

A magnetometer utilizing quantum superconductivity as the basis for the flux sensor element has been designed and used for biochemical susceptibility measurements in the temperature range from 1.5°K to 300°K. The sensitivity and reproducibility of this instrument have been tested by measurements on small amounts of material of well-known susceptibilities. Using this instrument the temperature dependence of the magnetic susceptibilities of oxy- and metaquochemerythrin have been measured and for the first time their anti-ferromagnetic components have been unambiguously resolved. From this data the exchange coupling constants between the two high-spin iron (III) atoms in each subunit have been determined to be -77 and -134 cm⁻¹ respectively.

I. Introduction

Magnetic susceptibility measurements for years have been a standard technique used in chemical investigations of metal ion containing substances providing information about structure, oxidation states and bonding. A need exists, however, for improved sensitivity for demanding experiments on weakly magnetic biochemical compounds.

After discovery of the Josephson effect and quantum phase coherence in superconductors, it was soon realized that a new type of sensitive instrumentation could be developed which would detect very small changes in magnetic fields. Although at one time this possibility was drawn to the attention of workers in the chemical fields^{1,2}, the development of these capabilities has remained within the realm of low temperature physics.

A cryogenic quantum magnetometer system has been developed which employs the principles of quantum superconductivity for the sensor as well as superconducting magnets and shielding to provide the steady, noise free magnetic fields necessary for these sensitive measurements. Even in this early state of development, this scientific tool is already capable of measuring static susceptibilities over a broad temperature range with sensitivity and reproducibility unmatched by conventional techniques.

A description of the apparatus and its methods of use are given. Data taken with some previously characterized chemical substances are presented which demonstrate some of the special features of the system. Also the application of the system to measurements of proteins containing low concentrations of metal ions is demonstrated.

II. Experimental Apparatus

A simplified schematic diagram of the experimental arrangement is shown in Fig. 1 which also includes the essential electronic detection circuit. The specimen (A) is placed inside a thin extension of a glass dewar (B) surrounded by a

superconducting solenoid (C) immersed in a liquid helium bath with its temperature stabilized to within 1 m°K. The details of the specimen region are shown in Fig. 2. The solenoid is enclosed by a superconducting shield and the dewars are mounted inside a Mu-Metal shield. The entire dewar system is shock mounted to minimize the effects of vibration. To provide adequate electromagnetic shielding, the entire experiment is carried out in an rf-shielded room.

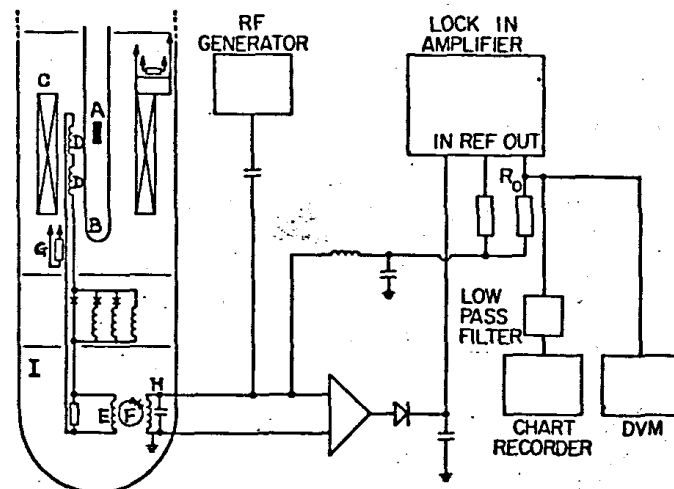


Fig. 1. Schematic diagram of the magnetometer including the electronic detection system: (A) sample, (B) inner glass dewar, (C) superconducting solenoid, (D) pick-up coils, (E) secondary coil of flux transformer, (F) superconducting sensor, (G) heat switch for flux transformer, (H) RF tank circuit, (I) superconducting sensor compartment. R_0 is the feedback resistor.

Any change in the magnetic flux in the sample compartment (caused by either the introduction of the sample or a change in its susceptibility) is detected by two superconducting pick-up coils (D) which are constructed to automatically compensate the magnetization changes due to sample holder and its surroundings to within 0.15%. These coils and the secondary coil (E) comprise a dc superconducting flux transformer which transforms any flux change in the sample (A) to the superconducting sensor (F). A heat switch (G) is provided in the flux transformer line to open up the transformer circuit and isolate the sensor during large changes in the magnetic field of the solenoid. In this way the magnetic field around the sensor can be kept less than 1 mG. The flux transfer ratio was optimized for a three coil-system composed of the primary gradiometer coil around the sample, the secondary coil around the sensor, and leads that connect the primary to the secondary with inductances L_1 , L_2 and L_3 respectively. From fluxoid conservation one can easily show that maximum flux transfer between sample and sensor occurs when

$L_2 = L_1 + L_3$ in the flux transfer relation

$$\frac{\phi_2}{\phi_1} = \frac{n_1}{n_2} \frac{L_1}{L_1 + L_2 + L_3}$$

where ϕ_1 and ϕ_2 are the fluxes through the primary and secondary coils respectively, and n_1 , n_2 are the numbers of turns of primary and secondary coils respectively. To insure optimal flux coupling between the sensor and the flux transformer secondary, the secondary coil is wound directly around the thin film sensor and the length of the secondary coil exceeds that of the sensor. Our maximum flux transfer ratio has been 22% which is within a few percent of the theoretical limit. The flux transfer ratio (sensitivity of the system) can be changed from 0.2 to 10^{-4} in four steps by successively breaking superconducting wires which short out inductors placed in series with the pick-up coils. The superconducting sensor consists of a thin, cylindrical, superconducting film with one weak link due to a non-superconducting (normal) metal underlayer deposited on a 3-mm diameter glass rod. Both this ring and an rf tank circuit (H) which is inductively coupled to the ring are placed in a separate superconducting compartment (I) which further shields the electronics from unwanted external fields.

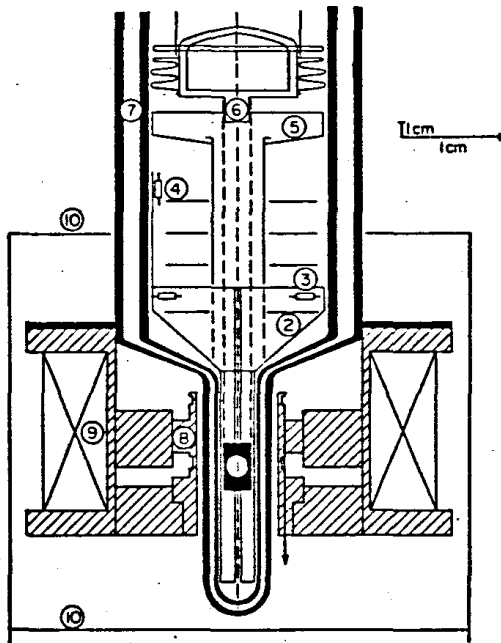


Fig. 2. Details of the sample region: (1) sample, (2) copper shield, (3) thermometer, (4) heater, (5) radiation shield, (6) connector for sample holder, (7) inner glass dewar, (8) flux transformer coils, (9) superconducting solenoid, (10) superconducting shield in He dewar.

The details of the operations of the sensor and the electronic detection system have been described elsewhere,^{3,4} but since this is a relatively unfamiliar device, we give a brief description of the operation of the complete system. We start with the case where the feedback loop is open, i.e., R_0 (in Fig. 1) is taken out. An rf current is introduced to the tank circuit at the resonant frequency ω . If, in addition, there is a

low frequency sample-related flux ϕ_{DC} enclosed by the ring, then the resultant Emf in the ring at frequency ω becomes a periodic function of the flux ϕ_{DC} and is given by:³

$$\text{Emf} \propto \phi_{rf} \omega J_1 \left(\frac{2\pi \phi_{rf}}{\phi_0} \right) \cos \left(\frac{2\pi \phi_{DC}}{\phi_0} \right) \sin \omega t$$

where J_1 is the first order Bessel function, ϕ_{rf} is the rf flux in the ring and the flux quantum $\phi_0 = hc/2e = 2.07 \times 10^{-7} \text{ G}\cdot\text{cm}^2$. In the usual mode of operation the Emf is maximized by choosing the drive flux ϕ_{rf} in such a way that the induced current in the superconducting sensor reaches the critical current of the weak link at the peak of each cycle, making $J_1 \approx 1/2$. This Emf is then amplified and rectified to produce a dc "signal voltage" which is only a function of the sample-related flux ϕ_{DC} :

$$V_{DC} \propto \omega \phi_0 \cos \left[2\pi \left(\frac{\phi_{DC}}{\phi_0} \right) \right]$$

Thus the signal is a periodic function of the sample-related flux with period ϕ_0 and the flux sensitivity is independent of the background field. Actually in order to eliminate dc drifts in the instrument the above dc signal is usually converted to low frequency ac by applying a small, low frequency modulation to the device. The amplitude of this ac signal is also periodic in ϕ_{DC} as above. In order to linearize the system this signal is used to drive a feedback loop in such a way as to exactly compensate for flux changes due to the sample. The magnetometer is thus "locked on" to some specific value of the magnetic flux enclosed by the sensor. A change in the flux $\Delta\phi_{DC}$ due to the susceptibility change in the sample is compensated by feeding the dc output signal of the lock-in amplifier back into the rf coil so as to generate an opposing field. The compensation current thus depends linearly on the flux change $\Delta\phi_{DC}$. A digital readout of the voltage across the feedback resistor R_0 can be accurately converted into the corresponding value of $\Delta\phi_{DC}$ in terms of the flux quantum ϕ_0 since the ϕ_0 -voltage ratio can be determined to within one part in 10^4 .

The response time of the sensor is roughly ω^{-1} , at least up to the gap frequency of the superconductor, $\omega \sim 10^{12}$, and thus is not a practical limitation. In the operation described here the response time was typically 1 sec and was set by requirements on the amplifier noise.

III. Experimental Procedures

At a fixed temperature and magnetic field, the total susceptibilities $\chi(T_0)$ and magnetic moments $\mu(T_0)$ are measured by moving the sample from one pick-up coil into the other with the phase and frequency of the signal remaining locked on. The output voltage of the lock-in amplifier is recorded. Digital data are taken at the initial and final positions of the sample. The signal can change beyond the range provided by the phase sensitive detector because a skip of an integral mul-

tiple of the flux quantum Φ_0 is allowed and can be counted.

Changes of susceptibility as a function of temperature can be determined by first adjusting the sample position to be within the pick-up coil with the aid of the digital voltmeter read-out and then the sample temperature is changed with the phase sensitive detector locked-on while the system records changes in the flux at the sample. The background effect of the sample enclosure can be measured when the sample is moved out of the pick-up coil area. However, the effect of the sample holder is automatically compensated by the use of a long uniform synthetic quartz holder extending through both compensated pick-up coils. Thus accurate relative changes in susceptibility $\Delta\chi(T)$ and magnetic moments $\Delta\mu(T)$ can be made. The total susceptibility of the sample does not enter into the measurement. Thus small changes in strongly magnetic samples can be measured at the same sensitivity as small changes in diamagnetic materials.

For measurements in the range of $1.3^\circ - 5.2^\circ\text{K}$, liquid helium is introduced into the inner dewar and the temperature is regulated by controlling the helium vapor pressure. For investigations in the temperature range of 2° to 300°K , a copper foil of high thermal conductivity and high purity surrounds the sample which provides a uniform temperature distribution around the sample area and allows one to make remote temperature changes and measurements. The sample is first cooled down to 2°K (approximately the temperature of the helium bath under working conditions) by first introducing about 10^{-3} torr of helium exchange gas into the vacuum space of the inner dewar and about 10 torr of purified coupling gas in the inner dewar. To raise the temperature of the sample, the helium exchange gas in the dewar vacuum space is removed by a helium cryogenic adsorption pump. Above 20°K an electrical heater is used to raise the temperature of the sample by warming the copper foil which surrounds the sample.

IV. Tests of the System

The flux transformer was initially calibrated with a superconducting sample which has the well established volume susceptibility, $-1/(4\pi)$ emu/cm³ characteristic of superconducting metals. The detection system is calibrated by determining the Φ_0 to voltage ratio.

Tests of the system have been performed with chemical compounds of well known susceptibilities to demonstrate the ability of the system in obtaining data with small quantities of materials. The temperature dependence of the susceptibility for a randomly oriented single crystal of 164 microgram of hydrated copper sulfate was determined in a magnetic field of 47 G (not kG). In figure 3, the susceptibility data for $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ are plotted versus temperature. The data points represented by solid circles are total susceptibility points while the open circles come from relative measurements with respect to the 38°K point. From a similar plot of inverse susceptibility versus temperature one can derive the magnetic moment $\mu(6^\circ\text{K}) = 1.81 \pm 0.02\mu_B$ /copper(II) ion

and a Curie-Weiss temperature $\theta = (0.6 \pm 0.2)^\circ\text{K}$ which compare favorably with data obtained with conventional systems for sample quantities four orders of magnitude larger.⁵

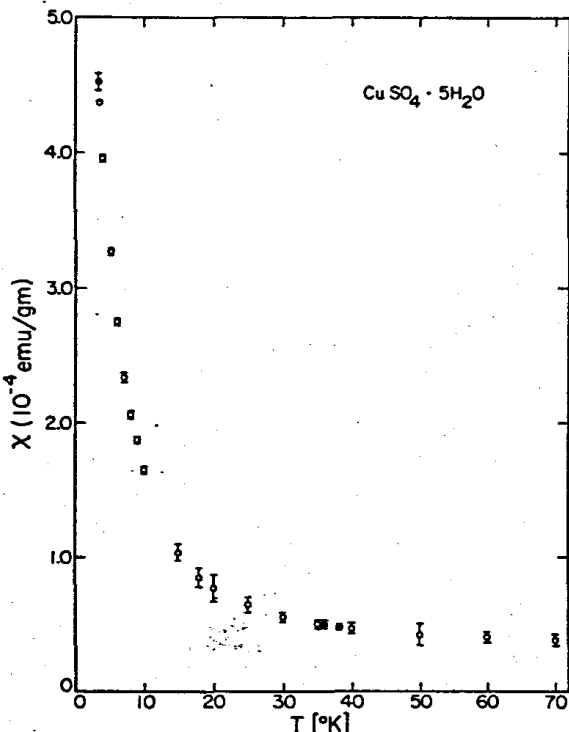


Fig. 3. Susceptibility versus temperature for 164 μg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in a magnetic field of 47 G. Data shown as solid and open circles are obtained from measurements of total susceptibilities and susceptibility changes respectively.

The ability to work with near-zero susceptibilities has been similarly demonstrated by measurements on dimeric copper(II) acetate which in the solid state consists of pairs of antiferromagnetically-coupled copper ions. The day to day reproducibility of our instrument lies within the experimental scatter of a given run.

V. System Performance

The present sensitivity and reproducibility of this magnetometer for a typical 0.050 cm^3 sample in a magnetic field ranging from 12 G to 800 G are as follows: In the temperature range of 2° to 120°K the reproducibilities in relative changes of the volume susceptibility, $\Delta\chi(T)$, and the magnetic moment, $\Delta\mu(T)$, are $\pm 1.5 \times 10^{-9}$ emu/cm³ and $\pm 1 \times 10^{-9}$ emu respectively for a sample whose total change in susceptibility is $\approx 1.2 \times 10^{-5}$ emu/cm³; for $\Delta\chi \geq 1.2 \times 10^{-5}$ emu/cm³ the reproducibility in $\Delta\chi(T)$ is $\pm [4 \times 10^{-4} \times \Delta\chi(T)]$. At higher temperature, the reproducibility is mainly limited by the background magnetism contribution due to construction material. For total absolute susceptibility measurements between 2° and 200°K where the sample is moved from one coil into another, the reproducibilities in $\chi(T_0)$ and $\mu(T_0)$ are $\pm 1.5 \times 10^{-8}$ emu/cm³ and $\pm 9 \times 10^{-9}$ emu respectively for $\chi(T_0) < 4 \times 10^{-5}$ emu/cm³; and for $\chi(T_0) > 4 \times 10^{-5}$ emu/cm³, $[\pm 4 \times 10^{-4} \times \chi(T_0)]$ and $[\pm 4 \times 10^{-4} \times \mu(T_0)]$ respectively. The uncertainties quoted here include all the background effects. The reproducibility is highest in the determination of

susceptibility changes $\Delta\chi(T)$. This feature is particularly useful in measuring materials which show small changes in a large susceptibility such as biological compounds. Metalloproteins usually have large diamagnetic backgrounds arising from the organic matter which do not contribute to the temperature dependent susceptibility one is interested in. Some examples of these are discussed below.

The present limitations on our system are related to the construction compromises which we have made in assembling this first apparatus and are not related to any intrinsic sensitivity limit. In fact, we are at least a factor of 10 less sensitive than the intrinsic sensitivity of our present sensor. And even this intrinsic sensitivity can be improved by modifying the amplifier-sensor system. Our design limits the sample size to a volume less than or equal to 0.2 cm^3 . And though, while the system was designed for use with small samples, there is no fundamental limit to the size of the sample such as exists with some of the conventional techniques.

The principal sources of noise and background effects which now limit the performance are vibrations, flux-creep and the magnetic contribution of the construction materials. Trace amounts of ferromagnetic impurity or oxygen are especially troublesome. To reduce thermal fluctuations and magnetic disturbance from gas bubbles we usually operate our sensor immersed in liquid helium below the λ temperature. This procedure also maintains thermal equilibrium of the sensor in the presence of large temperature differences between sample and sensor. To maintain thermal equilibrium independent of the level of liquid nitrogen a copper radiation shield is inserted at nitrogen temperature between the helium and nitrogen dewars. The sample dewar is covered with a cloth "sock" which protects and supports a helium II film over a constant area of dewar surface independent of helium level. In this way large temperature differences can be maintained between sample and sensor while preserving a constant temperature at both the sample and sensor. Our present field limit of 800 gauss is determined by ambient vibration and flux creep.

VI. Biochemical Studies

The applications of magnetochemical techniques to problems in biochemistry is of interest for a variety of structural and mechanistic reasons.^{6,7} The most obvious applications involve the study of the static susceptibility of metalloproteins which contain one or more transition metal atoms within a protein molecule whose molecular weight can approach 10^6 . Much of the primary activity of the molecule is centered at these atoms. From the examination of their magnetic properties one can obtain information about the oxidation state, the spin-state, the coordination environment, and about the existence of antiferromagnetic interactions among the metal atoms. Such studies require a susceptibility measuring system of high sensitivity because of the low concentration of paramagnetic ions in the diamagnetic pro-

tein host.

The iron containing protein, oxyhemerythrin, an oxygen carrier in lower invertebrates provides a challenging test of the magnetometer system. This protein has a molecular weight of 108,000 and contains only 0.81% iron distributed in eight identical subunits of the protein, each containing two iron atoms. Previous attempts to measure the susceptibility of oxyhemerythrin were unable to detect any temperature dependence in the susceptibility leading to the postulation of strong antiferromagnetic coupling between pairs of iron ions.^{8,9}

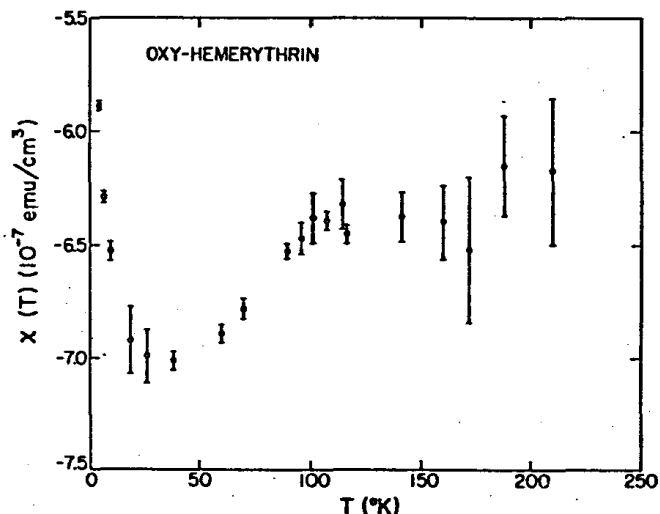


Fig. 4. Susceptibility versus temperature for 0.162 ml of an alcoholic suspension of oxyhemerythrin containing 460 μg of iron at 47 G. The meaning of the solid and open symbols is the same as in Fig. 3.

The temperature dependence of the susceptibility of 0.162 ml of a suspension of oxyhemerythrin containing a total of 460 μg of iron measured in a field of 47 gauss is presented in figure 4. Again the solid data points correspond to measurements of the total susceptibility, the others to relative measurements. The corresponding temperature dependence of the magnetic moment per Fe-ion is shown in Fig. 5 which is obtained by assuming that the rise in susceptibility below 40°K is due to paramagnetic impurities. The susceptibility contribution of these impurities has been subtracted out assuming Curie-law behavior with $C = 4.94 \times 10^{-7} \text{ }^\circ\text{K-emu/cm}^3$. The results clearly indicate the presence of antiferromagnetic contribution to the susceptibility. A similar antiferromagnetic behavior has also been measured in metaquoemerythrin. A full discussion and interpretation of the hemerythrin results in connection with chemical and optical properties appears elsewhere.¹⁰

VII. Conclusion

A new experimental technique for measurements of the temperature dependence of magnetic susceptibilities of biochemicals in the temperature range

of 1.5° to 300°K has been developed based upon new principles of measurement using superconducting electronics. A superconducting quantum magnetome-

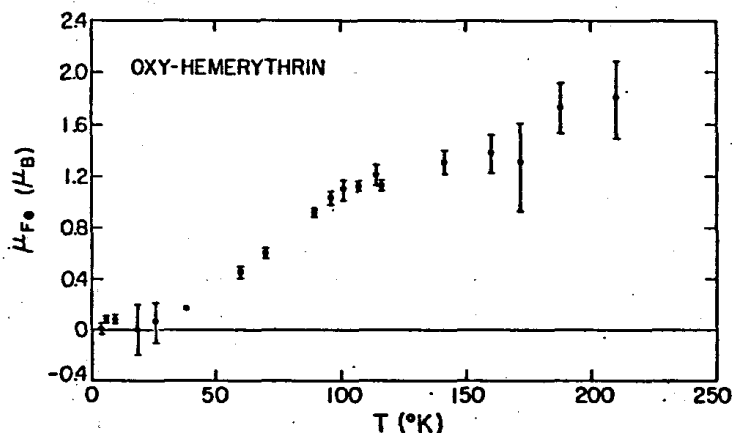


Fig. 5. Magnetic moment per Fe-ion versus temperature for 0.162 ml of an alcoholic suspension of oxyhemerythrin containing 460 μg of iron at 47 G assuming paramagnetic impurities with a Curie constant $C = 4.94 \times 10^{-7}$ $^{\circ}\text{K}\text{-emu}/\text{cm}^3$.

ter system using these techniques has been built which when applied to a variety of measurements provided results with an accuracy which compares favorably with those obtained by using conventional methods of magnetic susceptibility determination. The unsurpassed sensitivity of the system opens a new realm of magnetic measurements on weakly magnetic materials. Already data have been obtained on biochemical compounds which were unmeasurable with previously existing techniques. The method is still in its infancy and unlike conventional techniques shows great promise for considerable improvement in sensitivity and convenience.

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