Fabrication and Manipulation of Ionotropic Hydrogels Cross-Linked by Paramagnetic Ions

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This article describes the fabrication and manipulation of millimeter-scale spheres fabricated from ionotropic hydrogels that are cross-linked with paramagnetic metal ions (e.g., Ho^{3+}). These ionotropic hydrogels experience a force in a magnetic field gradient that correlates with the concentration of the paramagnetic cations cross-linking the polymer. In an externally applied magnetic field, the paramagnetic hydrogel spheres assemble into ordered arrays or confined geometrical structures in the regions of highest magnetic field. These spheres can be separated from heterogeneous mixtures of diamagnetic materials using a simple bar magnet. Two applications using these recoverable hydrogel spheres were demonstrated: (i) When prepared with embedded indicator dyes bound to paper, the spheres were used as colorimetric sensors for pH. (ii) When prepared with embedded activated carbon powder, they were used to remove organic materials from aqueous solutions.

Introduction

This article describes the fabrication and manipulation of millimeter-scale spheres made from ionotropic hydrogels that are cross-linked with paramagnetic metal ions (e.g., Ho^{3+}). These ionotropic hydrogels experience a force in a magnetic field gradient that reflects the paramagnetism of the ions cross-linking the polymer. (Most magnetically responsive hydrogels are made with embedded superparamagnetic particles in the polymer matrix.) Although the forces on paramagnetic materials are weaker than those on superparamagnetic materials, we can separate spheres that are cross-linked with paramagnetic ions from spheres cross-linked with diamagnetic ions (e.g., La^{3+} or Al^{3+}). The magnetic response allows for collection of the hydrogel spheres from aqueous or heterogeneous mixtures for further use or analysis. In an externally applied magnetic field, the paramagnetic hydrogel spheres assemble into ordered arrays or confined geometrical structures in the regions of highest magnetic field. We demonstrated two applications for these hydrogel spheres: (i) as colorimetric sensors for pH, when prepared with embedded paper fibers containing bound indicator dyes, and (ii) as sorbents for the removal of organic molecules from aqueous solutions, when prepared with embedded activated carbon powder. In both applications, the spheres can be recovered with a bar magnet and reused.

Ionotropic Hydrogels. Ionotropic hydrogels are polymers that form hydrated gel matrices in the presence of metal cations. Most multivalent cations will cross-link aqueous solutions of alginic acid (AA)—a linear copolymer of α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues—to form hydrogels.[1,2] A wide variety of ions form complexes with AA, including alkaline earth metals (e.g., Ca^{2+}, Sr^{2+}, Ba^{2+}), transition metals (e.g., Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Al^{3+}, Fe^{3+}, Sn^{4+}), and lanthanide metals (e.g., La^{3+}, Nd^{3+}, Eu^{3+}).[3,4]

Polymers other than AA also form ionotropic hydrogels.[5] Pectin[6] and carboxymethyl cellulose (CMC)[7] present carboxylate groups and gel in the presence of metal cations. The polysaccharide chitosan,[8] which bears an amino group on each residue, and κ- and ι-carrageenan,[6,9,10] both of which bear sulfate groups, also form ionotropic hydrogels. Aside from these polysaccharides, poly(bis(4-carboxyphenoxy)-phosphazene)[11] is a widely used polycarboxylate that gels in the presence of metal cations. These ionotropic hydrogels have been used as cell scaffolds (e.g., for islet cells),[12−14] delivery systems for pharmaceuticals,[7,15] sorbents for the

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sequestration of metals in contaminated aqueous solutions,[16] substrates for microfluidics,[17] and components in the production of foods and beverages.[18]

Structure of AA Hydrogels. Cozzi and co-workers developed three techniques to measure the affinity of AA for metal cations:[4] (i) The first monitored the change in pH upon the addition of a metal cation to a solution of AA.[19] (ii) The second determined the amount of metal cation required to form a precipitate from a solution of AA.[19] (iii) The third measured the relative rate of migration of the metal ion to the aqueous solvent front during thin-layer chromatography (TLC) through a film of AA.[20,21] These methods yield similar trends for the strengths of complexation of ions with AA: $Pb^{2+} > Ba^{2+} > Fe^{3+}, Al^{3+} > Cu^{2+}, Cd^{2+} > Ca^{2+} > Zn^{2+}, Co^{2+}, Ni^{2+} > Mn^{2+}, Mg^{2+} > K^{+} > Na^{+} > Li^{+}$.[4,19−21] Monovalent alkali earth metals (e.g., Na^+ and K^+) and some divalent cations—Mn^{2+} and Mg^{2+}—do not cross-link AA to form hydrogels.[4]

There is a growing body of research aimed at defining the structure of and the mechanism of cross-linking in alginate hydrogels.[1] The mechanism of cross-linking is the coordination of carboxylic acids and hydroxyl groups on the polymer to the metal ions. While the conformations of MM and MG blocks in AA are roughly linear, the GG blocks are “puckered”.[15,22] Rees et al. observed that these puckered sequences create cavities that can be occupied by cations.[22] This configuration is called the “egg-box model”, as the cross-linking cations (the “eggs”) sit in the cavities found in GG blocks of the polymer. It is the most widely accepted model for the structure of alginate hydrogels.

Hydrogels and Magnetism. Magnetically responsive hydrogels can be manipulated by an applied inhomogeneous magnetic field. A common approach for fabricating magnetically responsive hydrogels is to create a two-phase system in which ferromagnetic or superparamagnetic particles are either suspended in the polymer matrix before gelation[23−25] or synthesized in preformed hydrogels.[26,27] The magnetic particles decrease the transparency of the hydrogels and alter the porosity of the material.

Only a few examples exist in which ionotropic hydrogels incorporate paramagnetic cations as the cross-linking agents. One application of hydrogels cross-linked with paramagnetic ions (e.g., Cu^{2+} or Mn^{2+}) is in the determination of structures of the hydrogels by NMR spectroscopy.[28,29] Another application is the use of these magnetic hydrogels as coatings on implantable devices to enhance the contrast for magnetic imaging.[30,31] All reported uses of magnetic ionotropic hydrogels are passive—none take advantage of the positive magnetic response of the hydrogels for actuation or manipulation.

Experimental Design

We selected Ho^{3+} as the paramagnetic ion to cross-link the aqueous polymer solutions into hydrogels because it offers two distinct advantages over main-group paramagnetic cations. First, Ho^{3+} has one of the largest magnetic susceptibilities ($\chi \sim +0.44$ cm^3/mol) of any ionic species.[32] Because the magnetic susceptibilities of the organic polymer and water are negative and small, the positive magnetic response of these hydrogel spheres arises solely from the presence of Ho^{3+} cations. The strength of the magnetic force is linearly proportional to the concentration of the Ho^{3+} bound within the gel. Second, the affinity of AA for cations typically increases with charge. The Ho^{3+}−AA complex is strong enough for the hydrogel spheres to remain intact in aqueous solutions (pH $\sim$ 7) for long periods of time (over 1 year) and retain their positive magnetic response.

Results and Discussion

Cross-Linking and Formation of Paramagnetic Ionotropic Hydrogels. We produced millimeter-sized hydrogel spheres by cross-linking anionic polymers in aqueous solutions with Ho^{3+} cations based on a standard procedure.[13] We injected droplets of the solution of polymer (typically 2% w/v) from a 27-gauge needle into an aqueous solution of $Ho(NO_3)_3$ (concentrations 10−500 mM), buffered with 10 mM Tris, pH 7.5. The viscosity of the solution and the gauge of the needle determined the size of the droplet and, correspondingly, of the hydrogel sphere. The spheres we fabricated were 2−5 mm in diameter.

We investigated the effect of pH on the production of the hydrogel beads. Using either buffered solutions of polymers in water (pH 7.5 or greater) or unbuffered solutions of polymer (pH $\sim$ 6, for the sodium salt of AA), stable hydrogels could form so long as the polymer did not precipitate with the change in pH. At low values of pH ($\sim$1), some of the polymers were insoluble. The pH of the aqueous solution of Ho^{3+} (over the range 4−10) did not affect the formation of stable hydrogels.

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Table 1. Polymers that Formed Hydrogels in the Presence of Ho^{3+} and Those that Did Not[a]

Formed Gels with Ho^{3+}	Did Not Form Gels with Ho^{3+}
AA	hyaluronic acid
CMC	chitosan
ι-carrageenan	κ-carrageenan
PG	dextran sulfate
poly(bis(4-carboxyphenoxy)-phosphazene)	poly(styrene sulfonate)
	poly(vinyl phosphonic acid)
	poly(acrylic acid)
	chondroitin sulfate

[a] See Supporting Information Figure 3 for structures of the polymers.

We surveyed a number of ionomers—polymers that present bound ions and mobile counterions—for their ability to form stable hydrogels with Ho^{3+} (Table 1). Of the ionotropic polymers we investigated, AA, CMC, *ι*-carrageenan, and poly(galacturonic acid) (PG) formed stable hydrogel spheres in the presence of Ho^{3+}. Poly(bis(4-carboxyphenoxy)phosphazene) produced hydrogel particles, but instead of spheres, either shells or toroids formed depending on the height from which the droplets fell into the aqueous solution containing holmium cations. Unlike the other hydrogels, poly(bis(4-carboxyphenoxy)phosphazene) formed fragile gels that could not be manipulated without destruction of the material.

Not every polymer reported in the literature to form hydrogels in the presence of metal cations formed a stable hydrogel in the presence of Ho^{3+} (Table 1). Vercruysse et al. reported that hyaluronic acid formed a “reverse transition” hydrogel in the presence of several lanthanides, with a critical temperature of gelation dependent on the molecular weight of the polymer and on the metal cation.[33] We did not observe hyaluronic acid to produce this type of gel with Ho^{3+}. Also, *κ*-carrageenan—a polymer that gels in high concentrations of potassium—did not gel in the presence of Ho^{3+}, and neither did chitosan, a polymer that is soluble only when its pendent amine groups are protonated (i.e., $pH < 6.3$).[34]

The anionic polymers dextran sulfate, poly(styrene sulfonate), poly(vinyl phosphonic acid) (2−40% w/v), poly(acrylic acid) (2−35% w/v), and chondroitin sulfate (2−10% w/v) did not form stable hydrogels with Ho^{3+}. Dextran sulfate, poly(styrene sulfonate), and poly(vinyl phosphonic acid) precipitated out of solution in the presence of 100 mM Ho^{3+}, but we do not consider these precipitates to be gels as they do not maintain their structural integrity. Poly(acrylic acid) and chondroitin sulfate remained soluble in the presence of holmium, even for $[Ho^{3+}] > 1$ M.

Characterization of the Paramagnetic Hydrogels. In order to compare the affinity of AA for Ho^{3+} relative to other ions, we modified the procedure for TLC described by Cozzi et al.[20] by performing liquid chromatography (LC) on a column of acidified AA. Our use of LC instead of TLC offered the advantages of more accurate quantitative data, improved reproducibility by reusing the same column instead of homemade single-use plates, and easier analysis by substituting UV−vis detection for chemical developing reagents.

To compare our data to those of Cozzi, we calculated R_f values based on the ratio of eluant (0.1 M HCl) required to elute nitrate, a marker for the solvent front, to the volume required to elute a metal analyte. Chromatographic analysis of Ni^{2+}, Cu^{2+}, and Fe^{3+} gave R_f values of 0.79, 0.59, and 0.19, respectively (for an example chromatogram, see Supporting Information Figure 1). These data are similar to the results of Cozzi, et al. Analysis of Ho^{3+} gave an R_f value of 0.62. This result suggests that the affinity of AA for Ho^{3+} is markedly lower than for trivalent cations of transition metals such as Fe^{3+} (0.19, which we measured) and Cr^{3+} (0.39, as reported in the literature).[20,21]

Using UV−vis spectroscopy, we measured the concentration of holmium inside of the Ho^{3+}−AA spheres and calculated the magnetic susceptibility per sphere. First, we fabricated a set of AA spheres in three different concentrations of Ho^{3+}—10 mM, 100 mM, and 500 mM. The AA spheres were subsequently washed for several hours to extract any unbound Ho^{3+}. A 5-mL aqueous solution of ethylenediaminetetraacetic acid (EDTA; pH 7.4) dissolved 100 AA spheres from each of the three sets. Using the absorption peak for Ho^{3+} at 451 nm, we measured the absorbance of each solution and determined an average value for the $[Ho^{3+}]$ per sphere. The concentration of Ho^{3+} per sphere—roughly 100 mM—was similar for the spheres made from the 100 mM and 500 mM solutions. The 10 mM solution produced spheres with a lower concentration of Ho^{3+}—roughly 85 mM. These concentrations correspond to magnetic susceptibilities of $+0.042$ and $+0.037$, respectively.

Separation and Manipulation of Hydrogel Spheres. Rare-earth permanent magnets (e.g., NdFeB) attract paramagnetic hydrogel spheres suspended in diamagnetic media and allow the spheres to be manipulated. The magnetic force acting on a paramagnetic sphere in a diamagnetic medium is linearly proportional to the concentration of paramagnetic ions within the sphere (eq 1), where χ_s and χ_l are the magnetic susceptibilities of the sphere and the liquid,

$$\vec{F} = \frac{(\chi_s - \chi_l)V}{\mu_0}(\vec{B}\cdot\vec{\nabla})\vec{B} \tag{1}$$

respectively (we assume χ_l for a diamagnetic liquid to be negligible), V is the volume of the sphere, μ_0 is the magnetic permeability of free space, and $(\vec{B}\cdot\vec{\nabla})\vec{B}$ is the product of the magnetic field and its gradient.

To test the effect of Ho^{3+} incorporation on the magnetic response of the hydrogels, we cross-linked CMC in solutions containing both paramagnetic cations (Ho^{3+}) and diamagnetic cations (La^{3+}). These cations were selected because they are trivalent lanthanides, so we expect both to interact with CMC with similar binding affinities. We cross-linked CMC in six different solutions of Ho^{3+} and La^{3+} ions; each solution contained a different mole fraction of Ho^{3+} but an identical total concentration of cations ($[La^{3+}] + [Ho^{3+}]$). During fabrication, we left the spheres to soak for 12 h and assumed that the relative concentration of the cross-linking cations in the hydrogel was similar to that of the bulk solution. After washing the spheres in deionized water for 2 h, for each batch of AA spheres, we measured the furthest distance from

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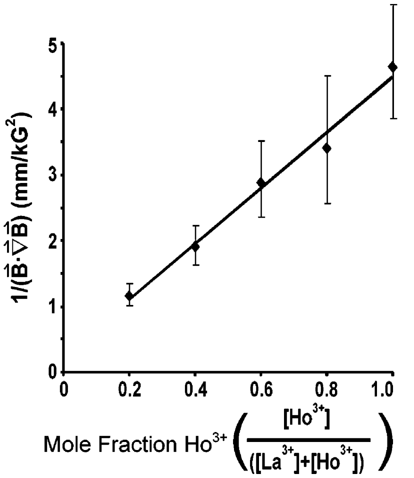

Figure 1. Magnetic susceptibility is controlled by the extent of incorporation of a paramagnetic ion into a hydrogel. A set of CMC spheres was cross-linked with different relative concentrations of paramagnetic Ho^{3+} and diamagnetic La^{3+}. The graph plots the inverse of the dot product of the magnetic field and its gradient against the mole fraction of Ho^{3+} cations. The inverse proportionality is linear, as predicted by eq 1. Error bars represent one standard deviation based on the data from seven measurements.

which a hydrogel sphere was attracted to the surface of the NdFeB magnet (Supporting Information Figure 2) when the orientation of the magnet was parallel to the benchtop (i.e., normal to the gravitational force). As a control, we cross-linked AA spheres with diamagnetic La^{3+} cations only; these spheres were not attracted to the surface of the magnet.

The distance from which a paramagnetic sphere is attracted to a magnet is a function of the orientation and strength of the magnet. We, therefore, assumed that the minimum force required to attract a paramagnetic sphere would scale linearly with the extent of Ho^{3+} incorporation in the sphere when the distribution of the magnetic field was held constant. The sphere will only move when the magnetic force is greater than the viscous drag force on the sphere, since the gravitational force can be neglected, as it is orthogonal to the magnetic force. We measured the magnetic field strength normal to the surface of the magnet with a hand-held magnetometer and calculated the magnetic field gradient from these measurements.[35] The graph of the inverse of the product of the magnetic field and its gradient versus the mole fraction of Ho^{3+} in the cross-linking solution shows a linear relationship (from eq 1), which confirms that the minimum force required to attract a paramagnetic CMC sphere is constant (Figure 1). The time that is required for a paramagnetic sphere to attach to the surface of a bar magnet will depend on the distance of the sphere from the magnet, the strength of the applied field, the gradient of the field, the magnetic susceptibility of the paramagnetic hydrogel, viscous forces (which depend on the effective radius of the particle and the viscosity of the solution), and the force of gravity.

Figure 2 shows two different types of separations (using a NdFeB bar magnet) of hydrogel spheres cross-linked with paramagnetic ions from hydrogel spheres cross-linked with diamagnetic ions. In the first type of separation, a magnet was immersed in a mixed suspension of (i) paramagnetic CMC spheres (2−3 mm in diameter) cross-linked with Ho^{3+} and (ii) diamagnetic AA spheres (3−4 mm in diameter) cross-linked with Al^{3+} (Figure 2A). When we brought the permanent magnet into contact with the mixture of spheres, only the paramagnetic Ho^{3+}−CMC spheres were attracted to the magnet and could be removed from the container (together with adhering water; Figure 2C). In order for the spheres to break the surface tension of the water without falling from the magnet, the magnets had to be withdrawn slowly and at an angle. The Al^{3+}−AA spheres were not attracted to the magnet. The paramagnetic hydrogel spheres formed no more than a monolayer on the surface of the magnet; its strength was too weak to enable spheres farther from the surface of the magnet to be lifted from the bottom of the chamber. We could release the magnetic spheres either by shaking the magnet in solution or by directing a stream of water from a wash bottle at the beads (Figure 2D). To transfer all of the paramagnetic CMC spheres, we repeated this process of attracting paramagnetic spheres to a magnet, transporting them once bound, and releasing them in a second chamber. Diamagnetic AA spheres did not transfer.

In the second type of separation, the magnet did not come in contact with either the solution or the hydrogel spheres. In this case, we used the same polymers as before but switched the cross-linking cations. We placed a suspension of a mixture of paramagnetic AA spheres (3−4 mm in diameter) cross-linked with Ho^{3+} and diamagnetic CMC spheres (3−5 mm in diameter) cross-linked with La^{3+} into a Petri dish (Figure 2E). After tilting the dish to allow for all of the spheres to settle near one edge (Figure 2F), we passed a NdFeB magnet along the underside of the Petri dish. Only the paramagnetic spheres followed the magnet, yielding an in-plane, spatially resolved separation of the two types of spheres (Figure 2G).

For the separation methods described above, the percent recovery was 100%, as we isolated all of the paramagnetic spheres from the mixture without contamination by any of the diamagnetic spheres. The number of attract-and-detach cycles required to transfer the paramagnetic spheres quantitatively depended on the surface area of the magnet and the strength of the magnetic field. The experiment depicted in Figure 2A−D required three to four cycles to transfer all of the paramagnetic spheres (∼50) from one chamber to the other.

We could also manipulate the paramagnetic hydrogels such that they assembled in the presence of an inhomogeneous magnetic field in the region of highest field strength. We placed several spheres into a Petri dish containing deionized water over a set of rare-earth magnets and gently agitated the dish by hand. Figure 3 depicts three different polymer hydrogel spheres (CMC, *ι*-carrageenan, and AA), each having assembled into a geometrical pattern under the influence of the external magnetic field. The size and spacing of the magnets can alter the regions of highest magnetic field and, thus, can be used to control the final position of the spheres. The highest field from an array of small magnets spaced several diameters apart is directly over each magnet (Figure

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(35) We assumed that the components of the magnetic field parallel to the surface were negligible and did not contribute significantly to either the field or the gradient.

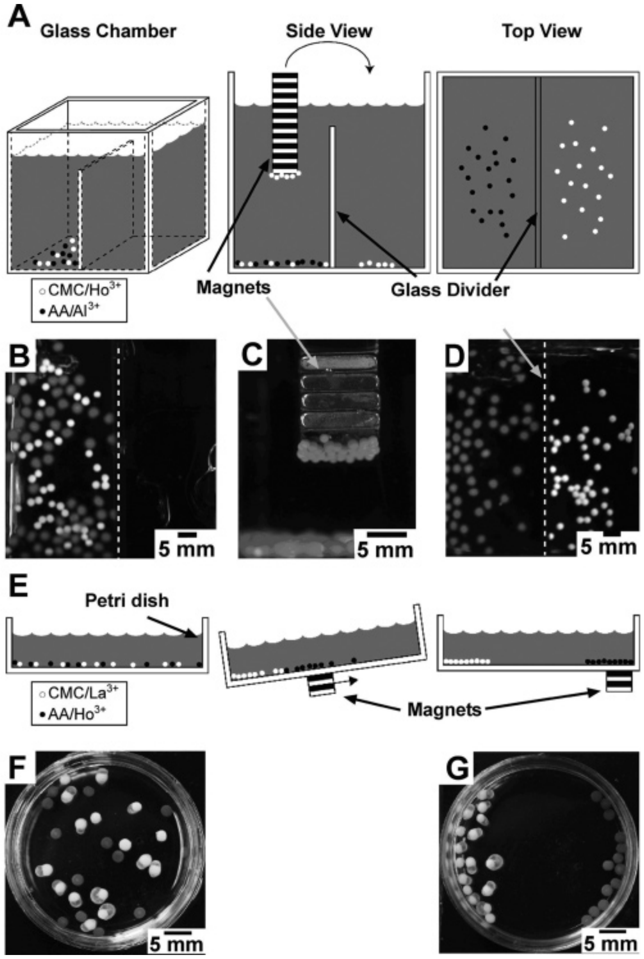

Figure 2. Separations of paramagnetic hydrogel spheres from diamagnetic hydrogel spheres using rare-earth magnets. (A) A set of schematics—three-dimensional, side-view, and top-view—illustrating the method of separation used in parts B−D in which the magnets contact the spheres in solution. (B) An optical image of the initial mixture of paramagnetic CMC spheres (white, Ho^{3+}) and diamagnetic AA spheres (gray, Al^{3+}) placed in a glass chamber (A). (B−D) Upon immersion of a magnet in the mixture, the paramagnetic spheres were strongly attracted to the surface of the magnets, from which they were transferred selectively to a second chamber. The dotted white line highlights the glass divider separating the two chambers. (E) A schematic illustration of the second type of separation, in which a magnet was placed under the dish where it does not contact the spheres. (F, G) Optical images of the mixture of paramagnetic AA spheres (gray, Ho^{3+}) and diamagnetic CMC spheres (white, La^{3+}) in a Petri dish (E) before and after separation by an external rare-earth magnet.

3A), whereas the highest field for similarly poled, large magnets adjacent to each other is over the gap between the magnets (Figure 3B). The geometrical constraints on the pattern of assembly appear to be limited only by the profile of the magnetic field that can be generated on a length scale comparable to the diameter of the hydrogel spheres.

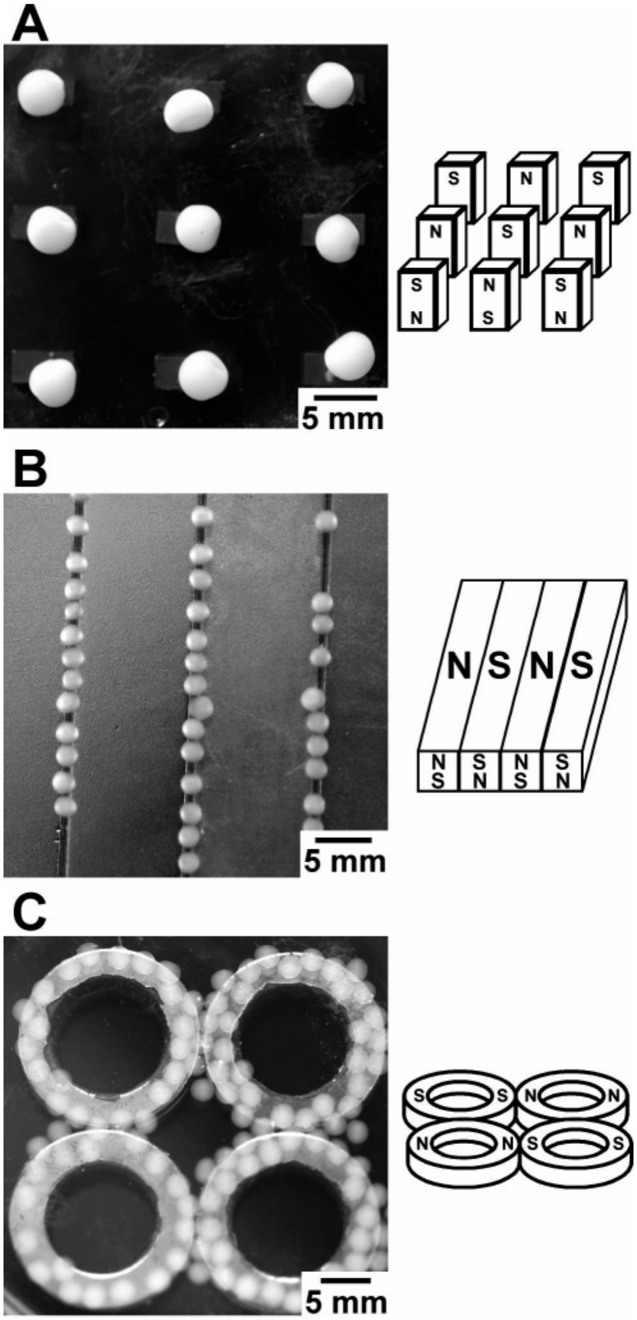

Figure 3. Self-assembly of the paramagnetic hydrogel spheres using different geometries and configurations of NdFeB magnets. Schematic drawings on the right illustrate the polarities of the magnets. The spheres collect over the region of highest magnetic field. (A) CMC spheres assembled into an open lattice structure of small magnetic posts. (B) Spheres of ι-carrageenan assembled into lines over the intersection between two adjacent magnets with opposite polarity. The magnets were immersed in the solution containing the spheres. (C) AA spheres aligned with a set of ring magnets only in the region directly above each magnet. In this geometry, there is essentially zero magnetic field in the center of each ring and in the center of the cluster of rings.

Colorimetic pH Detection Using Hydrogel Spheres. We produced paramagnetic hydrogel spheres with embedded colorimetric pH indicators that can register the pH of a medium and be recovered magnetically. A pulp of pH paper in which the indicators were covalently bound was prepared by pureeing a suspension of the paper in water. (In experiments where the indicator was not covalently attached to the paper (or the hydrogel), the indicator diffused into solution.) We dissolved AA (2% w/v) in the aqueous solution containing the pulp and then fabricated AA spheres using a 100 mM solution of Ho^{3+}. We made three sets of these spheres, each with a different pH indicator. These spheres change color when placed in aqueous solutions of various pH. We soaked the spheres in solutions having a pH of 0 (1 M HCl), 6.2 (1 M Bis-Tris), 9.9 (1 M CAPS), and 14 (1 M NaOH; Figure 4). The spheres changed color within seconds and retained their color when soaked in the solutions of pH 0, 6.2, and 9.9 for >100 h. In the pH 14 solution, the indicator dye degraded after $\sim$6 h.

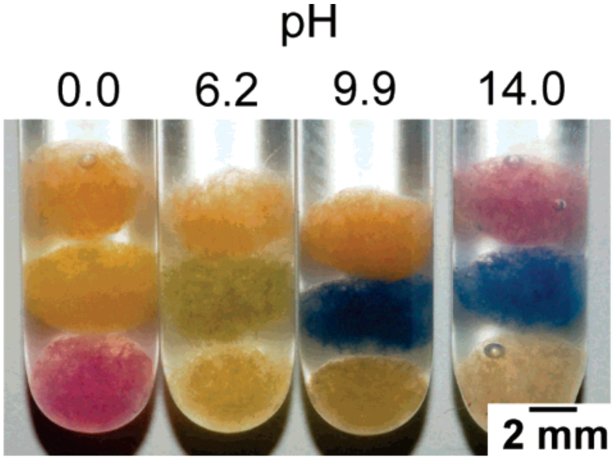

Figure 4. Optical image of the same set of three types of hydrogel spheres with embedded fibers of pH paper containing different covalently bound indicators in solutions titrated to four different values of pH: 0, 6.2, 9.9, and 14 (from left to right). Each tube contains three spheres (one of each set).

The presence of Ho^{3+} cations does not interfere with the color changes of the pH paper, and the presence of the embedded fibers disrupts neither the cross-linking nor the paramagnetic response of the hydrogel. These spheres, therefore, can be removed or separated by a rare-earth bar magnet as discussed earlier (Figure 2). This experiment demonstrates that magnetic hydrogels can be used to introduce sensors into an aqueous system and that the spheres can be retrieved and reused without contaminating the sample.

Hydrogel Spheres as Retrievable Sorbents. Paramagnetic AA spheres can host other materials with useful properties. Activated carbon powder is used as a decolorizing agent and it is a common sorbent for removing undesirable organic molecules from aqueous solutions. In such applications, the carbon must be removed from the system (usually) by filtration; however, in heterogeneous mixtures or large bodies of water, filtration is not possible. The paramagnetic hydrogels prepared with embedded carbon powder have three useful features: (i) The aqueous solution easily diffuses through the hydrogel matrix and exposes the carbon powder to the solution and its contaminants. (ii) The carbon powder, like the pH paper, does not interfere with the paramagnetism of the hydrogel. (iii) The hydrogel, along with the carbon powder and the adsorbed contaminants, can be removed by external magnets.

As a demonstration of this application of paramagnetic hydrogels, we placed three sets of fifteen 3−5 mm spheres into a 10-mL aqueous solution of crystal violet dye (0.1 mg/mL). The first set was a control containing AA spheres cross-linked by Ho^{3+} with no embedded carbon. The second set contained Ho^{3+}−AA spheres with 5% (w/v) carbon powder, and the third set contained Ho^{3+}−AA spheres with 10% (w/v) carbon powder. After shaking the three vials for 2 h, we measured the UV absorption of each solution (Supporting Information Figure 4) and compared their relative absorption at 590 nm. For the carbon-free control set, the relative ratio of the absorbance of the final solution to the initial solution was 0.945. The decrease in observed concentration of dye was most likely due to dilution resulting from adding spheres, which contained adhered water, to the solution of dye. The ratio for the solution containing the 5%-carbon-powder hydrogel spheres to the initial solution was 0.087, and the ratio for the solution containing the 10%-carbon-powder hydrogel spheres to the initial solution was 0.036. The embedded carbon powder efficiently removed the dye contaminant from the aqueous solution. This experiment demonstrates that paramagnetic hydrogels can be employed as a matrix for sorbents that remove organic contaminants from aqueous solutions.

Conclusion

We have demonstrated the ability to fabricate magnetically responsive hydrogels that are cross-linked with paramagnetic ions. Paramagnetic hydrogel spheres assemble in magnetic field gradients and can be used as a matrix to introduce recoverable sensors or reagents to aqueous mixtures.

In comparison to other magnetic hydrogels, these materials have several advantages, including that (i) they are translucent and, thus, simple to assay (unlike Fe_3O_4, which is black); (ii) they gel rapidly (in <1 s of contact with a solution of paramagnetic cations), which allows for simple, fast fabrication; and (iii) they are able to incorporate solid materials and be used as sensors or sorbents. These paramagnetic ionotropic hydrogels also have their disadvantages, including that (i) the magnetic response of the spheres is at least an order of magnitude weaker than that of hydrogel spheres incorporating superparamagnetic particles and (ii) some buffers (e.g., phosphate anions) precipitate the holmium from the spheres and cause them to dissolve slowly.

Owing to the simplicity of their fabrication, ease of use, and low toxicity, paramagnetic hydrogels cross-linked with Ho^{3+} represent a new class of functional materials available for use as environmentally friendly detection systems.

Experimental Section

Cross-Linking and Formation of Paramagnetic Ionotropic Hydrogels. The polymers (AA (Aldrich); CMC (Aldrich); PG (Aldrich); ι-carrageenan (Aldrich)) that successfully formed hydrogels were initially dissolved in water (2% w/v). The hydrogel spheres were formed by dropping the dissolved polymer solution (5−10 mL) through a 27-gauge needle (Becton Dickinson & Co.) into an aqueous solution of $Ho(NO_3)_3$ (10−500 mM, Aldrich) and leaving them to soak for 30 min. The spheres were subsequently soaked in 40 mL of deionized water for at least 2 h and rinsed to remove any free Ho^{3+}. This washing process was repeated at least three times.

Preparation of Acidified AA. This procedure was based on the work by Cozzi et al.[20,21] A solid portion of the sodium salt of AA (23 g, Sigma-Aldrich) was suspended in 150 mL of 3 M HCl and stirred at room temperature for 8 h. The liquid phase was decanted and replaced by 100 mL of 3 M HCl, and the mixture was stirred for an additional 3 h. The solid was isolated by vacuum filtration through a large frit of sintered glass and washed with deionized water. The acidified AA product was stored as an aqueous slurry.

Ion Exchange Chromatography of Metals on AA. All chromatographic experiments were conducted on a Varian Star model HPLC instrument equipped with a variable-pressure solvent delivery pump (Rainin Dynamax SD-300) and a UV−vis dual wavelength detector (Rainin Dynamax UV-D II). Chromatography was per-formed isocratically using 0.1 M HCl as the eluant. A glass column (length = 20 cm, i.d. = 1 cm) suitable for LC at low pressure (<30 psi) was packed with a slurry of acidified AA. The AA was compressed by setting the rate of flow at 0.8 mL/min at a pressure of 20 psi. Additional portions of AA were added until the final height of the packed stationary phase was 19 cm. The column was washed with 0.1 M HCl at a flow rate of 0.4 mL/min for 12 h prior to its initial use.

The procedure for loading the analyte onto the column involved opening the column, removing the excess mobile phase, and allowing the remaining eluant to drain until level with the top of the stationary phase. At this point, a 100 μL aliquot of a 50 mM solution of the metal ion in 0.1 M HCl spiked with 2 mM KNO_3 was injected onto the top of the column and allowed to drain until level with the top of the stationary phase. An additional 100 μL of eluant was added and allowed to drain, then the void space of the column was filled completely with eluant, and the HPLC instrument was programmed to deliver eluant through the column at 0.4 mL/min with a pressure of 15 psi. The elution of nitrate (NO_3^-) and the metal ions was monitored by UV−vis detection at 220 and 393 nm. Each metal ion was chromatographed in triplicate, and the resulting R_f values were associated with 90% confidence intervals of ±0.016 or less.

Determination of Magnetic Susceptibility of AA Spheres. Sets of AA spheres were cross-linked with aqueous solutions of 10 mM, 100 mM, or 500 mM Ho^{3+}. One hundred spheres of each set were dissolved in 5 mL of a 0.5 M solution of EDTA (Aldrich). The absorption spectrum of each solution was taken with a UV−vis spectrophotometer (HP 8453), and the baseline of each spectrum was corrected to zero absorption—we believe the dissolved alginate salt in the samples resulted in Rayleigh scattering that shifted the baseline up.

Separation and Manipulation of Hydrogel Spheres. A set of NdFeB magnets (Master Magnetics, Inc.) was placed in an aqueous solution such that the polarization of the magnetic field was parallel to the benchtop. A single hydrogel sphere was placed at a distance of 2 cm from the surface of the magnet and manually brought closer to the magnet until the magnetic force attracted the sphere to the surface of the magnet. The furthest distance at which the magnetic force could attract the sphere was recorded. The measurement was repeated at least seven times for each type of hydrogel sphere. The strength of the magnetic field was measured with a hand-held magnetometer (Sypris 6010).

Colorimetric Detection of pH Using Hydrogel Spheres. ColorpHast pH paper (EMD Chemicals, Inc.) was removed from 100 plastic strips and soaked in 25 mL of hot water for at least 2 h. Neither the paper nor the indicator dyes dissolved or leached into solution. The mixture containing the paper was shredded with an electric food processor (Cuisinart MM-2M) for 30 s to create pulp. AA was dissolved into the wet pulp (2% w/v) and cross-linked in a 100 mM Ho^{3+} solution. A 16-gauge needle (Becton Dickinson & Co.) was required to form the drops of the polymer/pulp solution in order to avoid clogging. After rinsing, the spheres were placed in each of four solutions having different pH values.

Hydrogel Spheres as Retrievable Sorbents. Carbon powder decolorizing agent (Eastman Kodak)—5% (w/v) or 10% (w/v)—was dispersed in a 2% (w/v) aqueous solution of AA. The solution of AA-containing carbon was cross-linked in a 100 mM solution of Ho^{3+} ($Ho(NO_3)_3$). Three sets of 15 spheres, each set containing a different carbon loading (0%, 5%, or 10%), were added to separate vials containing 10 mL of a 0.1 mg/mL aqueous solution of crystal violet dye (Sigma). The solutions containing the spheres were shaken in scintillation vials on a vortex mixer (VWR) for 2 h. UV−vis spectra of the supernatants were recorded on a UV−vis spectrophotometer (HP 8453) and compared to the absorption of the initial 0.1 mg/mL solution of crystal violet.

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Supporting Information Available: A sample chromatogram from the LC analysis of metal ions, data pertaining to the positive magnetic response of the spheres, chemical structures of the monomer units for the polymers used in this study, and UV−vis spectra demonstrating that the Ho^{3+}−AA spheres with embedded carbon powder function as sorbents are provided (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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