Magnetic Langmuir–Blodgett Films of Ferritin with Different Iron Contents

Miguel Clemente-León,*† Eugenio Coronado,*† Alejandra Soriano-Portillo,‡ Enrique Colacio,† José M. Domínguez-Vera,*‡ Natividad Gálvez,‡ Rafael Madueño,§ and María T. Martín-Romero§

Instituto de Ciencia Molecular, Universidad de Valencia, Polígono de la Coma s/n, 46980 Paterna, Spain, Departamento Química Inorgánica, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain, and Departamento Física y Termodinámica Aplicada, Universidad de Córdoba, Campus Universitario de Rabanales, C3 (Edificio Marie Curie), 2ª planta, 14014 Córdoba, Spain

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Magnetic Langmuir–Blodgett films of four ferritin derivatives with different iron contents containing 4220, 3062, 2200, and 1200 iron atoms, respectively, have been prepared by using the adsorption properties of a 6/1 mixed monolayer of methyl stearate (SME) and dioctadecyl(dimethylammonium) bromide (DODA). The molecular organization of the mixed SME/DODA monolayer is strongly affected by the presence of the water-soluble protein in the subphase as shown by π–A isotherms, BAM images, and imaging ellipsometry at the water–air interface. BAM images reveal the heterogeneity of this mixed monolayer at the air–water interface. We propose that the ferritin is located under the mixed matrix in those regions where the reflectivity is higher whereas the dark regions correspond to the matrix. Ellipsometric angle measurements performed in zones of different brightness of the mixed monolayer confirm such a heterogeneous distribution of the protein under the lipid matrix. Transfer of the monolayer onto different substrates allowed the preparation of multilayer LB films of ferritin. Both infrared and UV–vis spectroscopy indicate that ferritin molecules are incorporated within the LB films. AFM measurements show that the heterogeneous distribution of the ferritin at the air–water interface is maintained when it is transferred onto solid substrates. Magnetic measurements show that the superparamagnetic properties of these molecules are preserved. Thus, marked hysteresis loops of magnetization are obtained below 20 K with coercive fields that depend on the number of iron atoms of the ferritin derivative.

Introduction

Natural ferritin is the iron-storage protein of animals, plants, and bacteria. It is a roughly spherical protein about 12 nm in diameter composed of 24 subunits arranged around a 7.5-nm-diameter iron ferrihydrite-like species capable of accommodating up to 4500 iron atoms.¹ By a suitable chemical process, it is possible to use the empty protein shell, apoferritin, as a confined environment in which different nanoparticles can be built. Several groups have prepared nanoparticles of inorganic compounds within the protein core for potential magnetic, catalytic, and biomedical sensing applications.²⁻⁵

In view of the interesting properties of ferritin, its supramolecular organization is an important step in the search for possible applications. Most of the examples reported in the literature have been concerned with the adsorption of ferritin onto different electrodes such as tin-doped indium oxide (ITO) or bare gold electrode surfaces.⁶ Thus, Zapien et al. showed that ferritin can be immobilized by adsorption at submonolayer coverages on ITO.⁷ On the other hand, Yoshinobu et al. achieved immobilization of ferritin on oxide patterns prepared by anodic oxidation of Si surfaces by atomic force microscopy (AFM).⁸ More recently, self-assembled monolayer (SAM)-modified gold electrodes showing an electrochemically regulated uptake and release of iron atoms have been prepared.⁹ Another possible strategy is the organization of ferritin at the air–water interface by adsorption onto Langmuir films formed by a mixture of methyl stearate and a positively charged alkylammonium surfactant⁴,¹⁰ or by an amphiphilic β-cyclodextrin.¹¹ These monolayers have then been transferred onto solid substrates by using the horizontal touching method.

In all of these cases, the deposition of ferritin monolayers is limited to one monolayer. However, for some applications it may be useful to go beyond the monolayer arrangement. For instance, Beissenhirtz et al. very recently reported the preparation of multilayers of another protein, cytochrome C, by the layer-by-layer method. The presence of a higher amount of this

† Universidad de Valencia.
‡ Universidad de Granada.
§ Universidad de Córdoba.

Table 1. Ferritin Derivatives Used in This Work

<table>
<thead>
<tr>
<th>number of iron atoms</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td></td>
<td>4220</td>
<td>3062</td>
<td>2200</td>
<td>1200</td>
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Electroactive molecule has shown to be useful in biosensing applications.¹³

An elegant approach to arranging molecules into well-organized multilayered films is the Langmuir–Blodgett (LB) technique.¹⁴ This technique affords a level of control over the orientation and placement of the molecules that is not available with other techniques. For this reason, the LB technique has been widely applied to create ultrathin films with a specific architecture that can be used as chemical sensors, modified electrodes, or molecular electronic devices.¹⁴,¹⁵ We used this technique previously to organize inorganic complexes with interesting magnetic properties such as the polynoxometalates¹⁶ and the Mn₁₂ single-molecule nanomagnets. In this last case, LB films with a hysteresis of magnetization below 5 K were obtained.¹⁷ The use of ferritin can be of interest to generate magnetic LB films with a memory effect because it contains a much higher number of magnetic ions and exhibits superparamagnetic behavior at blocking temperatures higher than those observed in the Mn₁₂ clusters. Here we show that it is possible to prepare LB films of four ferritin derivatives 1, 2, 3, and 4 containing 4220, 3062, 2200, and 1200 iron atoms, respectively. We report the preparation of these LB films and their structural and magnetic characterization. The interest in using different ferritin derivatives is to tune the magnetic properties of the LB films (coercive fields and critical temperatures) and to prove that our method is valid not only for natural ferritin but also for other synthesized ferritin derivatives. A preliminary communication of some of these results has been reported very recently.¹⁸

Experimental Section

Iron-saturated 1 and nonsaturated 3 horse spleen ferritin were obtained from Sigma-Aldrich and were exhaustively dialyzed against water using a Spectra/Por Float-A-Lyzer with a molecular weight cutoff (MWCO) of 300 000 Da prior to use. 2 and 4 were obtained by partial core iron reduction of 1 and 3, respectively, using thioglycollic acid/sodium acetate (0.1 mL, 0.1 M, pH 4.5) in the presence of 2,2’-bipyridyl. After 1 h of treatment, the ferritin solutions were exhaustively dialyzed as for 1 and 3.

Atomic absorption analysis of ferritin samples 1–4 gave iron concentrations of 0.24, 0.17, 0.12, and 0.07 M, respectively. Apoferritin concentrations in 1–4 were determined on the fully demetalated samples by the Lowry total protein micromethod assay (Sigma diagnostic) and were confirmed by UV–demetalated samples by the Lowry total protein micromethod assay. Cutoff (MWCO) of 300 000 Da prior to use. Concentrations of 0.24, 0.17, 0.12, and 0.07 M, respectively. Transfer ratios close to unity were obtained. CaF₂ substrates were used for infrared (IR) spectroscopy, quartz substrates for UV–vis spectroscopy, hydrophilic glass substrates for atomic-force microscopy, and Mylar substrates for magnetic measurements.

Two types of Langmuir troughs were used: a NIMA 611 D (Nima Technology, Coventry, England), provided with a W/F, Easy-type system using a strip of filter paper for good wettability and one moving barrier (total spreading surface of ~505 cm²) with a compression velocity of 10–20 A² molecule⁻¹ min⁻¹, facilitating the recording of the surface pressure–area (π–A) isotherms and the preparation of the LB films for AFM measurements, and a KSV3000 trough used to prepare the samples for IR spectroscopy, UV–vis spectroscopy, and magnetism. Millipore water with a resistivity higher than 18 MΩ cm was used in all of the experiments.

BAM and Ellipsometric Measurements. The I-Elli2000 used for these experiments is equipped with an imaging device by which images of the film at the Brewster angle (Brewster angle microscopy, BAM) can be recorded with a lateral resolution of 1 μm. The image processing procedure included a geometrical correction of the image as well as a filtering operation to reduce interference fringes and noise. Furthermore, the brightness of each image was scaled to improve contrast. The microscope and the film balance were located on a table with vibration isolation (anti-vibration system MOD-2 S, Halcyonics, Göttingen, Germany) in a large class-100 clean room. The ellipsometric measurements were performed using a PULSA pulsed imaging ellipsometer (I-Elli2000 supplied by NFT, Nanofilm Technologie, Göttingen, Germany) with a solid-state nitrogen laser (wavelength 532 nm, 50 mW). The accuracy of the device is 0.02° in Δ and Ψ. For measurements of the films at the air–water interface, we used an angle of incidence of φ = 50°. The ellipsometric angles, Δ and Ψ, were measured on different regions of interest (ROI, minimum size of about 20 μm) avoiding any lateral structure or defect within the spot size of the beam, resulting in valuable results and retaining spatial information.

Infrared (IR) spectra were recorded on a FTIR 320 Nicolet spectrometer. UV–vis spectra were recorded on a Shimadzu UV-2401PC spectrometer.

The AFM instrument is a NanoScope IIIa Multimode scanning probe microscope equipped with a type “J” scanner (Digital Instruments, Santa Barbara, CA). The microscope was first calibrated by using a test sample with 10 × 10 μm² pitches that were 180 nm deep. Images were obtained at ambient temperature and humidity. The tapping mode was employed, using etched silicon cantilever probes of 125 μm nominal length, at a drive frequency of ~300 kHz and a spring constant of 40 nN/m. We generally use a root-mean-square voltage of approximately 2–2.5 V and adjust the set-point voltage for optimal image quality, which is generally ~0.5–1 V less than the root-mean-square voltage. Both height and phase data were recorded at a scan rate of 0.5–1 Hz and stored in either 256 × 256 or 512 × 512 pixel² format. Images were processed using Nanoscope version 4.20 software. For images to be used in measuring heights, the only image processing was zeroth-order flattening. For any given image, the height was analyzed in at least three distinct regions of the structure being analyzed. For optimal image quality in visualizing surface features (and therefore for use in figures), first order flattening was used unless otherwise stated. The only other image adjustments were setting the image height range, color contrast, and color offset for the best appearance of structural details.
The magnetic measurements were performed with a Quantum Design MPMS-XL SQUID magnetometer. For those experiments, 80 and 390 monolayers were deposited onto a diamagnetic Mylar sheet (0.075 x 5 x 15 mm²). The procedure followed for the magnetic susceptibility is described elsewhere.16

Results and Discussion

Preparation of Ferritin Derivatives with Different Iron Contents. We have used two commercially available ferritins, 1 and 3, containing 4220 and 2200 iron atoms, respectively, to prepare a series of ferritin derivatives. The native iron oxide cores of these two commercially available ferritins were emptied in a controlled manner by treatment for 1 h with thioglycollic acid. This procedure allows the preparation of ferritin derivatives with different iron contents without changing the native core structure. Ferritin derivative 2 containing 3062 iron atoms was obtained from iron-saturated ferritin derivative 1, and ferritin derivative 4 containing 1200 iron atoms was obtained from commercially available ferritin derivative 3 that contains 2200 iron atoms. Frankel et al. reported a similar method to prepare five ferritin derivatives with iron loadings between 2100 and 400 iron atoms, but these derivatives were characterized by only Mossbauer spectroscopy.19 Mann et al. developed a different method to prepare a series of artificial ferritin samples with different iron contents. This method is based on the chemical reconstitution of the iron oxide cores within the empty polypeptide shell of apoferritin.20

Preparation of Monolayers of Ferritin. The first step in preparing LB multilayers of ferritin is the preparation of a monolayer of ferritin at the water–air interface. Britt et al. developed a method of forming monolayers of commercially available horse spleen ferritin at the water–air interface.11 This method is based on the adsorption of ferritin dissolved in an aqueous subphase onto a mixed monolayer of dioctadecyldimethylammonium bromide (DODA) and methyl stearate (SME) in a 6:1 ratio. At the pH of the water subphase (5.5), ferritin is negatively charged. The adsorption of ferritin is enhanced in a mixed monolayer containing cationic and nonionic molecules such as DODA and SME with respect to the adsorption of either of the single molecules.11,21 These authors demonstrated that a 6:1 SME/DODA ratio is optimal to retain a maximum amount of ferritin under the matrix monolayer. We have seen that by using these conditions it is also possible to form Langmuir–Blodgett multilayer films by the vertical lifting method. Furthermore, we have extended this method to the four ferritin derivatives, 1–4.

A Isotherms. Prior to the transfer process, the surface pressure–area (π–A) isotherms of the different monolayers formed at the air–water interface were recorded. Figure 1 shows the isotherms corresponding to the mixed SME/DODA film fabricated on a subphase in the absence (short-dashed line) and presence of ferritin derivative 3 (solid line) as well as those of pure lipids (dotted-dashed and long-dashed lines for pure SME and DODA, respectively). The other ferritin derivatives used in this work gave similar results. The area is expressed per SME molecule for mixtures and pure monolayers and per DODA molecule per pure monolayer. The main points of the isotherms shown in Figure 1 are the following: (1) The SME/DODA mixture undergoes a negative deviation from the additivity rule (ΔA_{mixture})

\[
\Delta A_{mixture} = A_{SME/DODA} - (\chi_{SME}A_{SME}(\pi) + \chi_{DODA}A_{DODA}(\pi)) < 0
\]

This could be related to the miscibility between both components.22 (2) The presence of ferritin at the interface causes an area expansion at low surface pressure, although such an expansion does not correspond to the ferritin area (d ≈ 120 Å, \(A_{ferritin} \approx 11300 \text{ Å}^2/\text{molecule}\)). Hence, this could indicate that ferritin is located under the lipid matrix. Also, according to the additivity rule, a positive deviation is obtained. (3) A small kink is detected for all isotherms containing SME around 10 mN/m, probably because of a phase transition. Furthermore, a new slope around 25 mN/m for the mixed monolayer with ferritin in the subphase is observed.

Brewster Angle Microscopy. The mixed monolayers in both the absence and presence of ferritin derivative 3 in the subphase were observed directly by BAM at the air–water interface to obtain information about the morphological properties of the different films. Britt et al. have already shown some BAM images of these mixtures; however, some details of the lateral morphology cannot be appreciated.11

Figure 2 shows the morphological changes under pressure undergone by the lipid molecules in the mixed monolayer. The images of the 6:1 SME/DODA mixture on pure water obtained at different surface pressures display typical behavior of a lipid monolayer. Thus, at low surface pressure, for example, at \(\pi = 0.1 \text{ mN/m}\), a coexistence of gas and liquid expanded phases is observed. With increasing surface pressure, the lipid molecules organize domains with tilt-oriented alkyl chains (liquid condensed phase). Rotating the analyzer to 60° with respect to the polarizer, the domains changed their brightness, showing the anisotropy of the mixed monolayer (images at 0.8 and 5 mN/m). Further compression leads to solid-phase formation, where the anisotropy is lost (the alkyl chains are perpendicularly oriented) (10 mN/m). Finally, at high surface pressure the collapse is also observed. These images show the miscibility of the two lipids in the mixed monolayer at the interface, in good agreement with the deviation from the additivity rule. Also, they clearly reveal the phase transition at \(\sim 10 \text{ mN/m}\) corresponding to the kink detected in the isotherms.

In the case of the mixed monolayer at the air–water interface with ferritin in the subphase, the morphological characteristics of the film (Figure 3) are different with respect to the above system (i.e., in absence of ferritin). From the beginning of the

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compliance process, for example at $\pi = 0.1$ mN/m, the presence of small irregular domains with high reflectivity is observed. Such domains grow and tend to fuse as the surface pressure increases, covering almost all of the surface (image at 0.3 mN/m). Under compression, the film shows two regions with different optical properties: one with anisotropy (which may be due to domains of lipid in the liquid condensed phase) and another one without anisotropy (a region with higher brightness), as can be appreciated at 2 and 5 mN/m in Figure 3. These differences with respect to those recorded without ferritin in the subphase are indicative of the presence of the protein attached under the lipid matrix at the interface. However, these molecules of protein do not seem to form a full monolayer under the mixed film. In the sequence of images, the phase transition at 10 mN/m is also detected for the region that before showed anisotropy. Further compression of this system leads to an increase in the brightness of some parts of the film.

Figure 3 reveals the heterogeneity of the mixed monolayer at the air–water interface when the subphase contains ferritin (0.01 mg/mL). We think that the ferritin is located under the mixed matrix in those regions where the reflectivity is higher whereas the dark regions correspond only to the matrix. To prove such an idea, the ellipsometric angle, $\Delta$, under an angle of incidence of $50^\circ$ and at $\lambda = 532$ nm, was measured on those different regions of interest (ROI) (i.e., on the bright domains and on the surrounding dark regions) of the mixed monolayers in both the absence and presence of protein in the subphase. Figure 4 shows the variation of the ellipsometric angle, $\Delta$ ($\Delta = \pi - \Delta_0$, $\Delta$ is obtained in the presence of film at the air–water interface and $\Delta_0$ is obtained in the absence of it, for example, on an aqueous surface) with respect to the surface pressure.

The mixed monolayer in the absence of ferritin shows an increase in the values of $\Delta$ at low surface pressure, being almost constant from 5 to 10 mN/m when the condensed phase is achieved and the optical properties (refractive index and thickness) are invariable. $\Delta \approx 3^\circ$ at high surface pressure agrees with the result obtained for Tronin et al. for a DODA monolayer.23

In comparison, the presence of ferritin in the subphase causes changes in the values of the optical parameter $\Delta$. Thus, $\Delta$ increases as the surface pressure increases, and values higher than those for the mixed monolayer in the absence of ferritin are obtained. From 0 to 30 mN/m, the values shown in Figure 4 for this system correspond to the average overall focus, although at high surface pressure ROIs were used to measure the ellipsometric angle in the two regions with different brightness (Figure 3).

Figure 4. Variation of the ellipsometric angle, $\Delta$, of mixed monolayers containing SME/DODA = 6:1 both in the absence (circles) and presence (triangles) of ferritin molecules in the aqueous subphase, under compression. From 0 to 30 mN/m, the triangles correspond to the average overall focus, although at 35 mN/m (marked triangles) ROI were used to measure the ellipsometric angle in the two regions with different brightness (Figure 3).
The LB films present a strong red color.

**Infrared Spectroscopy.** The infrared spectra of LB films of 1–4 (19 monolayers deposited on CaF₂) are very similar. All of them present two strong bands associated with ferritin at 1654 and 1549 cm⁻¹ (Figure 5), besides the bands at 2915, 2849, and 1466 cm⁻¹, assigned to the CH₂ stretching or scissoring vibrations of DODA and SME alkyl chains, and the band at 1737 cm⁻¹, assigned to the C=O vibration of SME. These two bands are assigned to the N–H and C=O structural units of the protein together with the superposition of the –OH bending absorption on the band at 1654 cm⁻¹. All of these bands are narrower and slightly shifted in the LB films when compared to the spectrum of ferritin in the KBr pellet. This effect has been observed in other LB films of inorganic anions and can be explained by the nonrandom organization of the molecules within the LB films or the presence of positively charged amphiphilic molecules. We observe that the relative intensity of the DODA and SME bands with respect to the ferritin bands is similar for the LB films of 1–4. This indicates that the ferritin contents of these films are similar.

**UV–Vis Spectroscopy.** The UV–vis absorption spectra of LB films of the four ferritin derivatives on quartz substrates show the typical broad absorption centered below 500 nm of the ferritin iron core with a shoulder around 280 nm, characteristic of the apoferritin shell (Figure 6 for the LB film of 1). The absorbance changes at a given wavelength with the number of monolayers for LB films of 1 are shown in the inset of Figure 6. A linear dependence is found that indicates that the Langmuir film is transferred in a regular manner after each dipping cycle. Assuming that ferritin molecules present an isotropic distribution within the LB films, it is possible to estimate the number of molecules trapped within the LB films from the absorbance values at 420 nm. A concentration of (4 ± 2) × 10⁻¹³ mol/cm² per monolayer is calculated for the LB films of 1 with 21, 25, and 35 monolayers. The inverse of these values leads to a mean area per ferritin molecule of 30 000 ± 5000 Å². This value is higher than the area of a ferritin molecule (d = 120 Å, A_ferritin ≈ 11300 Å²). This result indicates that the number of ferritin molecules trapped within the LB film is not enough to form a continuous monolayer. This is consistent with BAM results that showed that at the water–air interface that there is not complete coverage of the SME/DODA monolayer by the adsorbed ferritin molecules. Therefore, the same effect is observed when the Langmuir monolayer is transferred onto solid substrates. This is also confirmed by AFM and magnetic measurements (see below). LB films of the other ferritin derivatives give similar results.

**Atomic Force Microscopy.** This technique was used to study the organization of the mixed system transferred onto the solid support. LB films of 3 with 11 and 19 monolayers were studied. Figure 7 shows some images from different regions of the film. These images show the irregular transfer process and three regions with different heights. Therefore, a nonhomogeneous distribution of the ferritin under the mixed monolayer is confirmed. Some analysis of such images can give us more information about the different regions.

In the case of the multilayer with 19 monolayers, besides those three regions one more is detected that corresponds to the clean glass surface. Thus, regions with heights of 72–62, 42–40, and 9–6 nm were measured. Considering the irregular distribution of the protein under the mixed matrix at the air–water interface (Figure 3 at high surface pressure) and supposing that the organization at that interface is conserved during the transfer process and constant transfer ratios, regions with ~266 nm (d_ferritin ≈ 12 nm, d_SMEDODA ≈ 2 nm) and ~38 nm, in the presence and absence of ferritin, respectively, will be expected. Therefore, we can estimate that the domains with a height of 9–6 nm correspond to zones with almost no film, which is probably due to defects in the substrate, those of 42–40 nm, to zones that contain only SME/DODA, and those of 72–62 nm, to multilayers containing ferritin. However, the height of this last zone does not fit with the expected value. This indicates the transfer of only about 25% of the protein retained at the air–water interface by its adsorption to the mixed monolayer. A more regular and homogeneous distribution of the ferritin in the film may improve the transfer success and the operation of the system. In that sense, films containing different cationic lipids should be studied.

**Magnetic Properties.** The iron oxohydride core of ferritin is antiferromagnetic below 140 K, but it presents a net magnetic moment arising from uncompensated iron spins largely at the surface of the core. It has been shown that ferritin behaves as a superparamagnet above 20 K. Below the blocking temperature (T_B ≈ 15 K), there is not enough thermal energy to allow the moments of the superparamagnetic particles of ferritin to oscillate across the magnetic anisotropy barrier, and the magnetic moments become frozen. As a result of this, zero-field-cooled (ZFC) and field-cooled (FC) susceptibility curves present

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different behaviors below \( T_B \). ZFC susceptibility presents a maximum proportional to \( T_B \), and the FC susceptibility curve increases continuously with decreasing temperature. Another consequence of the blocking of the magnetic moments below \( T_B \) is the appearance of frequency-dependent ac susceptibility peaks and a marked hysteresis loop of magnetization. Because two of the four ferritin derivatives used in the present work have been prepared by a new method, we will present first the magnetic properties of evaporated solutions of these ferritin derivatives. 1–4 present superparamagnetic behavior similar to that described in the literature. They show a maximum in the ZFC susceptibility curve whereas ZFC and FC curves are superimposed above 30 K. The maxima of the ZFC susceptibility curves are shifted toward lower \( T \) as the iron loading decreases. Thus, the maxima of the ZFC curves of 1–4 appear at 14.6, 14.0, 12.2, and 11.5 K, respectively (Figure 8). These \( T \) values are similar to those obtained for the artificially reconstituted ferritin derivatives of similar iron loadings with small differences of around 1 K.25 However, the number of derivatives prepared by us is not large enough to determine if there is a linear dependence of \( T_B \) on the particle size as described by Mann et al. for artificially reconstituted ferritin derivatives20,25 or a nonlinear dependence due to surface anisotropy as found by Mössbauer spectroscopy for a series of ferritin derivatives prepared by chemical reduction by Frankel et al.19 1–4 present frequency-dependent ac susceptibility peaks below 30 K with broad maxima between 10 and 15 K (Supporting Information).27 Finally, marked hysteresis loops of magnetization are obtained with coercive fields of 2500, 2700, 2400, and 2200 G at 2 K for 1–4, respectively. This hysteresis loop is characteristic of antiferromagnetic compounds and is very different from that in ferro- or ferrimagnets. It remains open up to much higher fields (around 20 000 G). Furthermore, saturation is not reached at high fields because of the field linear antiferromagnetic contribution.29

The magnetic properties of LB films of the four ferritin derivatives have been measured. A more complete magnetic characterization of the LB films of the ferritin derivative with intermediate composition, 3, was carried out. Monolayers (390) of this LB film were deposited onto a diamagnetic Mylar substrate. The temperature dependence of the susceptibility of this LB film is very similar to powder measurements (Figure 9a). The ZFC curve presents a maximum in the susceptibility around 11 K, and ZFC and FC curves are superimposed above 30 K (Figure 9b). This proves clearly that ferritin molecules are present within the LB films. Although \( \chi'' \) is too low to be detected, a marked hysteresis loop of magnetization is obtained with a coercive field of 2300 G (Figure 10). The shape of the hysteresis loop of magnetization is very similar to that of powder measurements. We have measured the hysteresis loop of magnetization at different temperatures, and we observed a decrease in the coercive fields with increasing temperature (inset of Figure 10) with a coercive field of 2200 G at 5 K that decreases by 1 order of

![Figure 7. AFM images of multilayers of the mixed SME/DODA monolayer, molar ratio 6:1, prepared on an aqueous subphase in the presence of ferritin 3 (0.01 mg/mL); \( \tau_{trans} = 35 \text{ mN/m} \). (Left) Multilayer of 12 layers. (Right) Multilayer of 19 monolayers with the analysis of the AFM image.](image)

![Figure 8. Thermal dependence of the zero-field cool (ZFC) and field cool (FC) susceptibility with an applied field of 50 G of evaporated samples 1–4.](image)

it confirms that the number of ferritin molecules trapped within the film is not enough to form a continuous monolayer, in agreement with AFM measurements. Similar values are obtained for the LB films of the other ferritin derivatives.

LB films of 80 monolayers of the other three ferritin derivatives also present a marked hysteresis loop of magnetization with coercive fields that decrease when the number of iron atoms is decreased. Thus, LB films of 1–4 present coercive fields of 3400, 2400, 2300, and 900 G, respectively. These coercive fields are of the same order of magnitude as those found for the powdered samples. Furthermore, the other magnetic data closely resemble those of the powdered samples. This is an expected result because the magnetic properties of this molecule have a molecular origin. The magnetic isolation provided by the diamagnetic apoferritin shell prevents any magnetic interaction between ferritin molecules.

Conclusions

We have demonstrated that it is possible to prepare Langmuir monolayers and LB multilayers of ferritin derivatives with different iron contents. Langmuir monolayers have been characterized by BAM microscopy and ellipsometric angle measurements that reveal the heterogeneity of this mixed monolayer at the air–water interface. Both infrared and UV–vis spectroscopy of the LB films indicate that ferritin, SME, and DODA molecules are incorporated within these LB films. Furthermore, AFM measurements showed that the heterogeneous distribution of the ferritin at the water–air interface is maintained when it is transferred onto solid substrates. Magnetic measurements show that the superparamagnetic properties of ferritin are preserved in the LB films.

This method is general and can be extended to ferritin derivatives containing other nanoparticles with interesting magnetic, electric, or optical properties. In the case of the ferritin derivatives used in this work, the main advantage of this method is that it permits the preparation of thin films with magnetic memory in a controlled manner. The thickness can be controlled monolayer per monolayer on the nanometer scale. Thus, LB films with thicknesses between 1 and 7 nm have been prepared. A more homogeneous distribution of ferritin molecules in these thin films could be achieved by using other amphiphilic molecules instead of DODA and SME, presenting stronger interactions with the ferritin molecules dissolved in the subphase. However, the use of ferritin derivatives with different iron loadings has been reported so far. They have been prepared following two approaches. One involves the preparation of structurally ordered monolayers of polymeric metal complexes in which the extended structure of the inorganic network facilitates the occurrence of cooperative magnetic phenomena. Some examples are LB films of divalent metal organophosphonates and cyanide-based extended networks. The problem of this strategy is the difficulty in controlling the formation of extended networks at the water–air interface. Another approach has been the use of molecular magnetic clusters exhibiting magnetic hysteresis, such as the well-known single-molecule magnets of the Mn12 family. Because ferritin molecules in solution present magnetic hysteresis, LB films of these superparamagnetic nanoparticles

Figure 9. (a) Thermal dependence of the ZFC susceptibility with an applied field of 50 G of evaporated sample 3 (empty circles) and the LB film of 3 (full circles) with 390 monolayers deposited on a Mylar substrate. (b) Thermal dependence of the ZFC (empty circles) and FC susceptibility (full circles) with an applied field of 50 G of the LB film of 3 with 390 monolayers deposited on a Mylar substrate. The molar susceptibility of the LB film is obtained by normalization to the evaporated sample.

Figure 10. Hysteresis loop of magnetization of evaporated sample 3 (empty circles) and the LB film of 3 (full circles) with 390 monolayers deposited on a Mylar substrate at 2 K. The magnetization of the LB films was normalized to that of the evaporated samples. (Inset) Temperature dependence of the coercive field for the LB film of 3 with 390 monolayers deposited on a Mylar substrate.

magnitude at 15 K ($H_c = 150$ G) and almost vanishes at 20 K ($H_c = 40$ G).

By comparison with powder samples, we have calculated the number of ferritin molecules trapped within the LB film of 3. A comparison of the susceptibility measurements and magnetization data gives the same results ($4 \times 10^{-13}$ mol of ferritin within the LB film of 3). Because the area of the substrate (1 cm$^2$) and the number of monolayers (390) are known, it is straightforward to estimate the density of ferritin molecules within the monolayer and the mean area per molecule of ferritin within the LB film. For LB films of 3 we have obtained a mean area of around 30 000 Å$^2$ that is consistent with the result obtained for the LB film of 1 from UV–vis spectroscopy. Because this value is higher than the mean area of a ferritin molecule (11 300 Å),
could be inscribed in this second group. A problem associated with this molecular approach to the magnetic LB films is that the blocking temperatures are still too low. Using these nanoparticles, an increase in the blocking temperatures from 5 (in Mn$_{12}$ films) to 20 K in ferritin has been achieved. Further increases in these values will be obtained in the future by playing with the possibility of replacing the antiferromagnetic iron oxohydride core of natural ferritin with other nanoparticles of magnets with higher $T_c$ (nanoparticles of Prussian-blue derivatives or metals such as cobalt). We are currently exploring this possibility.

Finally, the processing of ferritin in such transparent thin films is very convenient for light irradiation. A correct choice of the nanoparticle of the ferritin core could permit the preparation of thin films in which the magnetic properties could be tuned by light irradiation (photomagnetic thin films).

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**Supporting Information Available:** Thermal dependence of the zero-field cool and field cool susceptibility with an applied field and evaporated samples 1–4. Thermal dependence of the in-phase and out-of-phase susceptibilities of evaporated sample 4 at different frequencies. This material is available free of charge via the Internet at http://pubs.acs.org.