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# ANOMALOUS FIELD DEPENDENCE OF BLOCKING TEMPERATURE OF NATURAL HORSE-SPLEEN FERRITIN

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## Abstract

As the number of people who use computers grows each year, so does the need for better ways to record and preserve information. To meet that need, materials that can rapidly store and retrieve huge amounts of digital data need to be developed. The key to achieving reliable high-density memories lies in nanotechnology. For a recording material to move data in and out of storage speedily, its surface must have magnetic grains as small as 10 nanometers across. Such materials can be found in nature; the iron core in a ferritin protein is a perfect example. This study attempted to determine the magnetic properties of these iron cores, utilizing a magnetometer to test horse-spleen ferritin. It was found that the protein has several unique characteristics that make it a prospective material for the hard drives of the future.

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## 1. Introduction

A magnetic field applied to a paramagnet rotates the magnetization parallel to the field. However, this alignment is opposed by thermal fluctuations of the individual magnetic ions so that they become randomized when the field is removed. This behavior is called paramagnetic and the temperature-dependent paramagnetic *susceptibility* is given by

$$\chi_{\text{para}} = \frac{N\mu^2}{3kT},$$

where  $N$  is the number of atoms with unpaired electrons per cubic cm,  $\mu$  is the average magnetic moment per paramagnetic atom,  $k$  is Boltzmann's constant and  $T$  — the absolute temperature in kelvin [1].

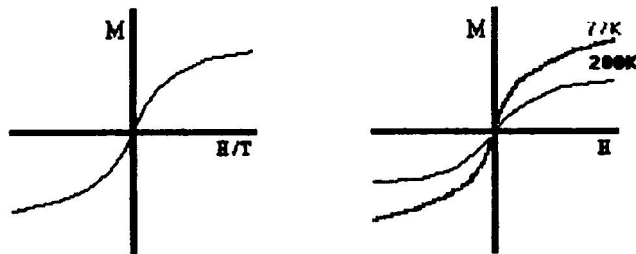
Some substances contain unpaired electrons that are magnetically coupled to neighboring atoms. This so-called exchange interaction results in a strong spontaneous magnetization in the absence of an external field, and in the remarkable property that the material retains the alignment imparted by an applied field after the field has been removed. This type of behavior is termed ferromagnetic and is referred to in everyday terms as "magnetic".

The effective magnetic moment of a ferromagnetic particle is determined by its size. A ferromagnetic sample with a volume greater than a critical value  $V_c$  divides into multiple magnetic domains, each magnetized along the local *easy axis* but in one of two opposite directions. The multiple domain structure is, however, no longer favorable below the critical volume, and the particle becomes a single domain with ferromagnetic alignment of all its moments along the easy axis in the same direction. Thermal fluctuations of the moment exist on a microscopic scale, but to reverse the direction of the single domain's magnetization requires an energy  $\Delta E$  to overcome the crystal-field anisotropy. Making the particle small enough releases moments from their

constraints, permitting the magnetization of the single-domain particle to fluctuate between the two easy-axis orientations, as in an ideal paramagnet. This is called superparamagnetism. The probability of such a reversal by thermal activation is proportional to  $\exp(-\Delta E/kT)$  [2, 3]. This differs from conventional paramagnetism because the effective moment of the particle is the sum of its ionic particles, which can be several thousand spins in a ferromagnetic particle small enough to show superparamagnetism.

Very fine ferromagnetic particles have very short relaxation times even at room temperature and behave superparamagnetically; that is, their behavior is paramagnetic but their magnetization values are typical of ferromagnetic substances. The individual particles have normal ferromagnetic moments but very short relaxation times so that they can rapidly follow directional changes of an applied field and, on removal of the field, do not hold any remanent moment [2].

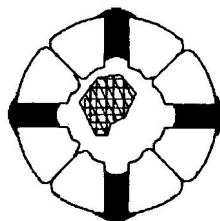
Superparamagnetism is characterized by two experimental features, also possessed by ordinary paramagnets (hence the name): there is no hysteresis in the field dependence of the magnetization ( $M$  versus  $H$  is a single-valued curve at a given temperature) and  $M$  is a universal function of  $H/T$ .



Superparamagnetism can be destroyed by cooling. This follows because the characteristic fluctuation time for a particle's moment varies exponentially with temperature, so the magnetization appears to switch sharply to a stable state as the temperature is reduced. The temperature at which this occurs is called the blocking temperature, and it depends linearly on the sample's volume and on the magnitude of the crystal-field anisotropy [4].

Some examples of superparamagnetic materials can be found in organic substances. Ferritin, an iron storage protein, is believed to be a model superparamagnet [5].

The ferritin molecule may be thought of as a protein cage with an iron core. The iron core is an inorganic complex originally assigned the formula  $(\text{FeOOH})_8(\text{FeOPO}_3\text{H}_2)$  and now often known by the name ferrihydrite. Ferritin's iron core is of variable size. Its maximum dimension, limited by the space inside the protein, is about 80 Å, and in a complete core there are between 4000 and 4500  $\text{Fe}^{3+}$  atoms. However, most ferritin preparations contain molecules with little or no iron core (apoferritin). The iron complex occurs as small microcrystalline particles. Within each ferritin protein cage there may be one, or even a few particles, together with some less well ordered material [6].



Ferritin's molecular architecture and its ability to take up and give up iron shows it to be well designed to act as an iron reservoir and a means of protection against *decompartmentalized* iron. Free iron may give rise to toxicity, mainly owing to its ability to catalyze the formation of reactive oxygen radical species [7].

Studies have shown that the nature of the magnetic interactions of iron in ferritin is relatively complicated. An early investigation of the magnetic susceptibility of ferritin at room temperature indicated that the effective moment of iron is 3.8 Bohr magnetons ( $\mu_B$ ), a value that is considerably lower than that predicted from the spin-only value of  $\approx 5.9\mu_B$ . A more recent investigation of the magnetic susceptibilities of ferritin over wide temperature ranges has shown that the iron atoms in ferritin exhibit antiferromagnetism. The Néel temperature, the critical temperature above which antiferromagnetic behavior disappears, has been determined to be  $\approx 240$  K [8].

The two main goals of the work presented herein were: (1) to determine the dependence of the blocking temperature on the applied field, and (2) to establish how the applied field affects the magnetic relaxation rate of ferritin.

## 2. Materials and methods

The material that was utilized in our experiments is commercial horse-spleen ferritin (100 mg/ml), obtained from Sigma Chemical Co. Commercial horse-spleen apoferritin (50 mg/ml) and 0.15 M NaCl solution were used to dilute the ferritin. In order to measure a sample, sample holders had to be prepared first. A non-magnetic epoxy (Stycast 1266) was filled into a soda straw and allowed to set. The set epoxy was extracted from the casing and cleaned in methanol, which etched the surface of the epoxy. Then the epoxy rod was cut into several  $\approx 0.5$  mm disks. A disk was inserted into an ordinary soda straw, and a small amount of silicon vacuum grease was used to hold it in place and make a watertight seal. 100  $\mu$ L sample of ferritin solution was placed in the vessel described above and flash frozen in liquid nitrogen. Other techniques, such as creating a vessel out of a piece of a soda straw were tried, but the sample holder made with epoxy proved to be the best since it could hold a sample of a desirable size. This vessel produced a very small diamagnetic background moment, indifferent to the moment of the sample.

### 2.1. Experimental procedure

All experiments were carried out in a Quantum Design MPMS5 magnetometer. Data taken included the applied field, temperature, magnetic moment of the sample, and time. However the independent variable for each experimental procedure was different.

#### 2.1.1. Moment vs. temperature

To obtain a temperature-dependent magnetic moment curve a sample was zero-field cooled (ZFC) according to the following procedure. After the sample was placed into the magnetometer, at  $\approx 40$  K, the magnetic field was turned to zero, and the temperature decreased to 5 K. Then a measuring field was applied to the sample. The temperature was the independent variable in this experiment: after the desired measuring field was attained, the temperature was systematically raised from 5 K to 100 K and measurements of the magnetic moment of the sample were taken at 0.25 K increments. The procedure was then repeated, but this time the sample was cooled in a field. The temperature was again increased from 5 K to 100 K, and measurements were taken at 0.25 K increments. The ZFC and field-cooled (FC) procedures were repeated for different measuring fields.

In order to establish how the intensity of interactions depends on the distance between the ferritin molecules, dilutions of the ferritin solution (50%, 25%, 13%, 6%, 3%, and 1% ferritin solution) were made by mixing the 100% solution with 0.15 M NaCl solution. Some samples were also diluted in apoferritin and freeze-dried. The ZFC and FC data showed that the diluted and freeze-dried samples behaved essentially similar to the 100% ferritin sample, indicating that interparticle interactions played little role in the temperature and field regimes in which we worked.

#### 2.1.2.. Hysteresis loops

To produce a hysteresis curve, a sample was cooled to a specific temperature. Once the desired temperature was achieved, measurements of the sample's moment as a function of field

began. The field was slowly swept from  $-5.5$  T to  $5.5$  T and then back to  $-5.5$  T. This experiment was performed at three temperatures: 5 K, 12 K, and 40 K.

### 2.1.3.. Magnetic relaxation

An external field of 5000 G was applied to a  $100 \mu\text{L}$  sample of ferritin solution, and the sample was cooled to 5 K. Once the desired temperature was reached the applied field was suddenly changed to a different, lower value. The magnetic moment of the sample was recorded every  $\approx 10$  seconds. The range of the lower field was 1000 G to  $-4000$  G. The relaxation experiments were also performed at 12 K.

## 3. Results and discussion

Figure 1 shows the zero-field-cooled and field-cooled magnetization curves as a function of temperature taken in an applied field of 100 G. The two curves show different behavior at low temperatures. As the temperature increases the magnetic moment in the FC curve decreases. However, as the temperature begins to rise from 5 K, the moment in the ZFC curve begins to increase. At a certain temperature the ZFC curve reaches a peak — that temperature is the *blocking temperature*<sup>1</sup> ( $T_b \approx 11$  K). Then the moment decreases as the temperature increases. At  $\approx 21$  K, the point of irreversibility, the two curves converge and show the same decreasing trend with the increasing temperature. At  $T \geq 30$  K the curves obey the Curie-Weiss law  $\chi = C/(T - T_c)$ , where  $C$  is the Curie constant and  $T_c$  is a temperature that characterizes the interactions between magnetic particles. By fitting the data above 30 K to this form, we have determined  $C = 2.23 \times 10^{-6} \text{ K} \cdot \text{emu} \cdot \text{G}^{-1}$  and  $T_c = -1.25$  K. The low absolute value of  $T_c$  confirmed that the interparticle interactions played an insignificant role in the temperature regimes in which we worked. Using

$$C = N\mu^2/3k$$

we calculated the average magnetic moment of one ferritin molecule to be  $\approx 173\mu_B$ , for a sample in which  $N = 3.71 \times 10^{14}$  ferritin molecules.

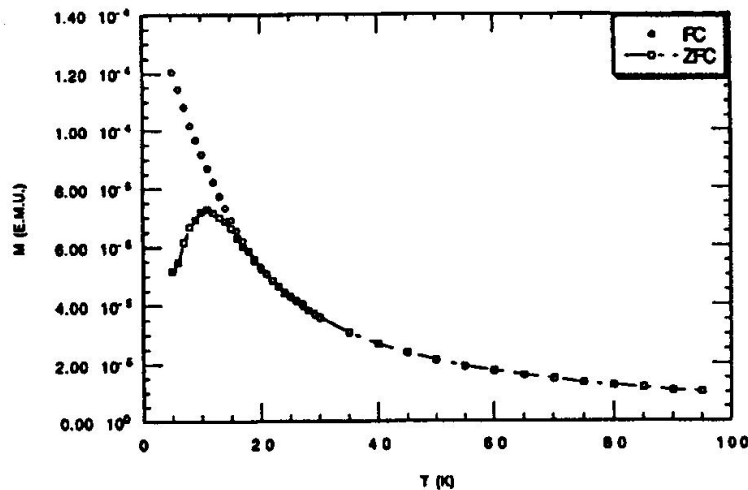


Fig. 1. ZFC and FC curves of  $10 \mu\text{L}$  sample at 100 G.

We attempted to determine the Fe content of our sample by utilizing an optical spectrometer to obtain the extinction coefficient (absorbance per mg/ml of iron in the ferritin sample) of the sample [9]. The results showed that the sample contained  $\approx 1/4$  of the iron estimated from assays forwarded with the sample. The lower extinction coefficient indicates a higher average magnetic moment per ferritin molecule. Therefore, the moment per ferritin could be as high as  $346\mu_B$ .

<sup>1</sup>Temperature at which the time to be thermally activated over the energy barrier becomes comparable to the measurement time.

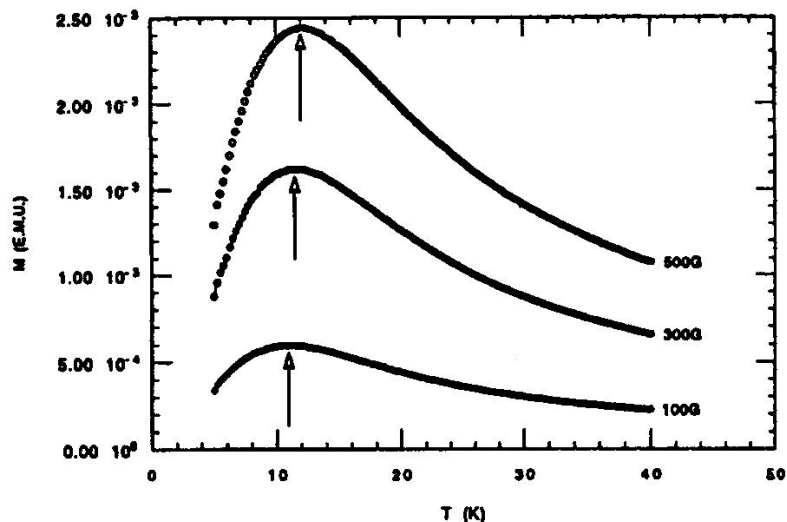


Fig. 3. Moment vs. temperature at different fields

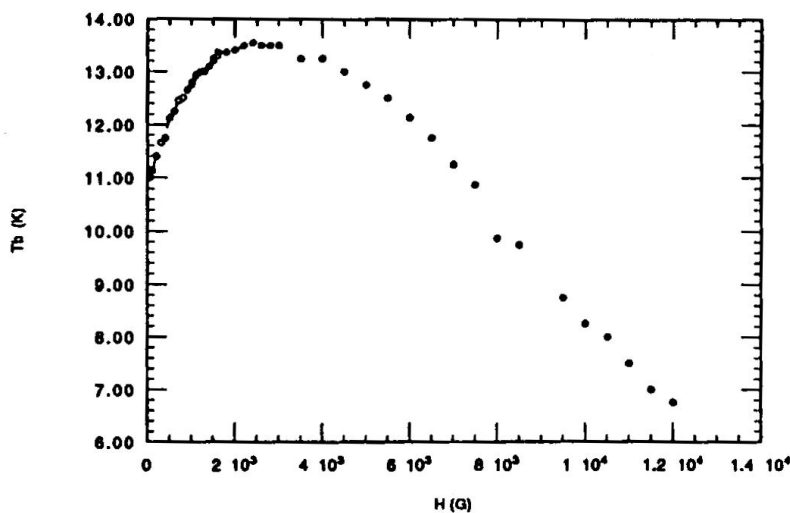


Fig. 3. Blocking temperature vs. field.

In theory, the blocking temperature of a substance should decrease with the increasing applied field and eventually disappear when the field reaches a critical value ( $H_c$ ). The higher field is expected to lower the barriers between the two easy axis orientations. However, our experimental data show that this is not strictly the case for the iron cores of ferritin. Figure 2 shows the zero-field-cooled curves at three different measuring fields, as indicated. The blocking temperature (indicated by the arrows) increases as the field increases, implying that the effective energy barriers between the two easy-axis orientations are increasing with field. Figure 3 describes in detail how the blocking temperature depends on the applied field.  $T_b$  increases, peaks at  $\approx 3$  kG and then decreases. The anomalous data is real; it is easily reproducible, and from Fig. 3 one can see that consistent results arise from two distinct samples, both with the same concentration.

We had also observed the same phenomena with a 1% ferritin/99% 0.15 M NaCl sample. The same qualitative effect occurs:  $T_b$  rises with  $H$  for low fields. However the peak occurs at a slightly lower field and the blocking temperatures are on the whole a bit lower. Our hysteresis experiments have also produced interesting results. Figure 4 shows an obvious anomaly in the hysteresis loop at 12 K. At zero field, the region where an ordinary hysteresis graph is the widest, the graph looks as if it is pinched closed at 12 K. The points are not identical, but they are certainly closer than expected. This aberration, though less obvious, is also present in the hysteresis loop at 5 K.

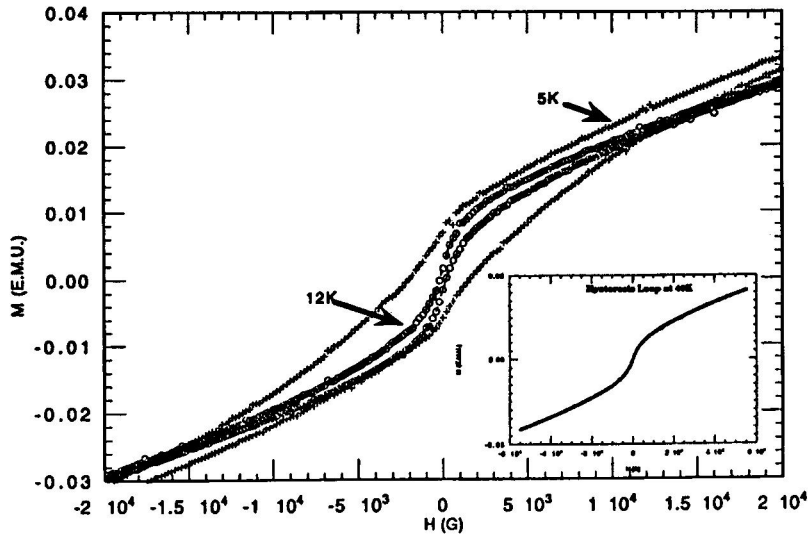


Fig. 4. Hysteresis loops at 5 K and 12 K.

Based on both, the anomalous  $T_b$  and the hysteresis data, we expected to observe the fastest relaxation rate at near zero field. The blocking temperature has a minimum at zero field, and the hysteresis curves show that the magnetization responds rapidly to a change in the applied field at around zero field.

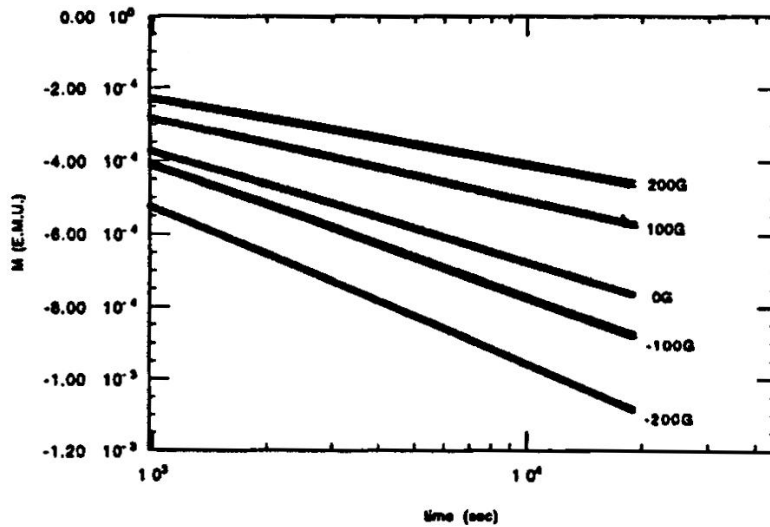


Fig. 5. Relaxation rate at different fields at 5 K.

The relaxation rate (magnetic viscosity) for every field was obtained by determining the slope of the graph of magnetization vs. time in the linear regime on a semi-log plot (Fig. 5). The viscosity ( $S$ ) was normalized by the difference between the initial and asymptotic (equilibrium) magnetization values =  $(dM/d\ln \text{time}) / (M_i - M_f)$ .  $M_f$  was determined from the equilibrium hysteresis curve for the 12 K hysteresis loop, calculated by rescaling the 40 K hysteresis curve. The points on that curve were assumed to be the final magnetization values ( $M_f$ ) at a specific field. The value used for the initial moment ( $M_i$ ) of the sample was the first magnetization measurement, during the relaxation experiment, after the applied field was changed.

In spite of our previous results we did observe any extraordinary behavior in the region around zero field, as shown in Fig. 6. The magnetic viscosity decreases as the measuring field increases. At  $\approx -600$  G  $|dS/dH|$  begins to sharply increase. The point of inflection appears to be at  $\approx 0$  G. As the field becomes positive,  $|dS/dH|$  starts to decrease until  $\approx 600$  G; beyond 600 G the magnetic viscosity still shows a decreasing trend, however, at a much slower rate.

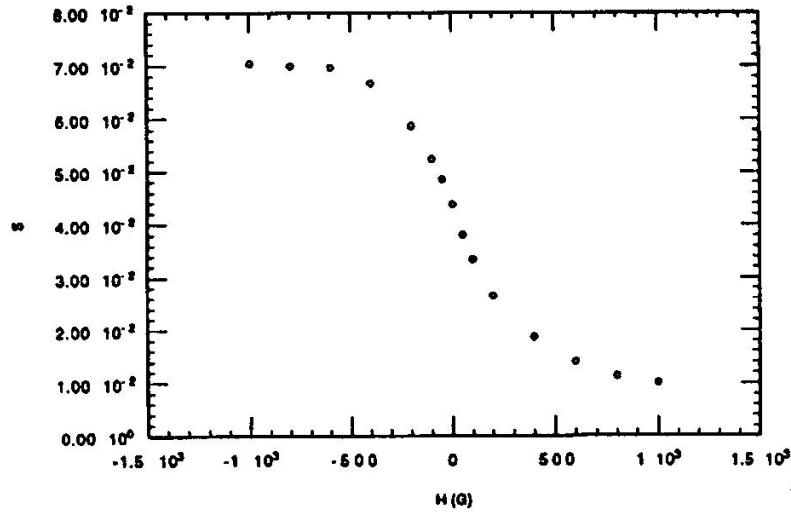
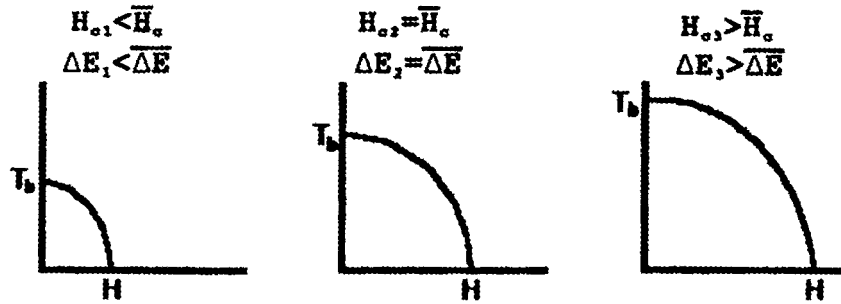


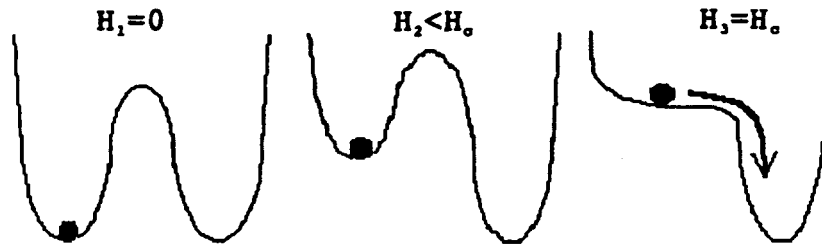
Fig. 6. Magnetic viscosity at 5 K.

The remarkably symmetric magnetic viscosity graph at 5 K appears to be smooth in all regions and shows no apparent anomalies at around zero field. We observed similar behavior at 12 K.

A possible explanation for the anomalous blocking temperature behavior (Fig. 3) is that the sample is composed of several species of ferritin, each categorized by an  $H_c$  and  $\Delta E$ . For simplicity, let us suppose that there are three distinct groups of ferritin



As shown in the diagram above, each group responds ideally to the increase in the applied field. As the field increases the value of  $T_b$  decreases. This response is due to the decrease in the energy barrier between the two easy axis orientations (ground states).



Since a particle needs less energy to overcome the crystal-field anisotropy, a smaller amount of thermal energy would reverse the particle’s magnetization. Thus the blocking temperature should decrease with increasing field. However in our model, the sample is composed of the three groups, and in combination they can behave quite differently.

At a low field ( $< H_{c1}$ ) all particles are frozen at low temperatures. The blocking temperature of the whole sample is the average of the blocking temperatures of the three groups. When  $H > H_{c1}$ , the first group is completely unblocked at all temperatures. The blocking temperature is then the average of the second and third groups, a larger value. In reality, there are not three

groups but a myriad of them. Each molecule has its own  $H_c$  and  $\Delta E$ ; thus the curve appears to be smooth, instead of having steps, as it would if the system had definite groups. Generalizing, at low fields  $|dN/dH|$  ( $N$  is the number of molecules having  $H_c$  greater than the applied field) is larger than  $|dT_b/dH|$ . The sample's  $T_b$  increases at all fields where  $|dN/dH| > |dT_b/dH|$ . The blocking temperature begins to decrease only when  $dN/dH \approx dT_b/dH$ . Since  $T_b$  of the sample begins to decrease at 3 kG, this is approximately the median  $H_c$ . Thus we can conclude that  $\overline{H_c} \approx 3$  kG in this system. Note that the low-temperature (5 K) hysteresis has a coercive field of  $\approx 1.7$  kG, and would be higher at lower temperatures.

#### 4. Conclusion

As the number of people who use computers grows each year, so does the need for better ways to record and preserve information. To meet that need, materials that can rapidly store and retrieve huge amounts of digital data have to be developed. The key to achieving reliable high-density memories lies in nanotechnology. For a recording material to move data in and out of storage speedily, its surface must have magnetic grains as small as 10 nanometers across [10]. With this kind of material, Stephen Y. Chou, a material scientist at the University of Minnesota in Minneapolis, is planning to make disks capable of recording 65 billion bits of data per square inch. To record on the new disks, one need only “flip” the magnetic orientation of each grain. To store a bit of data, a recording head magnetizes a tiny particle with its north pole pointing up. To erase it, the same head reverses the magnetic polarity. Because each grain has only two possible magnetic states, these magnetic disks do not require a recording head to track as precisely as current systems do, an advantage that would make the new disks faster and more accurate [10].

Ferritin's magnetic properties make it a contender to be the material utilized in the hard drives or tape drives of tomorrow. It is easily and cheaply synthesized. However more studies have to be conducted, to take significant steps toward the creation of the ferritin drive. In addition, the ferritin protein could serve as a biomagnetic coating for imaging structural defects in ferrous materials. Ferritin can also be utilized in enhancement of magnetic resonance image and the orientation of biological assemblies [11].

This project will continue. Further research will focus on ferritin solutions containing iron cores of almost identical size in order to have better controlled data and study the dependence of the blocking temperature anomaly on particle size.

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## References

1. D.H. Tarling, in: *Principles and Applications of Palaeomagnetism*, Santype Ltd., Great Britain 1971, p. 71
2. *Ibid.*, p. 15
3. L. Gunther, *Phys. World* **3**, 28 (1990)
4. C.M. Hurd, *Contemp. Phys.* **23**, 469 (1982)
5. S.H. Kilcoyne, R. Cywinski, *J. Magn. Magn. Mater.* **140-144**, 1466 (1995)
6. T.M. Loehr, in: *Physical Bioinorganic Chemistry Series: Iron Carriers and Iron Proteins*, Vol. 5, 1989, p. 125
7. *Ibid.*, p. 127
8. *Ibid.*, p. 158
9. M.E. May, W.W. Fish, *Arch. Biochem. Biophys.* **190**, 720 (1978)
10. R. Lipkin, *Science News*, April **22**, 245 (1995)
11. S. Gider, D.D. Awschelom, T. Douglas, S. Mann, M. Chaparala, submitted for publication