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Preparation of carboxylated temperature-responsive magnetic nano composite polymer particles

M.A.Alam[1], T.Sultana[1], M.A.Rahman[1], M.A.Nuri[2], M.A.J.Miah[1], H.Ahmad[1]*

[1]Department of Chemistry, Rajshahi University, Rajshahi 6205, (BANGLADESH)

[2]Department of Conservation Laboratory, Bangladesh National Museum, Shahbag, Dhaka, (BANGLADESH)

E-mail : samarhass@yahoo.com

ABSTRACT

Magnetic (Fe_3O_4) nano particles are very promising materials for many biomedical applications because of their ability to be manipulated and detected with an external magnetic field. In the present investigation a systematic approach has been proposed to prepare temperature-sensitive magnetic nano composite polymer particles. Nano-sized Fe_3O_4 particles were first prepared by co-precipitation of Fe^{2+}(aq) and Fe^{3+}(aq) in presence of aqueous NaOH solution. Fe_3O_4 nano particles were then functionalized with a silane coupling agent followed by two step polymerization. In the first step radical copolymerization of styrene and N-isopropyl acrylamide (NIPAM) was carried out in presence of functionalized Fe_3O_4 as seed particles. The produced latex particles were named as Fe_3O_4/P(S-NIPAM). Then seeded copolymerization of NIPAM, methacrylic acid (MAA) and N,N'-methylene-bis-acrylamide (MBAAm) was carried out in presence of Fe_3O_4/P(S-NIPAM) composite seed particles. The final composite polymer particles named as Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) were characterized by electron micrographs, FTIR, and thermogravimetric analyses. The magnetic nano composite polymer particles containing carboxyl groups exhibited temperature-sensitive phase transition at temperature close to 30°C.

KEYWORDS

Temperature-sensitive;
Magnetic nano composite particles;
PNIPAM;
Functional groups.

INTRODUCTION

Magnetic nano particles have attracted much interest, as they have some peculiar characteristics, such as quantum size effects and magnetic quantum tunneling. The future applications of magnetic nano particles are expected to be many, not only in high-density magnetic recording but also as a marker or labeling material in the medical field, whereby magnetic nano particles are linked to DNA, antibodies, and other biological molecules. Attempts to introduce magnetic particles into living cells are beginning to bear fruit, especially for drug delivery systems[1,2]. For biological applications, it is very important to conjugate magnetic nano particles with amino groups of biological molecules.

Magnetic nano particles encapsulated with stimuli-responsive polymers are expected to offer controlled and self regulated drug delivery to demonstrate an optimum drug release according to the physiological needs[3]. Among stimuli-sensitive polymers, temperature-sensitive polymers are the most commonly studied class and have played a successful role as a representative model to demonstrate how stimuli-sensitive properties can be modulated and utilized for drug delivery technology. Preparation of temperature-sensitive microgels based on poly(N-isopropyl acrylamide) (PNIPAM) was first reported in the late 1990s[4]. Since then considering the low mechanical strength, poor dimensional stability and slow response to temperatures, the researchers are focusing their attention on the preparation of rigid polymer particles[5-15]. These polymer particles demonstrated an abrupt phase transition at a temperature commonly known as lower critical solution temperature (LCST). A few reports are available on the encapsulation of nano-sized Fe_3O_4 particles by temperature-sensitive microgel layer. M. Omer *et al.* reported a complex four step process to prepare nano-sized magnetic temperature-sensitive microgel particles comprising crosslinked PNIPAM shell[16]. In another work S. Purushotham *et al.* prepared the same polymer microspheres by a relatively simple method and discussed the drug loading and release behavior[17].

In this investigation attempt was made to fabricate caroxylated temperature-sensitive magnetic nano composite polymer particles (MNCPs). Magnetic nano particles (Fe_3O_4) prepared by coprecipitation of Fe^{2+} and Fe^{3+} were functionalized with 3-(methacryloyloxy)propyltriethoxysilane (MPS), a silane coupling agent. Polymerization of styrene and NIPAM was then carried out in presence of functionalized Fe_3O_4 as seed particles to obtain Fe_3O_4/P(S-NIPAM) composite seed particles. Finally seeded emulsion copolymerization of NIPAM, methacrylic acid (MAA) and crosslinking agent N-N'-methelyne–bis-acrylamide (MBAAm) was carried out in presence of Fe_3O_4/P(S-NIPAM) composite seed particles. The temperature-responsive MNCPs produced were named as Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm). The presence of carboxyl functionality would be useful for potential coupling of biorecognizable ligands as well as this would impart pH-sensitive phase transition.

EXPERIMENTAL

Chemicals and instruments

Styrene from Sigma Chemical Company, USA, and MAA from Fluka Chemika, Switzerland were distilled under reduced pressure and preserved in the refrigerator. MBAAm from Sigma Chemical Company, USA, was preserved in the refrigerator and used without purification. NIPAM purchased from Acros Organics, USA, was recrystallized from a mixture of 90% hexane and 10% acetone, dried at a low temperature under vacuum and was preserved in the refrigerator. Potassium persulfate (KPS) from LOBA Chem. India was recrystallized from distilled water before use. Oil soluble initiator 2,2'-azobis(isobutyronitrile) (AIBN) from LOBA Chem. India, was recrystallized from distilled methanol. Poly(vinyl pyrrolidone) (PVP) from Fluka Chemika, Switzerland, of molecular weight 3.6×10^5 $gmol^{-1}$ was used as a polymeric stabilizer. MPS from Alfa Aesar, UK, was used as a coupling agent. Ethanol was dehydrated and distilled before use. Ferric chloride hexahydrate ($FeCl_3.6H_2O$), ferrous sulphate ($FeSO_4$), NaOH, oleic acid and other chemicals were of analytical grade. Deionized water was distilled using a glass (Pyrex) distillation apparatus.

Transmission electron microscope or TEM (JEM-1230, JEOL, Tokyo, Japan) was used to see the particle size distribution. IR Spectrophotometer (PerkinElmer, FTIR-100, UK), Centrifuge machine from Kokuson Corporation, Tokyo, Japan, pH-meter (Mettller Toledo MP 220, Switzerland), NICOMP 380 Particle Sizer (Santa Barbara, California, USA) and thermogravimetry analyzer (TGA) (EXSTAR-6000 TGA), from Seiko Instruments Inc. Japan were used for the characterization of latex particles.

Preparation of Fe_3O_4 particles

Magnetic (Fe_3O_4) nano particles were produced by co-precipitation of Fe^{2+} and Fe^{3+} from their aqueous solution (molar ratio1: 2) using 5M NaOH. The precipitation was carried out in a three necked flask under a nitrogen atmosphere at 85°C for 2 h. Oleic acid was slowly added towards the end of the process to stabilize the Fe_3O_4 dispersion. The produced Fe_3O_4 particles were washed by serum replacement with deionized distilled water, followed by 0.05M HCl aqueous solution and again by deionized distilled water in order to remove the residual electrolyte and excess oleic acid.

Functionalization of Fe_3O_4 particles by MPS

Fe_3O_4 (3.0 g) particles and MPS (4.5 g) were taken in a three necked round flask. The dispersion media comprises 12.5% (w/w) water containing water-ethanol mixture (180 g). The reaction mixture was mechanically stirred at ambient temperature for 48 h.

Preparation of Fe_3O_4/P(S-NIPAM) composite seed particles

The copolymerization of styrene (1.64 g) and NIPAM (0.06 g) was carried out in presence of MPS functionalized Fe_3O_4 (0.4 g) as seed particles in ethanol-water dispersion media containing 60% (w/w) ethanol using oil soluble AIBN (0.06 g) as initiator. PVP (0.08 g) was used as a polymeric stabilizer. The copolymerization was carried out in a three necked round bottomed flask dipped in a thermostat water bath at 70°C for 36 h. During the polymerization an inert atmosphere was maintained and the reaction mixture was magnetically stirred at 150 rpm.

Preparation of temperature-responsive MNCPs by seeded copolymerization

Temperature-responsive MNCPs named as Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) were prepared by seeded copolymerization of NIPAM (0.1425 g), MAA (0.0045 g) and MBAAm (0.003 g) in presence of 0.3 g of Fe_3O_4/P(S-NIPAM) composite seed particles using KPS as initiator under a nitrogen atmosphere in a three necked round bottomed flask. The reactant mixture was mechanically stirred at 90 rpm and the copolymerization reaction was carried out at 70°C for 8 h.

Thermogravimetric analysis (TGA)

Thermal properties of the dry powdered samples of Fe_3O_4, functionalized Fe_3O_4, Fe_3O_4/P(S-NIPAM) composite seed, and Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite particles were measured by heating samples under flowing nitrogen atmosphere from ambient temperature to 600°C at a heating rate of 20°C/min and the weight loss was recorded.

RESULTS AND DISCUSSION

Figure 1 shows the TEM photographs of Fe_3O_4, functionalized Fe_3O_4, Fe_3O_4/P(S/NIPAM) composite seed and Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles. The latex particles irrespective of their composition are in the range of nano scale and thereby difficult to make comments on the exact shape and morphology. The magnified images shown in the inset indicates that the particles are approximately spherical in shape and the appearance changed after each step. The presence of clusters of variable sizes reflects the aggregation phenomena accompanying the drying process although there are also possibilities of aggregated forms during synthesis. A similar morphology of MPS and polymer coated magnetic nano particles were observed by others[18,19]. The average diameters of functionalized Fe_3O_4, Fe_3O_4/P(S/NIPAM) composite seed and Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles are 22.59, 32.90 and 57.51 nm respectively. The average size of Fe_3O_4 particles did not change much after functionalization with MPS. The average size of the latex particles increases after each step of respective polymer coating. This observation indicates that deposition of polymer on Fe_3O_4 occurs during polymerization.

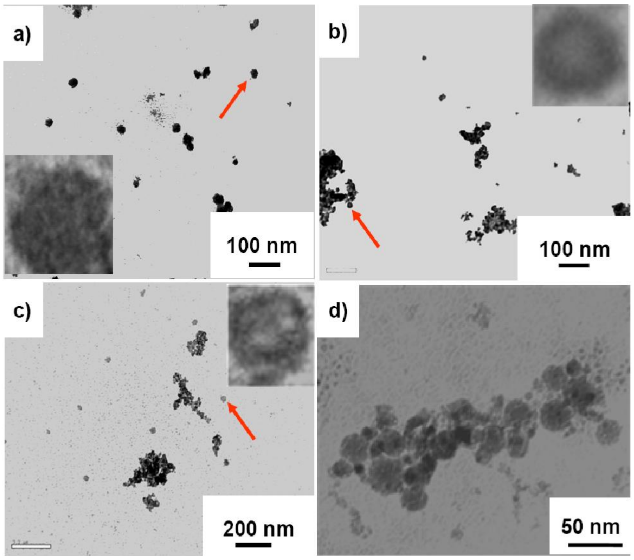

Figure 1 : TEM photographs of a) Fe_3O_4, b) functionalized Fe_3O_4, c) Fe_3O_4/P(S-NIPAM) composite seed, and d) Fe_3O_4/P(S-NIPAM)/P(NIPAm-MAA-MBAAm) composite polymer particles

The FTIR spectra of Fe_3O_4/P(S/NIPAM) composite seed and Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles are illustrated in Figure 2. The particles were washed and dried at 80°C prior to the recording of FTIR spectra in KBr pellets. In the spectrum of Fe_3O_4/P(S/NIPAM) composite seed particles a medium signal due to C=O stretching vibration for –CONHR group appears in the region 1690-1550 cm^{-1}. While Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles produce a sharp peak with a slight deviation in the region 1700-1540 cm^{-1}. The broad O-H stretching appears at 3650-2900 cm^{-1} in Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles represents absorbed water molecules, carboxyl and N-H groups. In contrast, in Fe_3O_4/P(S/NIPAM), this same peak represents the combined stretching vibration for N-H and -OH groups derived from substituted amide, and surface water molecules at almost in the same region but the signal shape and intensity changed slightly.

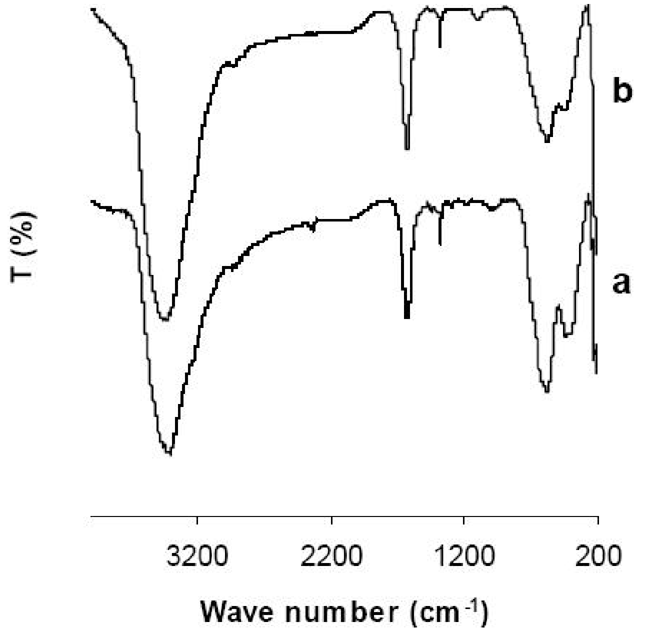

Figure 2 : FTIR spectra of a) Fe_3O_4/P(S-NIPAM) composite seed, and b) Fe_3O_4/P(S-NIPAM)/P(NIPAm-MAA-MBAAm) composite polymer particles recorded in KBr pellets

TGA was used to confirm the coating of inorganic Fe_3O_4 by organic polymer. It is expected that as the composite particles are heated from ambient temperature to 600°C, the organic part of the composite would be burned off and the percentage of the remaining part after calcination would give the content of inorganic component. Figure 3 represents TGA thermograms of Fe_3O_4, functionalized Fe_3O_4, Fe_3O_4/P(S-NIPAM) composite seed and Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles. It is observed that relative to Fe_3O_4 the functionalization with MPS reduces the weight loss by around 3%. This behavior may support the inclusion of inorganic silicon materials. Compared to this the coating by organic P(S-NIPAM) layer increases the weight loss by around 2% and successive seeded copolymerization increases the weight loss by further 18%. This increase in weight loss confirmed that Fe_3O_4 particles have successfully been covered with organic polymer.

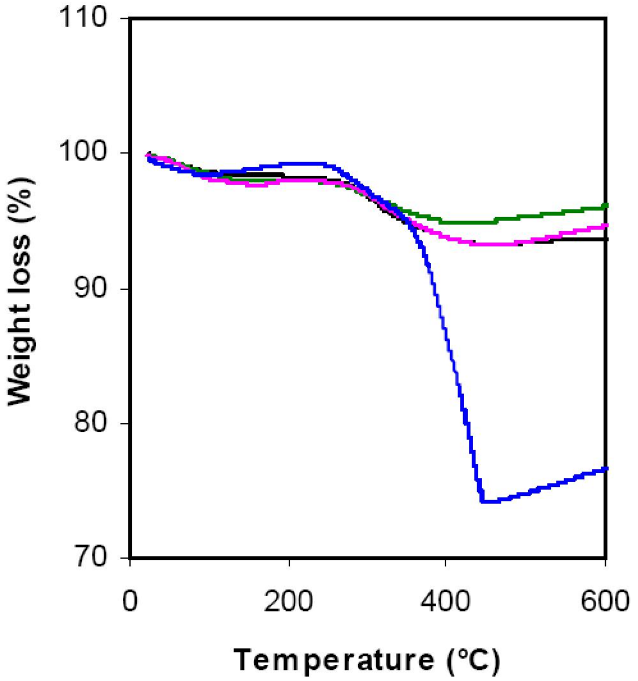

Figure 3 : TGA thermograms of Fe_3O_4 (black line), functionalizd Fe_3O_4 (green line), Fe_3O_4/P(S-NIPAM) (pink line) composite seed, and Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) (blue line) composite polymer particles

Figure 4 shows the variation of hydrodynamic diameters of Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles at pH 4 and pH 9. The hydrodynamic diameters decrease sharply at around 30ºC which is close to the LCST 32°C of PNIPAM aqueous solution. Hence, P(S-NIPAM)/P(NIPAM-MAA-MBAAm) copolymer layer deposited on Fe_3O_4 particles dehydrated and collapsed with increasing temperature above the LCST. The lower hydrodynamic diameter at lower pH value indicates that

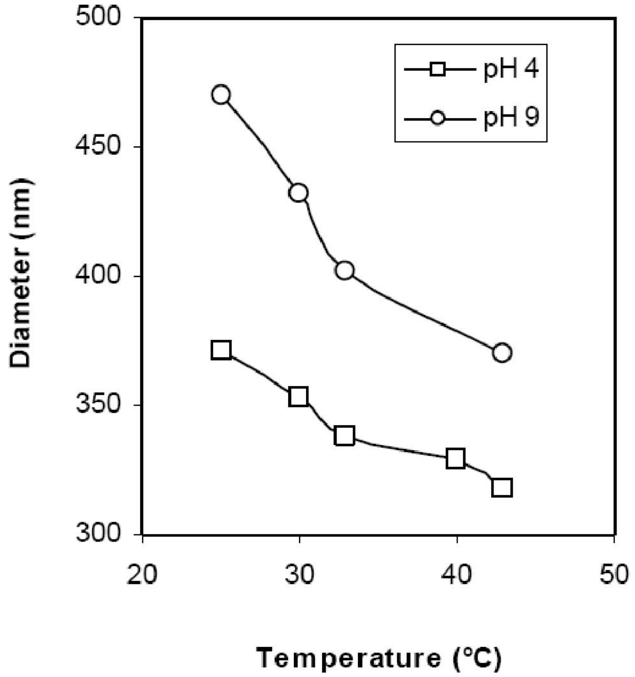

Figure 4 : Variation of average hydrodynamic diameter of washed Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles at pH 4 and 9 against temperature

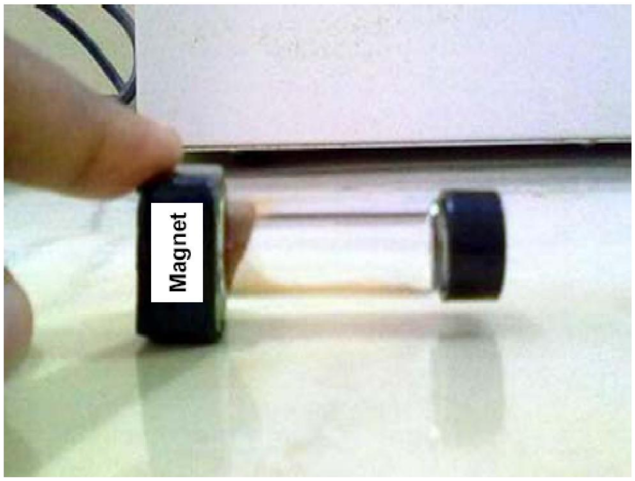

Figure 5 : Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite particles being attracted by a magnet

latex particles are relatively hydrophobic as the majority of carboxyl group derived from MAA component is protonated. Compared to that at higher pH value more carboxyl groups are deprotonated and thereby enhanced the swelling of latex particles through hydrogen bonding with water. Relative to the average diameter (57.51 nm) of Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite particles measured from the TEM photograph, the higher hydrodynamic diameter (~375.0 nm) at 45°C implied that particles dispersed in aqueous medium are still enough hydrophilic. The particle coagulation may also affect the ultimate diameter. This result suggests that the prepared composite polymer particles possess temperature-sensitive phase transition and additionally the presence of carboxyl group induces pH-sensitive swelling at higher pH value.

Figure 5 shows the image of dried Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm) composite polymer particles under the influence of external magnetic field. It is evident that the particles are attracted towards the magnet. So, we can say that temperature-sensitive MNCPs have successfully been prepared.

CONCLUSIONS

A simple four step process was used to prepare temperature-sensitive MNCPs named as Fe_3O_4/P(S-NIPAM)/P(NIPAM-MAA-MBAAm). The particles were attracted towards the magnetic field. The presence of PNIPAM and carboxyl group from MAA in the shell layer induced dual phase transition property in response to temperature and pH. The carboxyl functionality at the surface can also be used to conjugate biomolecules.

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