Magnetic Nanoparticles Encapsulated Within a Thermoresponsive Polymer

A. K. Gaharwar¹,², J. E. Wong¹,*, D. Müller-Schulte³, D. Bahadur², and W. Richtering¹

¹Institute of Physical Chemistry, RWTH Aachen University, Landoltweg 2, 52056 Aachen, Germany
²Department of Metallurgical Engineering and Materials Science, Indian Institute of Technology–Bombay, Mumbai 400076, India
³Lionex GmbH, Martelenberger Weg 8, 52066 Aachen, Germany

This study describes a facile two-step approach to modify the surface of nanoparticles, thereby imparting a core–shell structure to the system. The core consists of magnetic nanoparticles and the shell is composed of thermoresponsive hydroxypropyl cellulose, using a coupling agent to covalently bind the core to the shell. Hydroxypropyl cellulose is known for its biocompatibility and biodegradability, and its thermoresponsive properties make it an excellent candidate for fabricating biocompatible stimuli-responsive magnetic nanoparticles. We report the synthesis of magnetic nanoparticles and the successful binding of the polymer to them. X-ray diffraction studies show that the surface modification of the magnetic nanoparticles does not result in any phase change and the size of the magnetic core thus calculated (7 nm) reveals that such hybrid core–shell system is superparamagnetic in nature, as further confirmed by magnetization measurements. The size obtained by X-ray diffraction is in good agreement with that obtained by transmission electron microscope. Evidence of binding is given by Fourier transform infrared spectroscopy and a quantitative analysis of the polymeric content obtained by thermogravimetry analysis. Dynamic light scattering as a function of temperature reveals the thermoresponsive behavior of the particles with a lower critical solution temperature around 41°C, which is also the temperature at which cellulose undergoes a coil-to-globule transition.

Keywords: Magnetic Nanoparticles, Superparamagnetic, Hybrid Core–Shell, Thermoresponsive, Hydroxypropyl Cellulose, Biocompatible.

1. INTRODUCTION

Magnetic nanoparticles (MNP) have unique size-dependent properties and MNP based on iron oxides are attractive candidates in the field of biomedical applications.¹⁻⁵ For in vivo applications, MNP should not form any agglomerates; hence, to prevent this, the idea is to modify their surfaces by either coating or encapsulating them in organic or inorganic materials. One common approach is to embed nanoparticles in silica⁶ because the latter can be readily functionalized to impart the protective shell. Such inorganic/organic nanocomposites⁷,⁸ have attracted much attention lately in an attempt to exploit new hybrid properties derived from the various components.

Stimuli-responsive core–shell systems can be achieved by choosing one of the components to be sensitive to an external stimulus, such as pH or temperature.⁹ One particular class of stimuli-responsive materials that has generated much attention is temperature-sensitive polymers: Thermoresponsive polymers possess a release-trigger mechanism when they undergo fast, reversible structural changes from a swollen to a collapsed state by expulsing the solvent, and have recently been exploited as remote controlled drug delivery vehicles.¹⁰ We previously reported surface modification via layer-by-layer technique of a thermoresponsive poly(N-isopropylacrylamide) PNIPAM microgel core with polyelectrolyte multilayers and magnetic nanoparticles while preserving the reversible thermoresponsive behavior of the hybrid core–shell.¹¹⁻¹⁵ Such core–shell systems exhibit a lower critical solution temperature (LCST) of ~32 °C, when they undergo a transition from a swollen hydrophilic to a collapsed hydrophobic state. However, although PNIPAM is non-hazardous and soluble in water, biocompatible polymers are usually preferred for any potential biomedical applications.

Herein, we report the synthesis of a biodegradable hybrid core–shell system whereby the shell is thermoresponsive and the core is the magnetic nanoparticle. For this
work, the choice of the thermoresponsive shell is dictated by the bio-compatibility aspect of the polymer with an LCST nearing (but slightly higher than) our body temperature. Hydroxypropyl cellulose (HPC) is a water-soluble natural cellulose, with an LCST of ~41 °C, and with an excellent bio-compatibility as well as bio-degradability as approved by the United States Food and Drug Administration (FDA). The surface of the MNP is first modified through a silanization reaction, followed by HPC coupling. These new hybrid core–shell sytems are characterized by X-ray diffraction (XRD), thermogravimetric analysis (TGA), Fourier Transform Infrared (FTIR), dynamic light scattering (DLS), and magnetic measurements to show the successful surface modification of MNP.

2. EXPERIMENTAL DETAILS

2.1. Materials

Ferrous chloride tetrahydrate (FeCl₂·4H₂O), ferric chloride hexahydrate (FeCl₃·6H₂O), 25% ammonium hydroxide solution, 37% hydrochloric acid (HCl) solution, hydroxypropyl cellulose (HPC, average Mw ~100 000 g/mol), (3-aminopropyl)trimethoxysilane (APTMS) and sodium periodate (NaIO₄) were purchased from Sigma-Aldrich. All chemicals from commercial origin were used without purification. The water used in all experiments was double-distilled ultrapure water (Milli-Q-plus system, Millipore).

2.2. Synthesis of MNP

The synthesis of MNP was carried out via a controlled chemical co-precipitation approach, as described in detail in a recent paper. Briefly, 0.81 g FeCl₂·6H₂O in 5 ml water and 0.4963 g FeCl₃·4H₂O in a mixture of 1 ml water and 0.25 ml 37% HCl solution was prepared separately. Both solutions were mixed in an ultrasonic bath for 5 min to homogenize the mixture. The mixture of Fe³⁺ and Fe²⁺ solutions was then added drop wise to 40 ml of water and 10 ml of 25% NH₃ solution in a three-necked round bottom flask under an inert N₂ atmosphere and constant stirring for 30 min. Aggregates were first separated from the reaction mixture using a Nd–Fe–B magnet, and then washed three times with 0.3 M NH₃. Three cycles of centrifugation at 5000 rpm for 10 min were carried out and the precipitate was dissolved in 20 ml of water to obtain a stable ferrofluid. Exposure of this ferrofluid to a magnetic field revealed no phase separation, confirming the complete redisssipation of the MNP.

2.3. Surface Modification of MNP

A solution of 10 μl APTMS in 20 mL water was added drop wise, under N₂ atmosphere, to a 2 mL 5% MNP solution in 20 mL of water in an ultrasonic bath, and mixing carried out with a mechanical stirrer at 500 rpm for an additional hour. Ultrasonication is vital in reducing the particle size and size distribution by promoting the mixing of MNP and the APTMS. Initial attempt of this step without sonification lead to aggregations. The reaction mixture was purified by two centrifugation cycles (20 and 15 min) with removal of the supernatant and redisssipation in water between each run. The surface modified MNP, hereafter denoted as MNP/APTMS, was finally collected and redisssipated in 20 ml water overnight.

2.4. Surface Attachment of HPC to MNP/APTMS

A saturated NaIO₄ solution was prepared by dissolving 2 g (an excess) of NaIO₄ in 10 ml water using ultrasonication, kept in the dark for 1 hr, and finally filtered to separate the excess NaIO₄. To partially oxidize HPC polymer chains, a 10% wt solution of HPC was added to the saturated NaIO₄ solution and mixed for 18 hr in the dark at room temperature. To attach HPC onto APTMS modified MNP surface, 10 ml of the MNP/APTMS solution was added to partially oxidized HPC solution in an ultrasonic bath. Like in the previous step, ultrasonication proved to be important in reducing the particle size and size distribution by promoting the mixing of MNP/APTMS and the HPC. The solution was mixed for 12 hr. Impurities and unreacted polymers were removed from reaction mixture by three centrifugation cycles at 40 000 rpm (25, 15, 10 min). The surface-modified MNP/APTMS, hereafter denoted as MNP/APTMS/HPC, was finally collected and redisssipated in 20 ml water.

2.5. Characterization

Photon correlation spectroscopy (PCS) was used to determine hydrodynamic size and size distribution of the particles using a Zetasizer 3000HS (Malvern, UK) at 25 °C with a dilute dispersion of the particles in pure water. The particle size of the thermoresponsive MNP was measured as a function of the temperature by dynamic light scattering (DLS) on highly diluted samples with an ALV goniometer (ALV 5000E correlator). Temperature was varied from 20 to 60 °C, in steps of 2 °C. Scattered light was detected at 60° and particles size was calculated by cumulant fits. Fourier transform infrared spectroscopy (FTIR) was carried out with a Nicolet spectrometer (Magna IR, 550) within the wave range of 4000–400 cm⁻¹. A crystallographic study of the particles was performed on a rotating anode STOE STADI-P X-ray diffractometer system using CoKα radiation (λg = 0.179026 nm). X-ray diffraction (XRD) graphics were compared to the JCPDS standard data in order to deduce the crystal structure of the product. The size and morphology of particles were determined by a Transmission Electron Microscope (TEM) (Philips, CM 200) at 200 kV. Magnetic properties were measured by Vibrating Sample Magnetometer (VSM) (Lake Shore,
3. RESULTS AND DISCUSSION

The surface modification of the MNP core with silane coupling agent (APTMS)\textsuperscript{20,23} and a thermoresponsive polymeric shell (HPC) is illustrated in Scheme 1. First, MNP are prepared via co-precipitation technique, followed by surface modification by APTMS to introduce the desired functional groups, in this case, amino groups, onto the surface.\textsuperscript{20,23} Partially oxidized HPC was then covalently linked to the amino groups present on the APTMS-surface modified MNP to get core–shell particles with a magnetic core and a polymeric shell.

3.1. Surface Modification of MNP: An XRD Evaluation

Figure 1 shows the XRD patterns of MNP and MNP/APTMS/HPC. The XRD patterns of MNP and MNP/APTMS/HPC show five characteristic peaks of magnetite or maghemite marked by their indices ((220), (311), (400), (422) and (511)).\textsuperscript{24} The peaks from MNP/APTMS/HPC are slightly broader than those obtained for MNP due to the amorphous polymeric (HPC) or amine (APTMS) coating around the nanoparticles. The volume average relative particle size of the crystal, $D$, was calculated using the Scherrer formula giving a $D \approx 7$ nm for the magnetic core, indicating its superparamagnetic behavior. No additional peak, after surface modification with APTMS, suggests that the coating does not result in any phase change.

3.2. TEM

The TEM micrographs for the MNP, MNP/APTMS and MNP/APTMS/HPC are shown in Figure 2 together with an electron diffraction pattern of the MNP. Before modification, MNP showed a relatively narrow size distribution with a mean diameter of $\sim 7$ nm. After binding to APTMS and HPC respectively, the magnetite cores were still monodisperse with a similar size range. Polymeric components (APTMS and HPC) possess a too low electron density to contribute to the contrast in the image compared to MNP which have a high electron density. The size obtained is an indication that the surface modification did not result in agglomeration or a dramatic change in size of the MNP particles. This could be attributed to the fact that the reaction occurred only on the surface of the particles. The electron diffraction pattern of MNP (Fig. 2) consists of concentric rings consistent with a cubic inverse spinel structure of magnetite. Similar electron diffraction patterns were obtained for MNP/APTMS and MNP/APTMS/HPC. The characteristic $d$ spacing corresponds to the hkl values of (220), (311), (400), (440) and (533), which are in excellent agreement with the results obtained by XRD.\textsuperscript{24}

3.3. FT-IR

Further evidence of the successful surface modification of MNP is provided by FTIR spectra as shown in Figure 3 for MNP, MNP/APTMS and MNP/APTMS/HPC. For as
prepared MNP, a small absorption band between 3000 and 3600 cm\(^{-1}\) with peak at around 3427 cm\(^{-1}\) and a band at 1624 cm\(^{-1}\) are associated with the fundamental valence stretching vibrations of the O–H; the band at 1380 cm\(^{-1}\) is due to bending modes of O–C–H, C–C–H, and C–O–H of carbonate present on the surface of the MNP (as reaction was done in ambient conditions). Bands at 577 and 637 cm\(^{-1}\) are due to stretching vibration of Fe–O at tetrahedral site. The attachment of APTMS onto the MNP was monitored by the following bands in the spectra: 
(a) the broadening of the absorption band between 3000 and 3600 cm\(^{-1}\) with peak at around 3437 cm\(^{-1}\) is due to stretching vibrations of the N–H group together with contribution from O–H bond; 
(b) absorption bands at 2854 and 2926 cm\(^{-1}\) are due to stretching vibrations of the alkyl C–H bond; 
(c) transformation of band present at 1380 cm\(^{-1}\) to a very narrow and sharp band is due to the presence of strong amine group in APTMS; and 
(d) bands at 1023 cm\(^{-1}\) and at 1122 cm\(^{-1}\) are assigned to Si–O–Si bonds. The decrease in intensity of absorption band of Fe–O (stretching vibration) at 638 and 585 cm\(^{-1}\) indicates that APTMS is bound to the surface of the MNP.

MNP/APTMS/HPC particles have almost similar bands as MNP/APTMS, the major difference between the two are 
(a) a decrease in the intensity of the band at 1390 cm\(^{-1}\) indicating that N–H bonds have been used during the conjugation of APTMS with HPC and 
(b) the appearance of new bands at 1754 and 1464 cm\(^{-1}\), characteristics of the presence of aldehyde groups in partially oxidized HPC. The band at 1754 cm\(^{-1}\) is due to the aromatic C–H out-of-plane bending vibrations while that at 1464 cm\(^{-1}\) is due to C–H bending.

### 3.4. TGA

TGA was carried out to confirm the shell formation and also to quantify the amount of APTMS and HPC adsorbed on the surface of the MNP. Figure 4 shows TGA curves of MNP, MNP/APTMS and MNP/APTMS/HPC. As expected, the TGA curve for MNP shows almost no significant weight loss. The slight weight loss is due to loss of residual water. TGA results for MNP/APTMS, revealed a two-step weight loss. The initial weight loss is due to the loss of residual water in the sample while the significant weight loss (of about 7%) between 250 and 500 °C can be attributed to the degradation of APTMS. There was no significant weight change between 500 and 600 °C, implying the presence of only iron oxide within the temperature range. The TGA curve for MNP/APTMS/HPC shows a three-step weight loss. The first step corresponds...
Magnetic Nanoparticles Encapsulated Within a Thermoresponsive Polymer

3.5. Magnetic Measurements

Figure 5 shows the magnetization curves of MNP, MNP/APTMS, MNP/APTMS/HPC nanoparticles. No coercivity and remanence are observed before and after surface modification indicating that the MNP retain their superparamagnetic nature. The saturation magnetization of MNP, MNP/APTMS and MNP/APTMS/HPC, measured in a field of 2 T, are 52, 48 and 40 emu/g respectively. The pronounced weight loss (of about 20%) between 200 and 550 °C is an overlap of two degradation curves (one due to APTMS and another due to HPC). The results confirm that APTMS and HPC were bound to the MNP, and the more pronounced weight loss observed for MNP/APTMS/HPC shows that more non-magnetic, polymeric, materials are bound to its surface than in the case of MNP/APTMS.

![Magnetization curves obtained at room temperature for MNP, MNP/APTMS and MNP/APTMS/HPC.](image)

**Fig. 5.** Magnetization curves obtained at room temperature for MNP, MNP/APTMS and MNP/APTMS/HPC.

![Size distribution of MNP, MNP/APTMS and MNP/APTMS/HPC. DLS study of the thermoresponsive behavior of MNP/APTMS/HPC core–shell particles. Inset shows visual evidence (with the help of a Nd–Fe–B magnet) of agglomerated particles when temperature is increased above the LCST of HPC.](image)

**Fig. 6.** (a) Size distribution of MNP, MNP/APTMS and MNP/APTMS/HPC. (b) DLS study of the thermoresponsive behavior of MNP/APTMS/HPC core–shell particles. Inset shows visual evidence (with the help of a Nd–Fe–B magnet) of agglomerated particles when temperature is increased above the LCST of HPC.
presence of non-magnetic materials on the surface of MNP (APTMS and HPC) leads to a decrease in the saturation magnetization. Successive surface modification leads to a ~7% and ~20% decrease which is in very good agreement with the amount of non-magnetic materials obtained independently by TGA. The additional decrease in saturation magnetization of MNP/APTMS/HPC is a clear indication of additional non-magnetic materials on the surface of the MNP (APTMS + HPC).

3.6. DLS

Figure 6(a) shows the hydrodynamic size and size distribution of particles as evaluated using PCS after each modification step. The average hydrodynamic size of the particles (scattering angle = 90°) of MNP, MNP/APTMS and MNP/APTMS/HPC was 29, 40 and 102 nm, respectively. Hydrodynamic size is always an overestimation of the real core size, but comparing the values obtained by PCS with that obtained from TEM, this difference is quite pronounced which could be an indication that in solution we have formation of some clustering of the nanoparticles. However, from the size distribution, although the APTMS modification step introduces some polydispersity (while the HPC modification step produces fairly monodisperse particles), all the samples (MNP, MNP/APTMS and MNP/APTMS/HPC) are reasonably monodisperse.

Figure 6(b) shows that the hydrodynamic size of the MNP/APTMS/HPC as a function of temperature using DLS (scattering angle = 60°). The DLS curve also gives an indication of the stability of the particles in solution at different temperatures. At 20 °C MNP/APTMS/HPC are stable and do not show any agglomeration. The stability is due to the steric repulsion between neighboring particles owing to the presence of the HPC network around the particles. The stability is further confirmed when no sedimentation is observed even after 6 hours exposure to a magnet as illustrated in the inset of Figure 6(b). As the temperature is increased to around 40 °C (~LCST of HPC) the size of the core–shell particles increases. At temperatures higher than the LCST, there is a dramatic increase in size, contrary to the usual thermoresponsive behavior of HPC gel. Such anomalous behavior was also reported by Dou et al. who observed an increase in the size but only at temperatures around and higher than the LCST (41 °C). At temperatures greater than the LCST the hydrophilicity to hydrophobicity transition of HPC chains in water may induce self-association, leading to the formation of metastable nanoparticle aggregates. Gao et al. also reported that HPC chains may form different metastable aggregates depending on how the system is brought to that temperature. A rapid increase of temperature from room temperature to the LCST resulted in fast self-association of the HPC chains (and smaller nanoparticles) while incubating at intermediate temperatures gave HPC chains more time to associate into larger particles. The same author also found that increasing the temperature above 45 °C would lead to even larger and denser particles. The DLS curve in Figure 6(b) was obtained by increasing the temperature from 20 °C to 60 °C in steps of 2 °C with time intervals sufficiently long for the temperature to equilibrate. Indeed, stable and larger particles are observed. Only on exposure to a magnet for about 6 hours do the particles at 60 °C sediment, Figure 6(b) inset, revealing that larger and denser aggregates are formed. Once larger particles were formed, the thermoresponsive behavior is no longer reversible.

4. CONCLUSION

A facile two-step surface modification of magnetic nanoparticles has been successfully carried out for the fabrication of monodisperse core–shell hybrid particles with a bio-compatible and thermoresponsive hydroxylpropyl cellulose shell. The modification steps involve silanization of magnetic nanoparticles followed by covalent binding of the cellulose through amino groups. The physico-chemical characteristics of the core–shell system have been thoroughly carried out and reveal that such hybrid systems retain the individual properties of each component they are made up of, namely, superparamagnetic behavior of the native magnetic nanoparticles and the thermoresponsive behavior of the cellulose. Such unique combination of thermoresponsivity and magnetism could open up novel prospects in the field of nanomedical applications such as remote controlled drug carriers.

Acknowledgment: A. K. Gaharwar would like to thank the Deutscher Akademischer Austauschdienst (DAAD) for a scholarship. Support by the Fonds der Chemischen Industrie (Germany) and the Department of Science and Technology (Government of India) are gratefully acknowledged.

References and Notes

Gaharwar et al. Magnetic Nanoparticles Encapsulated Within a Thermoresponsive Polymer


Received: 20 February 2008. Accepted: 15 December 2008.

Delivered by Ingenta to:
PURDUE UNIVERSITY LIBRARY
IP : 128.46.89.153