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Hydrophilic magnetic nanoclusters with thermo-responsive properties and their drug controlled release

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ABSTRACT

Synthesis and drug controlled release properties of thermo-responsive magnetic nanoclusters grafted with poly(N-isopropylacrylamide) (poly(NIPAAm)) and poly(NIPAAm-co-poly(ethylene glycol) methyl ether methacrylate) (PEGMA) copolymers were described. These magnetic nanoclusters were synthesized *via* an *in situ* radical polymerization in the presence of acrylamide-grafted magnetic nanoparticles (MNPs). Poly(NIPAAm) provided thermo-responsive properties, while PEGMA played a role in good water dispersibility to the nanoclusters. The ratios of PEGMA to NIPAAm in the (co)polymerization in the presence of the MNPs were fine-tuned such that the nanoclusters with good water dispersibility, good magnetic sensitivity and thermo responsiveness were obtained. The size of the nanoclusters was in the range of 50–100 nm in diameter with about 100–200 particles/cluster. The nanoclusters were well dispersible in water at room temperature and can be suddenly agglomerated when temperature was increased beyond the lower critical solution temperature (LCST) (32 °C). The release behavior of an indomethacin model drug from the nanoclusters was also investigated. These novel magnetic nanoclusters with good dispersibility in water and reversible thermo-responsive properties might be good candidates for the targeting drug controlled release applications.

1. Introduction

Magnetite nanoparticles (MNPs) have recently attracted increasing attention because of their unique physical properties and good magnetic sensitivity [1]. Several potential applications of MNPs in bio-nanotechnology have been widely reported such as magnetic resonance imaging (MRI) enhancing agents, targeted drug delivery, hyperthermia treatment of tumors and biomolecular magnetic separation [2] and diagnosis [3]. Because of high surface area and strong inter-particle attractive interactions such as Van der Waals force, magnetic force and gravitational force, MNPs tend to aggregate and essentially agglomerate to form uncontrollable large clusters, leading to the loss in their nanoscale-related properties [4] and limiting their applications [5]. Coating MNPs with polymers is one of an efficient strategy to prevent MNP agglomeration. Long chain polymers coated on MNP surface provided steric stabilization mechanism and can be designed in an attempt to improve their stability and dispersibility in media, and also served as a platform for conjugation with functional bio-entities for use in various applications [6].

In particular, many attempts have been made to develop effective drug delivery systems by taking advantage of magnetically guidable properties of MNPs. The conjugation of a drug to MNP surface grafted with polymers is an efficient approach to deliver the drug to targeted cells, tissues or specific organs [7]. The drug-conjugated MNP vehicles must have high saturation magnetization, so that they can rapidly respond to an applied magnetic field. Preparation of micron-sized magnetite particles resulted in uncontrollable agglomeration in aqueous dispersions, leading to the loss in nano-scale related properties, such as superparamagnetism and high surface area-to-volume ratio. Another promising approach in enhancing magnetic responsiveness of MNPs without sacrifice in these desirable properties was to assemble them into nanoclusters. Unlike micron-sized particles, formation of MNP nanoclusters significantly increased magnetic sensitivity [8] as opposed to individual MNPs and also maintained their dispersibility and stability in water [9]. Importantly, their superparamagnetic behavior might also be maintained as long as the individual MNP cores can be distinguished from each other after agglomeration [10], meaning that there was not one polycrystalline particle but a cluster with distinguishable MNPs smaller than

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20 nm. Various approaches have been recently reported in an attempt to control the formation of MNP nanoclusters, including physical or physicochemical interaction between pre-synthesized MNPs and polymer particles [5,7,11–13], *in-situ* polymerization of monomers in the presence of MNPs [14–19] and *in-situ* precipitation of MNPs in the presence of polymer microspheres [20–22].

In-situ polymerization of thermo-responsive poly(N-isopropylacrylamide) (poly(NIPAAm)) in the presence of MNPs to form magnetic nanoclusters was studied in the present work. Poly (NIPAAm), a well-known thermo-responsive polymer, can exhibit phase separation in aqueous solution at 32 °C, the so called lower critical solution temperature (LCST) [23–28]. At the temperature below its LCST, poly(NIPAAm) is hydrophilic and soluble in water because it swells and extends due to the intermolecular hydrogen bonding between the polymer chains and water molecules. Conversely, the polymer chains collapse at a temperature higher than its LCST, resulting in an increase in hydrophobicity due to the formation of intramolecular hydrogen bonding among the polymer chains.

In this work, thermo-responsive MNP nanoclusters grafted with poly(NIPAAm) and poly(NIPAAm-co-PEGMA) copolymers were synthesized *via* a free radical polymerization using acrylamide-functionalized MNPs as active nanocrosslinkers (Fig. 1). Poly (NIPAAm) played a role in thermo-responsive properties for drug controlled release, while polyPEGMA provided good water dispersibility to the nanoclusters. The ratio of PEGMA to NIPAAm in the clustering reactions was fine-tuned such that the MNP nanoclusters with good water dispersibility, good magnetic sensitivity and thermo responsiveness were obtained. Transmission electron microscopy (TEM) was used to investigate size and size distribution of the nanoclusters. Thermo-responsive properties and controlled release of an indomethacin model drug of the MNP nanoclusters were investigated. The effect of the copolymer composition on the drug release behavior was also investigated.

2. Experimental

2.1. Materials

Unless otherwise stated, all reagents were used without further purification: iron (III) chloride anhydrous ($FeCl_3$, 98%, Acros), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 99%, Acros organic), ammonium hydroxide (NH_4OH, 28–30%, J.T. Baker), sodium hydroxide (NaOH, 98%, Aldrich), oleic acid (68%, Carlo Erba), (3-aminopropyl) trimethoxysilane (APTES, 98%, Acros), triethylamine (TEA, 97%, Carlo Erba) and ammonium persulfate (APS, 98%, Carlo Erba) were used as received. N-isopropylacrylamide (NIPAAm, 99%, Acros) was recrystallized twice from hexane. Poly(ethylene glycol) methyl ether methacrylate (PEGMA, Aldrich) with an average molecular weight of 300 g mol^{-1} was purified by passing through basic alumina column and stored at −4 °C until used. Acryloyl chloride was synthesized *via* a coupling reaction between acrylic acid and benzoyl chloride at 75 °C to give a colorless liquid; 60% yield. Indomethacin (99%, Sigma) was used as received.

2.2. Syntheses

2.2.1. Synthesis of acrylamide-grafted MNP as a crosslinker

Synthesis procedure for acrylamide-grafted MNP as a crosslinker consisted of three consecutive steps: (1) synthesis of MNP *via* a co-precipitation method, (2) functionalizing the MNP surface with amino groups and (3) coupling between amino-grafted MNP and acryloyl chloride to obtain acrylamide-grafted MNP.

In the first step, aqueous solutions of $FeCl_3$ (1.66 g in 20 mL deionized water) and $\cdot 4H_2O$ (1 g in 20 mL deionized water) were mixed together with stirring. Black precipitant was observed once 25% NH_4OH (20 mL) was added into the solution. The dispersion was continuously stirred for another 30 min to complete the reaction. The dispersion was centrifuged at 5000 rpm for 20 min to precipitate large aggregate and the aqueous supernatant was discarded. An oleic acid solution in hexane (2.0 mL in 20 mL hexane) was then introduced into the MNP dispersion with stirring. Oleic acid-coated MNP was then re-precipitated in acetone.

In the second step, oleic acid-coated MNPs (0.03 g) were re-dispersed in toluene, followed by an addition of TEA (0.4 mL) in a round bottom flask. Then, (3-aminopropyl) triethoxysilane (APTES) (0.4 mL) was added dropwise to the mixture with stirring for 24 h at room temperature under N_2 atmosphere. Amino-grafted MNPs were separated using an external magnet and washed with ethanol and deionized water.

Lastly, the amino-grafted MNPs (0.1 g) were re-dispersed in a NaOH solution (3 g in 10 mL deionized water). Acryloyl chloride (4.0 mL) was added dropwise to the dispersion in an ice-water bath and the mixture was continuously stirred at room temperature for 24 h. The product was separated by applying an external magnet, thoroughly washed with water and stored in the form of dispersions in water having 0.02 g of the MNP/ml water.

2.2.2. Synthesis of poly(NIPAAM-CO-PEGMA)-grafted MNP nanocluster

Poly(NIPAAm-co-PEGMA)-grafted MNP nanoclusters having 100 mole equivalent of NIPAAm and various mole equivalents of

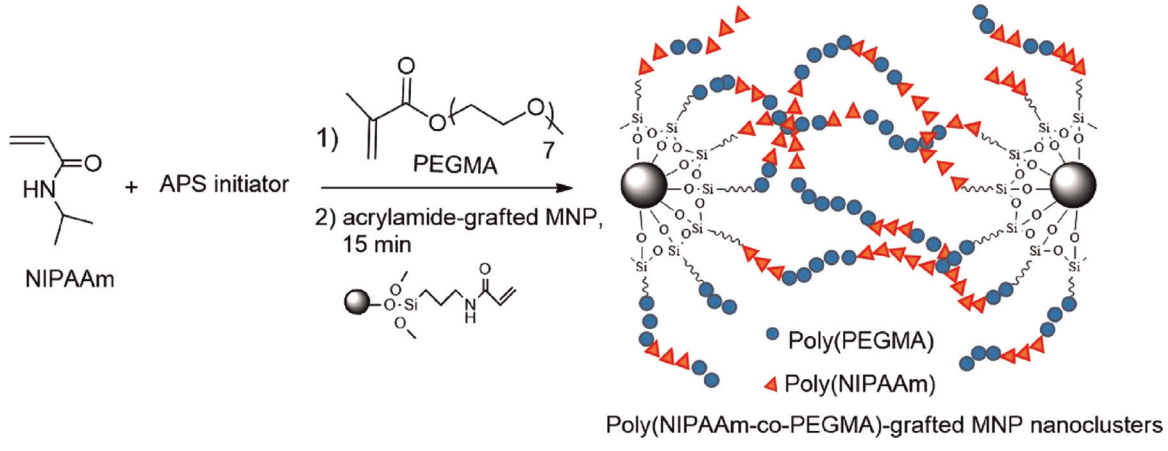

Fig. 1. Synthesis of poly(NIPAAM-co-PEGMA)-grafted MNP nanoclusters *via* a free radical *in situ* (co)polymerization in the presence of acrylamide-functionalized MNPs.