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## MAGNETIC SUSCEPTIBILITY OF LYSOZYME

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We have measured the magnetic susceptibility of hydrated powder of lysozyme by the Faraday method at different values of the applied magnetic field, and of lysozyme aqueous solutions by a superconducting magnetometer at different temperature. We find lysozyme to behave as a normal diamagnetic substance, and not in the abnormal way detected by previous authors.

A recent paper [1] in this journal called our attention on the abnormal magnetic behaviour of lysozyme, both in solution and as hydrated powder. Therefore we have investigated the susceptibility of lysozyme hydrated powder at different values of the magnetic field and of two lysozyme aqueous solutions at different temperatures.

We have measured the magnetic susceptibility  $\chi$  of the protein powder by the Faraday method. The field gradient  $dH/dz$  was applied to the sample by suitable Bruker pole expansions and the force  $F_z$  was measured by a Cahn balance connected to a digital voltmeter. Using this method, it is easily seen that if some oxygen gas is present in the cell region, besides its possible paramagnetic contribution to the absolute  $\chi$  value of the sample, it will also alter the measurement because the paramagnetic molecules create a pressure gradient, due to the field gradient, which produces on the sample an Archimedes' like force. We have checked the relevance of this effect by measuring the different susceptibilities of a glass sphere in pure nitrogen gas and in air. In order to avoid this contribution to  $\chi$ , we have been careful to avoid any oxygen contamination in and around the sample, by continuous flushing of high purity nitrogen gas.

The water content in the sample of hydrated protein powder was measured in absence of magnetic field by its weight difference relative to the same sample after several days of flushing of dry nitrogen (about 1 l/h). Infrared measurements carried out on lysozyme  $\ddagger$  films in similar experimental conditions con-

firm that no water is left in the sample following this procedure. When measuring the magnetic susceptibility, in order to reduce the experimental error, a computer controlled apparatus was designed to switch on and off the field and to average the data until the statistical error was sufficiently low. The temperature of the sample was kept constant within  $\pm 0.1^\circ\text{C}$ .

In one run the hydration of the lysozyme powder was kept constant at about 30% weight hydration, and the field was varied by one order of magnitude as shown in fig. 1. Absolute values of  $\chi$  were derived by comparison with data obtained on samples of known  $\chi$ , like liquid water [2]. This result indicates that  $\chi$  does not show any dependence from the magnetic field within the experimental error, besides the low

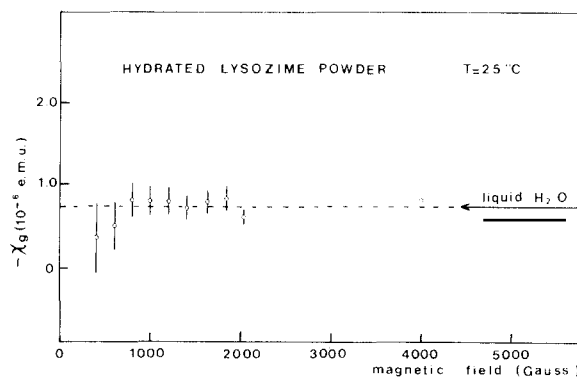


Fig. 1. The magnetic susceptibility per gram of about one gram of hydrated lysozyme powder, contained in a quartz cell and under flow of humid nitrogen gas, measured by the Faraday method at different values of the magnetic field. For further details see text. The value of liquid  $\text{H}_2\text{O}$ , measured in the same apparatus, is shown for comparison.

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<sup>‡</sup> Lysozyme salt free, purchased from Worthington Biochemical Corporation Freehold, New Jersey, USA.

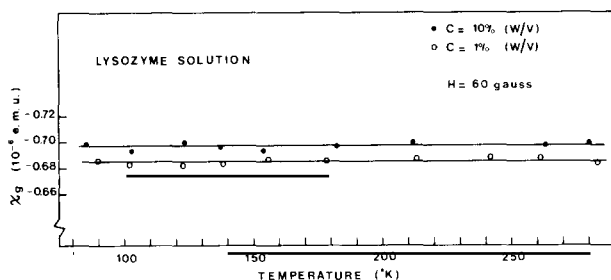


Fig. 2. The magnetic susceptibility per gram of two aqueous lysozyme solutions, at the concentration indicated in grams per volume, measured by the Josephson magnetometer at different temperatures.

field region where  $F_z$  becomes too small to be measured with sufficient accuracy.

The magnetic susceptibility of the lysozyme solutions at low magnetic field has been measured by a Josephson effect magnetometer, which has been described elsewhere [3, 4]. This apparatus has a sensitivity of  $2 \times 10^{-9}$  e.m.u., and can be operated at a well constant magnetic field (created by a superconducting solenoid) over a wide range of temperature. In fig. 2 we report the results for two lysozyme aqueous solutions of different concentration in weight, in the same experimental volume  $V = 0.2 \text{ cm}^3$ . The susceptibility

of these solutions is found to be temperature independent, and its value is  $\chi_g = 0.697 \pm 0.004 \times 10^{-6}$  e.m.u. for the 10% solution, and  $\chi_g = 0.686 \pm 0.004 \times 10^{-6}$  e.m.u. for the 1% solution. These figures are well in agreement with the  $\chi_g$  value reported [5, 6] for ice,  $\chi_g = 0.683 \times 10^{-6}$  e.m.u., pointing out that lysozyme has a susceptibility per gram about 10% larger than ice. This figure is consistent with the less precise data offered by the Faraday method for the hydrated powder reported in fig. 1.

The two above reported results taken together, namely the magnetic field independence of the hydrated powder and the temperature independence of the two aqueous solutions, and the absolute value of the measured susceptibilities, show that lysozyme behaves as a normal diamagnetic substance.

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