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Monodispersed polymer encapsulated superparamagnetic iron oxide nanoparticles for cell labeling

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ABSTRACT

Polymer encapsulation of raw ferrofluids was carried out by free radical emulsion polymerization using macro-RAFT copolymers as stabilizers. The iron oxide cores with positively charged surface at the pH of encapsulation were dispersed in a solution of negatively charged random macro-RAFT copolymer by ultrasonication. Uniform polymer encapsulated super paramagnetic iron oxide nanoparticles (SPIONs) were produced by feeding the mixture of methyl methacrylate (MMA) and *n*-butyl acrylate (BA) monomers into the dispersion containing water soluble initiator 4,4'-azobis (4-cyanopentanoic acid) (V501). Shell thickness could be controlled by adjusting the amount of coating monomer added. Polymer encapsulation of SPIONs aligned in a magnetic field produced polymer encapsulated SPION rods. Monodispersed sterically stabilized crosslinked polymer encapsulated SPIONs were synthesized using diblock and random macro-RAFT copolymers as stabilizers. Rhodamine labeled SPIONs were found to be non-cytotoxic and suitable for cell labeling.

1. Introduction

Superparamagnetic iron oxide nanoparticles (SPIONs) are the most studied nanoparticles due to their relatively low cost and simple synthesis process [1]. They are normally in the form of magnetite [1], maghemite [2] or iron oxides doped with other metal oxides [1–3] which can enhance the particles overall magnetic properties. SPIONs are superparamagnetic nanoparticles which consist of a single magnetic domain and display magnetic properties only in the presence of an external magnetic field [1,2]. These attributes have led to SPIONs finding extensive applications in biomedical science from cell labeling [1,2,4,5], hyperthermia [1–3] to drug delivery [1,2,6]. However, iron oxide nanoparticles in their original form have a weakly charged surface which makes them susceptible to aggregation [1,7]. Surface modification involving attachment of stabilizers such as surfactant-like organic substances [1,2] or hydrophilic polymers [1,2] can increase their stability. The provision of steric stabilizers makes the SPIONs more stable, especially in high salt biological media [1,2].

KCPC has extensive experience in modifying SPIONs with macro-RAFT copolymers for cell labeling and drug delivery towards cancer treatment [8–12]. The modification process simply involves the dispersion of SPIONs in a macro-RAFT stabilizing solution. The macro-RAFT stabilizers are diblock in nature and consist of a short block that contains acid groups such as carboxylic [11] or phosphonic [9,13] which anchor the macro-RAFT copolymer to the SPION surface while the second block comprises relatively long blocks of polyethylene oxide or polyacrylamide, which serve as steric stabilizers for the SPIONs [9–11]. The modified SPIONs have been found to be extremely stable in highly concentrated salt solutions and biological media. However, this dispersion process still has an inherent drawback which is the exposure of the SPION

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surface to the dispersed media [9–14]. Furthermore, Jain et al. reported desorption of the polymer stabilizers when macro-RAFT diblocks of poly (acrylic acid) and polyacrylamide at pH greater than 12 and lower than 4 [11]. He attributed this desorption to full protonation of carboxylic groups at pH lower than 4. For pH greater than 12, the desorption was thought to be due to electrostatic repulsion between negatively charged surface and negatively charged diblock copolymers. This lead to reduced particle stability, affecting performance of the modified SPIONs when macro-RAFT diblocks of poly (acrylic acid) and poly (acrylamide) are used as stabilizers at such pH ranges [11]. One possible solution for desorption of this particular type of diblocks is to chain extend the living ends of macro-RAFT stabilizers, making them less labile on the particle surface. The chain extension process can be carried out using polymer encapsulation [15] which may also form a uniform thin polymer shell insulating the iron oxide surface from dispersing media.

Polymer encapsulation of iron oxide nanoparticles has been carried out by non-RAFT free radical emulsion [16,17], miniemulsion [18] and microemulsion [19] polymerization but suffered from complexity, low efficiency, uncontrolled polymer shell thickness and only applicable on a small scale. Therefore, it is in our interests to develop a ready to scale-up method which can efficiently encapsulate SPIONs. In this work, we will explore the application of our previously developed polymer encapsulation techniques to encapsulate SPIONs within polymer shells [6,15]. The encapsulation process is based on RAFT mediated emulsion polymerization using macro-RAFT copolymers as stabilizers. In this process, nanoparticles are first dispersed in the macro-RAFT copolymer solution by sonication or milling followed by free radical emulsion polymerization in which controlled chain extension of adsorbed macro-RAFT stabilizers leads to formation of uniform polymer shells encapsulating nanoparticles in the centers of polymer particles. Throughout the process, particle stability is maintained by anchored charges or hydrophilic groups from the macro-RAFT stabilizers. The process is simple, versatile and has been successfully adapted to encapsulate a range of particulate materials [20–26]. In this work, polymer encapsulation of SPIONs will be carried out using macro-RAFT copolymers as stabilizers. The superparamagnetic properties will be taken advantage of to synthesize polymer encapsulated SPION nanorods. Incorporation of steric stabilizers to produce encapsulated SPIONs which are stable in high salt and biological media will be demonstrated. Such particles will be further modified by fluorescent dye attachment for cell labeling.

2. Experimental

2.1. Reagents

Milli RO water was used in the synthesis of latexes and acrylic acid-containing RAFT agents. Acrylic acid (AA) (Aldrich) was purified by distillation under reduced pressure. Butyl acrylate (BA) (Aldrich) and methyl methacrylate (MMA) (Aldrich), divinyl benzene (80%, DVB) (Aldrich) and styrene (Sty) (Aldrich) had inhibitor removed by passing them through an inhibitor-removal column (Aldrich). Rhodamine B isothiocyanate (Aldrich), PBS buffer solution (Aldrich), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (Aldrich), n-hydroxy succinimide (NHS) (Aldrich), 2,2'-(ethylenedioxy)bis-(ethylamine) (Diamine) (Aldrich), ammonium hydroxide (NH_4OH) (Aldrich), carbon disulfide (Aldrich), acetone (Aldrich), tetrabutylammonium bromide (Aldrich), 2-bromopropanoic acid (Aldrich), dimethyl sulfoxide (DMSO) (Aldrich), diethyl ether (Aldrich, Univar), hydrochloric acid (Aldrich, Univar), sodium sulfate (Aldrich), 2,2'-azobisisobutyronitrile (AIBN) (Wako), acrylamide (AAm) (Aldrich) and 4,4'-azobis (4-cyanopentanoic acid) (V-501) (Wako) were used as received. Dioxane (Aldrich) was distilled under reduced pressure before use. Super paramagnetic iron oxide nanoparticles (SPIONs) dispersion (5.25%) were obtained from by Sirtex Medical Ltd and used as supplied.

The RAFT agent, 2-([(butylsulfanyl)carbonothioyl]sulfanyl) propanoic acid (PABTC), was synthesized as previously described [27].

Synthesis of short chain poly (BA-*co*-AA) macro-RAFT PABTC-(BA_7-*co*-AA_{10}) copolymers using PABTC.

The amphiphilic macro-RAFT copolymers were prepared as reported in our previous work [15]. PABTC (1.86 g, 7.8 mmol), AIBN (0.064 g, 0.39 mmol), AA (5.63 g, 78.1 mmol), BA (7.51 g, 58.6 mmol) in dioxane (15.00 g) was prepared in a 50 mL round-bottomed flask. 1,3,5-trioxane (0.09 g, 0.9 mmol) was added as an internal standard for proton NMR. This was stirred magnetically and sparged with nitrogen for 10 min. The flask was then heated at 70 °C for 7 h under constant stirring. The final copolymer solution was 50.7% solids. The copolymers were characterized by ^{1}H NMR (300 MHz, Acetone-d6) and size exclusion chromatography (SEC). Based on ^{1}H NMR, the conversion of BA and AA was around 97% and therefore the theoretical molecular weight is 1869.8 g mol^{-1}. The copolymers were characterized by MALDI (Bruker Daltonics Apex Ultra 7 T Fourier Transform Ion Cyclotron Resonance (FTICR) Mass Spectrometer and found to be narrowly distributed in a range from 1000 to 3000 (g/mole), peaked at 2000 (g/mole).

2.2. Synthesis of poly((acrylic acid)-block-poly(acrylamide)) macro-RAFT PABTC-(AA_5-block-AAm_{60}) using PABTC

A solution of PABTC (0.74 g, 3.1 mmol), V501 (0.05 g, 0.2 mmol), acrylamide (13.40 g, 188.6 mmol) in dioxane (17.2 g), 1,3,5-trioxane (0.01 g) and water (20.5 g) was prepared in a 100 mL round bottom flask. This was stirred magnetically and sparged with nitrogen for 15 min. The flask was then heated at 70 °C for 4 h. By ^{1}H NMR, conversion was determined to be at 99.5% after 4 h. At the end of this period, AA (1.12 g, 15.6 mmol) and V501 (0.05 g, 0.2 mmol) was added to the flask. The mixture was deoxygenated and heating was continued at 70 °C for a further 3 h s. The copolymer solution had 28.8% solids and conversion was found to be 96% by ^{1}H NMR.

2.3. Encapsulation of SPIONs with poly(MMA-co-BA) using PABTC-(BA_7-co-AA_{10}) copolymers as stabilizers

Poly (MMA-*co*-BA) encapsulated SPIONs were synthesized using the following procedure. In a 100 ml beaker, macro-RAFT PABTC-(BA_7-*co*-AA_{10}) solution (1.20 g, 0.3 mmol) was dispersed in water (60.1 g) to yield a cloudy yellow solution of pH 4. Ammonium hydroxide (2.8% solution in water) was added to the macro-RAFT solution to raise the pH to 9.0. This was followed by 1 min ultrasonication using a Vibra-Cell Ultrasonic Processor (Sonics and Materials, Inc.) standard probe at amplitude of 30% to obtain a clear yellow solution with pH 7.0. To this solution, SPIONs (20.11 g) was added drop wise and thoroughly dispersed by ultrasonication using a Vibra-Cell Ultrasonic Processor (Sonics and Materials, Inc.) standard probe at amplitude of 30% for 30 min. After sonication, the dispersion pH was measured at 6.5 and it was then transferred to a 100 mL round bottom flask containing V501 (0.04 g, 0.15 mmol) which was subsequently sealed and purged with nitrogen for 10 min. The whole flask was then immersed in an oil bath with a temperature setting of 70 °C and was magnetically stirred. A deoxygenated 10:1 (weight ratio) solution (1 mL, 0.94 g) of methyl methacrylate (MMA), butyl acrylate (BA) was injected into the flask, while in the 70 °C oil bath, at a rate of 2.0 mL/hour. This feed rate