

Preparation and characterization of magnetic nanoparticles embedded in microgels

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Abstract

This article describes the magnetically responsive microgel that consists of a small iron oxide magnetic nanoparticles (~15 nm in diameter) embedded in a biocompatible microgel varying from ~65 to ~110 nm in diameter. These systems show great promise as active component of microscale and nanoscale devices and are expected to have wide applicability in various biomedical applications. Polymeric microgels have been prepared by emulsion free copolymerization of thermoresponsive *N*-isopropylacrylamide and acrylic acid with a water-soluble persulfate initiator. The obtained microgel magnetic composite particles possess a lower critical solution temperature (LCST) in water solutions, with a rapid decrease of the particle size being observed at elevated temperatures. The morphology and elemental composition of the composites were characterized by transmission electron microscopy, X-ray diffraction and Fourier transform infrared spectroscopy (FTIR).

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1. Introduction

There is a considerable interest in preparation of particles which can be manipulated in different systems by external stimuli such as thermal, electric or magnetic field. Polymeric particles can be easily prepared and their size, morphology and surface groups can be varied in the broad range. However, conventional polymers cannot provide some special properties like, for example magnetic response. Therefore, incorporation of magnetic iron oxides in polymeric particles can be an interesting route for preparation of hybrid particles which can provide this interesting feature.

Polymer shells encapsulating inorganic particles are also of great interest in pharmaceutical and biotechnological industries for producing drug release products [1,2]. Magnetically controlled drug targeting is one of the various possibilities of drug targeting. This technology is based on binding targeted drugs with magnetic nanoparticles, which concentrate drugs in

the area of interest by means of magnetic fields. Recently, the development of magnetically responsive microspheres has brought an important driving force into play [3–13].

Various strategies have been proposed for synthesis of magnetic polymer particles [14]. However, recent studies have underlined the major difficulty to encapsulate inorganic particles homogeneously, especially in the case of magnetic particles in polymer microgels [3–6].

In the field of drug delivery, many types of colloidal carriers have been tried out, which actively respond to external stimuli such as temperature, pH, electric or magnetic field [7–9]. Among the good candidates, one finds microgels that are cross-linked latex particles swollen by a good solvent. Different inorganic or polymeric materials have been proposed as carriers of magnetic materials. A considerable advantage of the polymeric carriers is the presence of a variety of functional groups, which is able to modulate the carrier properties for the desired applications.

Aqueous magnetic fluids are colloidal dispersions of magnetic nanoparticles in water, exhibiting a giant paramagnetic behavior. They can be used in biosciences, namely as contrast agents for magnetic resonance imaging (MRI) [10], for magnetic guidance of drugs or radioisotopes and for cell sorting processes. The

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encapsulation of magnetic particles inside organic swellable particles made of polystyrene or polymethylmethacrylate has been extensively described [11–13].

This paper describes a general method to encapsulate magnetic nanoparticles (core) homogeneously inside a thermo and pH responsive microgel shell. Under the influence of external magnetic field, the core warms up due to the magnetic induction and causes a thermal transition in the shell and/or by shifting the LCST of the polymer to anywhere from 32 °C to 60 °C by introducing more hydrophilic acrylic acid (AAc) on to the polymer backbone. This behavior is of interest for biomedical applications in targeted drug delivery. The usual way to produce magnetic polymer particles consists in coating the magnetic material by a surfactant and embedding it in the polymer using processes such as suspension, emulsion or precipitation polymerization. Here we use the emulsion free polymerization process to disperse the aqueous magnetic fluid and the hydrophilic monomer localized together in water droplets just prior to polymerization. As the magnetic particles are themselves hydrophilic, no surface treatment is required before the encapsulation process. This is a key factor since there is neither surfactant nor polymer molecules in the initial magnetic fluid that can interfere with the surfactant used for the emulsification or with the polymer matrix.

2. Experimental section

2.1. Materials

The monomer *N*-isopropylacrylamide (NIPAAm) was obtained from Sigma (99%), recrystallized in hexane, and dried under vacuum before use. Acrylic acid (AAc, Sigma, 99.5%), *N,N*-methylene bis-acrylamide (MBA, Sigma), ammonium persulfate (APS, Merck), ammonium hydroxide (Merck, 25%) and oleic acid (Sigma) were all used as received from the indicated suppliers. Water used in all experiment was purified to a resistance of 10 M Ω (Milli-Q Reagent Water System, Millipore Corporation) and filtered through a 0.2 μ m filter to remove any particulate matter. In the preparation of magnetite nanoparticles, ferrous chloride hexahydrate (FeCl₃·6H₂O, Sigma, 99%) and ferric chloride tetrahydrate (FeCl₂·4H₂O Sigma, 99%) were used as received.

2.2. Synthesis of colloidal magnetic nanoparticles

Dissolved 23.5 g FeCl₃·6H₂O and 8.6 g FeCl₂·4H₂O in 600 ml deionized water under N₂ with mechanical stirring at 600 rpm (revolution per minute) and 85 °C and then quickly added 30 ml of 7.1 M NH₄OH. To the resulting suspension 16 ml oleic acid was added dropwise over a period of 30 min. After several minutes, the magnetic precipitate was separated by magnetic decantation and washed with deionized water several times, two times with ethanol and finally evaporated to dryness to get iron oxide (Fe₃O₄) powder. It was further modified with 4 ml of 7.1 M NH₄OH to form the hydrophilic magnetic nanoparticles and suspended in distilled water leading to a stable colloidal dispersion (pH 7) after repeated washing.

2.3. Preparation of microgel magnetic particles (MMP)

A typical recipe for the preparation of microgel magnetic particles: in a three-necked round-bottom flask equipped with a reflux condenser and an inlet for nitrogen gas, oleic acid coated iron oxide nanoparticles (5 ml, 2 mg/ml) were diluted with pure milli-Q water and placed in an ultrasonicator (Elma Transsonic T 460H, 285 W, 35 kHz) for 10 min to get an aqueous solution of colloidal magnetite (Fe₃O₄). The solution was purged with nitrogen for 30 min and was bubbled through the solution for the duration of the reaction to remove any oxygen, which can intercept radicals and disrupt the polymerization. The solution was agitated using a mechanical stirring of 250 rpm. An approximately 94:6 wt.% ratio of NIPAAm (26.1 ml, 0.01 M): AAc (1.6 ml, 0.01 M), MBA (300 μ l, 0.049 g/ml) was then added and stirred for 15 min to give homogeneity. The solution was heated to 70 °C in an oil bath, and then APS (50 μ l, 20% w/w) was added to initiate the polymerization. The reaction was continued for 4 h. At the end of the period, the solution was cooled and the MMP were easily separated from the suspension under a magnetic gradient (a strong Nd–Fe–B magnet) and washed several times with ethanol finally with water.

2.4. Characterizations

Transmission electron microscopy (TEM) images were obtained using a JEOL 100 CX II transmission electron microscope operated at 80 kV accelerating voltage. A drop of an aqueous dispersion of magnetic nanoparticles and MMP were placed on a Formvar-coated copper TEM grid (300 mesh size) and allowed to air-dry.

FTIR spectra were measured on a Perkin-Elmer Spectrum-One spectrophotometer. The magnetic nanoparticles and MMP were dried, and the powders were mixed with KBr and pressed to a plate for measurement.

X-ray diffraction (XRD) pattern of the dried colloidal particles (by depositing the colloidal particles on a microscope glass slide (after solvent evaporation) followed by drying in a vacuum) was recorded using a Bruker Advance D8 XRD machine (Cu α source with 1.5406 wavelength), in thin film mode.

The size of the aqueous microgel magnetic particle solution was determined by Dynamic Light Scattering (DLS) Particle Size Analyzer (HORIBA LB-550, Japan) from 25 to 45 °C, the measuring range was from 1 nm to 6 μ m, and the light source was a 650 nm Laser diode of 5 mW. The samples of about 20.0 ml aqueous microgel solutions were measured directly without any pretreatment.

3. Results and discussion

Magnetic nanoparticles were prepared by coprecipitation method. The displacement of air by N₂ gas during preparation prevented oxidation of ferrous ion in the aqueous solution and also controlled the particle size. It is well known that magnetic iron oxide prepared by the coprecipitation method has a large number of hydroxyl groups on its surface in contact with the aqueous phase. The –OH groups on the surface of Fe₃O₄ particles react readily with carboxylic acid head of oleic acid molecules. Excess oleic acid is then adsorbed to the

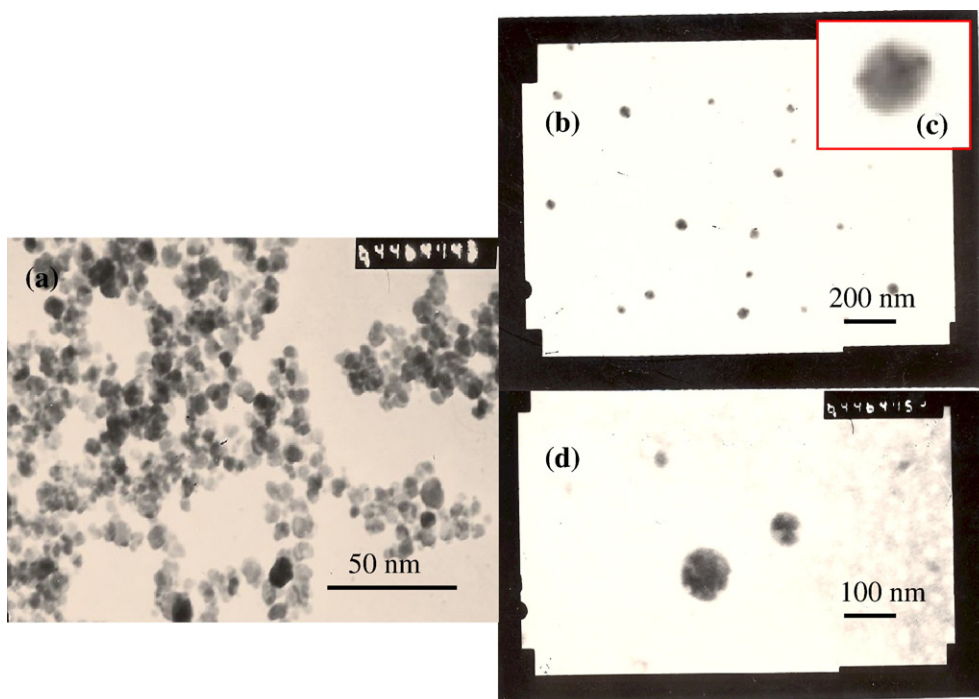


Fig. 1. TEM images of magnetic nanoparticles (a), microgel magnetic particles showing dark core iron oxide nanoparticles surrounds by grayish shell of polymer microgel (b) and (d). (c) is an expanded view of (b).

prebound oleic acid layer to form a hydrophobic shell. When the magnetic nanoparticles are put into an NH_4OH solution, the outer layer of oleic acid on the Fe_3O_4 surface is transformed into an ammonium salt of oleic acid, which modifies the magnetic nanoparticles so that they exist in a dispersed state in an aqueous phase as their surfaces are now hydrophilic.

Colloidal iron oxide/microgel particles of core-shell (iron oxide-core and microgel-shell) were prepared by emulsion polymerization reaction. The copolymer microgels were prepared which compose of pNIPAAm (which renders thermo sensitivity) and polyacrylic acid (which renders the pH sensitivity) to the polymeric micelles. To render the micellar

aggregates more stable, crosslinking of the polymeric chain was done by using MBA during the vinyl polymerization process and polymerization was carried out by free radical polymerization reaction using ammonium persulphate. The reaction was run at 70°C , which is much higher than the LCST of copolymer NIPAAm/AAc (ca. $\sim 38^\circ\text{C}$), because the pNIPAAm chains are well soluble in water when the temperature is lower than LCST. At a temperature higher than LCST, the copolymer precipitated spontaneously on the magnetite-core surface and copolymerization with the cross-linked MBA leads to the formation of a compact thermosensitive shell.

It further pursued TEM investigation to learn about the particle sizes and morphology of the microgel magnetic particles as formed in the aqueous solution in the present method. The TEM pictures were

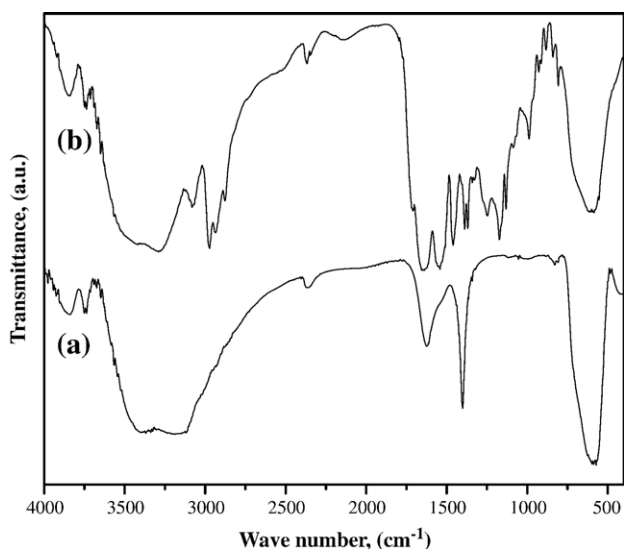


Fig. 2. FTIR spectra. (a) Magnetic nanoparticles and (b) microgel magnetic particles recorded in KBr pellets.

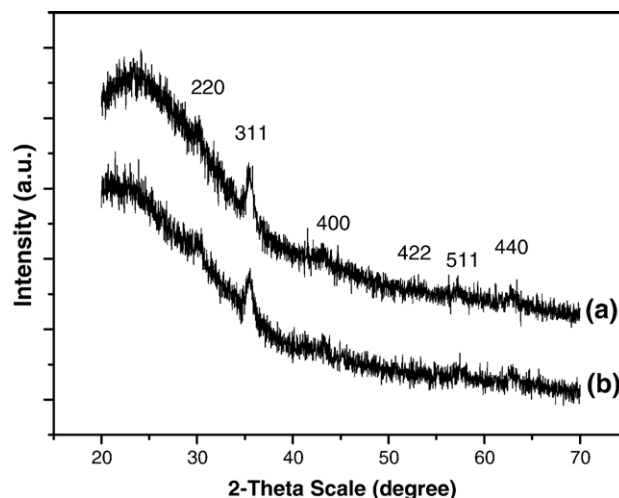


Fig. 3. X-ray diffraction results of the (a) magnetic nanoparticles and (b) microgel magnetic particles.

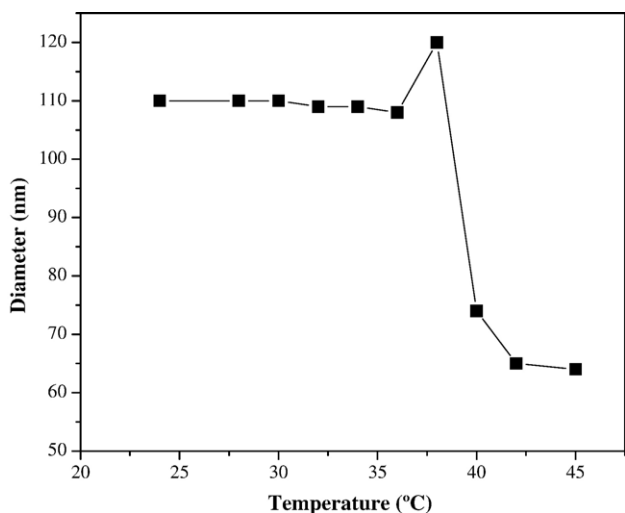


Fig. 4. The results of variable temperature DLS: diameter of the microgel magnetic particles vs. temperature.

recorded of the samples dried on a copper grid. The micrographs are shown in Fig. 1. The micrograph in Fig. 1a indicates that the particle sizes ranged from 10 to 15 nm for iron oxide only. The magnetic nanoparticles in suspension did not settle in over a day of storage. On the other hand, the TEM studies of MMP (Fig. 1b and d) revealed the formation of monodisperse microgel particles, with microgel layer formation surrounding a cluster of aggregated iron oxide nanoparticles, resulting in greater than 60–85 nm diameter clusters of composite particles. A closer look into TEM micrograph indicates the formation of iron oxide/copolymer microgel core–shell nanocomposites with assembled iron oxide nanoparticles distinguishable by dark cores surrounded by grayish shells of copolymer composed of NIPAAm/AAC (Fig. 1c and d). In brief, TEM micrographs showed that individual iron oxide–microgel core–shell composite particles were formed in the present set of experiments.

Fig. 2 shows a comparison between the FTIR spectra of the iron oxide nanoparticles and microgel magnetic particles. Previously, it was reported that the characteristic absorption band of Fe–O bond of bulk Fe_3O_4 was at 570 and 375 cm^{-1} [15]. However, when the size of Fe_3O_4 particles was reduced to nanoscale dimensions, the surface bond force constant increased due to the effect of finite size of nanoparticles, in which the breaking of large number of bonds for surface atoms resulted in the rearrangement of nonlocalized electrons on the particle surface [16].

Therefore, the FTIR spectrum of iron oxide nanoparticles and microgel magnetic particles would exhibit a blue shift and the characteristic absorption bands of Fe–O bond were shifted to higher wavenumber of about 584 cm^{-1} , confirmed that the main phase of iron oxide in both samples, as shown in Fig. 2a and b. The IR spectrum of microgel magnetic particles shows deformation of two methyl groups on $-\text{C}(\text{CH}_3)_2$ at 1368 and 1386 cm^{-1} , $-\text{CH}_3$ and $-\text{CH}_2$ deformation at 1460 cm^{-1} , $-\text{CH}_3$ symmetric stretching at 2877 cm^{-1} , $-\text{CH}_3$ asymmetric stretching at 2936 cm^{-1} , amide I and amide II peaks at 1646 and 1549 cm^{-1} , respectively. In addition to these peaks, the carbonyl stretching bond attributed to the carboxylic acid group of AAc units is observed at 1715 cm^{-1} in microgel magnetic particles. The results above revealed that microgel was coated on the iron oxide nanoparticles successfully.

The crystallinity of magnetic nanoparticle-embedded microgels was investigated by powder XRD and the results are shown in Fig. 3. The results show that the iron oxide nanoparticles prepared in the absence of copolymer had six diffraction peaks at 2θ of 30.1, 35.4, 42.9, 52.7, 57.5 and 62.7, representing corresponding indices (220), (311), (400), (422), (511) and (440), respectively of iron oxide. This revealed that the magnetic particles were pure Fe_3O_4 with spinel structure. The microgel coated magnetic nanoparticles (Fig. 3b) exhibited the same peaks as iron oxide nanoparticles, indicating a crystalline structure. This finding confirmed that the microgel coating did not influence in crystallinity structures of the magnetic nanoparticle embedded in microgels, i.e. MMP.

The thermoresponsiveness of microgel magnetic particles was studied by DLS at various temperatures increasing from 25 °C to 45 °C. Fig. 4 shows the relationship between the hydrodynamic diameter of the microgels and temperature. The diameter did not change much with the temperature increasing from 25 °C to 38 °C. But from 38 °C to 45 °C, the diameter decreased from 118 nm to 64 nm, indicating that the temperature of phase transition was about 38 °C. We can also find that the diameter measured by DLS below 38 °C was bigger than that measured by TEM, while at 40 °C (65 nm) was almost the same as that measured by TEM (60 nm). This is because at the temperature of 40 °C, pNIPAM chains exhibited hydrophobic characteristic, they can hardly dissolve in the water and shrank around the magnetite nanoparticles leading to the same result measured by TEM.

The superparamagnetic property of magnetic microgel particle is critical for their application in biomedical and bioengineering fields, which prevents magnetic microgels from aggregation and enables them to redisperse rapidly when the magnetic field is removed [17]. The variation of the magnetization with the applied magnetic field provides the information on the magnetic properties of the microgel magnetic particles. Fig. 5 illustrated the separation and redispersion process of

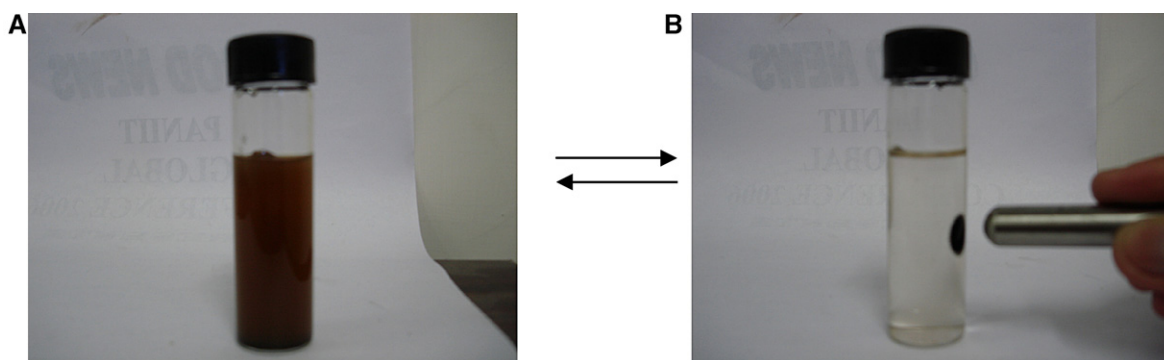


Fig. 5. Photographs of the separation (A to B) and dispersion (B to A) of the microgel magnetic particles (MMP): (A) without external magnetic field, (B) with external magnetic field (the magnetic field strength of the magnet is 2000 G). A color change from saddle brown to transparent was observed when an external magnetic field is applied.

the microgel magnetic particles. In the absence of an external magnetic field, the dispersion of the microgel magnetic particle was saddle brown and homogeneous (Fig. 5A). When the external magnetic field was applied, the microgel magnetic particles were enriched, leading to transparency of the dispersion (Fig. 5B).

4. Conclusion

Magnetic iron oxide nanoparticles/microgels composite microspheres prepared by emulsion free polymerization of NIPAAm and AAc in the presence of iron oxide stabilized by oleic acid exhibit a spherical shape and size in the range 65–110 nm. Iron oxide nanoparticles were successfully encapsulated in the polymeric microgels. The obtained microgels magnetic particles showed temperature dependent behaviours and superparamagnetic properties. The high sensitivity of these microgels to small changes in external stimuli suggests that they could be useful in biotechnology and magnetically-guided drug delivery applications more effectively than with a single stimulus.

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