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PHYSICAL CHARACTERIZATION OF IRON OXIDE NANOPARTICLES IN MAGNETOFERRITIN

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Introduction. Natural ferritin is the iron-storage protein of animals, plants, and bacteria. It is a spherical biomacromolecule of external diameter about 12 nm composed of 24 protein subunits arranged as a hollow sphere of approximately 8 nm in diameter. Inside the sphere, iron is stored in the ferric oxidation state as a complex molecule with a crystallographic structure similar to mineral ferrihydrite. By a proper chemical process, it is possible to use the empty protein shell of ferritin, i.e. apoferritin, as a confined environment, in which magnetic iron oxide nanoparticles can be synthesized forming a biocompatible ferrofluid called magnetoferritin [1]. The latest studies show that brain ferritin [2] in patients with the Alzheimer's disease [3] has a polyphase structure, incorporating also magnetite. The structure, quality and quantity of the iron core composition in the brain ferritin have not been fully determined yet, and it has not been established whether they are related to the origin of neurodegenerative diseases or to their consequences [4, 5]. For these reasons, by combining different techniques it is necessary to fully characterize the structure and the physico-chemical properties of ferritin and distinguish between magnetic structures, especially for understanding the role of magnetite presence in the development of neurodegenerative diseases. Of particular interest is the search for methods allowing detection of magnetite inside ferritin proteins in vivo and inside magnetoferritin as a model system in vitro, respectively. Magnetoferritin is a relatively new biocompatible nanomaterial with continuously increasing popularity in many fields of science from medicine through nanotechnology up to physics. If compared with physiological ferritin, magnetoferritin contains magnetic nanoparticles (Fe_3O_4, γ-Fe_2O_3) surrounded by the empty protein shell (apoferritin). The problem of toxicity and side effects of magnetic nanoparticles in organs and tissues is minimized due to the protein nature of this unique material, which is important for many possible applications in clinical practice as a drug carrier, contrast medium in radiodiagnostics, or in

magnetic hyperthermia therapy. In addition to biocompatibility, another advantage of magnetoferritin for biotechnological applications is a relatively short time of controlled synthesis adapted to the formation of magnetite specifically inside the protein cavity and creation the magnetoferritin molecule.

1. Experimental methods and results. The mechanism of in vitro chemical synthesis of magnetoferritin could be summarized in three basic steps. Ferrous ions first pass along the hydrophilic pathway through the protein channels of apoferritin and during the transition are driven by an electrostatic gradient followed by the oxidation of iron ions. Through the regulation of the ratio of iron and apoferritin, it is possible to prepare electrostatically stable, homogeneously dispersed magnetoferritin molecules in a colloidal solution with a different loading factor, i.e. the iron content per molecule of protein. Generally, the loading factor of the magnetoferritin molecules and the kind of iron oxides in the composition of the mineral core could be modified by a specific set up of conditions for synthesis. The loading factor of the prepared samples was determined spectrophotometrically. The hydrodynamic diameter of the magnetoferritin molecules was determined by dynamic light scattering. It increases with the amount of iron inside apoferritin. The results from transmission electron microscopy (TEM) show that the size distribution depends on the loading factor. Electron diffraction of the magnetoferritin samples confirms a face-centered cubic mineral structure of the magnetic nanoparticles inside apoferritin, but it is not possible to distinguish between magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). The first preliminary small angle neutron scattering (SANS) measurements for the samples of magnetoferritin to investigate the composition of the iron core of protein molecules were made using contrast variation technique at different ratios of H_2O/D_2O, which is characterized by a residual intensity because of the distribution over the content of the magnetic component in the protein cavity. The scattering data indicate the presence of well-formed and almost non-interacting apoferritin in the solution. The form factor of such a shell has a pronounced side peak with maximum at $0.8\,\mathrm{nm}^{-1}$ (Fig. 1). The scattering data of iron oxide loaded apoferritin dispersions show that the spherical shell-like molecules are preserved in the solution. The relative height of this maximum is lower; this can be related to the fact that it is filled with iron oxide and is not anymore perfectly spherical. Magnetic properties of magnetoferritin were investigated using a SQUID magnetometer in magnetic fields up to 50 kOe. The magnetic measurements show superparamagnetism of the prepared magnetic

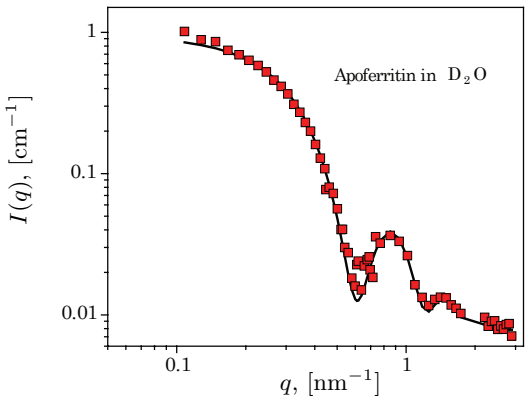

Fig. 1. The SANS measurements of apoferritin.

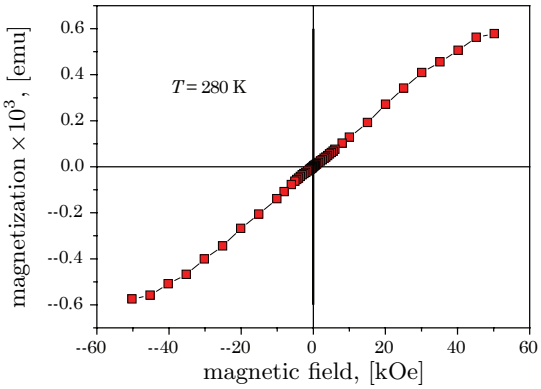

Fig. 2. Field dependence of magnetoferritin magnetization measured at room temperature.

particles without hysteresis at room temperature (Fig. 2). The hysteresis loop at room temperature is described by a Langevin function:

$$M = M_{\mathrm{s}} \left[\mathrm{cth} \frac{\mu_0 m H}{k_{\mathrm{B}} T} - \frac{k_{\mathrm{B}} T}{\mu_0 m H} \right].$$

The particles saturation magnetization of about 8 emu/g was calculated when we used particles sizes from TEM. These results indicate that our particles are mixed hematite and magnetite with a very large amount of hematite. The magnetization loops measured below T_{b} show the hysteresis with a coercive field from 200 to 450 Oe, which is dependent on the loading factor. The magnetization measured at 5 K undergoes a slow approach to saturation at fields, which we can achieve. This result needs a further investigation.

The different magnetic iron oxide nanoparticles show the different magneto-optical Cotton–Mouton effect behavior under an external magnetic field, which allows to characterize the iron core of magnetoferritin [6]. This fact has led to the first study of the magneto-optical properties of magnetoferritin with various

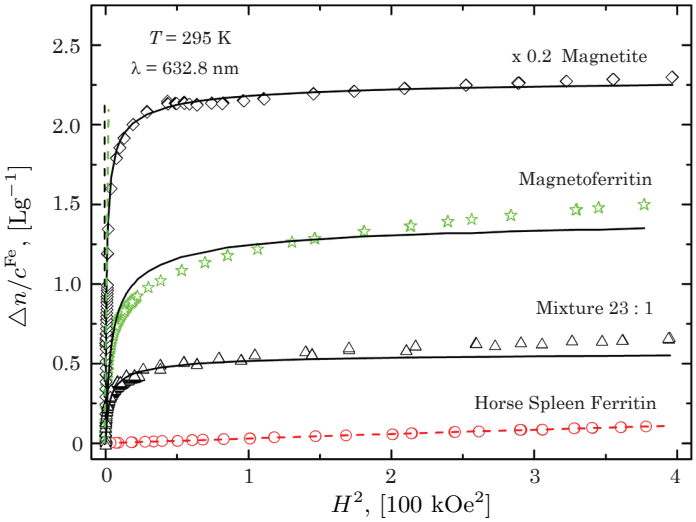

Fig. 3. Comparison of the reduced magnetic birefringence as a function of the square of the applied magnetic field.

loading factors in comparison to horse spleen ferritin and magnetite aqueous suspensions. Different behavior of reduced magnetic birefringence as a function of the applied magnetic field for suspensions of ferritin, magnetoferritin, and nanoscale magnetite can be observed (Fig. 3).

Conclusion. Differences in magnetically induced optical birefringence measurements of the prepared magnetoferritin, horse spleen ferritin and magnetite aqueous solutions allow discrimination between a maghemite and a magnetite core in synthetic magnetoferritin or ferritin, which could be a very powerful method in the determination of the oxidation state of iron oxide useful for biomedicine.

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