

Nanostructures

Polymer–Monomer Pairs as a Reaction System for the Synthesis of Magnetic Fe₃O₄–Polymer Hybrid Hollow Nanospheres***Yin Ding, Yong Hu, Xiqun Jiang,* Leyang Zhang, and Changzheng Yang*

Recently, hollow spheres have attracted increasing interest because of their application in drug delivery,^[1–3] cell and enzyme transplantation,^[4] gene therapy,^[5] separation in biomedicine, and as contrast agents in diagnostics.^[6,7] Despite significant advances in the preparation of hollow inorganic or polymeric spheres,^[8–11] the construction of nanosized hybrid hollow spheres with both inorganic component and organic polymers as the shells remains a major challenge. Currently, a widely used approach for the synthesis of hybrid micro or submicron hollow spheres is the core-template-based strategy in which the inorganic components are coated on the polymer particles by sol–gel chemistry^[12] or layer-by-layer deposition.^[13] One advantage of such an approach is that the thickness of the coating layers is controllable. Although successful in the preparation of hollow hybrid spheres, the core-template-based approach faces limitations involving the selection in core composition and nanosized core templates. Furthermore, the core must be removed to create the hollow center. Hence, a core-template-free strategy for the production of hollow hybrid spheres is of particular interest. Previous efforts towards the preparation of hollow hybrid spheres with micrometer dimensions and in the absence of a core template include the assembly of inorganic nanoparticles into hollow spheres by using block copolymers,^[14] the

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assembly of positively charged polyelectrolytes and negatively charged inorganic nanoparticles,^[15] and liquid-phase deposition of inorganic nanoparticles on polymeric capsules.^[16] In previous work,^[17] we introduced a core-template-free approach to construct nanosized hollow polymeric spheres. The basis of this approach is that the reaction system is composed of water-soluble polymer–monomer pairs containing a cationic polymer and an anionic monomer. Depending on their concentration in aqueous solution, such polymer–monomer pairs can self-assemble to form micelles through electrostatic interactions, and hollow polymeric nanospheres are obtained by the polymerization of the anionic monomers inside the micelles.

As a significant advance in attempts to produce inorganic–organic hybrid hollow nanospheres, we present herein a robust, core-template-free method to prepare magnetic hollow Fe_3O_4 –polymer hybrid nanospheres in completely aqueous solution. The preparation procedure is schematically illustrated in Scheme 1. We selected chitosan (CS), which bears amino groups, as the cationic polymer and acrylic acid (AA), which bears acid groups, as the anionic monomer. The CS and AA polymer–monomer pair and Fe_3O_4 nanoparticles stabilized by poly(vinyl alcohol) (PVA) were mixed and formed micelles loaded with Fe_3O_4 nanoparticles; the cores consist of the polyionic complexes of CS and AA (i.e. positively charged protonated CS chains and negatively charged dissociated AA) and the shells consist of protonated CS chains. The formation of CS–AA micelles was confirmed by fluorescent probe, dynamic light scattering (DLS), and transmission electron microscopy (TEM).^[17] Initiation of the polymerization of AA with potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) followed by cross-linking of the shells with glutaraldehyde (GA) at the end of polymerization led to the formation of magnetic hollow Fe_3O_4 –polymer hybrid nanospheres.

The size and morphology of the hybrid spheres were investigated by DLS in aqueous solution, and by TEM and magnetic force microscopy (MFM) in the solid state. The mean-number-average hydrodynamic diameter (D_h) for the synthesized hybrid spheres in aqueous solution was measured to be 76 ± 6.1 nm by DLS and the zeta potential to be 35 ± 2.6 mV by electrophoretic light scattering, suggesting that chitosan molecules with ionized amino groups are at the outermost layer of the hybrid spheres. Figure 1 shows representative TEM images of the synthetic materials. After the addition of Fe_3O_4 nanoparticles (4–6 nm in size) into an aqueous solution of CS–AA followed by polymerization of AA and selective cross-linking of CS at the end of polymerization, spheres with incorporated Fe_3O_4 nanoparticles were formed (Figure 1a). The average diameter of such a structure is 58 ± 11 nm, smaller than that determined by DLS in

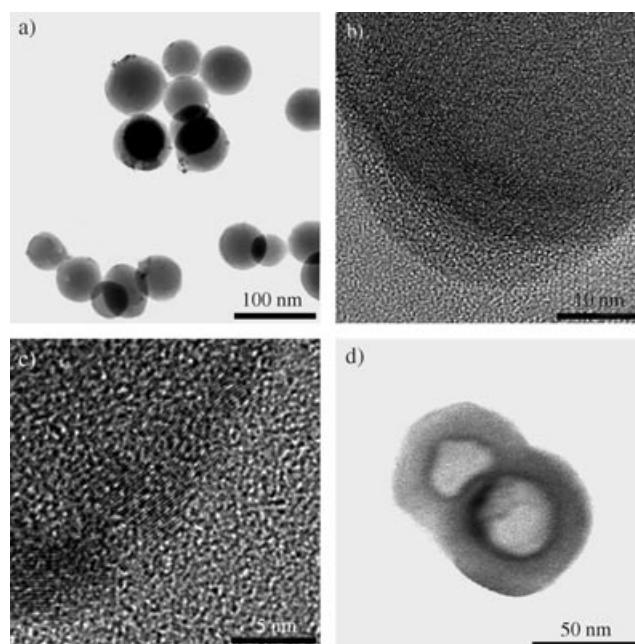
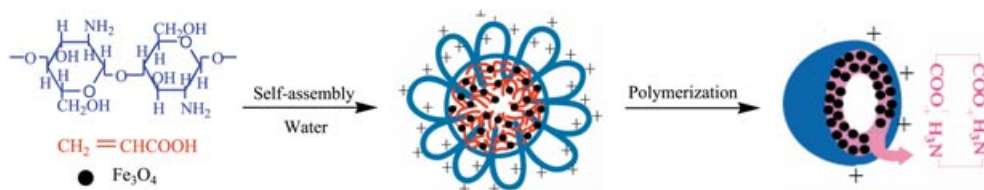


Figure 1. a) Direct-view TEM image of the synthesized Fe_3O_4 –polymer hybrid nanospheres; b) and c) Direct-view HRTEM images of the synthesized Fe_3O_4 –polymer hybrid nanospheres; d) Cut-section TEM image of the Fe_3O_4 –polymer hybrid nanospheres after microtomy at room temperature.

aqueous solution as a result of the sample being dry for TEM analysis. The electron-diffraction pattern recorded from these spheres confirms that magnetite nanoparticles with a minor amount of Fe_2O_3 were loaded into the nanospheres. A salient feature of Figure 1a is that these nanospheres have an intense dark circle within the shells of the spheres and dark spots at the surface of some spheres, which suggests that the distribution of the Fe_3O_4 nanoparticles is not concentrated in the core of the spheres. This is confirmed by high-resolution transmission electron microscopy (HRTEM) observation (Figure 1b,c). The lattice fringe planes of Fe_3O_4 nanoparticles arranged in the dark stripe region, indicative of the crystalline region of the Fe_3O_4 particles, are clearly observed inside the shell of the spheres or at the surface of the spheres. The size of the lattice fringe planes of individual Fe_3O_4 nanoparticles is estimated to be 4–6 nm, which corresponds well to the size of Fe_3O_4 nanoparticles used. The occurrence of Fe_3O_4 nanoparticles in the inner shell of the spheres indicates that the magnetic nanoparticles are trapped inside the micelles before polymerization by the interaction of AA and PVA attached to the Fe_3O_4 nanoparticles, as verified by FTIR. The presence of the magnetic nanoparticles the surface of the spheres suggests



Scheme 1. Illustration of the preparation process of hollow hybrid nanospheres.

that partial adsorption of Fe_3O_4 nanoparticles onto the spheres occurs during the reaction process. The most interesting feature of these nanospheres is their hollow interior, as revealed by the TEM image in Figure 1d obtained by microtomy of a hybrid nanosphere sample at room temperature which had been prepared by depositing hybrid nanospheres on a polyimide sheet followed by embedding in epoxy resin. The image shows the rings of sectioned spheres and the cavity in the interior, thus providing compelling evidence for a hollow sphere structure. Although the Fe_3O_4 nanoparticles are too small to be distinguished, elemental analysis (energy-dispersive spectrometer) provides evidence for the existence of Fe_3O_4 nanoparticles in the hollow spheres.

MFM imaging provides further evidence for the morphology of the hollow hybrid nanospheres and the distribution of magnetite nanoparticles in the nanospheres. As seen from the atomic force microscopy (AFM) image in Figure 2a, the hybrid nanospheres appear as intact spheres with an average height of 12 ± 2.5 nm and an average width of 47 ± 6.6 nm. In the MFM image (Figure 2b), a significant magnetic signal

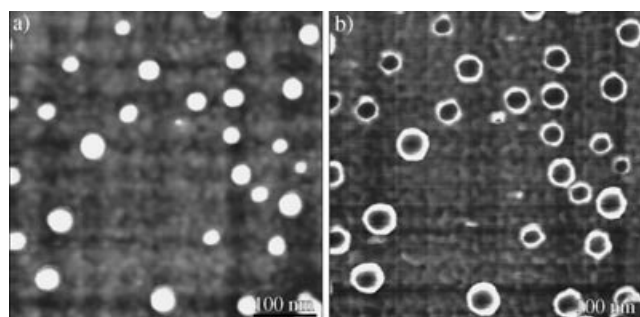


Figure 2. a) AFM and b) MFM images of hollow Fe_3O_4 -polymer hybrid nanospheres. Vertical scales (from dark to bright): 0 to 15 nm and -160° to -206° , respectively.

from the hollow hybrid nanospheres is detected. The rings, which correspond exactly to the positions of spheres in the AFM image, have diameters of 52 ± 4 nm; the thickness of the circular shells is 15 ± 2 nm. No magnetic signal in the center of the spheres is detected. Moreover, in an additional control experiment, no bright ring was detected in the MFM analysis of Fe_3O_4 -free hollow chitosan-poly(acrylic acid) nanospheres. This result indicates again that the magnetite nanoparticles are distributed in the shell wall of the hollow hybrid nanospheres.

We now turn to the formation of the hollow Fe_3O_4 -polymer nanospheres. Based on the $\text{p}K_a$ values of 4.26 for AA and 4.8 for poly(acrylic acid) (PAA),^[18] the conversion of AA monomers inside the micelles into PAA results in some dissociated carboxylic groups becoming protonated carboxylic groups. The original micellar core comprised of positively charged protonated CS and negatively charged dissociated AA gradually disintegrates owing to the polymerization of AA. On the other hand, the residual dissociated carboxylic groups in the produced PAA will interact with the protonated amino groups in CS to form water-insoluble polyelectrolyte complexes between CS and PAA, which causes a decrease in the size of the micelles, as determined by DLS.^[17] This process

experiences two kinds of force: The electrostatic attraction between CS and PAA shrinks the spheres, whereas the electrostatic repulsion from the shell tends to expand the spheres. The equilibrium between the two opposing forces results in the formation of the cavity in the center of the nanospheres, with CS-PAA polyelectrolyte complexes as the inner shell and protonated CS chains as the outer shell. As PVA attached to the Fe_3O_4 nanoparticles interacts with PAA by hydrogen bonding (as revealed by the shift of the carbonyl absorption band of PAA from 1753 to 1713 cm^{-1} in FTIR measurements), the Fe_3O_4 nanoparticles should occur in the inner shell where polyelectrolyte complexes between CS and PAA exist. This is confirmed by the TEM image shown in Figure 1b.

The hollow hybrid nanospheres were calcined as part of our efforts to use these materials. The polymers were removed at elevated temperatures (550°C) under argon for 1 h to afford the completely hollow inorganic nanospheres (Figure 3). The electron-diffraction pattern confirms that these hollow inorganic nanospheres are composed of Fe_3O_4 . The diameter and wall thickness of these hollow Fe_3O_4

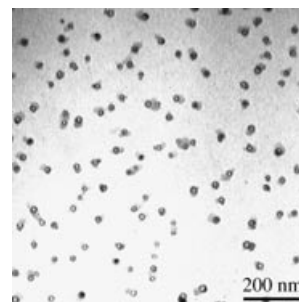


Figure 3. TEM image of hollow Fe_3O_4 nanospheres produced by calcination of hollow Fe_3O_4 -polymer hybrid nanospheres.

nanospheres are 20 ± 2 nm and 5 ± 1 nm, respectively, which are much smaller than those of uncalcined hollow hybrid spheres. Apparently, the hollow Fe_3O_4 nanospheres undergo significant shrinkage during the formation. In contrast to the shrinkage rate (below 30%)^[8] of hollow inorganic spheres prepared by colloidal templates, the shrinkage rate is more than 50% in our case. This may be due to the hollow interior of synthesized hybrid nanospheres and the distribution of Fe_3O_4 nanoparticles in the walls of hybrid nanospheres not being compact. Furthermore, the low polydispersity in the size of the hollow Fe_3O_4 nanospheres can be considered as indirect evidence that the synthesized hollow hybrid nanospheres have a relatively uniform size.

In summary, we have illustrated a successful synthesis of hollow Fe_3O_4 -polymer hybrid nanospheres by the addition of Fe_3O_4 nanoparticles to an aqueous solution of polymer-monomer pairs composed of the cationic polymer CS and the anionic monomer AA, followed by polymerization of AA and selective crosslinking of CS at the end of polymerization. The process is simple and allows the large-scale preparation of the nanospheres. There is also the possibility of extending this approach to other systems that involve different inorganic nanoparticles and different polymer-monomer pairs.

Experimental Section

Synthesis: The hollow Fe_3O_4 -polymer hybrid nanospheres were prepared by adding 10 mL of a PVA-stabilized magnetite nanoparticle suspension ($\approx 1 \times 10^{18}$ Fe_3O_4 particles mL^{-1}) to 50 mL of an aqueous solution of CS-AA, which consisted of 0.5 g of purified CS ($M_w = 200$ kDa, degree of deacetylation = 90%) and 0.22 g of AA, with a stoichiometric molar ratio of 1:1.1 (glucosamine unit/acid). Polymerization was then initiated by $\text{K}_2\text{S}_2\text{O}_8$ at 70 °C under a nitrogen stream. The concentration of $\text{K}_2\text{S}_2\text{O}_8$ in the reaction system is about 5 mM. As the reaction system appeared opalescent, the reaction was allowed to proceed for another 100 minutes at 50–60 °C. Afterwards, 0.5 mL of glutaraldehyde, a bifunctional crosslinker, was added to the reaction system at 40 °C to crosslink chitosan selectively. The resultant suspension was filtered to remove any possible aggregation and treated by repeated ultracentrifugation and washes. The nonloaded magnetite nanoparticles were separated in the ultracentrifugation process. The Fe_3O_4 loading content was determined to be 11% by thermogravimetric analysis.

Characterization: The mean particle size and zeta potential of the spheres were determined by DLS on a Brookhaven BI-9000AT instrument and a Zetaplus (Brookhaven Instruments Corporation, USA), respectively. All the measurements were repeated three times. TEM analysis was carried out on a JEOL JEM-2010 instrument at an acceleration voltage of 200 kV. For microtoming samples, an aqueous suspension of the crosslinked hollow Fe_3O_4 -polymer hybrid nanospheres was deposited on a polyimide sheet, and the sample was dried naturally. The polyimide sheet deposited with hollow Fe_3O_4 -polymer hybrid nanospheres was embedded in an epoxy resin, and sections approximately 70 nm thick were obtained by microtoming the resin sample at room temperature. MFM analysis was performed on a scanning-probe microscope (SPI3800, Seiko Instruments Inc, Japan) by using magnetic-moment MFM tips. Soft-tapping conditions were used. The lift mode (while tapping) was used to record the magnetic signal in the phase-detection mode. The samples for MFM analysis were coated with a 5-nm gold layer to avoid attachment of the nanospheres to the tip.

Calcination of hollow Fe_3O_4 -polymer hybrid nanospheres was carried out in a high-temperature oven under argon at 550 °C for 1 hour.

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