Preparation of Uniform Magnetic Microspheres through Hydrothermal Reduction of Iron Hydroxide Nanoparticles Embedded in a Polymeric Matrix

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A novel method is described for the preparation of nearly monodispersed and highly magnetic responsive microspheres with magnetite nanocrystals formed in a polymeric matrix by hydrothermal reduction. The method is based on the formation of iron hydroxide/polymer composite microspheres by acid-catalyzed condensation polymerization of urea and formaldehyde in the presence of colloidal iron hydroxide. The iron hydroxide colloids entrapped in the polymer matrix are then in situ converted to magnetite nanocrystals by reaction with sodium borohydride under hydrothermal conditions. Characterization of the resulting microspheres with electron microscopy and vibrating sample magnetometry confirmed that these particles possessed a uniform spherical morphology, narrow particle size distribution, and high magnetic susceptibility. More interestingly, the magnetic nanoparticles embedded in the polymer matrix are of cubic shape and highly crystalline structure. While the growth of uniform composite microspheres is accounted for by the well-known LaMer model, the formation of the cubic magnetite nanocrystals appears to involve a dissolution−recrystallization process. After being coated with silica by the sol−gel approach, the magnetic particles were used as adsorbents for isolation of genomic DNA from biological samples, with results comparable to those obtained by magnetic silica microspheres. Incorporation of iron hydroxide colloids into polymer microspheres coupled with chemically induced phase transformation represents a new cost-effective approach to the preparation of uniform magnetic microspheres that is more controllable with respect to particle properties and more amenable to large-scale production.

1. Introduction

The inherent ability of magnetism has been used to separate ferrous objects from mixtures for many decades; however, the benefits of this separation technique for life science applications have not been fully realized until recently due to advances in materials chemistry and automated instrumentation. Biomagnetic separation utilizes magnetically responsive particles as a solid support for nucleic acid isolation,[1–3] protein purification,[4–6] cell sorting,[7–9] immunoassays,[10–12] and enzyme-immobilized magnetic microreactor.[13] These particles are typically coated with an affinity ligand for selective binding to targets in biological samples such as DNA, proteins, viruses, and cells. When they are placed in a sample solution, a ligand−substrate complex is formed on the surface of the magnetic particles. Separation of the target-bound magnetic particles is achieved by applying an external magnetic field to the suspension. If desired, the targets bound on the magnetic particles can be recovered by elution with an appropriate buffer. Since the binding, washing, and elution steps involving magnetic particles can be carried out in an automated liquid processor, the biomagnetic separation offers a highly efficient high-throughput approach for processing a large volume of samples that is attractive for applications in genomics, proteomics, and diagnostics. The magnetic particles developed for use in these applications are typically composed of magnetite or maghemite nanocrystals distributed in a polymer matrix. These particles are coated with a polymer shell that protects the iron oxide from oxidation or dissolution and provides chemical groups for covalent attachment of biomolecules.

Several approaches have been developed for the preparation of magnetic microspheres, which may be classified into three categories: in situ formation, core−shell process, and copolymerization. Ugelstad et al. were the first to report the preparation of highly uniform micrometer-sized magnetic

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polymer microspheres by in situ formation approach.[14] These particles were obtained by oxidation of ferrous ions adsorbed on macroporous, functionalized polystyrene beads, resulting in the formation of maghemite nanoparticles within the pores of these particles. Kumacheva and co-workers recently used this approach to prepare microspheres containing not only magnetic but also metal and semiconductor nanoparticles.[15] The core−shell processing involves self-assembly of alternating layers of polyelectrolytes and colloidal magnetic nanocrystals on polymer microspheres of micrometer size.[16] The magnetite loading can be increased by a multiple layer-by-layer deposition process. The third approach is based on heterogeneous polymerization of a polymer precursor adsorbed on colloidal magnetic particles. Examples in this category include copolymerization of cobalt particles with poly(dimethylsiloxane),[17] copolymerization of methyl methacrylate with oleic acid-modified magnetic particles,[18] and emulsion-templated polymerization of monomers in the presence of magnetite nanoparticles.[19–21] Despite the success of these approaches in producing micrometer-sized magnetic polymer microspheres, each has its own pros and cons with respect to the implementation of the synthetic methods and the properties of the particles produced. For instance, highly uniform magnetic microspheres can be obtained by Ugelstad's method; however, the multistep reactions involved make the preparation tedious and time-consuming. The core−shell process has the same drawback as the workload increases considerably with the multilayer deposition. By contrast, the copolymerization process is easy to implement and readily scaled up for bulk quantity production. However, the magnetic particles produced by this approach generally have broad size distribution.

To address these problems, we developed a novel two-step procedure for preparation of monodispersed magnetic polymer microspheres, which was based on condensation polymerization coupled with hydrothermal reduction. The first step of the synthesis involves polymerization of urea and formaldehyde (UF) in the presence of colloidal iron hydroxide, which is also known as ferrihydrite, resulting in monodispersed, micrometer-sized ferrihydrite/UF composite microspheres. The as-synthesized microspheres are then subjected to reaction with sodium borohydride under hydrothermal conditions to facilitate the phase transformation of the ferrihydrite to magnetite. The morphology, iron loading, and magnetic properties of the composite microspheres were characterized as a function of the concentration of the ferrihydrite sol and molar ratio of B/Fe used in the preparation using scanning electron microscopy, atomic absorption spectrometry, and vibrating sample megnetometry, respectively. A unique feature of this method is that the morphology and magnetic properties of the particle products could be controlled through the factors that affect the polymerization and subsequent reduction. The biotechnological applications of such magnetite/UF microspheres were demonstrated using silica-coated particles for the isolation of genomic DNA from *Saccharomyces cerevisiae* cells and maize kernels.

2. Experimental Section

2.1. Materials. Formaldehyde (37 wt %, AR), urea (AR), and tetraethoxysilane (CP) were obtained from Tianjin Chemical Reagent Company (Tianjin, China). Iron hydroxide or more specifically ferrihydrite (which are used interchangeably in the present work) sols were synthesized by fast hydrolysis of ferric salt solution at room temperature as described previously.[2,22] Briefly, a total of 160 g of $NaHCO_3$ powder was slowly added to a 1 L of distilled water in which 250 g of $FeCl_3 \cdot 6H_2O$ was dissolved. The mixture was stirred continuously for 1 h, yielding a reddish brown ferrihydrite (nominal formula $5Fe_2O_3 \cdot 9H_2O$) sol at 140 mg/mL $Fe_2O_3 \cdot 1.8H_2O$. The as-synthesized ferrihydrite forms single spherical particles, approx 4−6 nm in size, consistent with that reported in the literature.[23] Magnetic silica microspheres used as a reference material for comparative studies were obtained from BaseLine Chromtech Research Centre (Tianjin, China). Wild-type *Saccharomyces cerevisiae* cells were a gift from Mr. Yonggang Zhang of Tianjin University. Fresh corn used to provide maize kernels was obtained from a local fruit market. Agarose of molecular biology grade, molecular weight marker 1 kb DNA stepladder, and snailase were purchased, respectively, from Lianxing Biotechnology (Tianjin, China), Dingguo Biotechnology (Beijing, China), and Hope Biotechnology (Tianjin, China). Other reagents used in DNA isolation and analysis were of analytical grade and were used without further purification.

2.2. Preparation of Magnetic Microspheres. Magnetic microspheres were prepared by a two-step procedure involving the formation of ferrihydrite/UF composite microspheres and subsequent reduction of the ferrihydrite to magnetite. In a typical preparation, a total of 17.5 mL of stock ferrihydrite sol (140 mg/mL) and 3 mL of deionized water were homogenously mixed to give a working ferrihydrite sol at 120 mg/mL $Fe_2O_3 \cdot 1.8H_2O$. Then, 1.05 g of urea was added, and the pH of the resultant suspension was brought to 2 with 2 M nitric acid. This was followed by addition of 1.57 mL of aqueous formaldehyde (37 wt %) with stirring. After the addition was complete, the mixture was left without agitation at ambient temperature. Within 10 min, a yellowish gel was formed, which was composed of uniform microspheres as shown under an optical microscope. The microspheres generated were allowed to age overnight, and the product was collected by filtration, washed with deionized water, and dried under vacuum at 60 °C. Finally, the particle sample was suspended in 130 mL of 0.1 M sodium borohydride solution (freshly prepared with deionized water, pH 9), and the suspension was transferred to an autoclave. The reduction reaction was carried out in H_2 atmosphere at 4 mPa and 80 °C for 2 h. At the end of the reaction, the initial yellowish microspheres were turned into black ones, which were readily

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attracted by a hand-held magnet. The reduction product was magnetically retrieved, thoroughly washed, and vaccuum-dried.

2.3. Surface Modification of Magnetic Microspheres. The magnetic microspheres prepared above (0.5 g) were placed in a three-necked flask containing 4 mL of deionized water and 20 mL of ethanol. Under continuous mechanical stirring, 0.5 mL of aqueous solution of ammonium hydroxide and 0.125 mL of tetraethoxysilane were sequentially added to the reaction mixture and the reaction was allowed to proceed for 3 h at ambient temperature. The resulting microspheres were magnetically retrieved, thoroughly washed, and vaccuum-dried.

2.4. Isolation of Genomic DNA. The protocols used to extract genomic DNA from wild-type *Saccharomyces cerevisiae* and maize kernels were the same as previously described.[2] Briefly, 40 mL of *Saccharomyces cerevisiae* culture solution was centrifuged in a 50 mL tube to yield a 0.6 g of wet pellet. Into the tube was added 3 mL of 0.1% ME-PB buffer (0.1% β-mercaptoethanol, 25 mM Na_2HPO_4, 175 mM NaH_2PO_4, 0.8 M sorbitol, pH 5.8). Following 10 s of vortexing, the mixture was incubated at 30 °C for 30 min. The mixture was centrifuged at 3500 rpm for 5 min, and the supernatant was discarded. Into the tube was added 2.5 mL of 1% snailase-PBS buffer (pH 7.5), and the suspension was gently agitated at 30 °C for 1 h. The resulting suspension was centrifuged at 2000 rpm for 5 min, and the supernatant was removed again. Into the tube were added 2.5 mL of lysis buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, 1% Triton-X-100, pH 8.0) and 0.5 mL of 10% sodium dodecylsulfate (SDS), and the mixture was incubated at 55 °C for 10 min to yield a crude lysate. A 0.5-mL aliquot of the crude lysate was transferred into a 1.5 mL microcentrifuge tube. Then 1 mL of binding buffer (20% PEG_{8000}, 2 M NaCl) was added, followed by 0.2 mL of silica-coated magnetite/UF microsphere suspension (0.05 g/mL). The suspension was agitated gently at ambient temperature for 10 min, and the magnetic particles were immobilized using a magnetic separator (BaseLine Chromtech Research Centre, Tianjin, China). The supernatant was removed, and magnetic particles were washed twice with 70% ethanol. After the removal of the supernatant, the adsorbed DNA was eluted from the magnetic particles by addition of 0.2 mL of TE buffer (10 mM Tris-HCl, pH 7.8, 1 mM EDTA, pH 7.8) and incubation with gentle agitation at 25 °C for 10 min. The magnetic particles were immobilized again, and the eluate was collected and analyzed by UV spectrometry. A 10-μL aliquot of the eluted DNA was assayed by gel electrophoresis on a 1% agarose gel in Tris-acetate buffer. The above procedures were repeated when magnetic silica microspheres were used as adsorbents.

A different cell lysis procedure was employed in the isolation of genomic DNA from maize kernels. After treatment of liquid nitrogen, 2 g of maize kernels was thoroughly ground with a pestle in a mortar. Into the ground maize kernels was added 10 mL of lysis buffer (100 mM Tris-HCl, 50 mM EDTA, 0.5 M NaCl, 1.5% SDS, pH 8.0). The suspension was vortexed and incubated at 65 °C for 30 min with occasional shaking. The crude cell lysate was then centrifuged at 5000 rpm for 10 min. The supernatant was collected and stored at 4 °C until use. Extraction and analysis of genomic DNA from the cleared cell lysate using magnetic microspheres were the same as describe above for yeast.

2.5. Characterization. The size and shape of the microspheres were studied using a Philips XL30 environmental scanning electron microscope equipped with Oxford ISIS300 energy-dispersion spectroscopy (EDS) operating at 20 kV. The scanning electron microscopy (SEM) was performed on mechanically polished and unetched copper plate, and all samples were coated with a thin gold film under vacuum prior to microscopy. All EDS analyses were conducted at 20 kV, and test regions were selected on the multilayer surface of the magnetic particles to avoid recording of Cu peaks. All the samples for transmission electron microscopic (TEM) studies by FEI Tecnai G^2 F20 at 200 kV were prepared by directly transferring the suspended particles to the standard copper grid coated with an amorphous carbon film. The selected area electron diffraction (SAED) patterns of the magnetic particles were recorded on a JEOL 100 CXII TEM. The crystalline type of the iron species present in the composite microspheres was characterized by powder X-ray diffraction (XRD) on a Rigaku D/max 2500v/pc diffractometer with Cu Kα radiation at 40 kV and 200 mA. The magnetic hysteresis loops of the particle samples were recorded on a LDJ 9600-1 vibrating sample magnetometer (VSM) operating at room temperature with applied fields up to 10 kOe. The iron contents of the composite microspheres were determined using acid dissolution followed by atomic absorption spectroscopy (AAS) on a Hitachi 180-80 atomic absorption spectrometer. The thermogravimetric analysis (TGA) of the samples was performed on a Shimadzu TA-50, in air, with a temperature ramp of 10 °C/min. Characterization of the pore structures of the particles was performed using a Sorptometer NOVA 2000 operating at an adsorption temperature of −195.6 °C (liquid nitrogen). The UV absorbances of the DNA solutions were recorded on a Shimadzu UV-2450 spectrophotometer.

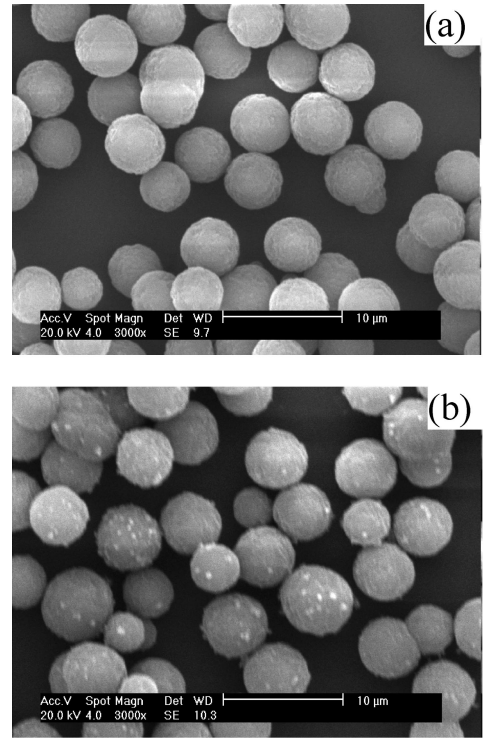

Figure 1. SEM images of (a) ferrihydrite/UF microspheres synthesized from iron hydroxide sol at 120 mg/mL $Fe_2O_3 \cdot 1.8H_2O$ and (b) their borohydride reduction product.

3. Results and Discussion

3.1. Preparation of Magnetic Microspheres. The morphology of the composite microspheres was studied by SEM, and the SEM images of the samples are shown in Figure 1. The SEM images a and b correspond to the particle sample prepared from a ferrihydrite sol at 120 mg/mL $Fe_2O_3 \cdot 1.8H_2O$ and its borohydride reduction product. Both of them exhibit good monodispersity and regularly spherical morphology. However, detailed inspections of these images reveal several distinct differences. First, the particles before reduction show

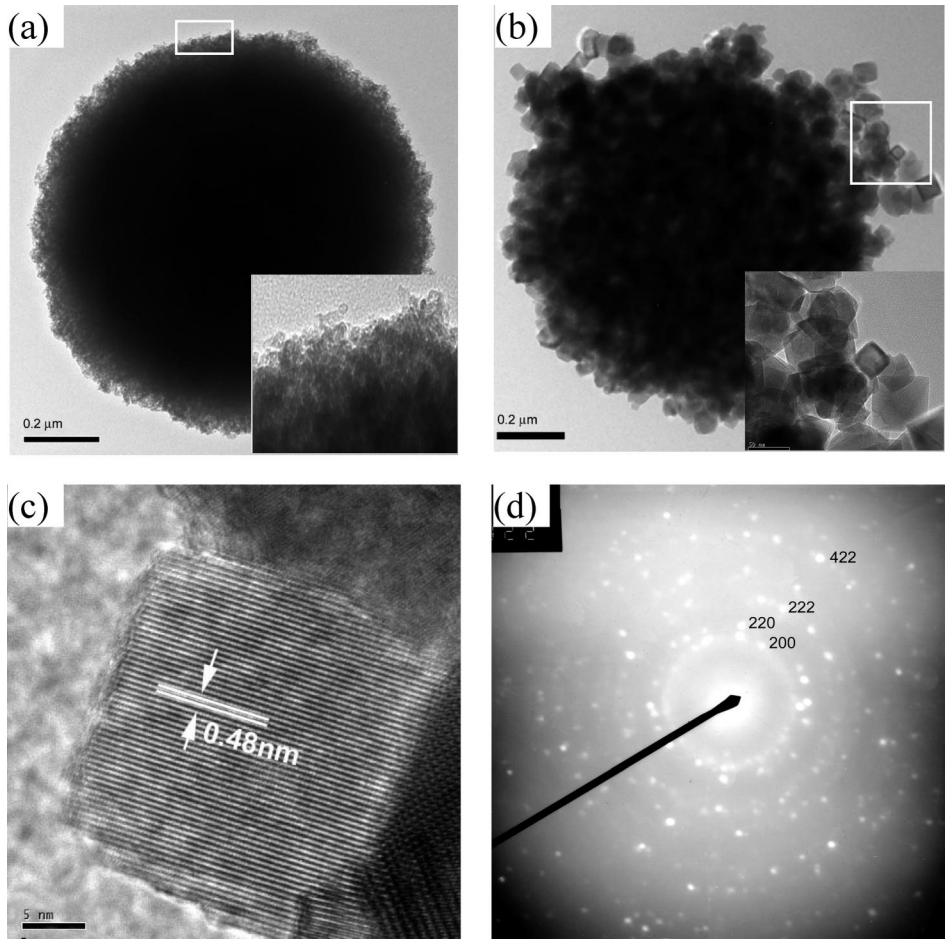

Figure 2. HRTEM images of (a) ferrihydrite/UF microspheres synthesized from iron hydroxide sol at 120 mg/mL $Fe_2O_3 \cdot 1.8H_2O$ and (b) their reduction product. (c) Magnified view of the individual Fe_3O_4 nanocrystals embedded on a magnetic UF microsphere. (d) SAED pattern of an individual magnetic UF microsphere.

an average particle diameter of 3.8 ± 0.19 μm whereas the reduction product has a corresponding value at 4.5 ± 0.17 μm, as determined from measurements of over 300 particles in different regions of SEM images using Image Pro 6.0 software. This increase in particle size for the reduction product indicates that particle growth occurs during hydrothermal reduction. Another noticeable contrast is associated with the texture of the composite microspheres. The borohydride treatment turned the initially rather smooth surfaces of the composite microspheres into rough ones with a large number of tiny particles embedded on the surfaces.

High-resolution transmission electron microscopic (HRTEM) studies of the particle samples confirmed changes in morphology of the ferrihydrite nanoparticles brought about by borohydride reduction. The TEM images a and b in Figure 2 correspond to a ferrihydrite/UF composite microsphere and its reduction product. The original composite microsphere consists of poorly crystallized, several nanometer sized colloids embedded in a UF resin matrix, and therefore it appears to possess smooth surfaces. By contrast, the reduction product shows that highly crystallized, much larger nanoparticles in cubic shape are present both on and inside the polymer matrix. The cubic nanocrystals possess particle sizes generally below 50 nm, and their lattice spacing is about 0.48 nm (Figure 2c), which is consistent with the lattice spacing of (111) planes of the Fe_3O_4 crystal structure (JCPDS 87-2334). The SAED pattern collected from the reduction product (Figure 2d) is also consistent with a cubic iron oxide phase of magnetite. The formation of nanocrystal clusters on the surface of the microsphere is indicative of the migration of ferric ions from the inner core to the outer surface, leading to the particle growth during the chemical reduction.

To verify the phase transformation of the ferrihydrite in the composite microspheres upon treatment with sodium borohydride, powder XRD analysis was performed on the particle samples, and the XRD patterns are shown in Figure 3. The XRD patterns a and b in Figure 3 correspond to the particle samples before and after borohydride reduction. The ferrihydrite in the original composite microspheres exists in poorly crystallized form, consistent with characteristics of synthetic ferrihydrite.[22,23] However, a remarkable increase in crystallinity is observed with the reduction product. The discernible peaks (Figure 3b) can be indexed to (220), (311), and (440) planes of a cubic unit cell, which correspond to Fe_3O_4 with cubic structure (JCPDS 87-2334) (Figure 3c). The mean crystallite diameter of the magnetite is 23.7 nm, as calculated from the half-width of (311) reflection peaks of the XRD patterns using the Scherrer formula where the k value was taken as of 0.94 for cubic shaped particles.[24]

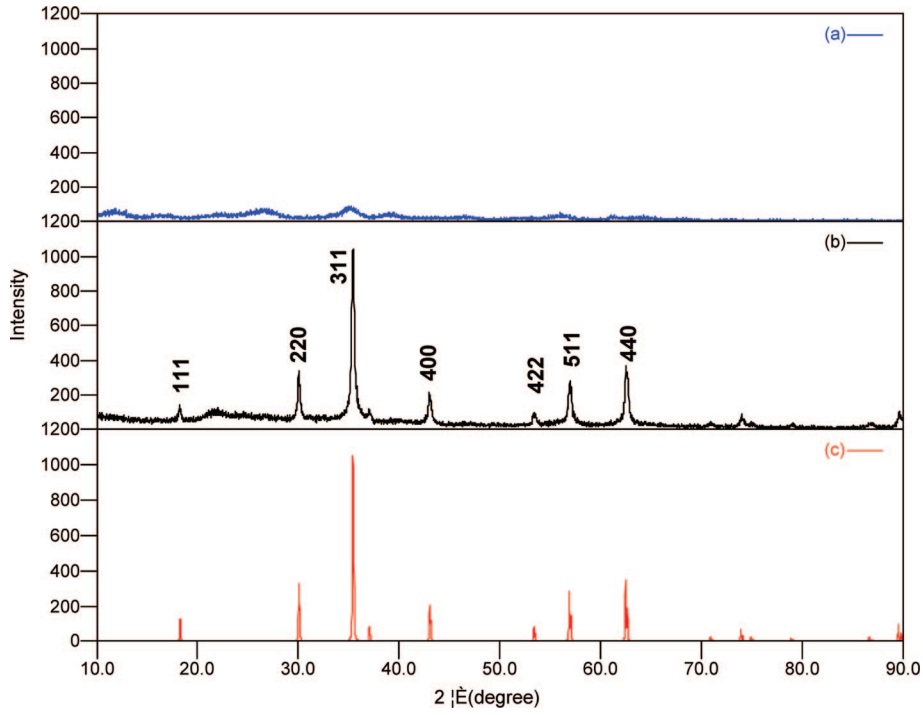

Figure 3. XRD patterns of (a) ferrihydrite/UF microspheres and (b) their reduction product. The bottom pattern (c) was drawn according to JCPDS 87-2334 by software Cyrstallographica Searh-Match V. 2.1.1.0.

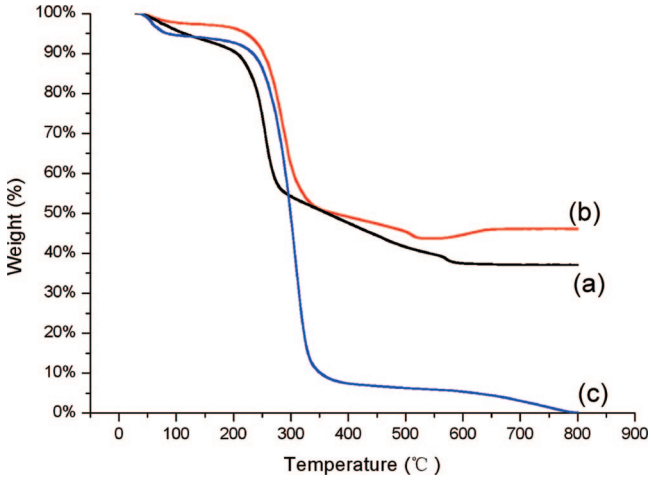

Figure 4. TGA plots of (a) ferrihydrite/UF microspheres, (b) their reduction product, and (c) pure UF microspheres.

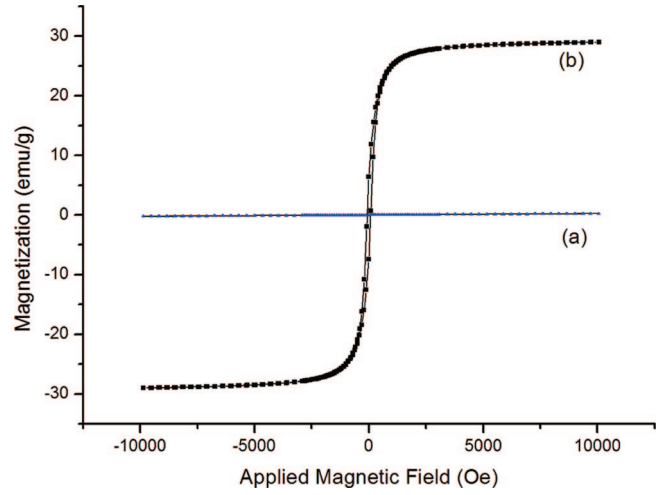

Figure 5. Room temperature magnetization curves of (a) ferrihydrite/UF microspheres and (b) their reduction product.

The iron loading of the composite microspheres was determined using atomic adsorption spectrometry. The composite microspheres before reduction contained 28 wt % Fe. After reduction, however, the iron content increased to 34 wt % Fe. This suggests that partial dissolution of the polymer matrix occurs during the hydrothermal reduction. To verify this finding, thermogravimetric analysis (TGA) was employed to study the composition of the composite microspheres before and after reduction in comparison with that of pure UF microspheres prepared in the absence of iron hydroxide sol, and the results are shown in Figure 4. The TGA plots a, b, and c correspond to the ferrihydrite/UF microspheres, their reduction product, and the pure UF particles, respectively. Two stages of weight loss were identified in these plots, 25−200 °C and 200−700 °C. At temperatures below 200 °C, the loss of weight is attributed to the gasification of the small molecules such as water and low-molecular-weight polymer. The steep change in this region for the iron hydroxide/UF microspheres indicates that more low-molecular-weight species desorbed from the ferrihydrite/UF microspheres than from the magnetite/UF counterparts. The second weight loss observed at temperatures higher than 200 °C is attributed to the slow decomposition/vaporization of the higher-molecular-weight species present in the microspheres and the release of water molecules from colloidal iron hydroxide. It is noted that a slight gain in weight at about 600 °C occurs for magnetite/UF microspheres, suggesting a phase transformation sets in due to oxidation of the Fe_3O_4 to Fe_2O_3. The remaining iron oxide fractions were found to be 37.6 wt % Fe_2O_3 (or

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equivalently 26.31 wt % Fe) and 46.06 wt % Fe_2O_3 (or equivalently 36.62 wt % Fe), respectively, for ferrihydrite/UF microspheres and their reduction product. The iron contents determined with TGA are in good agreement with those obtained using AAS. The increased percentages of the iron species in the reduction product suggest that the UF resin, the organic component of the composite microspheres, may be decomposed partially under the hydrothermal conditions. Considering the alkaline nature of the reaction media, the pH of which measured at the end of the reaction was around 12, it becomes apparent that cleavage of amide bonds in the UF polymer is most likely responsible for the loss of a portion of the organic component during the borohydride reduction.

The magnetic properties of the magnetite/UF microspheres obtained by borohydride reduction were investigated by VSM with applied field up to 10 kOe at 300 K. Figure 5 shows the hysteresis loops of the ferrihydrite/UF microspheres and their reduction product. The ferrihydrite/UF microspheres are essentially nonmagnetic, whereas the reduction product displays characteristic behavior of paramagnetic materials. The measured saturation magnetization (M_s), the coercivity (H_c), and the remanence (M_r) of the magnetite/UF microspheres are 28.97 emu/g, 74.54 Oe, and 6.18 emu/g, respectively. Since the magnetite contents in the composite microspheres are 46.95 wt % Fe_3O_4 based on AAS or 44.52 wt % Fe_3O_4 based on TGA, the saturation magnetizations normalized over the percentages of magnetite are 61.70 and 65.07 emu/g, respectively. These measured values are well below the corresponding ones (84−92 emu/g depending upon the particle size) for bulk magnetite,[25,26] suggesting that a complete conversion from ferrihydrite to magnetite was not realized under the reaction conditions employed and further enhancement in magnetization could be achieved through optimization of the reaction parameters. Nevertheless, the magnetization of these particles is 1.5−2.5 times higher than that of commercially available magnetic polymer microspheres such as DynaBeads M280 and Sera-Mag.[27]

Two factors were found to be critical in determining the morphology and magnetic properties of the reduction product. One is the concentration of iron hydroxide sol used in the fabrication of ferrihydrite/UF microspheres and another is the molar ratio of sodium borohydride to the iron species (B/Fe) in the microspheres used in the subsequent reduction of iron hydroxide incorporated. Figure 6 shows the particle size and iron loading of the composite microspheres as a function of the concentration of the iron hydroxide sol used in the initial synthesis solution. With the sol concentration increased from 28 to 140 mg/mL $Fe_2O_3 \cdot 1.8H_2O$, the spherical morphology of the product is essentially unchanged but the particle size is decreased and the iron loading is increased. When the concentration is lower than 28 mg/mL, the amount of the magnetic nanocrystals obtained with reduction treatment is insufficient to support their spherical structure, giving rise to severely deformed particles. This is ascribed to the corrosive effect of the alkaline media on the amide bond of the polymer resin that caused a partial loss of the organic component. With the iron sol concentration greater than 140 mg/mL, more tiny magnetic nanocrystals are produced during reduction, which cannot be completely incorporated into the composite microspheres. Apparently, this is associated with the migration of iron species from the inner core to outer surface during the phase transformation and subsequently the formation of magnetite clusters on the surface of the microspheres.

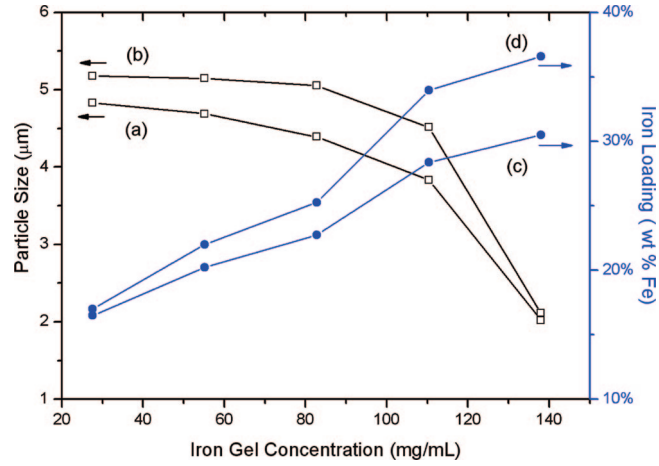

Figure 6. Particle size and iron loading of (a and c) ferrihydrite/UF microspheres and (b and d) their reduction product as a function of concentration of iron hydroxide sol used in the reaction mixture.

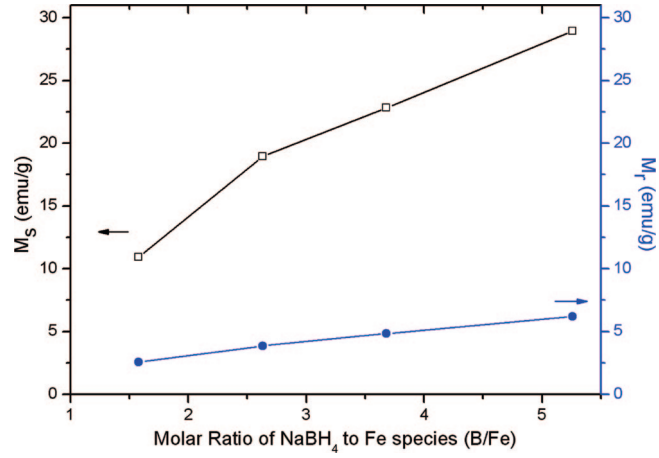

Figure 7. (a) Saturation magnetization and (b) remanence of magnetic UF microspheres obtained by reaction of ferrihydrite/UF microspheres with sodium borohydride in varying molar ratios.

The second key factor for the preparation of magnetic microspheres is the B/Fe molar ratio in the reduction reaction. The moles of Fe(III) in the composite microspheres were obtained from the data for wt % Fe determined by AAS. Figure 7 shows the variations of the saturation magnetization (M_s) and remanence (M_r) of the magnetic microspheres with the B/Fe ratio used in the reduction reaction. Both M_s and M_r increase in virtually linear fashion with the B/Fe ratio from 1.58 to 5.26; however, the ratio of M_r/M_s is almost constant at about 0.2. Clearly, the phase change is facilitated by increasing amount of the reducing agent in the reaction mixture. To understand the effect of the molar ratio on the

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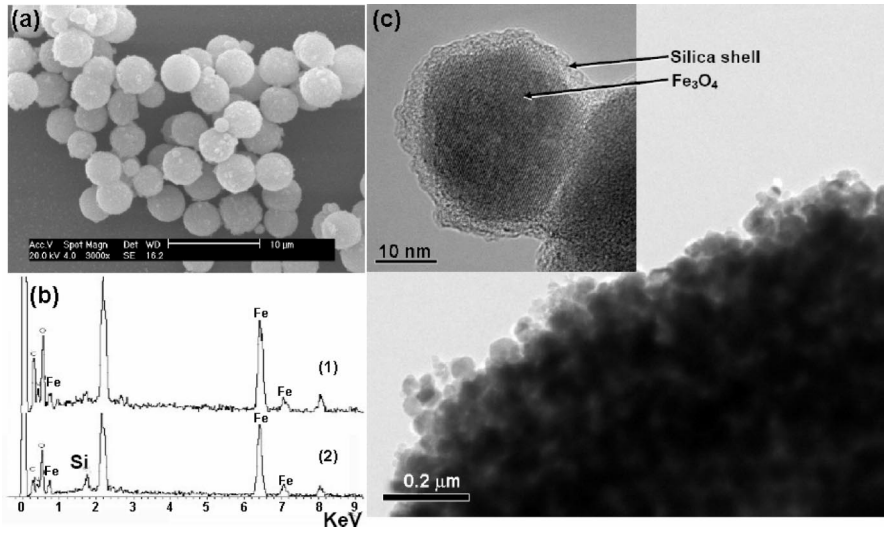

Figure 8. (a) SEM image of silica coated magnetite/UF microspheres. (b) ESD spectra of magnetite/UF microspheres without (1) and with (2) silica coating. (c) HRTEM images of silica coated magnetite/UF.

magnetic properties of the product, we proceed to consider the stoichiometry of the overall reaction. In alkaline media (pH 9−12) containing sodium borohydride, the transformation of ferrihydrite to magnetite can be described by the following equation:

$$12Fe_2O_3 \cdot 1.8H_2O(s) + NaBH_4(aq) \rightarrow 8Fe_3O_4(s) + NaBO_2(aq) + 23.6H_2O(l) \quad (1)$$

Conversion of 1 mol of ferrihydrite to magnetite requires consumption of only 1/12 mol of sodium borohydride, and this translates into the B/Fe molar ratio of 0.042. The B/Fe molar ratios explored in the present study are greater than that required by order of magnitude, but a complete convention has yet to be achieved. The excess consumption of the reducing agent is probably caused by (i) the hydrolysis of sodium borohydride as a side reaction[28] and (ii) the reduction of formaldehyde present in the UF matrix. Suppressing or inhibiting the fast hydrolysis of the borohydride is considered necessary to induce the phase transformation. No magnetic microspheres were obtained when the reduction was performed under ambient pressure in an open system. The presence of H_2 pressure in the hydrothermal system limits the hydrolysis of the sodium borohydride and therefore favors its reaction with the ferrihydrite nanoparticles embedded in the polymer matrix. On the other hand, however, the use of the reducing agent in greatly stoichiometric excess could transform the ferrihydrite and its related iron oxides into zero valented iron, resulting in amorphous iron boron alloys as primary products.[29–31]

According to the experimental results presented above, mechanisms underlying our synthetic route to the magnetite/UF composite microspheres may be outlined as follows. The formation and growth of monodispersed iron hydroxide/UF composite microspheres appears to follow the LaMer model.[32] Under the catalytic action of an acid, precursors including urea and formaldehyde undergo condensation reaction giving rise to urea−formaldehyde oligomers. The oligomers extract iron hydroxide colloids from the reaction solution resulting in oligomer-coated nanoparticles. When the solution reaches a critical supersaturation, a short single burst of nucleation results. This nucleation step may arise from cross-linking induced by intermolecular dehydration of linear or branched oligomers formed in a prior step. The resulting nuclei then grow uniformly and isotropically by fusion of oligomer-coated nanoparticles until the final size is attained. Thus, the diameters of these microspheres can be tuned in the range from 2.5 μm to 8 μm by controlling the growth parameters. The ferrihydrite nanoparticles embedded in the polymer matrix were converted into magnetite nanocrystals via a redox reaction under hydrothermal conditions.[33,34] This phase transformation is believed to occur through a dissolution−reduction−recrystallization mechanism. In alkaline media containing sodium borohydride, the ferrihydrite nanoparticles in the polymer matrix are hydrolyzed to form $Fe(OH)_x^{3-x}$ capable of dissolution and reaction with borohydride in solution to form $Fe(OH)_x^{2-x}$. As the dissolution and reduction proceed continuously under hydrothermal conditions, the concentrations of Fe(III) and Fe(II) contained in the polymer matrix increase and reach critical points where nucleation of magnetite occurs. The magnetite nuclei grow at the expense of ferrihydrite and form cubic crystals in the pores and the outer surface of the polymer microspheres as well. The migration of the iron species from the inner pores to the outer surface of the particles as evident from an increase in the average particle size suggests that magnetite crystals are formed in the bulk solution (homogeneous nucleation) rather than originated at the ferrihydrite substrate (heterogeneous nucleation).

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Table 1. Physical Characteristics of Magnetic Microspheres Used in Isolation of Genomic DNA[a]

adsorbent	M_s (emu/g)	d_p (μm)	S (m^2/g)	pore size (Å)	pore volume (mL/g)
magnetic silica microspheres	21.45	5	249.85	80	0.49
silica coated magnetite/UF microspheres	28.97	4.5	91.66	5	0.11

[a] M_s is the saturation magnetization, d_p is the average particle diameter, and S is the BET surface area.

3.2. Surface Modification of Magnetic Microspheres. To prevent the oxidation and leaching of the magnetite embedded in the UF matrix, a sol−gel approach was employed to provide the magnetic microspheres with a silica shell. The SEM image (Figure 8a) shows that the silica coated magnetic microspheres retain the morphology of the original particles. To confirm the chemical composition of the surface modified particles, energy-dispersive spectroscopy (EDS) was performed on the particle samples before and after surface modification. As can be seen from Figure 8 b, a peak corresponding to silicon is clearly identifiable in the surface modified particle sample. The strong Au peaks (unannotated peaks at about 2.1 and 8.0 keV) were derived from gold film as the image enhancer in SEM. HRTEM images (Figure 8c) of a silica coated particle show that magnetite nanocystals are coated with silica shells in the thickness range 2−4 nm.

3.3. Purification of Genomic DNA with Magnetic Microspheres. Isolation and purification of DNA from a biological sample is an essential procedure in many fields of biotechnology and biomedical research. In this work, the as-prepared silica coated magnetite/UF microspheres were tested against magnetic silica microspheres as adsorbents for isolation of genomic DNA from yeast cells and corn kernels. The magnetic silica microspheres used as a reference material consist of magnetite nanoparticles uniformly dispersed in a silica matrix, which were shown to be superior in terms of yield, purity, and integrity of the DNA recovered.[2] The physical characteristics of these two types of magnetic microspheres are listed in Table 1. With a binding buffer containing poly(ethylene glycol) and sodium chloride, both magnetic particles are capable of extracting genomic DNA from wild type *Saccharomyces cerevisiae* cells and maize kernels. The purified DNA samples were characterized by agarose gel electrophoresis and UV spectrometry. Figure 9 shows the ethidium-stained gel images of DNAs from (a) yeast and (b) corn, from which it is evident that the extracted genomic DNAs are of length greater than 20 kb. Table 2 shows that the yields and A_{260}/A_{280} ratios of the genomic DNAs isolated with silica-coated magnetite/UF microspheres are comparable with those using magnetic silica. This suggests that both types of magnetic particles are of similar quality in terms of integrity, yield, and purity and well suited for purification of genomic DNA.

In summary, uniform magnetic polymer microspheres have been successfully synthesized through a hydrothermal reduction of preformed iron hydroxide/urea−formaldehyde resin composite microspheres with sodium borohydride in H_2 atmosphere at 4 mPa and at 80 °C and for 2 h. The gas pressure applied in the hydrothermal system could inhibit the decomposition of borohydride and be favorable for the reactions between borohydride and ferric ions dissolved from iron hydroxide colloids, which result in the formation of highly magnetizable polymer microspheres. SEM images show that there is a growth in the average particle size of the composite microspheres upon the hydrothermal reduction, suggesting a dissolution−recrystallization mechanism operating in phase transformation. TEM images and XRD patterns confirm that the original poorly crystallized iron hydroxide nanoparticles embedded in the composite microspheres are converted into magnetite nanocrystals when the molar ratio of B/Fe is about 5. VSM measurements reveal that the reduction products show characteristic behavior of paramagnetic materials with apparent saturation magnetizations of 29 emu/g, which are translated to effective magnetizations of 62 emu/g when normalized over the percentage of magnetite in the composite microspheres. The magnetic polymer microspheres coated with silica shells have been successfully applied to purification of genomic DNA from yeast cells and corn kernels, demonstrating their potential for biochemical and biomedical applications. Taken together, this work provides a simple, effective, and low-cost synthetic method to prepare uniform magnetic microspheres, which is more controllable with respect to the particle properties and amenable to large-scale production.

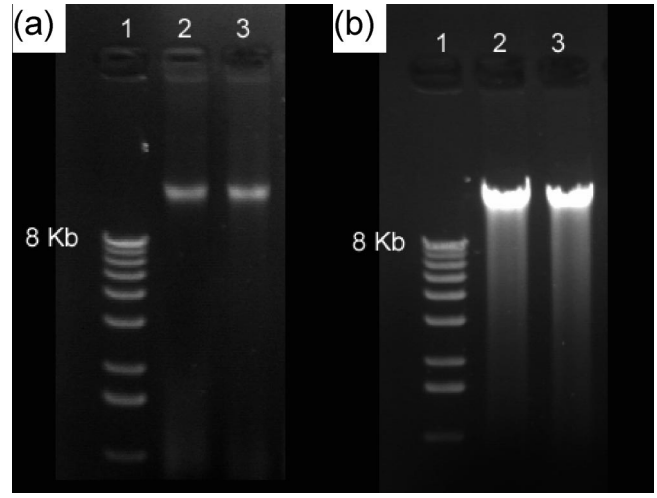

Figure 9. 1% agarose gel electrophoresis of genomic DNA isolated from (a) *Saccharomyces cerevisiae* and (b) maize kernels. Lane 1, DNA ladder; lane 2, DNA isolated with magnetic silica; lane 3, DNA isolated with silica coated magnetite/UF microspheres.

Table 2. Yields and A_{260}/A_{280} Ratios of Genomic DNA Isolated from Maize Kernels and Yeast Cells

	maize kernels		yeast cells	
adsorbent	yield (μg/mg)	A_{260}/A_{280}	yield (μg/mg)	A_{260}/A_{280}
magnetic silica microspheres	0.994	1.86	3.639	1.82
silica coated magnetite/UF microspheres	0.846	1.82	3.580	1.85

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Supporting Information Available: TEM image of the as-synthesized iron hydroxide nanoparticles and SEM images of pure UF microspheres prepared in the absence of iron hydroxide and their hydrothermal reduction products (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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