Protein-based microgels containing magnetic iron oxide nanoparticles

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Microgels, micrometer-sized polymer hydrogel particles, are attractive functional materials for a number of medical applications, including controlled drug delivery. Microgels are most often comprised of industrially produced polymers that have been shown to be biologically compatible. Natural polymers (carbohydrates and proteins) are attractive macromolecules for producing microgels due to their innate biocompatibility and the multiple functional groups that can be used for synthetic modification or crosslinking. Even with these apparent advantages, there are only a handful of synthetic methods for producing protein microgels. We present a
novel synthesis in which hemoglobin and Fe\(^{2+}\) react, in the presence of KNO\(_3\) and KOH, to produce protein microgels that contain magnetic iron oxide nanoparticles. The synthesis results in microgels with polymer properties (denaturing and glass transition temperatures) that are consistent with the dried protein. The iron oxide nanoparticles exhibit an average diameter of 22 nm, are ferrimagnetic, and display properties consistent with Fe\(_3\)O\(_4\). The microgels are capable of adsorbing 98% of a dye in a 200 \(\mu\)M solution. The multiple functional capabilities displayed by these materials: biocompatibility, magnetism, and properties archetypal of hydrogels, will make the magnetic hydrogels attractive for a number of biomedical applications.

**Introduction**

Polymer hydrogels are being investigated for a number of medical applications: from tissue transplants to controlled drug release.\(^1\) Hydrogels are produced when hydrophilic polymers are crosslinked, resulting in a larger, water-absorbent structure. Microgels, or hydrophilic polymer microspheres, are a subclass of these substances that are classified by their size (with diameters ranging from 1 to 100 \(\mu\)m). While microgels have been most commonly studied as controlled drug delivery devices\(^2\), they have also been implicated as having potential use in medical diagnostics\(^3\) and tissue engineering\(^4\).

Because of their size, the preparation of microgels is often more complicated than for larger hydrogels. Templated synthesis is often used to control and define the ultimate size of the microgel.\(^5\) In one example, polymers are formed within an emulsion where the size of the microgel is defined by the dispersed phase.\(^6,8\) Another example of size control occurs when the synthesis is carried out in a microfluidic device.\(^9,10\) Some, more brute-force methods involve producing a larger hydrogel and manually cutting this material into smaller sizes.\(^11\)
To be useful in medical applications, the polymers that are used in these syntheses must be biologically compatible. That is, they should provide an interface that can be recognized by an organism, they cannot be rejected by an organism, and the polymers must eventually be broken down into components that are non-toxic. While industrial polymers are often used to prepare microgels because their physical and chemical properties are more easily tailored, another approach is to use naturally produced polymers, such as polysaccharides or proteins. These macromolecules provide a platform that intrinsically promotes multiple aspects of biocompatibility. While polysaccharides have been widely investigated in microgel formation and function, there are only a few synthetic procedures that produce protein microgels. Further, most of the protein microgels that have been studied are found in food sources (casein and whey).

We present a technique for preparing protein-based microgels that contain magnetic iron oxide nanoparticles. The nanoparticles are formed concurrently with, and incorporated into, the microgels. These two processes appear to be coupled. That is, in the absence of iron, no microgels are formed. And, in the absence of protein, nanoparticles are not formed. The incorporation of magnetic nanoparticles within the microgel adds an extra functionality to the material that could potentially be optimized and exploited for use in controlled drug delivery or magnetic resonance imaging (MRI). The synthesis involves addition of an aqueous solution containing Fe$^{2+}$ to a solution containing a protein (bovine hemoglobin, in this case), KOH, and KNO$_3$. The thermal transitions and size of the microgels were analyzed. The structure of the microgels was interrogated with Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The composition and structure of the iron oxide nanoparticles were probed with transmission electron microscopy (TEM), powder X-ray diffraction (XRD).
measurements, and FTIR. The magnetic properties of the nanocomposite material were measured using SQUID. The ability of the microgels to adsorb dye molecules was assessed. These materials offer a very promising platform from which to tailor new functional materials for medical applications.

2. Experimental

**Materials** FeSO$_4$$\cdot$6H$_2$O and KOH were purchased from Amresco. KNO$_3$ was purchased from Alfa-Aesar. Bovine blood hemoglobin (Hb) and rhodamine 6G were purchased from Sigma Aldrich. HPLC grade water was purchased from BDH.

**Microgel Synthesis** Stock solutions were made for all of the reagents: 0.30 M FeSO$_4$, 0.9 M KNO$_3$, 1 M KOH, and 15 μM Hb. The FeSO$_4$ solution was made with freshly degassed water (N$_2$ was bubbled through water for 30 minutes) and was prepared immediately before addition to the reaction mixture. An IKA Eurostar 20 overhead stirrer was used to mix the reaction solution. The stirring shaft was wrapped in Teflon tape to prevent the shaft from damage during the reaction. A 50 mL conical polypropylene centrifuge tube was clamped in a position such that the shaft of the overhead stirrer reached to the 5 mL graduation mark inside of the tube. A hot water bath, set to 80 °C was placed around the centrifuge tube and the stirrer was set to 2000 rpm (mixing speeds greater than 3000 rpm cause the sample to foam and overflow). 8.9 mL of KNO$_3$, 2 mL of KOH, Hb (typically 2.4 mL), and water (so that the final reaction volume was 20 mL) were added to the tube. Freshly prepared FeSO$_4$ (typically 2 mL) was added to the tube and the mixture was allowed to react for 90 minutes. This mixture was centrifuged (Sorvali R6C+ centrifuge with an SH 3000 swinging bucket rotor) for 5 minutes at 3000 rpm. The supernatant was removed and the microgels were re-suspended in HPLC grade water. This synthesis was based off of a
previous literature protocols for producing iron oxide nanoparticles\textsuperscript{22,23} coupled with another procedure that results in gold nanoparticles trapped within fibers of proteins.\textsuperscript{24} The iron concentration in the reaction mixture was varied from 0 mM to 50 mM. The Hb concentration was varied from 0 μM to 5 μM.

**Analytical measurements** UV-Vis absorption spectroscopy was performed on a Shimadzu UV-2550 spectrophotometer. For the measurements that required a dried sample, a solution of suspended microgels was heated (to 40 °C) and dehydrated in a Shel Lab vacuum oven. FTIR spectra of dried samples were recorded with Bruker Alpha spectrometer using a platinum attenuated total reflection sample holder. Differential scanning calorimetry measurements were performed with a Q2000 Calorimeter from TA Instruments. For these measurements, 3mg of dried sample was added to a TZero sample pan (TA Instruments) and sealed with a hermetic lid. The DSC temperature cycling was performed in the following manner: the temperature was equilibrated to 0 °C, increased to 150 °C at 10 °C/minute, held at 150 °C for 2 minutes, and then decreased to 0 °C at 5 °C/minute. This process was repeated twice. XRD measurements were recorded using a Rigaku Miniflex II equipped with an NaI scintillation counter detector, a 450 W Cu Kα (λ = 1.540562 Å) X-ray source, and a diffracted beam monochrometer. The sample was mounted on an aluminum holder.

**Light microscopy and Microgel Size Analysis** Hydrogel images were collected using an Olympus Bx61 microscope with 2.5X, 10X, and 40X objective lenses. A microscope calibration slide (AmScope 4-Scales Stage M calibration slide) was used to convert image width in pixels to micrometers. Measurements of microgel area were performed using ImageJ software. As part of the size assessment, a solution of microgels were filtered through a 0.2 μm-pore nylon filter, observed with a light microscope, and analyzed with ImageJ.
Assessment of dye uptake 1 mL of suspended microgels were placed in a 1.5 mL Eppendorf tube. Rhodamine 6G was added to this mixture such that the final concentration was between 20 and 200 μM. The microgels were exposed to the dye for 24 hours. After this period, the samples were centrifuged (Forma Scientific centrifuge with rotor 851) at 12,000 rpm for 5 minutes. The supernatant was drawn off and 1 mL of water was added to the sample. The sample was vortexed to resuspend the microgels. The dye content of each supernatant was analyzed using UV-Vis absorption spectroscopy. A mass balance analysis in which the mass of dye in the supernatant was subtracted from the original mass of dye added yielded the mass of dye that remained within the microgels.

Results

Synthesis During the synthesis of the iron oxide containing microgels, Fe$^{2+}$ (in a freshly made, degassed solution) is added to a stirred reaction mixture at 80 °C that contains a protein (Hb) along with KOH and KNO$_3$ and allowed to react for 90 minutes. We observed the immediate formation of both microgels and nanoparticles upon addition of Fe$^{2+}$ to the reaction solution. However, we notice that there are some changes to the average microgel size and appearance over the first hour of the reaction (data not shown). Figure 1 shows the results of a synthesis of the iron oxide nanoparticle containing microgels. These images are fairly representative of the products over the concentrations of iron and Hb we used in this study. We conducted two sets of syntheses where either the iron or Hb concentrations were varied. In one set, the Fe$^{2+}$ concentration was held at 30 mM and the Hb concentrations had the following values: 0.01 μM, 0.05 μM, 0.1 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM, 1.2 μM, 1.8 μM, 2.0 μM, 3.0 μM, 4.0 μM, and 5.0 μM. Microgels were not observed for Hb concentrations less than 0.1 μM. In the second set, the Hb concentration was kept constant at 1.8 μM and the Fe concentration took the
following values: 50 mM, 40 mM, 30 mM, 20 mM, 2 mM, and 1 mM. Microgels were not observed for the 1 mM Fe sample.

Figure 1. Results of microgel synthesis. These products were the outcome of a reaction that included 30 mM Fe$^{2+}$ and 1.8 M μM hemoglobin. The microscope image (B) was taken using a 40X objective lens.

**Microgel size analysis** A size distribution of the microgels in solution was produced by analyzing multiple images from the light microscope for each reaction condition studied. The sizes of the observed particles ranged in size from 0.7 μm$^2$ to over 1000 μm$^2$. **Figure 2** shows this analysis in multiple formats. The table indicates how the average and median particle sizes change as a function of reactant concentrations. The histograms give more detail of what a typical size distribution actually looks like. The vertical grouping of graphs is meant to highlight what these distributions look like for smaller particle areas, which constitute the majority of particles produced. The table and the middle set of histograms correspond to images of microgels post-synthesis. We also used a 0.2 μm-pore nylon filter to filter the samples. The size analysis for one set of reaction conditions is shown in the red histograms. The blue and red histograms in **Figure 2** show the data for the same reaction products (30 mM Fe$^{2+}$ and 1.8 M μHb).
Figure 2. Analysis of microgel sizes. The table shows the average area and median area of the microgels produced for each of the reaction conditions. The histograms in the middle (blue) show the size distribution for a reaction that used 30 mM Fe$^{2+}$ and 1.8 μM Hb. The histograms on the right (red) show the size distribution for the same sample but after filtering through a nylon filter with 0.2 μm pores. For each set of histograms, multiple graphs are shown to highlight the size distribution at smaller particle areas.

**Microgel composition** From the light microscopy images (see Figure 1), the microgels appear to as mostly a clear substance that contain areas of darker material. It is readily apparent that the microgels are magnetic; a magnet attracts the microgels in a suspension, leaving a clear solution.

To further probe the physical and chemical composition of the microgels, we analyzed them using TEM, SEM, XRD, and FTIR.

Figure 3 shows TEM images of two samples (A: 30 mM Fe/2 M μHb, B: 50 mM Fe/1.8 μM Hb) along with the average nanoparticle diameters and standard deviations for each synthetic condition. Most of the reactions yield similar results with average nanoparticle widths between 20 and 30 nm. The standard deviations for these widths are on the order of 35% for all of the
reactions. The two reaction conditions that yield different results are for iron concentrations of 50 and 40 mM.

Figure 3. TEM images for different reaction conditions. A: 30 mM Fe^{2+} and 2 μM Hb. B: 50 mM Fe^{2+} and 1.8 μM Hb. Scale bar: 100 nm. The table shows the average nanoparticle width and standard deviation for each reaction condition studied.
Whereas TEM highlights the iron oxide nanoparticles, SEM images (Figure 4) draw attention to the macro-structure of the protein arrangement within the microgel. At lower magnifications, the appearance of the dried microgel samples is reminiscent of a head of broccoli, with clumped spheroids dominating the view. At higher magnifications, the spheroids can be described as nanoparticles within a mesh-like material.

Figure 4. SEM images of microgels. Both images are taken from a sample produced by 30 mM Fe$^{2+}$ and 1.8 μM Hb. The primary image (scale bar: 100 nm) shows nanoparticles within a mesh-like structure. The inset (scale bar: 2 μm) shows the microgels at lower magnification.

FTIR and XRD (Figure 5) were used to determine the identity of the iron oxide species within the samples. FTIR and DSC were used to better understand the protein network that gives support to the microgels. (DSC data are shown in the supporting information.)

The XRD spectrum shows scattering peaks at 2θ = 39°, 45°, 65°, 78°, and 82.5° that come from the aluminum sample holder. The primary sample peaks are found at 2θ = 39°, 45°, 65°, 78°, and 82.5°. The peaks at 2θ = 18°, 71°, 74°, and 87° appear to be from a minor reaction product. FTIR spectra are highlighted by the amide I and amide II absorption features of the protein along with the Fe–O absorption peak from the nanoparticles. The amide I feature, which is indicative of
protein structure, is present in hemoglobin but not the microgel. The amide II feature is still observed in the microgel. There is only one Fe–O absorption feature observed for the microgels (at 550 cm\(^{-1}\)).

Figure 5. FTIR and XRD spectra. The top graph shows the FTIR spectra for hemoglobin (red) and a dried microgel sample (blue) (produced from 30 mM Fe\(^{2+}\) and 1.8 μM Hb). The amide I, amide II, and Fe–O features are highlighted. The bottom graph shows the XRD spectra of the same microgel sample. Primary sample features are marked with their corresponding assignments. Other features include the scattering peaks from the aluminum sample holder (Al) and a minor reaction product (*).

The DSC data for the microgels show two melting features and a glass transition. In the first scan, there are two observed melting features (at 54.5 °C and 55.5 °C). In successive scans, only
the melting process that occurs at 55.5 °C is detected. A glass transition process is observed at 69 °C. These observations are consistent across each reaction condition studied. The results mimic the thermal transitions of powdered hemoglobin with the only difference being that hemoglobin only shows a single melting transition in the first thermal scan (54.5 °C). (Data shown in the supporting information.)

Magnetic Properties The microgels clearly show magnetic properties as they can be easily manipulated with a magnet. To further explore these properties, we employed SQUID magnetometry. The maximum magnetism (per gram of sample) and coercivity (at 5 K) are fairly constant across all samples studied (Figure 6). These values deviate from this average only for the 40 and 50 mM Fe²⁺ samples.
Figure 6. SQUID magnetometry data. These graphs show the maximum magnetization per gram of sample (blue squares) and coercivity at 5K (red circles) for the microgels as a function of hemoglobin concentration (top) and Fe$^{2+}$ concentration (bottom).

**Dye adsorption** We studied the dye uptake for the 30 mM Fe$^{2+}$, 1.8 $\mu$M Hb sample. The reaction with these starting conditions produces 2.6 mg of solid microgel per mL of reaction volume. Using UV-Vis absorption we measured the amount of dye adsorbed by a 1 mL aliquot of microgels. We allowed the samples to come to equilibrium and rinsed off any dye that might have been interacting with the exterior surface of a microgel. Except for the lowest concentration of dye added, the microgels retained nearly 80% of the rhodamine added to the solution.

Table 1. Dye uptake by microgels.

<table>
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<tr>
<th>Concentration of dye in solution ($\mu$M)</th>
<th>Mass of dye in microgel ($\mu$g)</th>
<th>% of dye adsorbed</th>
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**Discussion**

This microgel synthesis is attractive for the preparation of functional microparticles for a number of reasons. First, the supporting polymer for the microgel is a protein, and as such, the particles should be biocompatible and facilitate biological recognition. While we used hemoglobin in this
study, we have used our procedures with other proteins and have observed similar outcomes (data not shown). Though the microgel size analysis yields a broad range of areas, the filtered samples indicate that the individual particles are likely smaller than the microscope images suggest. The procedure results in the incorporation of magnetic iron oxide nanoparticles within the microgel. The magnetic properties, intrinsic to this material, suggest that the microgels could eventually be optimized for use as MRI contrast agents, magnetically induced controlled drug delivery, or magnetically controlled bio-affinity separations. Magnetic polymer microgels, or ferrogels, have been previously described.\textsuperscript{25-30} To our knowledge the materials here constitute the first example of a protein-based ferrogel. This discussion will further explore some of the salient properties of the microgels while keeping an eye towards potential functional applications.

When observed by eye, the microgel particles appear to be random precipitates. The resulting reaction solution becomes dark and cloudy by the end of the procedure (Figure 1). However, under the microscope, the microgel particles are noticeably transparent with areas that contain a darker material. The TEM and SEM images (Figures 3 and 4) further clarify this picture by showing nanoparticles trapped within a fibrous mesh of polymeric material. We expected the procedure to result in the formation of some iron oxide, as our protocols are similar to previously reported protein-templated syntheses of iron oxide nanoparticles.\textsuperscript{22,23} Significantly, our methods are different from this earlier report in two primary ways. Our protocol is optimized to rapidly produce nanoparticles trapped within a supporting protein-based microparticle as opposed to individual nanoparticles with a protein-based coating. Second, our previous experiments have indicated the importance of metal to protein ratios for determining the identity of the reaction product in protein-templated metal nanoparticle synthesis.\textsuperscript{24} We performed several preliminary experiments to determine the optimal iron:hemoglobin ratio for producing magnetic microgels.
As described earlier, we expected our protocols to yield some form of iron oxide nanoparticle. The XRD spectra show peaks at $2\theta = 30^\circ, 36^\circ, 43^\circ, 53^\circ, 57^\circ,$ and $62.5^\circ.$ These peaks can either be attributed to Fe$_3$O$_4$ or $\gamma$-Fe$_2$O$_3$ as these both share a similar spinel crystal structure. The FTIR spectra only show a single Fe–O absorption feature (at 550 cm$^{-1}$). As $\gamma$-Fe$_2$O$_3$ FTIR spectra show two Fe–O features between 400 and 600 cm$^{-1}$ and Fe$_3$O$_4$ only displays one, we can accurately identify the nanoparticles as Fe$_3$O$_4$.

We have anecdotal evidence suggesting the importance of properly degassing the Fe$^{2+}$ starting solution. When this solution is made without, first, thoroughly degassing the water, the Fe$^{2+}$ rapidly oxidizes to Fe$^{3+}$, which is insoluble in water and forms a yellow/brown precipitate. We contend that we can observe insufficient degassing in microgel products as well. These samples result in iron oxide particles that are more amorphous and clumped together. These particles display a higher coercivity and lower maximum magnetization than the other samples described in this study.

Other systems that use proteins to template the synthesis of iron oxide nanoparticles are usually performed in oxygen free environments. We contend that, for our synthesis, the reaction products are formed so quickly (due to the high concentrations of KOH and KNO$_3$), that proper degassing of just the Fe$^{2+}$ starting solution is the only necessary precaution. For the preparation of some magnetic microgels, where it is only necessary for the particles to show ferromagnetism, this precaution may be enough to secure a good result. However, fine-tuning the magnetic properties of the nanoparticles in future experiments will require extra control steps, including a tighter control over removing oxygen from the system. As one example, iron oxide materials that are being investigated as MRI contrast agents are noted for being superparamagnetic and having
nanoparticle diameters that are under 20 nm.\textsuperscript{32,33} To extend our procedures to MRI-active materials, we will need to alter the protocol to reduce the overall nanoparticle size.

Some of the iron oxide nanoparticles found in our microgels appear to have a cubic shape (Figure 3). This is expected due to the cubic spinel packing structure of $\text{Fe}_3\text{O}_4$. However, it is easy to see how changing the reaction conditions (like increasing the initial $\text{Fe}^{2+}$ concentration from 30 to 50 mM) can affect the size of the nanoparticles that are produced. The change in nanoparticle size noticeably alters the magnetic properties of the nanoparticle (Figure 6). Other studies have noted the importance of the protein identity in determining nanoparticle structure. As an example, Matsunaga’s group have shown that a small peptide, implicated in biomineralization processes in magnetotactic bacteria, can facilitate the production of cubic-octahedral crystal geometries.\textsuperscript{23} We will continue to explore control parameters such as these for optimizing our microgels for specific functions where nanoparticle homogeneity (in both size and crystal packing) needs to be tightly maintained.

The transparent portion of the hydrogels appears to be a network of unfolded proteins. We argue that the protein is certainly incorporated into the microgel. Any soluble protein left over in the reaction mixture would be eliminated after the centrifugation step and removal of the supernatant. Also, the absence of observed microgels for the products in the absence of either iron or hemoglobin, indicate that both are necessary for microgel formation. We contend that any protein-related signals in the dry sample are consistent with protein that is incorporated within the microgel. The FTIR data for hemoglobin show strong absorbance features for both the amide I and amide II frequencies. In the microgel, the amide II peak is present while the amide I peak is not. The position of the amide I absorption feature corresponds to the conformational state of the protein. For hemoglobin, this peak denotes the alpha helical nature of the protein. That the amide
I feature does not show up in the FTIR spectra for the microgels, while the amide II feature does, indicates that the protein structure has changed substantially upon forming the microgel. This should come as no surprise given the reaction conditions (0.1 M KOH and 80 °C) and the unfolding temperature for hemoglobin in solution (3° structure: 50 °C, 2° structure: 65 °C).

The DSC data indicates that hemoglobin plays the predominant role in dictating the dynamic polymeric-motional properties of the microgels. Dried hemoglobin shows a single melting (unfolding) process during the initial DSC scan (at 54.5 °C). In the consecutive scans the unfolding process is observed at 55.5 °C. This observation indicates that one structure unfolds at 54.5 °C and refolds into a new structure with an unfolding temperature of 55.5 °C. This unfolding transition (55.5 °C) loses intensity in consecutive scans, suggesting that the unfolding process is largely irreversible. The dried hemoglobin sample shows a glass transition temperature near 70 °C. The DSC data for the microgels is very similar to that for dried hemoglobin. The primary difference is that the microgels display two unfolding transitions for the first scan (at 54.5 °C and 55.5 °C). In the consecutive scans, only the 55.5 °C transition appears. This indicates that hemoglobin in the microgel is less structured than in the dried hemoglobin sample.

Importantly, the microgels also show a glass transition temperature near 70 °C. A glass transition occurs when a polymer changes from a rigid amorphous phase to a more fluid phase. The glass transition may be critical for enabling controlled drug delivery or facilitating microgel motion through confined spaces such as blocked veins and arteries or capillaries. Future experiments may seek to alter this glass transition temperature (by using different proteins or incorporating industrial polymers) and determine its effect on microgel tensile and rheological properties.

While we have observed some microgel deformation in the presence of a magnetic field, we
surmise that lowering the glass transition of these materials can maximize the ability of the microgel to deform under external stimuli.

The absolute size of the polymer microparticles and their polydispersity are often critical components in the analysis of new microgel preparation protocols. Our observations reveal a very broad range of sizes for produced microgels (0.7 to over 1000 μm² with an average size of around 25 μm² and median size under 10 μm²). As a comparison, the average and median areas for these microgels are less than those measured for red blood cells, which have areas of roughly 50 μm². Our microgels also appear to have an irregular shape. That is, they do not seem to be spherical.

We argue that a size analysis for these microgels is complicated by the fact that they are magnetic. Clumping from magnetic interactions would lead to larger observed microgel sizes. TEM and SEM images (Figures 3 and 4) show that clusters of nanoparticles do exist, but that those clusters are surrounded by what appears to be an organic, protein matrix. It is difficult to estimate particle size from these dried samples, as it is impossible to discern clumping from extended microparticle size. Our best evidence that the microgels are smaller than the size analysis suggests is the particle size distribution of the sample that has been passed through a 0.2 μm-pore nylon filter.

The unfiltered samples show particle sizes that range between 0.7 and 2500 μm². The majority of these particles are less than 10 μm². Upon passing these particles through a 0.2 μm-pore nylon filter (using vacuum filtration), two primary observations can be made. First, all resultant particle sizes are less than 500 μm². This certainly may be due to the fact that particles larger than the pore size will encounter difficulties passing through the filter. And this does happen. However,
the distribution of filtered particle sizes (between 5 and 350 μm², considerably larger than the filter’s pores) indicates that the particles might be aggregates of smaller particles or that the particles are able to distort their geometries in order to fit through the pores. The truth is most likely some combination of the two. Our observations of microgel distortion under stimulation by magnetic fields indicate that individual particles are capable of changing their geometry without breaking apart into multiple fragments. The larger microgels are most likely to just be collections of smaller microgels that are clumped together due to magnetic interactions. The second critical observation from the filtered particle size data is that there are no observed microgels with areas less than 5 μm². The best explanation for this observation is that small particles are forced to pass through small channels within the filter. The small particles interact within the filter and clump together. The result from this close-proximity confinement is that the small area microgels are not present in the image analysis.

The overall picture that the size analysis paints is that the magnetic microgels are capable of deforming their shape and capable of coming together, through magnetic interactions, to form larger particles. One aspect of this framework that is unclear is if the polymer mesh observed in the SEM (Figure 4) is static or dynamic. That is does the polymer network redistribute itself when one microgel comes in contact with another microgel, or is the polymer network set once it has been synthesized? We are inclined to think that the latter is a more reasonable assessment. We have observed and reported on a similar static protein network in a system where gold nanoparticles are trapped within fibers of unfolded proteins.24

Along with size control, another aspect of polymer microparticles that is most often studied is their ability to uptake dye and release drug molecules. To simulate this process, we have tested the microgels’ ability to adsorb dye molecules. We do observe dye uptake by the microgels at
different concentrations of dye (Table 1). The highest capacity for dye uptake that we measured was 36 μg of dye per mg of microgel, which accounts for 98% of the total dye in solution. As yet, we have been unable to determine the optimal process to induce dye removal from these hydrogels, mimicking other controlled drug delivery experiments. Typical controlled delivery stimuli have been: changes in pH and changes in temperature. We have preliminary data hinting that some dye removal occurs in the presence of a magnetic field. However this process has not been optimized and is the focus of continuing experimentation. It may be that a lower glass transition temperature is required for efficient release of dye from the microgel. We will continue to explore how changes to this parameter affect the functional properties of the microgels.

**Conclusions**

We have described a one-pot synthesis of a protein-based microgel that contains magnetic iron oxide nanoparticles, in which the protein templates the synthesis of the nanoparticles concurrently with the formation of the microgel. The microgel retains the melting and glass transition characteristics of the dried protein. The iron oxide nanoparticles display properties consistent with Fe₃O₄. While the microgels appear to display a large variety of sizes, we contend that the microgels seem larger than they are due to magnetically induced clumping. Based on the multiple practical aspects of these protein-based microparticles: biocompatibility, biological signaling capabilities, dye uptake, and magnetic properties we contend that this novel microgel preparation will help to guide the development of novel functional materials.

**ASSOCIATED CONTENT**
Supporting Information. The supporting information contains TEM images, DSC data, and dye adsorption data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. †These authors contributed equally.

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ABBREVIATIONS

CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; TLC, thin layer chromatography.

REFERENCES


