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Polymeric nanostructures with pH-labile core for controlled drug release

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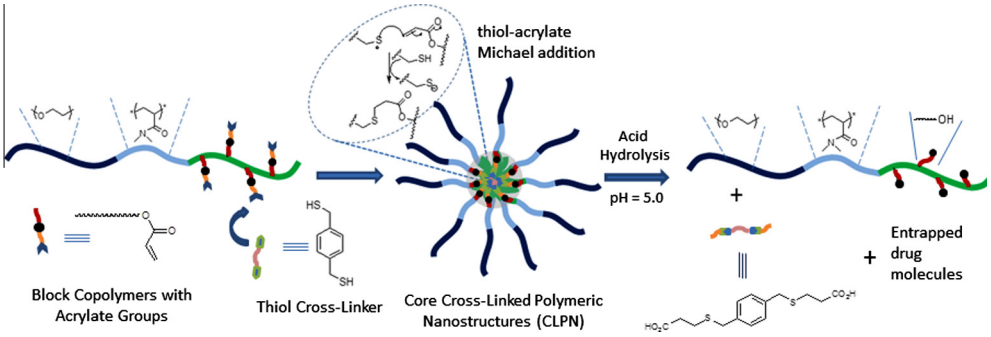
GRAPHICAL ABSTRACT



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ABSTRACT

Efficient and stimuli-triggered controlled delivery of therapeutics is one of the important issues in modern advanced therapy. In the present work, a versatile route for the synthesis of core cross-linked polymeric nanostructures (CLPN) through thiol–acrylate Michael addition reaction via the formation of β-thiopropionate has been described. The acid groups of the poly(acrylic acid) block of poly(ethylene glycol)-*b*-poly(N-isopropylacrylamide)-*b*-poly(acrylic acid) triblock copolymer were reacted with 2-hydroxyethyl acrylate (HEA) to yield the corresponding acrylate-functionalized copolymer (**P1**). Following this, **P1** was reacted with a thiol functionalized cross-linker (**CL**) resulting in the formation of core cross-linked polymeric nanoparticles through acrylate–thiol Michael reaction. The ability of these nanoparticles to encapsulate drug molecules inside their core and their effective release following a pH-triggered controlled degradation of the core were demonstrated. The temperature sensitive release behaviour of the system was also studied. The non-toxic nature of the precursor polymers and the core cross-linked polymeric nanoparticles was also established, that further substantiated their potential as carriers for controlled release of drugs.

1. Introduction

Nano sized cross-linked polymeric nanoparticles have emerged as promising drug carriers due to their outstanding performance in the field of controlled and site-specific delivery of potent lipophilic anticancer drugs. Cross-linked star polymers have been extensively studied owing to numerous potential applications, such as carriers of small molecules, chemical sensors, tissue engineering, cosmetics, catalysis and others [1–4]. Temperature- and pH-triggered disassembly of the cross-linked nanostructures have been particularly interesting due to their relevance to biomedical sciences. The two main approaches for the synthesis of cross-linked star polymers that have been explored till now are – (i) shell cross-linking [5–10] and (ii) core cross-linking [11–21], Fulton et al. [19–21] and McCormick et al. [22,23] reported the synthesis of a wide

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range of thermoresponsive and pH-degradable shell and core cross-linked polymer assemblies using poly(N-isopropylacrylamide) (PNIPA) as thermoresponsive polymer and acid cleavable immine bond. Development of core cross-linked micelles or nanogels with labile core that de-crosslink under weakly acidic and reductive environment [24–30], thereby releasing the encapsulated drug molecules, has found significant interest in the field of chemotherapy [31,32]. β-Thioester formed via thiol–acrylate Michael addition reaction for a variety of organic molecules as well as polymers was shown by Li et al. [33]. Likewise, phosphine catalyzed nucleophile mediated thiol–acrylate Michael addition reaction and the acid sensitive properties of such β-thiopropionate have been applied for the development of pH-sensitive soft materials [34].

In this work, we have aimed at establishing a simple synthetic approach for the synthesis of core cross-linked polymer nanostuctures (CLPN) utilizing thiol–acrylate Michael addition reaction between an acrylate functionalized triblock copolymer and a thiol functionalized two-arm cross-linker. The synthesized linear triblock copolymer consisted of a thermo-responsive PNIPA block, a biocompatible PEG block, as well as a reactive block containing pendant acrylate functionalities which participated in the cross-linking reaction. Presence of the non-reactive or inert blocks was expected to prevent macroscopic phase separation of the cross-linked nanostructures. We have further investigated the pH-induced disaggregation of the cross-linked networks, which is important attributes necessary for applications involving controlled release of drug molecules.

2. Experimental

2.1. Materials and methods

Nile red, Prednisolone and α,α′-dichloro-p-xylene were purchased from Sigma–Aldrich and used as received. All the other chemicals and solvents were purchased from SRL and Spectrochem (India) and purified by standard procedure. ^{1}H NMR spectra were obtained on a Bruker DPX-200 and 400 MHz NMR spectrometer and spectra were calibrated using TMS as the internal standard using $CDCl_3$, d_6-DMSO as NMR solvents. Molecular weight and PDI of the polymers were determined by a GPC instrument (Viscotek) using RI detector and THF as eluent at a flow rate of 1 ml/min. The molecular weights were calculated relative to polystyrene standards. The absorption and fluorescence spectra were collected using a UV–vis spectrophotometer (Shimadzu UV-1601) and spectrofluorometer (Hitachi F-7000) respectively. A negative staining technique was used for the transmission electron microscopy (TEM) studies of the CLPN. A drop of the sample solution was allowed to settle on a carbon coated copper grid for 1 min. Excess sample was wiped away with filter paper, and a drop of 1% uranyl acetate solution in methanol was allowed come in contact with the sample for 1 min. The samples were air dried and analyzed using a JEOL JEM 100CX microscope operating at 80 kV. The hydrodynamic size of the nanoaggregates were obtained from dynamic light scattering at 22 °C using a Malvern Nano ZS instruments employing a 4 mW He–Ne laser operating at wavelength of 633 nm.

2.2. Synthesis of (4-mercaptomethyl phenyl)-methanethiol (*CL*)

1 g (5.883 mmol) of α,α′-dichloro-p-xylene was dissolved in 10 mL of ethanol and 20 mL of water was added to it. Thiourea (0.89 g, 11.7 mmol) was mixed with the solution and the reaction mixture was then refluxed for 2 h. A solution of NaOH (0.5 g, 12.5 mmol) in 5 mL of water was added to it and it was further stirred under refluxing condition for another 3 h. The mixture was then allowed to cool, ethanol removed by evaporation and the aqueous part acidified with concentrated HCl followed by extraction with diethyl ether. After three extractions, the ether portions were combined, dried with anhydrous sodium sulphate and dried to evaporation to yield the cross-linker molecule **CL** (Yield 0.78 g, 79%), ^{1}H NMR ($CDCl_3$): δ 1.75 (t, 2H, J = 7.6 Hz), 3.70 (d, 4H, J = 7.6 Hz), 7.2 (s, 4H). ^{13}C NMR ($CDCl_3$): δ 32.4, 128.2, 139.4. (Fig. S1).

2.3. Synthesis of poly(ethylene glycol)-b-poly(N-isopropylacrylamide)-b-poly(acrylic acid) triblock copolymer

The detailed synthesis of the triblock copolymer, poly(ethylene glycol)-*b*-poly(N-isopropylacrylamide)-*b*-poly(acrylic acid) via RAFT polymerization has been described in our earlier report [35]. In brief, PEG based CTA was used for the block copolymerization of tBA and then the synthesized poly(ethylene glycol)-*b*-poly (t-butyl acrylate) was used as a macro-CTA for the polymerization of NIPA to yield the corresponding triblock copolymer, followed by hydrolysis of the tBA units to form the said acrylic acid based triblock copolymer.

2.4. Synthesis of hydroxyethylacrylate functionalized triblock copolymer (*P1*, Scheme 1)

In a 100 mL two-neck round-bottomed flask equipped with a magnetic stirring bar, poly(ethylene glycol)-*b*-poly(N-isopropylacrylamide)-*b*-poly(acrylic acid) (1.0 g, 1.2 mmol) was added in 15 mL of dry THF and stirred for 30 min for complete dissolution. It was then mixed with 2-hydroxy ethylacrylate (HEA) (1.09 g, 4.0 mmol) and the resultant mixture was stirred in an ice bath at 0 °C for 15 min. Dicyclohexylcarbodiimide (DCC) (0.42 g, 2.0 mmol) in 5 mL dry THF was added very slowly for 45 min at the same temperature and the reaction mixture was further stirred at room temperature for 48 h. After that, the insoluble dicyclohexylurea (DCU) was removed by filtration, and the filtrate was concentrated on a rotary evaporator and precipitated into an excess of cold diethyl ether. The process was repeated thrice and the final precipitates were collected and dried under high vacuum for overnight to afford **P1** as a white solid powder (0.7 g, yield 62%). ^{1}H NMR (d_6-DMSO): δ 1.08 (br, CH(C**H**$_3$)$_2$ of NIPA), 1.47 (br, $CHCH_2$ polymer backbone), 1.84 (br, $CHCH_2$ polymer backbone), 3.56 (br, C**H**$_2$—C**H**$_2$O— PEG), 3.84 (br, C**H**(CH$_3$)$_2$ of NIPA), 3.42 (br, C**H**$_2$—C**H**$_2$O— of HEA), 5.89 (br, C**H**$_2$=CH— of HEA), 6.15 (br, CH$_2$=C**H**— of HEA), 6.34 (br, C**H**$_2$=CH— of HEA).

2.5. Synthesis of CLPN via thiol–acrylate Michael reaction (Scheme 1)

P1 (0.2 g) taken in a two-neck round bottomed flask, was dissolved in 2 mL of dry THF/DMSO and the flask was purged with nitrogen for 15 min. Different mol% of **CL** was then added with dimethylphenylphosphine (10 mol% with respect to **CL**) and the solution was stirred for 6 h under dry Ar atmosphere. The reaction was stopped and solvent was evaporated under reduced pressure. It was then precipitated in large excess of cold diethyl ether. The process was repeated thrice, the solid residue was washed several times with ether and hexane, collected and dried under high vacuum for overnight to afford the product as a white solid powder (yield = 74%). The formation of the CLPN was confirmed from ^{1}H NMR, GPC, DLS and TEM images.

2.6. Encapsulation of Nile Red in CLPN

Nile Red (9.56 mg, 30 μmol) was mixed with 10 mg of the **CLPN 1** in 1 mL of HPLC grade DMSO, followed by addition to 20 mL of milliQ water at the rate of 10 μL/min under vigorous stirring. This