experimental constraints of the EPR experiment where the SPION's Zeeman interaction dominates, the effective magnetic field at the SPION is the sum of three components

$$B_{eff}=B_{appl} + B_a + B_d$$

where B_{appl} is the product of the SPION's Zeeman interaction with the applied field while B_a and B_d are the vector projections of the SPION's anisotropic and demagnetizing contributions upon the applied field, B_o. The projections of B_a and B_d upon B_o are defined by the polar and azimuthal angles θ and ϕ[16, 21]. The angle dependence of B_a and B_d means that the B_{eff} provides

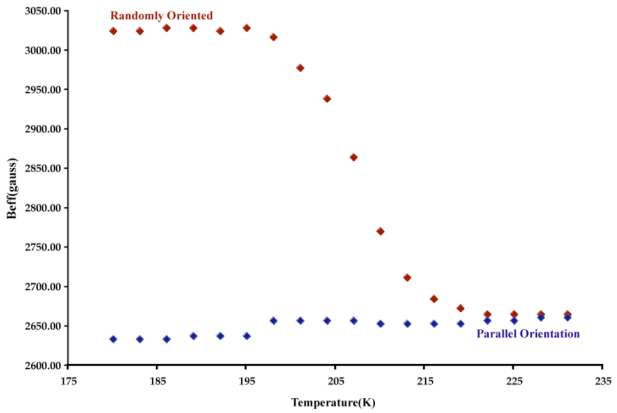

Figure 3 The B_{eff} (Gauss) as a function of temperature: warming from 180→231 K (red triangles) while cooling 231→180 K (blue triangles).

information about the orientation of the SPIONs. As a test of this, the SPIONs were pre-aligned using a 5 kG shaped permanent magnet prior to forming the glass. The shaped permanent magnet has its flux lines approximately parallel to the applied field of the EPR spectrometer. The ensemble of SPION magnetic moments should align roughly parallel to the spectrometer's applied field; however, due to field inhomogeneity, some distribution was expected with the SPION orientations. After 20 minutes in the field of the permanent magnet, the sample was converted to a glass and examined by EPR. A goniometer was used to probe the θ-dependence of B_{eff} (figure 4a) prior to melting the glass. The B_{eff} displays periodic behavior with a period of 120 degrees. The minimum value of the B_{eff} is only ~10 G lower than the value of the unaligned sample; however, the amplitude of B_{eff} variation is ~90 G as a function of θ. When the sample is warmed to 231 K and the θ-dependence of B_{eff} obtained, significant differences result (figure 4b). The period is greater than 180° and, interestingly, displays hysteresis behavior. The amplitude of the angle dependence has reduced to 30 G and the minimum B_{eff} is ~100 G lower than that obtained from the non-aligned sample (figure 4, blue). Efforts to fit the data using equations for the B_{eff} [17] were unsuccessful and suggest the B_{eff} is capturing both the ϕ- and θ-dependence. This suggests the ensemble average of the SPIONs orientation has not yet reached its minimum with respect to the applied magnetic field. The SPION's magnetic moment induces

the particles to move along the magnetic field gradients; a property useful for isolating SPIONS[5]. This property may also explain the increased time required for the SPIONs to reach their minimum with respect to the applied field. Localized concentration gradients may have occurred upon placement in the shaped magnet that effectively

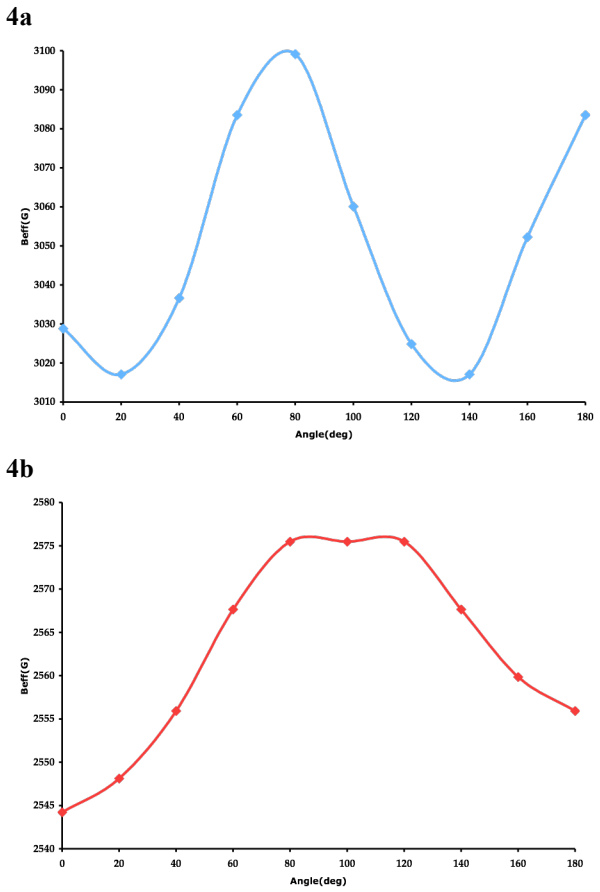

Figure 4. The B_{eff} as a function of θ (degrees) after being aligned in a magnetic field (a) and after melting the glass (b). In both figures, the line is intended only as a guide for the viewer.

overcome the magnetically dilute conditions established at the start of the experiment. One consequence of this would be cooperative magnetic interactions between SPIONs[22] that require longer times for thermal motion to overcome. An essential challenge facing SPION applications is monitoring SPION progress within the environment. We have demonstrated here that EPR is a useful tool for observing the state of the SPION environment in solutions.

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