Linear and nonlinear magneto-optics of ferritin

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(Received 2 February 2009; accepted 8 June 2009; published online 7 July 2009)

Measurements of Rayleigh light scattering and Cotton–Mouton (CM) effect are carried out at room temperature for 100 mM NaCl solutions of apoferritin/ferritin loaded with 0, 90, 100, 500, 700, and 1500 Fe atoms/molecule. Because of the spherical shape, ferritin macromolecule should not manifest magnetic anisotropy; however, in solution it shows the induced magnetic birefringence (CM effect) and changes in intensity of the scattered light components. The newly obtained data support the previously reported conclusions indicating that the deformation of linear optical polarizability induced in the ferritin by a magnetic field and the orientation of the induced magnetic dipole moment by this field are the main sources of the magneto-optical phenomena observed. Nevertheless, it is also found that the orientation of the permanent magnetic dipole moment contributes to both effects. The magnetic field induced changes in the light scattering and the CM effect theoretically depend on the linear magneto-optical polarizability, \( \chi \), on the nonlinear magneto-optical polarizability, \( \eta \), and square of the permanent magnetic dipole moment value of the macromolecule, \( \mu^2 \). On the basis of the theory describing both effects as well as the experimental data, the values of the anisotropy of linear magneto-optical polarizabilities components, the values of the linear optical polarizability and its anisotropy, nonlinear magneto-optical polarizability and its anisotropy, are estimated. Also the magnetic dipole moment of the ferritin macromolecule is found. Interestingly, not all iron atoms in the ferritin are indicated to be in the superparamagnetic state, some of them occur in the diamagnetic form. © 2009 American Institute of Physics.

[DOI: 10.1063/1.3159844]

I. INTRODUCTION

Most proteins are diamagnetic so the effect of a magnetic field on them is difficult to detect. In contrast, ferritins are a class of metalloproteins present in both plant and animal cells that show strong magnetic responses. The micelleous tertiary structures of these proteins allow iron accumulation in the form of hydrated oxides and phosphates of this metal. Thus ferritin is a large spherical macromolecular protein with iron compounds situated in the cavity created by a peptide shell.\(^1,2\) If needed for the organism, the iron is released through intermediacy of the iron reductase enzyme. In the human body, ferritin macromolecules are accumulated in the liver, spleen, and medulla cells.

Recently, the effective diffusion coefficients and hydrodynamic radius for monomers, dimers, and trimers of ferritins and apoferritins in solution have been determined on the basis of dynamic light scattering and small angle x-ray scattering.\(^3-5\) The atomic force microscopy, NMR, electron paramagnetic resonance (EPR), and the neutron scattering measurements have been used in investigation of ferritins.\(^6-8\) Ferritin molecule has been used by some authors as a paramagnetic label.\(^9\) Other authors have used the UV-visible spectroscopy, video microscopy, and x-ray diffraction for detection of iron (III) complexes formed in the iron core during the ferritin-apoferritin conversion.\(^10,11\) When considering the influence of different physical factors on a living organism, the effect of magnetic field cannot be neglected as, among others, it has recently been applied in many different medical diagnostic methods. The magnetization\(^12,13\) and Mössbauer measurements\(^14,15\) have been made on natural ferritin showing the antiferromagnetic feature of the molecular core responsible for the superparamagnetic behavior of the metalloprotein.\(^16-21\) Therefore, further determination of the magnetic field influence on ferritin has become a problem of increasing importance for analysis of its physiological functions.

Solutions of ferritin containing different numbers of iron atoms per molecule have been studied by the Cotton–Mouton (CM) effect and nonlinear light scattering in dc magnetic field. Both methods provide information on the macromolecule structure complementary to that obtained from EPR, magnetization, or Mössbauer measurements. A theory taking into account simultaneously the diamagnetic and paramagnetic futures of the biomolecule is proposed for the first time. Analysis of the results obtained allows identification of the deformation of the linear optical polarizability induced in the biomacromolecule by a magnetic field as the main source of the magneto-optical phenomena observed. The orientation of the permanent and induced magnetic dipole moments plays an important role in explanation of the observed effects. The ferritin molecules are proposed to have diamagnetic and superparamagnetic features simultaneously and the theory assuming this proposition brings a new understanding of the phenomena measured and a new interpretation of the previously collected data.
II. THEORY

A. Cotton–Mouton effect

Let us consider a Cartesian space in which the polarization plane of light incident onto a medium makes an angle of 45° with the z direction of an external magnetic field \( \vec{H} \). Fig. 1. In the following, the interaction of ferritin (symmetry axis 3) with an external dc magnetic field \( \vec{H} \) is considered. The electric field vector \( \vec{E} \) of the incident light intensity \( I \) is composed of the two components: parallel \( \vec{E}_p \) and perpendicular \( \vec{E}_\perp \) to \( \vec{H} \). The difference in the propagation velocity between these two components implies the appearance of the phase difference \( \varphi \) over a path \( L \) in the sample, which can be expressed as

\[
\varphi = 2 \pi \frac{n_p - n_\perp}{\lambda} L,
\]

where \( n_p \) and \( n_\perp \) are the light refraction indices of the light beam polarized in parallel and perpendicular to \( \vec{H} \), respectively; \( (n_p - n_\perp)L \) is the difference in the optical length of these beams, and \( \lambda \) is the light wavelength. According to the CM equation,

\[
\varphi = 2 \pi LCH^2,
\]

where \( C \) is the CM constant. The molar CM constant is given by the following expression:

\[
C^M = C \frac{6n M}{(n^2 + 2)^2 \rho},
\]

where \( n \) is the light refraction index of the medium, \( M \) is the molecular mass of the medium and \( \rho \) is its density. For non-interacting macromolecules the CM effect is related to the deformation of the linear optical polarizability of a macromolecule as well as the reorientation of the induced and permanent dipole moments, all three phenomena appearing under the influence of a magnetic field. In such a case, the total molar CM constant \( C^M_{tot} = C^M \) can be expressed in the following way:

\[
C^M_{tot} = \frac{2 \pi N_A}{15} \left\{ 15 \eta kT + \alpha k \left[ 3 \left( \chi_0 - \chi_\perp \right) + \frac{\mu^2}{k^2T^2} \right] \right\},
\]

In Eq. (4) \( N_A \) is Avogadro’s number, \( k \) is Boltzmann’s constant, and \( T \) is the absolute temperature in Kelvin, \( \mu \) denotes the value of the magnetic permanent dipole moment \( \mu \) aligned to the symmetry axis 3 of the macromolecule, while \( \alpha \) is the mean linear optical axes 3 of the macromolecule. For non-ideal systems the contribution of induced and permanent dipole moment orientation in this constant. It is due to the diamagnetic and paramagnetic/superparamagnetic futures of the macromolecule, simultaneously.

\[
\chi_0, \chi_\perp, \text{ stand for the linear magneto-optical polarizability components of the macromolecule along and perpendicular to its symmetry axis 3, and describe } \chi, \text{ the mean value of this polarizability as}
\]

\[
\chi = \frac{\chi_0 + 2 \chi_\perp}{3},
\]

and \( \kappa_\chi = \frac{\chi_0 - \chi_\perp}{3 \chi} \) the anisotropy of the components as

\[
\kappa_\chi = \frac{\chi_0 - \chi_\perp}{3 \chi}.
\]

In Eq. (4) the nonlinear magneto-optical polarizability \( \eta \) is defined as

\[
\eta = \frac{\eta_0 + 2 \eta_\perp}{3},
\]

where \( \eta_0, \eta_\perp \) are the components along and perpendicular to symmetry axis 3 of the macromolecule, respectively. The same components describe \( \kappa_\eta \) the anisotropy of the nonlinear magneto-optical polarizability as

\[
\kappa_\eta = \frac{\eta_0 - \eta_\perp}{3 \eta}.
\]

The total molar CM constant \( C^M_{tot} \) is composed of the deformational part, \( C^M_{def} \),

\[
C^M_{def} = 2 \pi N_A \eta \kappa_\eta,
\]

which refers to the deformation of the diamagnetic part of the macromolecule caused by the magnetic field and reorientational part, \( C^M_{reor} \),

\[
C^M_{reor} = \frac{2 \pi N_A}{15} \alpha k \left[ 3 \left( \chi_0 - \chi_\perp \right) + \frac{\mu^2}{k^2T^2} \right],
\]

which describes the contribution of induced and permanent dipole moment orientation in this constant. It is due to the diamagnetic and paramagnetic/superparamagnetic futures of the macromolecule, simultaneously.
B. Static and nonlinear light scattering in dc magnetic field

The parameters $\alpha$, $\kappa_\alpha$, $\alpha_\parallel$, and $\alpha_\perp$ are usually determined from the static light scattering. In such a case we consider a Cartesian space in which the incident light of intensity $I_0$ is polarized perpendicularly to the plane of observation $xy$ and the external dc magnetic field $\vec{H}$ is applied along the $z$ direction, Fig. 2.

Small $H_v$ and large $V_v$ intensity components of the scattered light (with the electric vector polarized parallel and perpendicular to the plane of observation $xy$, respectively) are measured at $90^\circ$ along the $x$-axis. For the axially symmetric ferritin macromolecule with its mean linear magneto-optical polarizability $\gamma$ and the anisotropy $\kappa_\gamma$ of the linear magnetic-optical polarizability components, the distinguished symmetry axis (axis 3) makes the angle $\theta$ with $\vec{H}$. We shall refer to $H_v^H$ and $V_v^H$ as the small and large components of the scattered light intensity, respectively, when a magnetic field $\vec{H}$ is applied, and to $H_v^0$ and $V_v^0$ in the absence of this field. Then one can define the measured relative changes in the components as

$$
\Delta H_v^H = \frac{H_v^H - H_v^0}{H_v^0},
$$

$$
\Delta V_v^H = \frac{V_v^H - V_v^0}{V_v^0}.
$$

For small values of $H = |\vec{H}|$ (when $U < kT$, where $U$ is the potential energy of the diamagnetic macromolecule in the presence of $\vec{H}$) the changes in the small $\Delta H_v^{\text{diam}}$ and large $\Delta V_v^{\text{diam}}$ components as a function of the magnetic field can be expressed in the following way:

$$
\Delta H_v^{\text{diam}} = \frac{2}{21} \left( \frac{\chi_\parallel - \chi_\perp}{kT} + \frac{\kappa_\parallel}{2 \alpha \kappa_\alpha} \right) H^2, \quad (15)
$$

$$
\Delta V_v^{\text{diam}} = \frac{4}{15} \left( \frac{\chi_\parallel - \chi_\perp}{kT} \kappa_\alpha + \frac{\eta}{\alpha} \right) H^2. \quad (16)
$$

The nonlinear magneto-optical polarizability $\eta$ describes the changes in the electric dipole moment induced by the electric field of the incident light beam due to the external magnetic field $\vec{H}$. Both equations, Eqs. (15) and (16), are composed of two terms. The first term contains the linear magneto-optical polarizability $\chi$ and its anisotropy $\kappa_\gamma (\chi_\parallel - \chi_\perp = 3 \chi_\perp)$, and describes the magnetic molecular orientation. The second term contains the nonlinear magneto-optical polarizability $\eta$, and describes the changes in the linear optical polarizability $\alpha$ due to the molecular deformations induced by the magnetic field. For superparamagnetic molecules of spherical symmetry (small $\kappa_\gamma$), with the permanent dipole moment $\vec{\mu}$ aligned to the symmetry axes 3, one obtains

$$
\Delta H_v^{\text{par}} = \frac{1}{21} \left( \frac{\chi_\parallel - \chi_\perp}{kT} + \frac{\mu^2}{k^2 T^2} \right) H^2, \quad (17)
$$

$$
\Delta V_v^{\text{par}} = \frac{4}{15} \kappa_\alpha \left( \frac{\chi_\parallel - \chi_\perp}{kT} + \frac{\mu^2}{k^2 T^2} \right) H^2. \quad (18)
$$

In this case, Eqs. (17) and (18) are also composed of two terms. The first one contains the linear magneto-optical polarizability $\chi$ and describes the induced dipole moment magnetic molecular reorientation. The second one contains the permanent magnetic dipole moment and describes its reorientation in the magnetic field.

As for the CM effect, the total changes in the light scattering component can be due to the diamagnetic and paramagnetic interactions. In such a case, the total change in the small component $\Delta H_v^{\text{tot}}$ is a sum of the diamagnetic and paramagnetic components,

$$
\Delta H_v^{\text{tot}} = \Delta H_v^{\text{diam}} + \Delta H_v^{\text{par}}. \quad (19)
$$

Introducing Eqs. (15) and (17) into Eq. (19) one obtains

$$
\Delta H_v^{\text{tot}} = \left[ \frac{\eta \kappa_\gamma}{2 \alpha \kappa_\alpha} + \frac{2 (\chi_\parallel - \chi_\perp)}{21 kT} + \frac{2 \mu^2}{21 k^2 T^2} \right] H^2, \quad (20)
$$

which is composed of the deformational part, $\Delta H_v^{\text{tot \, def}}$,

$$
\Delta H_v^{\text{tot \, def}} = \left[ \frac{\eta \kappa_\gamma}{2 \alpha \kappa_\alpha} \right] H^2, \quad (21)
$$

and the reorientational part, $\Delta H_v^{\text{tot \, reor}}$,

$$
\Delta H_v^{\text{tot \, reor}} = \frac{2}{21} \left( \frac{\chi_\parallel - \chi_\perp}{kT} + \frac{\mu^2}{k^2 T^2} \right) H^2. \quad (22)
$$

Analogous reasoning can be applied for the change in the large component of the scattered light,

$$
\Delta V_v^{\text{tot}} = \Delta V_v^{\text{diam}} + \Delta V_v^{\text{par}}. \quad (23)
$$

Substituting Eqs. (16) and (18) to Eq. (23) one obtains

$$
\Delta V_v^{\text{tot}} = \left[ \frac{\eta \kappa_\gamma}{15 \alpha} \left( \chi_\parallel - \chi_\perp \right) kT + \frac{4 \kappa_\alpha \mu^2}{15 k^2 T^2} \right] H^2, \quad (24)
$$

which is composed of the deformational part, $\Delta V_v^{\text{tot \, def}}$,

$$
\Delta V_v^{\text{tot \, def}} = \left[ \frac{\eta \kappa_\gamma}{15 \alpha} \right] H^2, \quad (25)
$$

and the reorientational part, $\Delta V_v^{\text{tot \, reor}}$,

$$
\Delta V_v^{\text{tot \, reor}} = \frac{2 \kappa_\alpha}{15} \left( \frac{\chi_\parallel - \chi_\perp}{kT} + \frac{2 \mu^2}{k^2 T^2} \right) H^2. \quad (26)
$$
III. MATERIALS AND METHODS

A. Preparation of solutions

Measurements were carried out for 100 mM NaCl solutions of ferritin molecules with a known number of iron atoms. Ferritin samples were prepared by filling with iron the apoferitin macromolecules obtained from the horse spleen (Sigma); the source of iron atoms was a portion of 15 mM ferrous ammonium sulfate from Sigma. The samples studied were prepared from the basic solution (the concentration of 51 g/l) in 100 mM NaCl in the following way. This basics solution was placed in a 4 ml cellulose dialysis column (Millipore). 20 mM MOPS-NaCl buffer (Sigma) was added and the solution was centrifuged at 8000 rpm over 30 min. The procedure was repeated several times and then, to the apoferitin in 20 mM MOPS-NaCl, pH 6.5, a portion of 15 mM ferrous ammonium sulfate was added in several steps. In each step 100 iron atoms per ferritin molecule were added. The solutions obtained were exposed to solute exchange in 100 mM NaCl.

Measurements were also carried out for solutions of ferritin from Boehringer Mannheim GmbH. This compound is an extract obtained from the horse spleen. The samples studied were prepared by dissolving the basic solution (the concentration of 36.5 g/l) in 100 mM NaCl.

The effective content of iron in apoferitin/ferritin molecule was measured by the atomic absorption spectroscopy at the Water and Soil Analysis Laboratory of A. Mickiewicz University and the concentration of the protein was estimated using the spectroscopic method of Lowry et al. The final protein concentrations, numbers of Fe per macromolecule, concentration range of ferritin solutions studied, and range of the protein effective volume fraction in solution are given in Table I.

In order to eliminate mechanic impurities, the water as well as all solutions was passed through a Millipore cellulose filter (pore diameter 0.22 μm) prior to use. The air bubbles were eliminated by centrifugation of the solution at 8,000 rpm for 30 min.

Repeatedly distilled organic liquids (benzene, nitrobenzene, cyclohexane and carbon tetrachloride) were used for testing the measuring device. The measurements were performed at 20 °C.

B. Apparatus and measurements

1. Light refractive indices

Light refractive indices of the ferritin solutions were measured at 20 °C for the light wavelength of 588 nm by means of the Pulfrich refractometer IRF-23. The values measured were converted to the increment at \( \lambda = 632.8 \) nm using the following formula:

\[
\frac{dn}{dc} = \frac{dn}{dc}_{588} \left(0.940 + \frac{2 \times 10^5}{\lambda^2}\right). \tag{27}
\]

2. Light scattering in the absence and presence of magnetic field \( H \)

Measurements of Rayleigh light scattering, light scattering, and dynamic light scattering in a dc magnetic field were carried out using the apparatus described in Ref. 27. The light beams were emitted in the y direction (Fig. 2) from a He–Ne laser \( \lambda = 632.8 \) nm or Ar+ ion laser \( \lambda = 514 \) nm (Carl Zeiss Jena, Germany). The sample was contained in a rectangular plane-parallel glass cell (1 × 1 × 3 cm³) placed between the pole pieces of the electromagnet. The scattered light emerged from the cell in the x direction at 90° to the y direction of the incident beam. The apparatus permitted measurements of the scattered light intensity of the horizontally and vertically polarized light by changing the positions of the analyzer at different magnetic field strengths. The device described was tested by performing measurements of the light scattering intensity and correlation function in the absence and presence of magnetic field of various strengths. The static light scattering data, i.e., depolarization ratios and absolute Rayleigh constants, obtained using some simple organic liquids (listed under Materials) were in good agreement to those reported in literature. Benzene was used as a working standard for testing. When calculating the absolute Rayleigh constants, we applied the absolute constant for benzene at \( \lambda = 632.8 \) nm and \( R_B = 8.765 \times 10^{-6} \) cm⁻¹. The intensities of light scattered by the ferritin molecules in solution were calculated as the differences in the light intensities scattered by the solution and the solvent.

3. Cotton–Mouton effect

Measurements of the CM effect were carried out using the apparatus described in details in Ref. 27. The light source was a He–Ne laser \( \lambda = 632.8 \) nm or an Ar+ ion laser \( \lambda = 514 \) nm (Carl Zeiss Jena, Germany), which produced beams aligned along the y direction. The electromagnet produced the maximum dc magnetic field strength of 2 T in the z direction and the holder for the sample cell was the same as used in the static light scattering studies. When the magnetic field was on, the ferritin solution became birefringent. The linearly polarized beam became elliptically polarized, which was detected as an increase in the light intensity. The linear polarization was changed from elliptical to linear at the angle

<table>
<thead>
<tr>
<th>( N )</th>
<th>Fe number per protein molecule</th>
<th>90</th>
<th>100</th>
<th>500</th>
<th>700</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>( c ), basic protein concentration in the sample (g/l)</td>
<td></td>
<td>15</td>
<td>26</td>
<td>14</td>
<td>2.5</td>
<td>36.5</td>
</tr>
<tr>
<td>( \Delta c ), concentration range of ferritin solutions (g/l)</td>
<td></td>
<td>2–15</td>
<td>1–26</td>
<td>0.6–14</td>
<td>0.16–2.5</td>
<td>0.3–7</td>
</tr>
<tr>
<td>( \Delta c \times 10^3 ), range of the protein effective volume fraction in solution</td>
<td></td>
<td>2.4–18</td>
<td>1.2–30</td>
<td>0.67–15</td>
<td>0.17–2.7</td>
<td>0.3–6.8</td>
</tr>
</tbody>
</table>
of ϕ/2 with respect to the incident light polarization by a quarter plate. The setup allowed birefringence measurements at different magnetic field intensities with the ϕ/2 angle determined to 0.01° accuracy. The device described was tested in the CM constant measurements for nitrobenzene, which determined to 0.01° accuracy. The device described was tested in the CM constant measurements for nitrobenzene, which gave a result in good agreement to literature values. When calculating the absolute CM constants, we applied the absolute constant for nitrobenzene at λ = 632.8 nm and C = 2.31 × 10⁻¹² Oe⁻² cm⁻¹.

IV. RESULTS AND DISCUSSION

A. Light refractive indices

Light refractive indices measured in 100 mM NaCl water solutions of apoferritin and ferritin with different contents of Fe/molecule at the light wavelength of 587.6 nm are shown in Fig. 3. On the basis of the plots and using relation (30), the refractive index increment dn/dc for λ = 632.8 nm was calculated (c is the concentration of protein in g l⁻¹), Table II. For the calculations the refractive index of 100 mM NaCl water solution was taken as n₀ = 1.3342.

B. Light scattering

Small H_u and large V_v intensity components of the scattered light were measured for a series of ferritin solutions of different concentrations using the geometry and the setup shown in Fig. 2 (see also Fig. 2 in Ref. 27). In Fig. 4 the depolarization ratio D_u measured as a function of ferritin concentration in 100 mM NaCl water solution for protein samples containing different numbers N (given in the inset) of iron atoms per molecule.

The depolarization ratio D_u values obtained for the proteins containing different numbers N of iron atoms are very small; they vary between 0 and 0.003. One can conclude that the increase in the iron in the core of ferritin does not influence the highly spherical shape of the protein and that the interactions between the solute and solvent are negligible.

The partial Rayleigh constants H_u and V_v obtained allowed a calculation of the absolute total Rayleigh constant R_u and the linear optical polarizability α_{scatt} of the ferritin macromolecule. These polarizabilities vary from 3.3 × 10⁻²⁰ to 7.0 × 10⁻²⁰ cm³. The results are shown in Fig. 5 along with the values of α_v, linear optical polarizabilities calculated by using the refractive index increment given in Table II [Eq. (8) in Ref. 27]. The α_v values change from 1.80 × 10⁻²⁰ to 3.57 × 10⁻²⁰ cm³ when one compares apoferritin with ferritin containing 1500 Fe atoms per protein molecule.

Table II presents the mean values obtained from both methods at a given iron content per molecule taken for further analysis. Knowing the D_u value one can calculate the square of the anisotropy of linear optical polarizability, κ²_{scatt} = 5D_u/(6−7D_u). Figure 6 shows the absolute values of |κ_{scatt}| as a function of ferritin concentration for different contents of iron atoms per molecule. The values obtained for a series of samples of equal Fe content per ferritin molecule in the

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**Table II.** Values of the refractive index increment obtained for λ = 632.8 at 100 and 15 mM NaCl water solutions of ferritin containing different numbers of iron atoms per protein molecule. The molecular mass M_v, linear optical polarizability α, calculated as mean of α_v and α_{scatt}, and the mean values of the absolute anisotropy of the linear optical polarizability |κ_{scatt}| are given.

<table>
<thead>
<tr>
<th>N=Fe/ferritin-iron atoms number per ferritin molecule</th>
<th>0</th>
<th>90</th>
<th>100</th>
<th>500</th>
<th>700</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.100 M NaCl dn/dc × 10⁴ [1/g]</td>
<td>1.58±0.02</td>
<td>1.65±0.05</td>
<td>1.78±0.03</td>
<td>1.83±0.06</td>
<td>1.89±0.05</td>
<td>2.53±0.03</td>
</tr>
<tr>
<td>0.015 M NaCl dn/dc × 10⁴ [1/g]</td>
<td>1.47±0.04</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>2.50±0.03</td>
</tr>
<tr>
<td>M_v × 10⁻³ (D)</td>
<td>450</td>
<td>456</td>
<td>457</td>
<td>486</td>
<td>500</td>
<td>557</td>
</tr>
<tr>
<td>α × 10⁻¹⁰ (cm³)</td>
<td>2.8</td>
<td>2.9</td>
<td>3.0</td>
<td>3.4</td>
<td>3.6</td>
<td>4.4</td>
</tr>
<tr>
<td>α × 10⁻⁶ (F m²)</td>
<td>3.1</td>
<td>3.2</td>
<td>3.3</td>
<td>3.8</td>
<td>4.0</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>κ_{scatt}</td>
<td></td>
<td>0.030</td>
<td>0.032</td>
<td>0.032</td>
<td>0.038</td>
</tr>
</tbody>
</table>
The polarizabilities are described in centimeter gram second system of units as a function of the effective index and from light scattering measurements, respectively, as a function of \( N=\text{Fe}/\text{ferritin} \), the number of iron atoms per ferritin molecule. The polarizabilities are described in centimeter gram second system of units (CGS) and SI units.

The mean values of the absolute anisotropy of linear optical polarizability \( |\kappa| \) obtained from the light scattering measurements at given iron content per molecule are plotted in Fig. 7 and the values estimated from the fit, used for further analysis are given in Table II.

Very small anisotropies obtained are characteristic of the spherical shape of the macromolecules studied. The small increase in \( |\kappa| \) can be due to uneven distribution of iron compound deposition inside the spherical macromolecule shell, when the mass of this deposition starts to be meaningful in comparison with the mass of the protein.

C. The Cotton-Mouton effect

The CM effect for different solute concentrations was measured in the geometry shown in Fig. 1 and the setup described in Ref. 27. The results of the magnetic birefringence are presented in Figs. 8–11. Figure 8 shows the phase difference \( \varphi/2 \) (deg) induced by the magnetic field in solutions of different ferritin concentrations as a function of the square of magnetic field intensity, \( H^2 \), Eq. (1).

The \( \varphi/2 \) angle was measured for ferritin containing \( N=90,100,500,700, \) and \( 1500 \) iron atoms per molecule. For all concentrations studied the dependence of \( \varphi/2=f(H^2) \) is linear, suggesting no interaction between the induced and permanent magnetic dipoles of the macromolecules.

Using the data presented in Fig. 8, one can calculate after Eq. (2), the CM constant, \( C \) (Oe cm\(^{-1}\)), for the concentration of ferritin studied, \( c(\text{g/l}) \). A linear dependence of \( C=f(c) \) was obtained for each studied content of Fe/molecule, Fig. 9.

Having the values of the CM constants \( C \), one can calculate from Eq. (3) the total molecular CM constants, \( C_{\text{tot}}^M \) (cm\(^3\) Oe\(^{-1}\) M\(^{-1}\)) [in International System (SI) of units (m\(^3\) T\(^{-2}\) M\(^{-1}\))], Eq. (5). The studies of dynamic light scattering in the absence and presence of magnetic field did not show any interactions of macromolecules at low concentrations in 100 mM NaCl water solution.\(^{4,5,27} \) Therefore, in the following calculations apoferritin and ferritin are considered as gaslike molecules. The total molecular CM constants \( C_{\text{tot}}^M \) as a function of the solute concentration for different iron contents per molecule are shown in Fig. 10.

As follows from this figure, the total molecular CM constant \( C_{\text{tot}}^M \) is independent of the concentration (in the limit of experimental error) for each \( N=\text{Fe}/\text{molecule} \). Therefore, it was useful to plot the total molecular CM constants as a function of this number and Fig. 11 presents the dependence of \( C_{\text{tot}}^M=f(N=\text{Fe}/\text{ferritin}) \).

The core of the ferritin molecule considered as the antiferromagnetic microcrystalline particle is responsible for the superparamagnetic behavior of an ensemble of randomly oriented particles. The knowledge of Fe content in the particle allows estimation of the net permanent dipole moment \( \mu \) of a single ferritin molecule resulting from uncompensated iron spins at the surface of the core.\(^{13-21} \) Taking into account the
spherical geometry of the ferritin molecule, reflected in a very small and nearly constant optical linear anisotropy, \( \langle \kappa_\alpha \rangle \), Fig. 6 and Eq. (4), one can write

\[
\frac{C_{M_{\text{tot}}}}{\alpha} = A + B \mu^2,
\]  

(28)

where

\[
A = \frac{2\pi N_A}{15} \left\{ \frac{15 \pi k T}{\alpha} + \frac{3k T}{\alpha}(\chi_1 - \chi_L) \right\},
\]  

(29)

and

\[
B = \frac{2\pi N_A}{15k^2 T^2} k_\alpha.
\]  

(30)

Figure 12 presents \( \frac{C_{M_{\text{tot}}}}{\alpha} = f(\mu^2) \) and the linear fit of the data gives \( A = -1.932 \times 10^{10} \text{ Oe}^{-2} \text{ M}^{-1} \) and \( B = 1.817 \times 10^{47} \text{ erg}^{-2} \text{ M}^{-1} \) values.

The positive \( B \) value indicates the positive sign of the optical anisotropy of the linear polarizability \( \kappa_\alpha \). Its value calculated using Eq. (28) is an average for all samples measured, \( \kappa_\alpha = 0.0012 \). It is a very small number which supports the earlier conclusion that ferritin molecule maintains the spherical symmetry irrespectively of the iron atoms number at the core.

\[\text{D. Light scattering in magnetic field}\]

Relative changes in the polarized \( \Delta V_v^H \) and depolarized \( \Delta H_v^H \) components of scattered light (defined in Eqs. (13) and

\[
\text{FIG. 9. CM constants, } C, \text{ calculated from Eq. (2) using the data shown in Fig. 8 as a function of ferritin concentration } c, \text{ for different numbers } N=\text{Fe/molecule. The axes are described in CGS and SI units.}
\]

\[
\text{FIG. 10. The total molecular CM constant } C_{M_{\text{tot}}} \text{ as a function of ferritin concentration } c \text{ for different } N=\text{Fe/molecule. The data are shown in the log-log scales. The straight lines indicate the average values of } C_{M_{\text{tot}}} \text{ constants equal to } 1.89 \times 10^{-8}, \ 6.89 \times 10^{-9}, \ 2.09 \times 10^{-9}, \ 8.46 \times 10^{-10}, \text{ and } 2.2 \times 10^{-10} \text{ Oe}^{-2} \text{ M}^{-1} (\text{in SI units: } 1.89 \times 10^{-6}, 6.89 \times 10^{-7}, 2.09 \times 10^{-7}, 8.46 \times 10^{-8}, \text{ and } 2.2 \times 10^{-8} \text{ m}^3 \text{T}^{-2} \text{ M}^{-1}) \text{ for } N=1500, 700, 500, 100, \text{ and } 90 \text{ Fe/ferritin, respectively.}
\]
Identical magneto-optical parameters describe the magnetic is described by the linear and nonlinear magneto-optical position of the macromolecule with the external magnetic field $H$.

$CM$ fulfils the equation using the data shown in Figs. 9 and 10 as a function of the iron atom number per ferritin molecule, $N=Fe/ferritin$.

Light scattering in magnetic field is theoretically described by Eqs. (20) and (24), in which the deformation $\Delta H^m$ and reorientation $\alpha$ interaction of the macromolecule with the external magnetic field $H$ is described by the linear and nonlinear magneto-optical polarizabilities and by the permanent magnetic dipole moment. Identical magneto-optical parameters describe the magnetic birefringence induced in a macromolecular solution, see Eq. (4). Having molecular values of $\chi_0 \chi_L = \chi_0 - \chi_L$ from the CM effect and from the measured changes in the small component in a magnetic field, the values of $\eta$ and $\kappa_\eta$ of the ferritin macromolecule can be found. Using Eq. (24) one can calculate $\eta$, used for calculation of the anisotropy of nonlinear magneto-optical polarizability, $\kappa_\eta$ from Eq. (21).

The measurements of dynamic light scattering in dc magnetic field excluded dimerization or any higher order aggregation of ferritin monomer in solution induced by a strong magnetic field. Both CM effect and light scattering changes observed in ferritin solution under the influence of the magnetic field can be interpreted as due to the following three phenomena. Two of them are connected with the reorientation of the permanent magnetic dipole moment and the second one with that of the one induced by the magnetic field. The third phenomenon is linked to the rearrangement of the inner iron core with increasing intensity of the magnetic field. Such a displacement can be connected with the deformation of the linear optical polarizability and with a change in the value and/or of the sign of its anisotropy. All three phenomena bring different contributions to the total CM effect and to the changes in the light scattering components.

Taking into account Eqs. (4), (11), and (12) describing the CM effect and using the magneto-optic parameters given above for $N=1500$ Fe/ferritin, one can find that the total molecular CM constant is determined by the permanent dipole orientation enlarged by about 5% due to the other phenomena. The contributions of $CM_{def}$ and $CM_{ind\,reor}$ are two orders of magnitudes larger than total CM constant, $CM_{tot} = 1.89 \times 10^{-8}$ $Oe\cdot M^{-1}$ $cm^3$ (in SI units $1.89 \times 10^{-6}$ $T^-2$ $M^-1$ $m^3$), have the opposite signs, and in summation with the $CM_{per\,reor}$ constant, describing the permanent dipole moment orientation, give a positive resultant value. Analysis of the CM effect gives $\mu_{exp} = 1.54 \times 10^{14}$ $erg\,Oe^{-1}$ ($1.54 \times 10^{21}$ $J\,T^{-1}$) for the permanent
magnetic dipole moment of the ferritin molecule containing 1500 iron atoms. This implies that $\mu_{\text{Fe}}^{\text{exp}} = 4.3 \mu_B$, for the magnetic dipole moment of an individual iron atom, where $\mu_B$ stands for the Bohr magneton. The result is in good agreement with the experimental values obtained from the measurements of the magnetic susceptibility of ferritin at room temperature, $\mu_{\text{Fe}}^{\text{RS}} = 3.8 \mu_B$, and it is consistent with the maximum predicted spin-only value of $\mu_{\text{Fe}} = 5.9 \mu_B$ and with the saturated magnetic dipole of Fe$^{3+}$, $\mu_{\text{Fe}} = 5.0 \mu_B$.\textsuperscript{18–20} If all iron atoms are in the $S = 5/2$ configuration (i.e., 100% iron ions are in the Fe$^{3+}$ state), the theoretically predicted saturated magnetic moment of such an ion is $\mu_{\text{Fe}}^{\text{theor}} = 5.0 \mu_B$.\textsuperscript{20} Then, taking into account the low temperature condition for $N = 1500$ Fe/ferritin, the resultant dipole moment of ferritin is $\mu_{\text{ferritin}}^{\text{theor}} = 1.8 \times 10^{-18} \text{ erg Oe}^{-1}$, but as follows from CM measurements this moment is $\mu_{\text{ferritin}}^{\text{exp}} = 1.54 \times 10^{-18} \text{ erg Oe}^{-1}$, as if the number of atoms contributed to the resultant dipole moment of ferritin. In the simplest approach it can be assumed that $\mu_{\text{ferritin}}^{\text{exp}}$ is produced by a number $N_{\text{theor}} = (\mu_{\text{ferritin}}^{\text{exp}} / \mu_{\text{Fe}}^{\text{theor}})^2 \approx 1100$ atoms in the Fe$^{3+}$ state, that is $\approx 74\%$ of all atoms. On the other hand, the measurements of light scattering in ferritin solutions in a magnetic field imply that diamagnetic interaction is responsible for the effect observed. As in apoferritin no changes in the scattered light induced by magnetic field are observed, the protein is not responsible for measured diamagnetic behavior and the orientation of the permanent magnetic dipole moment.\textsuperscript{20} Ferritin is a large spherical macromolecular protein with iron compounds situated in the cavity created by a peptide shell. Very small anisotropy of linear optical polarizability obtained from the static light scattering measurements and even smaller from the CM effect indicates the spherical shape of the ferritin for all samples irrespective of the number of iron atoms in the core. In a 100 mM NaCl water solution (like in 15 mM) no aggregation of the macromolecules is observed. Also no aggregates appearing due to interactions of the permanent or induced magnetic dipole moments in the magnetic field have been detected. The optically isotropic spherical macromolecule (with a very small anisotropy of linear optical polarizability components, $k_i$) should not show anisotropy of the magneto-optical polarizabilities. However, in solution it shows the induced magnetic birefringence (the CM effect) and the changes in intensity of the scattered light components induced by the magnetic field.

In view of the theory developed, only nonlinear interaction of the ferritin protein with the magnetic field can be responsible for the phenomena observed. Such nonlinearity is proposed to be a result of the displacement or deformation induced by a strong magnetic field in the iron compounds containing core. The percent contribution of all discussed effects to the entire birefringence and changes in the light scattering are calculated and discussed. They are different in the phenomena studied; however, they indicate that the diamagnetic behavior of the ferritin core plays an important role. In a ferritin molecule containing about 1500 iron atoms, $\approx 74\%$ of these atoms contribute to the superparamagnetic behavior and the orientation of the permanent magnetic dipole moment. The remaining iron atoms contribute to the nonlinear diamagnetic deformation of the protein core.

**ACKNOWLEDGMENTS**

This work was partially supported by Grant No. 2 PO3B 117 25 of the Ministry of Science and Higher Education in Poland.