Fabrication of Ordered Mesoporous Silica Films with Encapsulated Iron Oxide Nanoparticles using Ferritin-Doped Block Copolymer Templates

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Ordered mesoporous silica glasses containing encapsulated magnetic nanoparticles were fabricated by the 3D replication of preorganized block copolymer templates doped with ferritin. Solutions of Pluronic F127 (PEO_{105}-PPO_{70}-PEO_{105}) containing horse spleen ferritin and *p*-toluene sulfonic acid were spin-coated onto silicon test wafers. Phase selective deposition of silica within the ferritin-containing block copolymer was conducted by exposure of the template films to solutions of tetraethylorthosilicate in supercritical carbon dioxide. Silica network formation occurs exclusively in the hydrophilic block due to partitioning of the acid catalyst to the PEO rich domains during spin coating. Calcination of the resultant composite at 400 °C removes the polymer template and protein shell of the ferritin nanoparticles, yielding a robust mesoporous film as evidenced by electron microscopy. X-ray diffraction showed that after processing the crystalline structure of the iron oxide ferritin core was maintained. Magnetization measurements indicate that the magnetic properties of the ferritin cores are unaffected by the silica infusion and calcination steps. This approach provides a simple and general way to fabricate functionalized mesoporous materials with defined pore structures.

Introduction

Recently there has been a great deal of interest in the fabrication of defect-free mesoporous silica thin films for potential device applications including ultralow dielectric constant thin films for microelectronics, catalyst supports, sensors and separations media.[1−3] The incorporation of functionality within a mesoporous silica thin film through encapsulation of active materials significantly increases the utility of these materials.[4] For example, the immobilization of enzymes encapsulated in silica particles and thin films has been investigated for improved enzyme efficiency and stability. Horseradish peroxidase (HRP) encapsulated in silica has been shown to have an increased stability in organic solvents.[5] Trypsin and acid phosphatase have also been encapsulated in an inorganic silica network.[6,7]

Here we demonstrate a straightforward strategy for the encapsulation of active nanoparticles within mesoporous silicas using horse spleen ferritin as a model system. Ferritin is a spherical iron storage protein typically found in the spleen or liver of living organisms that has been widely studied for its interesting magnetic properties.[8−10] Structurally, ferritin comprises a complex protein membrane shell, apoferritin, surrounding an iron-oxide-like core. The apoferritin shell consists of twenty-four subunits that cooperatively self-assemble forming microchannels that enable iron ions to enter its core.[11,12] Within the protein shell, iron ions diffuse to active sites where the ions oxidize and condense to form an iron oxide, Fe_2O_3 bound with water, that closely resembles the structure of ferrihydrite [Fe(III)O·OH]. Commercially available horse spleen ferritin typically contains a distribution of core sizes; the average dimensions of the protein are a core diameter of 8 nm surrounded by an apoferritin shell of 12 nm in diameter.[11] In the bulk, the iron oxide core is ferrimagnetic; however, on the size scales present in the ferritin cluster, interactions between particles are minimized and thermal energy overcomes magnetic anisotropy, making the material superparamagnetic.[8,9] Such materials have been investigated for sensor and drug delivery applications.[10]

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Recently, our group has reported the three-dimensional replication of block copolymers with silica using supercritical carbon dioxide as a delivery medium.[13−18] Supercritical carbon dioxide selectively dissolves metal alkoxide precursors while swelling the block copolymer templates, enabling efficient precursor transport without disrupting microphase segregation.[19,20] After calcination, the resultant film contains well-ordered mesopores of the replicated block copolymer morphology. Our method separates the formation and organization of the template from precursor condensation, thus eliminating the need for mutual solubility.[13] Moreover, such an approach provides opportunity for doping of the template with functional material prior to silica network formation. This is beneficial for many biological systems, because they are usually soluble in aqueous media. In the case of ferritin, the hydrophilic protein shell selectively drives encapsulation in the hydrophilic domain of the amphiphilic block copolymer. Finally, because alcohols are highly soluble in supercritical carbon dioxide, they are readily removed from the incipient composite, minimizing their effect on the secondary structure of polypeptides.[13]

Our recent templates of choice include Pluronic poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers. Given the hydrophilic nature of the apoferritin shell, it is expected that ferritin will partition with the acid catalyst to the hydrophilic poly(ethylene oxide) domains (Figure 1). Moreover these templates are good candidates for doping with ferritin because interaction of the apoferritin shell with the copolymer reduces the likelihood of particle aggregation during template preparation and infusion. Electrophoretic experiments suggest a strong interaction between Pluronic F127 and apoferritin. Under acidic conditions, ferritin does not readily diffuse through a Pluronic medium.[21] Upon precursor infusion, silica will condense in the hydrophilic domain, encapsulating the ferritin. If the copolymer template and apoferritin protein shell are thermally removed, then the remaining structure is composed of mesoporous silica containing isolated magnetic ferritin cores.[22−24]

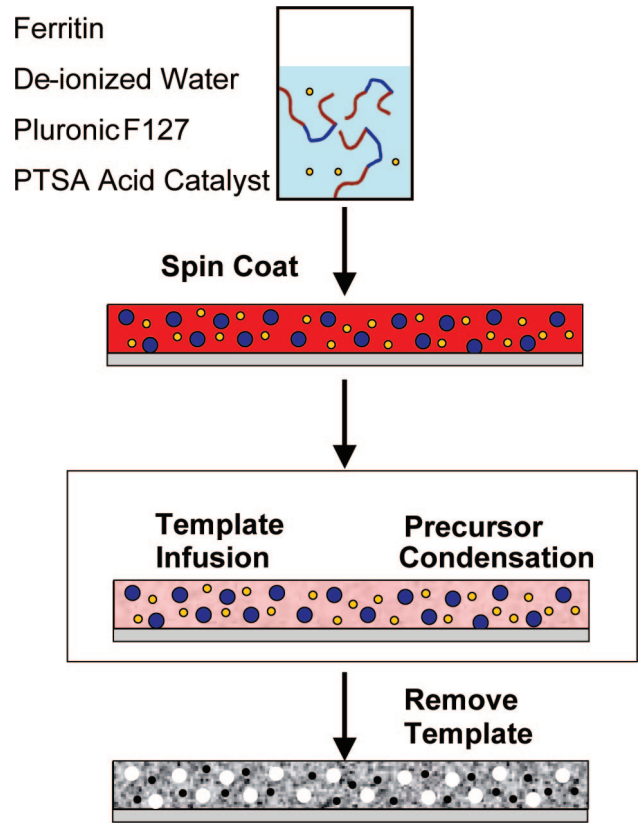

Figure 1. Schematic of the supercritical infusion process. The ferritin-doped Pluronic F127 template is first prepared in aqueous solution and spin-coated onto a suitable substrate. The sample is then exposed to a solution of supercritical carbon dioxide and TEOS, after which the template is removed by calcination. The red continuous domain represents PEO, which is doped with ferritin (yellow spheres). The blue PPO domains are removed during calcinations as is the apoferritin shell, leaving magnetic iron oxide particles (black) embedded in the mesoporous silica film.

Experimental Section

Materials. Horse spleen ferritin (sterile filtered solution in 150 mM NaCl) was purchased from Biochemica, Pluronic F127 (PEO_{105}-PPO_{70}-PEO_{105}) triblock copolymer was donated by BASF, and *p*-toluenesulfonic acid monohydrate (purity >98.5%) and tetraethyl orthosilicate were purchased from Sigma-Aldrich. Coleman grade carbon dioxide (99.99% minimum purity) was obtained from Merriam Graves.

Preparation of Mesoporous Magnetic Glasses. Templates were prepared by dissolving the Pluronic template and p-toluenesulfonic acid (2 wt % with respect to the polymer) in deionized water. Once the template was completely dissolved, suspensions with ferritin-to-template weight ratios of 0.01:1, 0.05:1, and 0.10:1 were prepared. The suspensions were red after mixing. In all cases, the pH of the suspensions was >2.5 to maintain the structural integrity of the ferritin.

The suspensions were spin-coated onto silicon test wafers. Each wafer was then placed into a stainless steel reactor with 5 μL of tetraethyl orthosilicate and two drops of deionized water separated by a watch glass to prevent mixing, and heated to 40 °C from room temperature over a period of 1 h. The reactor is constructed of two stainless steel blind hubs (Grayloc) with a graphite-coated stainless steel seal ring, has an internal volume of ~160 mL, and has machined ports for the temperature and pressure measurement and for the introduction and release of CO_2. Coleman grade carbon dioxide (99.99% purity) was slowly introduced to a final pressure of 131 bar. At the temperatures used, ferritin does not degrade.[11] After reaction for 3 h, the vessel was depressurized overnight and the sample was removed.

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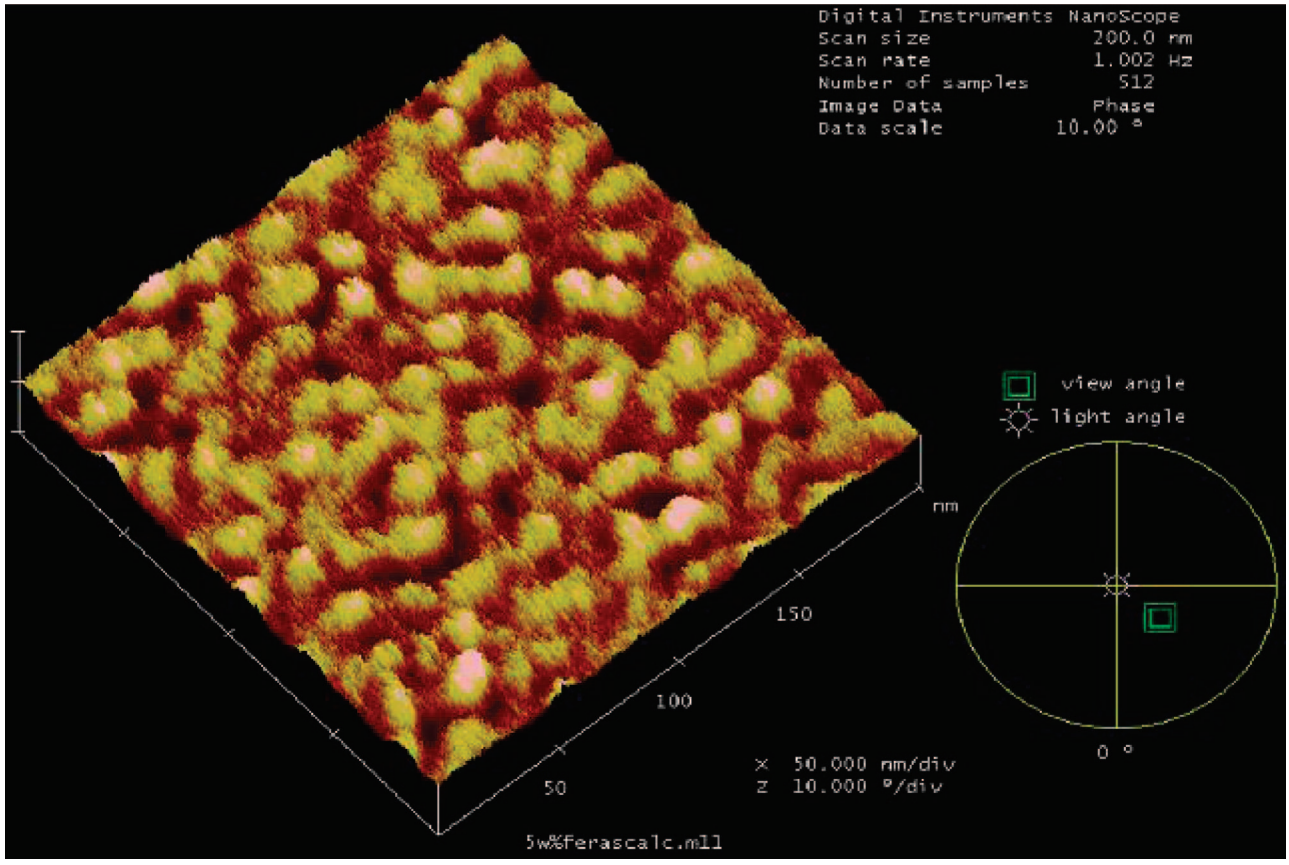

Figure 2. Magnetic force microscopy (MFM) of as spun 5 wt % ferritin-containing template film. This image indicates that the magnetic ferritin nanoparticles are well-dispersed.

Silica condensation was confined to the template domain where the acid catalyst was present, and therefore unreacted precursor and alcohol byproducts were removed with the supercritical phase during depressurization. There were no additional washing or aging steps necessary because all byproducts were vented. The samples were calcined at 400 °C for 6 h. The temperature ramp cycle was from room temperature to 400 °C over 4 h. Calcination efficiently removes the polymer template, the ferritin protein shell, and promoted additional silica network condensation (see below). Final film thickness was ranged between 200 and 300 nm.

Characterization. FTIR was performed in transmission on a BioRad Excalibur Series Spectrometer. TEM was performed on a JEOL 100CX 100 kV microscope. Samples were calcined, scraped from the silicon substrate, crushed with a mortar and pestle, diluted with ethanol, and then deposited on a Formvar coated copper TEM grid. XRD was performed on a Philips X'Pert X Ray Diffractometer. Magnetic force microscopy (MFM) was performed on a Digital Instruments atomic force microscope with a magnetic detection head. Magnetic measurements were performed on a Quantum Design MPMS XL-7 SQUID magnetometer.

Results and Discussion

Dilation of the block copolymer template films with CO_2 enables the enhanced diffusion of small molecules[25,26] within the block copolymer matrix without disruption to microphase segregation.[19,20] Consequently, the reaction and infusion within the template are conducted without disrupting the established morphology of the block copolymer template. The acid catalyst and ferritin remain partitioned to the hydrophilic domain while TEOS readily diffuses into the polymer and undergoes hydrolysis/condensation with the acid catalyst. MFM indicates that there is a well-distributed magnetically active material within the as-spun film (Figure 2). There is little observable aggregation in MFM imaging.

FTIR analysis confirms infusion of silica into the template and complete removal of the template after calination (see Figure S1 in the Supporting Information). After infusion, a broad band appears at about 3400 cm^{-1} corresponding to silanol stretching and additional bands appear at about 1170 and 810 cm^{-1}, which correspond to Si−O−Si stretching modes. The presence of silanol is indicative of incomplete network condensation. However, after calcination, the silanol stretch largely disappears while the Si−O−Si bands remain, which is consistent with further condensation of the silica network. Also, the prominent C−H stretching bands (2800−3000 cm^{-1}) are eliminated in the calcined sample, indicating that the block copolymer template and protein were removed.

The addition of ferritin or other nanoparticles to the block copolymer template could influence microphase segregation.[27−34] Recently, it was shown that addition of silica nanoparticles to a Pluronic F127 gel increased order as the silica particle

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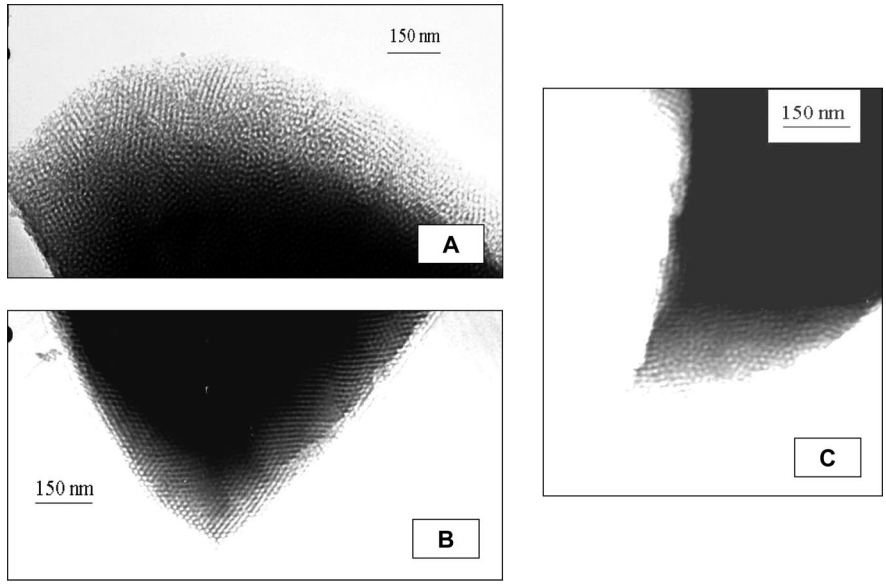

Figure 3. TEM of ferritin-containing samples: (A) 1 wt % ferritin loading, (B) 5 wt % ferritin loading, (C) 10 wt % ferritin loading. Reaction conditions: TEOS infused at 40 °C, 126 bar. Calcined for 6 h at 400 °C.

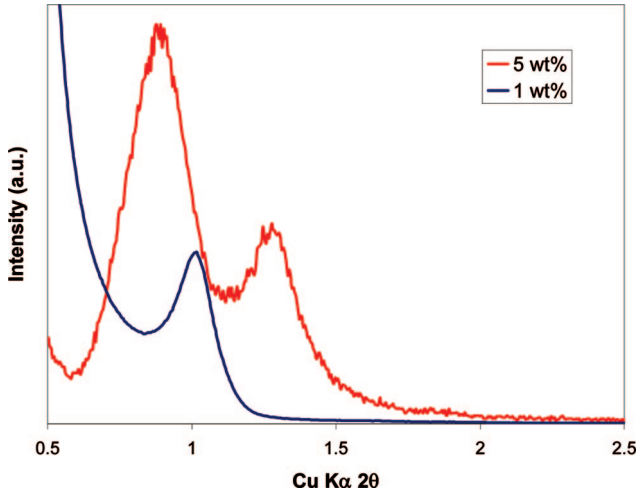

Figure 4. Small-angle XRD of 1 and 5 wt % concentration ferritin-containing silica films. Reaction conditions: TEOS, 40 °C, 126 bar. The *d*-spacing increases as concentration increases. The second-order reflection in the 5% film indicates a higher degree of order relative to the 1% film.

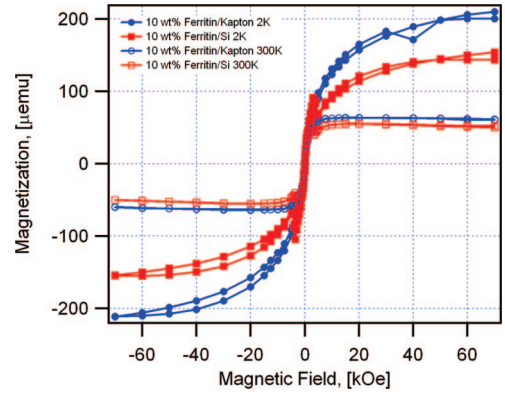

Figure 5. Magnetization curves for 10 wt % ferritin films as spun on Kapton tape (circles) and after silica infusion and calcination on a Si wafer substrate (triangles), at 2 K (filled symbols), and 300 K (open symbols).

concentration increased to the point at which the number of added particles was approximately equal to the number of interstitial sites available in the gel.[27,28] Further increases in particle concentration disrupted order. Nanoparticle assembly and distribution have also been directed by block copolymer thin films, where a match of the hydrophobic character of the nanoparticle and one block of the copolymer dictates the structure.[29] Typically, as large molecules of uniform size and shape are added to a single domain of a block copolymer, the altered domain swells and the particles concentrate in the center of the domain.[30,31]

Figure 3 shows the morphology of calcined mesoporous film samples prepared using three concentrations of ferritin. In the 1 wt % film (Figure 3A), it appears that pore order has been disrupted slightly relative to undoped mesoporous films prepared previously using a similar protocol.[13] The films prepared using templates containing 5 wt % appear to exhibit better order than those prepared with 1 wt % ferritin. This observation is supported by small-angle XRD analysis, where the appearance of a second order reflection as the ferritin concentration is increased from 1 to 5 wt % is apparent (Figure 4). The XRD data also show that when ferritin concentration in the film is increased, there is a distinct increase in *d*-spacing that arises from expansion of the domains of the template film to accommodate the ferritin particles. The ratio of the peak positions of first and second

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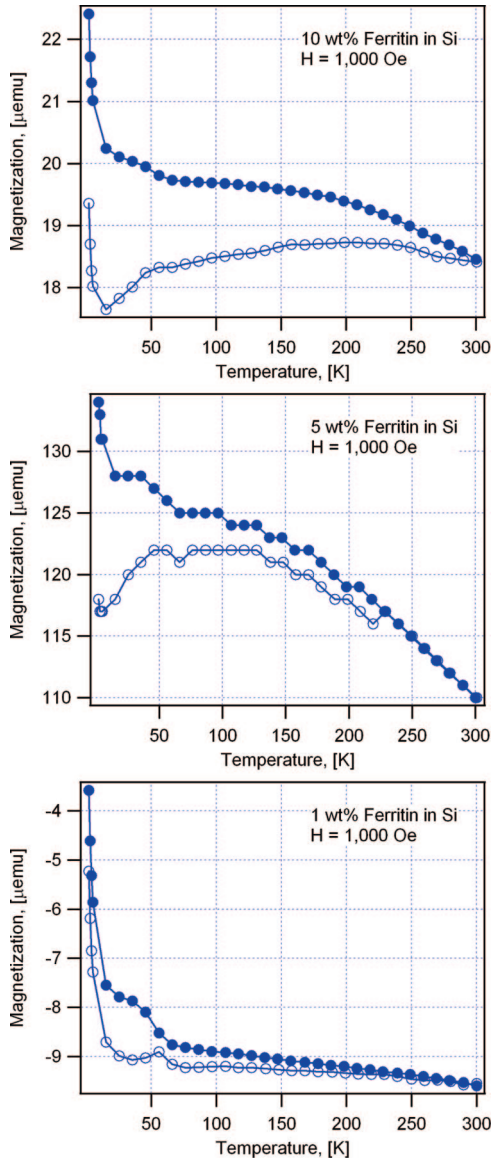

Figure 6. Zero-field-cooled (open circles) and field-cooled (closed circles) magnetization curves for ferritin films infused with silica and calcined on a Si wafer substrate, at an applied field of 1000 Oe.

order reflections $1:\sqrt{2}$ suggest that a cubic phase cylindrical morphology[32] is maintained within the films, which is consistent with the TEM micrographs. Further increase in ferritin concentration to 10 wt % appears to yield less-ordered structures. This may be due to frustration of template order because of high concentrations of the ferritin particles. It may also be possible that selective addition of high concentrations of particles to one block increases the apparent volume fraction of that block and the apparent disordering is the onset of an order−order transition. We are currently investigating the phase behavior of the block copolymer−ferritin composites in more detail to fully understand microphase segregation in these systems.

After calcination, there was no change in X-ray diffraction patterns attributed to Fe_2O_3 indicating that the iron oxide cores remain intact (see Figure S2 in the Supporting Information). As films were heated to 400 °C, both the polymeric template and the ferritin protein shell were removed, as evidenced by the disappearance of the C−O and C−H alkane peaks (see Figure S1 in the Supporting Information).

Magnetization measurements conducted at 2 and 300 K for the as-spun, ferritin-doped template films prior to silica encapsulation on Kapton tape and the calcined films in Si wafers show that the magnetic properties of the ferritin nanoparticles are preserved during processing. As an example, Figure 5 shows the corresponding results for films containing 10 wt % ferritin, after subtracting the diamagnetic background due to the sample holder, substrate, and infused silica. Magnetization is reported in emu as the deposited film mass was not determined experimentally for these films. However, the films were deposited under similar conditions and the sample had similar film area, hence the amount of ferritin in both samples should be similar. The observed magnetization magnitudes are in agreement. The as spun and calcined samples had a coercivity of 300 Oe at 2 K and ∼50 Oe at 300 K. The remanence in both samples was $\sim 1 \times 10^{-5}$ emu at 2 K and $\sim 1 \times 10^{-6}$ emu at 300 K. These observations indicate that the magnetic properties and core−core interactions are unaffected by the silica infusion and calcination steps. The observed coercivity at 300 K is consistent with ZFC/FC measurements with a field of 1000 Oe conducted between 2 and 300 K, as shown in Figure 6. These measurements indicate that at 300 K, the sample still has some ferromagnetic character, as the ZFC and FC curves at this temperature have not superimposed on each other. We believe that this is indicative of magnetic interactions between the ferritin cores at 10 wt %, as similar measurements made for 5 and 1 wt % ferritin on Si (also shown in Figure 6) yield superimposed ZFC and FC curves above temperatures of ∼225 K, indicating superparamagnetic behavior. In the case of the 1 wt % sample the reported magnetization is negative owing to the effect of diamagnetic background, which is not subtracted in the ZFC/FC data.

Conclusions

Phase selective deposition of silica within block copolymer templates doped with nanoparticles is a convenient approach for the preparation of functional mesoporous materials. In this report, ferritin has been successfully encapsulated in robust and crack-free mesoporous films. The apoferritin protein shell appears to prevent aggregation of iron oxide nanoparticles prior to calcination by limiting the interaction between the cores. The magnetic properties of the ferritin cores was preserved during processing and there was evidence of magnetic interactions between the cores at high loadings (10 wt %) of ferritin.

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Supporting Information Available: Additional figures and information (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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