



Preparation and Characterization of Poly(PEGMA) Modified Superparamagnetic Nanogels Used as Potential MRI Contrast Agents

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ABSTRACT

A novel synthesis method of biocompatible polymers-coated magnetic nanogels was developed. Poly(PEGMA) modified superparamagnetic nanogels were synthesized by in-situ polymerization using poly(ethylene glycol) methacrylate (PEGMA) as monomer and *N,N*-methylene-bis-(acrylamide) (MBA) as cross-linking agent in magnetite aqueous suspension under UV irradiation. The surface functional groups, structure, particle size, Zeta potential and magnetic property were characterized, respectively. The Fourier transform infrared spectroscopy (FTIR) results indicated that the poly(PEGMA) modified magnetic nanogels were synthesized successfully under UV irradiation. The analyses of transmission electron microscopy (TEM) and photon correlation spectroscopy (PCS) indicated that the poly(PEGMA) modified magnetic nanogels were essentially monodisperse, nearly spherical in shape, the mean diameter in aqueous solution was 68.4 nm, and the size distribution was narrow. The thermogravimetric analyzer (TGA) result indicated that there was a high Fe₃O₄ content of 53.4% in the magnetic nanogels, which endowed the magnetic nanogels with a strong magnetic response under external magnetic field. Magnetic measurement revealed that the saturated magnetization of the poly(PEGMA) magnetic nanogels reached 58.6 emu/g and the nanogels showed the characteristics of superparamagnetic. The Zeta potential was -17.3 mV at physiological pH (pH 7.4) which helped maintain stability in blood. The transverse relaxation time (T₂) was also performed for potential use of magnetic resonance imaging (MRI) contrast agents. The preliminary experiment verified the feasibility of the magnetic nanogels for potential MRI contrast agents.

Key Words:

magnetic nanogels;
photochemical polymerization;
poly(ethylene glycol) methacrylate;
magnetic resonance imaging (MRI);
superparamagnetic.

INTRODUCTION

Magnetic nanogels are nanosized particles with core/shell structure, which are composed of Fe₃O₄ (or γ -Fe₂O₃) as the core and cross-linked polymer as the shell. With the properties of magnetic response, biocompatibility, swelling property and surface functionality, magnetic nanogels are widely

used in biochemistry and biomedicine fields such as magnetic resonance imaging (MRI) [1-3], targeted drugs [4,5], protein immobilization [6,7] and DNA detection [8-10].

Several methods have been developed to prepare magnetic micro- and nanogels, such as micro-

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emulsion polymerization, emulsion polymerization and in-situ polymerization [11,12]. However, the processes usually involve surfactants and initiators, which pose limitations on practical biomedical applications. Our group has dedicated all its efforts to the synthesis of biocompatible magnetic nanogels with "green" methods and its bioapplications for years.

Among the hydrophilic monomers, poly(ethylene glycol) monomethacrylate (PEGMA), a poly(ethylene glycol) (PEG) derivative which contains one pendant hydroxyl group in every monomer, is one of the most attractive monomers for future bioapplication [13,14], the modifying poly(PEGMA) will not only prevent rapid clearing by macrophages, but also provide more sites for further biofunctionalization. Recently, Fan et al. have successfully synthesized P(PEGMA)-grafted magnetic nanoparticles via atom transfer radical polymerization method, and confirmed its good biocompatibility [15]. In this paper, poly(PEGMA) modified superparamagnetic nanogels were synthesized by in-situ polymerization using poly(ethylene glycol) methacrylate (PEGMA) as monomer and *N,N'*-methylene-bis-(acrylamide) (MBA) as cross-linking agent in magnetite aqueous suspension under UV irradiation, and the magnetic nanogels were characterized by FTIR, TEM, TGA, PCS and vibrating sample magnetometer (VSM), respectively. The results showed that this novel "green" method which avoided the surfactants and emulsifying agents, was an excellent technique for preparing monodispersed magnetic nanogels. Through the analysis of nuclear magnetic resonance (NMR) analyzer, we investigated the feasibility of the magnetic nanogels as a potential MRI contrast agent.

EXPERIMENTAL

Materials

Poly(ethylene glycol) methacrylate (PEGMA, $M_n \sim 360$), analytical grade, was purchased from Sigma Chemical Co., USA. *N,N'*-Methylene-bis-(acrylamide) (MBA), analytical grade, was purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China) and recrystallized prior to use. Nitrogen (99.99%) was available from Dezhou Long Li Co. All the other chemicals were of analytical grades.

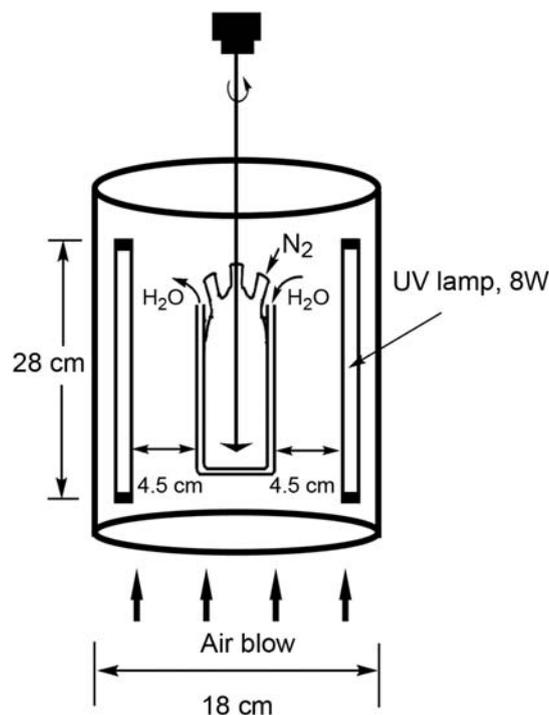


Figure 1. Schematic illustration of the photochemical reaction device.

Stirrer (IKA Co, Germany) was used to synthesize Fe_3O_4 nanoparticles. A photochemical reaction device equipped with two 8 W low-pressure mercury lamps was used as the UV source to synthesize magnetic nanogels. The photochemical reactor with the maximal emission wavelength at 253 nm was illustrated in Figure 1.

Synthesis of Poly(PEGMA) Modified Superparamagnetic Nanogels

Superparamagnetic Fe_3O_4 nanoparticles were prepared by partial reduction method [16,17]. The poly(PEGMA) modified magnetic nanogels were synthesized in a 150 mL interlayer quartz flask equipped with a stirrer and a N_2 inlet cooling with cycled water. Fe_3O_4 nanoparticles (10 mg) were dissolved in 60 mL of distilled water, mixed with 1.86 g of PEGMA for 10 min, added 0.8 mL of 1 wt% MBA, then the mixture was exposed under UV irradiation for 20 min. N_2 was bubbled throughout the preparation process. The poly(PEGMA) modified magnetic nanogels were collected with a permanent magnet and washed with distilled water for several times and redispersed in distilled water.

MRI Experiment of Poly(PEGMA) Modified Magnetic Nanogels

NMR Measurements of nuclear transverse relaxation time (T_2) was performed at room temperature in the centre frequency of 22.648 MHz, by means of pulse-FT spectrometers on samples at different concentrations of magnetic nanogels contained in a quartz vial. The NMR signal was collected by a $\pi/2$ - π Hahn echo sequence.

Characterization

FTIR Spectra of poly(PEGMA) modified magnetic nanogels were analyzed with a Nicolet Avatar 370 Fourier transform infrared (FTIR) spectroscopy in a wavenumber range of 4000-500 cm^{-1} . Morphology and structure of the magnetic nanogels were observed with transmission electron microscopy (TEM, Hitachi 600), the size of the magnetic nanogels was detected by TEM and photon correlation spectroscopy (PCS, Zetasizer Nano ZS, Malvern Instruments Ltd.). Thermogravimetric analysis (TGA) was performed by a thermal analyzer (DTG-60, Shimadzu) by heating the sample from room temperature to 700°C under N_2 atmosphere at a heating rate of 10 $\text{K}\cdot\text{min}^{-1}$. The samples for FTIR, TEM and TGA were vacuum dried at room temperature. The sample for PCS was diluted to 10^{-5} mol/L. Zeta potential of the magnetic nanogels was determined by PCS (Zetasizer Nano ZS, Malvern Instruments Ltd.) at pH from 2 to 12 adjusted with HCl and NaOH.

RESULTS AND DISCUSSION

Preparation and Constituent Analysis of Poly(PEGMA) Modified Magnetic Nanogels

Based on our study, when Fe_3O_4 nanoparticles were dispersed in aqueous solution containing PEGMA and MBA, monomer and cross-linker molecules would be adsorbed on the surface of Fe_3O_4 nanoparticles due to high surface energy [18]. As photon cross-section of Fe_3O_4 nanoparticles was much larger than of PEGMA monomer and MBA cross-linker, the Fe_3O_4 nanoparticles were thought to absorb a majority of photons during the photochemical reaction process. Valence band holes and conduction band electrons were generated on the surface of Fe_3O_4 nanoparticles [18,19].

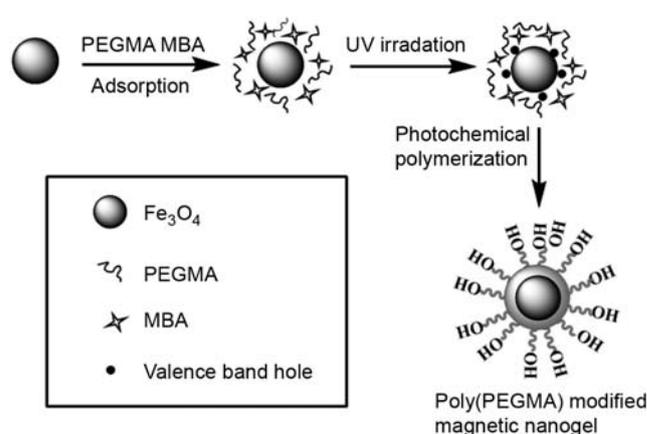


Figure 2. Schematic illustration of photochemical polymerization by UV irradiation.

Valence band holes had intensity liability to capture electrons and the PEGMA monomer adsorbed on the surface of Fe_3O_4 nanoparticles was initiated by valence band holes to produce PEGMA free radicals, which induced photochemical polymerization, then the Fe_3O_4 nanoparticles were coated by a cross-linked poly(PEGMA) shell with further cross-linking between poly(PEGMA) chains. In addition, the photochemical polymerization would take place homogeneously by high-speed stirring and symmetrical irradiation, and at last the poly(PEGMA) modified magnetic nanogels were prepared. As the hydrophilic PEG chains would extend to the outer space in aqueous solution, we inferred the magnetic nanogels were composed of Fe_3O_4 nanoparticles as the core, the poly(PEGMA) hydrogels as the shell, and the PEG chains extended outside the nanogels (Figure 2).

In order to confirm the coating of poly(PEGMA) hydrogel onto the surface of Fe_3O_4 nanoparticles via photochemical method, FTIR spectra were performed and the result was shown in Figure 3. In curve b, the bands of 1726 cm^{-1} and 1113 cm^{-1} were respectively assigned to the C=O and C-O stretching vibrations for carbonyl group of carboxylic ester, the wide band around 3423 cm^{-1} to the O-H stretching vibration, the bands of 2871 cm^{-1} and 1470~1380 cm^{-1} to the stretching and bending vibrations of methylene ($-\text{CH}_2-$), and the band of 1640 cm^{-1} to the characteristic vibrations of $-\text{CONH}-$ group. Furthermore, the peak of 584 cm^{-1} in curves a and b was the characteristic band for Fe_3O_4 nanoparticles. The coating of

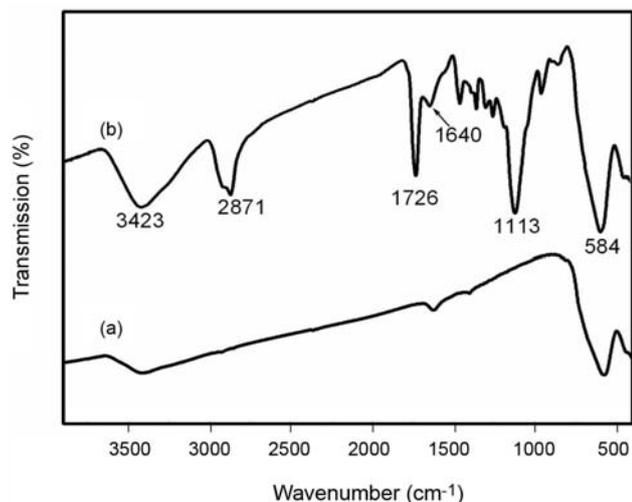


Figure 3. FTIR Spectra of the Fe_3O_4 nanoparticles (a) and magnetic nanogels (b).

poly(PEGMA) endowed the magnetic nanogels with excellent biocompatibility and hydrophilicity, which was good for its application in medicine and biology. The thermogravimetric analysis (TGA) was carried out to calculate the content of poly(PEGMA) shell in the magnetic nanogels after vacuum drying at room temperature for 48 h. As shown in Figure 4, 1.1% weight loss of magnetic nanogels was observed when the temperature rose from room temperature to 120°C , which means a small amount of water in the sample. A 45.5% weight loss was observed from 120 to 450°C , which presumed to be poly(PEGMA) gels component. A platform appeared after 500°C ,

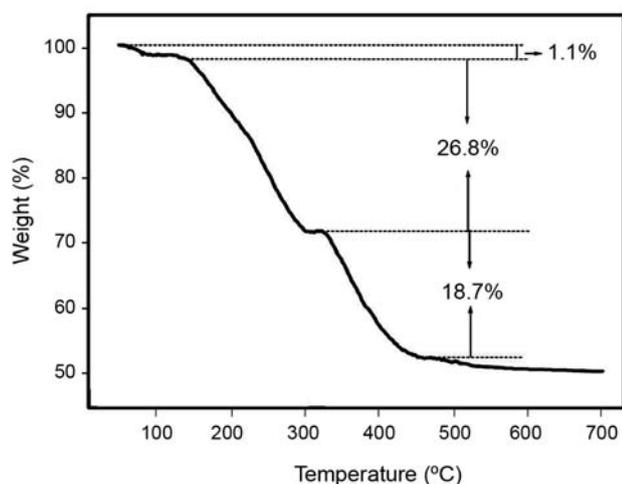


Figure 4. TGA of the magnetic nanogels.

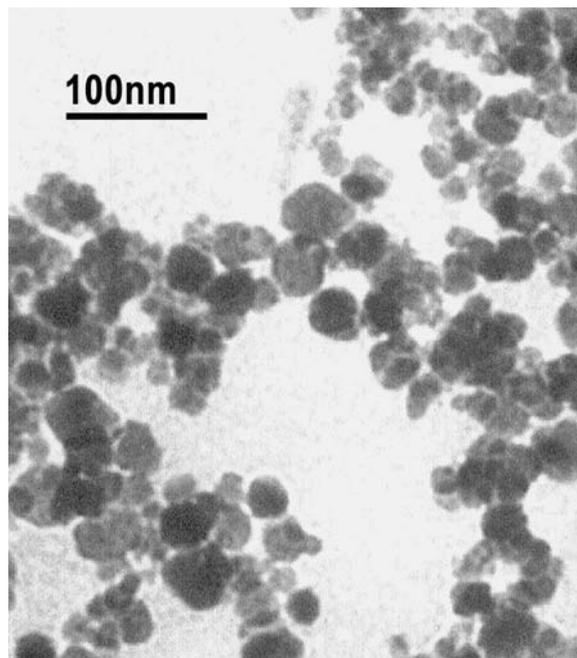


Figure 5. TEM micrograph for the poly(PEGMA) modified magnetic nanogels.

indicating that there was a high Fe_3O_4 content of 53.4% in the magnetic nanogels, which endowed the magnetic nanogels with a strong magnetic response under external magnetic field.

Characterization of Poly(PEGMA) Modified Magnetic Nanogels

The typical TEM micrograph for the poly(PEGMA) modified magnetic nanogels was shown in Figure 5. The magnetic nanogels are essentially monodisperse, nearly spherical in shape with a diameter of 38.2 nm. The average diameter of poly(PEGMA) modified nanogels measured by PCS was 68.4 nm in aqueous system (Figure 6), and the size distribution was narrow, which was good for its *in vivo* biomedical evaluation [20]. The particle size of a poly(PEGMA) modified magnetic nanogel in aqueous suspension was much bigger than determined by TEM. This is because the poly(PEGMA) hydrogel shell of magnetic nanogel swelled in aqueous suspension, the water encapsulated in hydrogel shell would evaporate and magnetic nanogel shrunk remarkably in dry state for measurement with TEM.

The magnetic property of Fe_3O_4 nanoparticles and the poly(PEGMA) modified magnetic nanogels

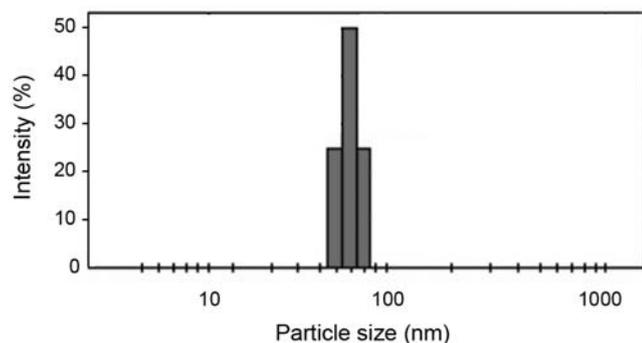


Figure 6. Particle size of the magnetic nanogels measured by PCS.

determined by VSM were shown in Figure 7. The result indicated that the magnetic nanogels showed no magnetism in zero magnetic fields, and behaved superparamagnetic. The saturation magnetization (M_s) was 58.6 emu/g, which was appreciably less than that of the Fe_3O_4 nanoparticles (65.46 emu/g). Therefore, the magnetic nanogels had high magnetic response in the presence of an external magnetic field.

Zeta potential was a significant parameter of nanoparticles, which was correlated with the stability of nanoparticles within the animal body, especially within the blood, so the Zeta potential of the poly(PEGMA) modified magnetic nanogels against different pH was investigated. As shown in Figure 8, the isoelectric point (pI) of the magnetic nanogels was 5.8, and the Zeta potential was -17.3 mV at physiological pH (pH 7.4). The higher absolute value of zeta

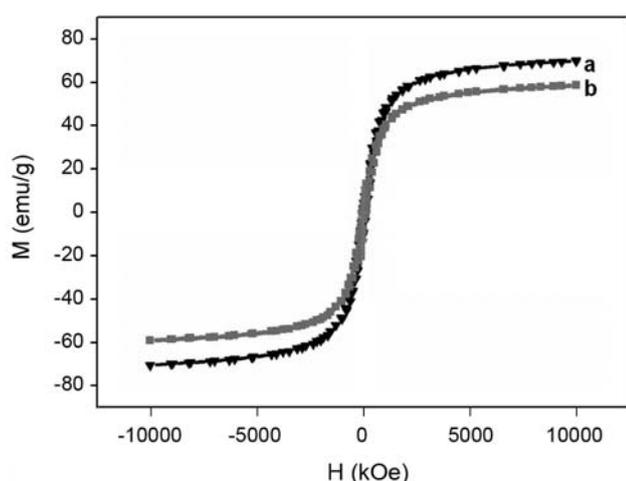


Figure 7. The magnetization of Fe_3O_4 nanoparticles (a) and magnetic nanogels (b) versus applied field.

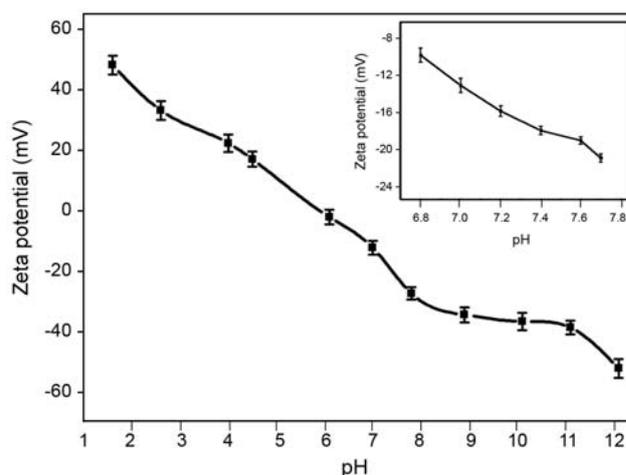


Figure 8. Zeta potential of the magnetic nanogels dependent on pH.

potential could significantly decrease the aggregation of the nanogels, and the negative Zeta potential could reduce the possible combination with haemoglobin, which played an important role in improving the stability and blood compatibility.

Measurement of Poly(PEGMA) Modified Magnetic Nanogels for Potential MRI Contrast Agents

We performed MRI experiments of three sample tubes containing poly(PEGMA) modified magnetic nanogels at different concentrations (C: 1, 0.5, 0.25 mg/mL). Figure 9 shows clearly the effect of the

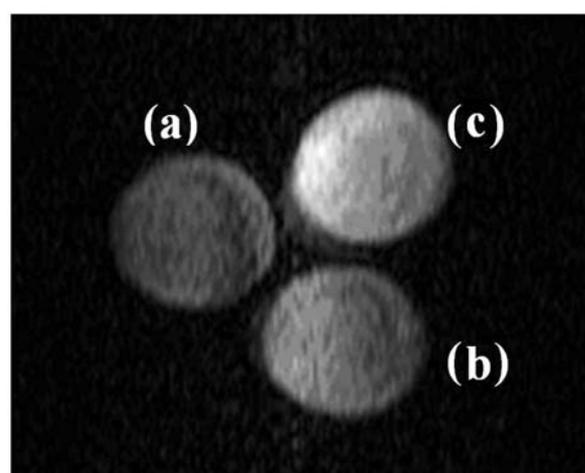


Figure 9. MRI image on the poly(PEGMA) magnetic nanogels at different concentrations: (a) 1.0 mg/mL, (b) 0.5 mg/mL, (c) 0.25 mg/mL.

concentration increase (the higher the C, the darker the image). Nuclear transverse (T_2 , 6 μ s) time of the magnetic nanogels at concentration of 1 mg/mL was obtained, which confirmed the feasibility of the nanogels used as potential MRI contrast agents.

CONCLUSION

A novel poly(PEGMA) modified superparamagnetic nanogels were synthesized by in-situ polymerization using poly(ethylene glycol) methacrylate (PEGMA) as monomer and *N,N'*-methylene-bis-(acrylamide) (MBA) as cross-linking agent in magnetite aqueous suspension under UV irradiation. The analyses of TEM and PCS indicated that the poly(PEGMA) modified magnetic nanogels were essentially monodisperse, nearly spherical in shape, and the mean diameter in aqueous solution was 68.4 nm. The saturated magnetization of these novel magnetic nanogels could reach 58.6 emu/g and the nanogels showed the characteristics of superparamagnetism. The negative Zeta potential and shorter T_2 times promised the magnetic nanogels would be an ideal candidate for potential MRI contrast agents.

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ABBREVIATIONS

MRI	Magnetic resonance imaging
PEGMA	Poly(ethylene glycol) methacrylate
MBA	<i>N,N'</i> -Methylene-bis-(acrylamide)
PCS	Photon correlation spectroscopy
VSM	Vibrating sample magnetometer
FTIR	Fourier transform infrared spectroscopy
TGA	Thermogravimetric analyzer
NMR	Nuclear magnetic resonance analyzer

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