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A novel starch-based stimuli-responsive nanosystem for theranostic applications

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ABSTRACT

The aim of this study was to synthesis and characterization of a novel stimuli-responsive polymeric nanosystem for theranostic applications. For this purpose, starch was modified by itaconic anhydride to afford an itaconat-functionalized starch macromonomer (starch-IA). This macromonomer with carboxylic functional groups was subsequently adsorbed onto the surface of iron oxide nanoparticles (Fe_3O_4 NPs), and then copolymerized with *N*-isopropylacrylamide (NIPAAm) monomer *via* a 'free' radical initiated polymerization technique to produce a temperature-responsive magnetic nanohydrogel (MNHG). The chemical structures of all samples as representatives were characterized by means of Fourier transform infrared (FTIR) spectroscopy. The lower critical solution temperature (LCST), thermal responsibility, morphology, elemental composition, thermal stability, and magnetic properties of the synthesized MNHG were investigated. In addition, the methotrexate (MTX)-loading capacity (~74%) and stimuli-responsive drug release ability of the synthesized MNHG were also evaluated. As results, we envision that the synthesized starch-*g*-PNIPAAm/Fe_3O_4 MNHG may be find theranostic applications, in part due to its smart physicochemical properties.

1. Introduction

It is an unquestionable fact that design and development of *de novo* drug delivery systems for enhanced delivery of therapeutic agents is one of the most important challenges for biomedical research [1,2]. In this respect, naturally occurring polymers-based functional biomaterials are attracted a great deal of attention as vehicles for the storage or transport of active species. These polymers are mainly divided into three classes including polysaccharides, polyamides, and polyesters [3–5]. Among these polysaccharides are considerable of interest mainly due to their low toxicity, biocompatibility, biodegradability, abundances, low cost, and multivalent-binding ability that qualified them for a wide range of biomedical applications such as, wound healing, tissue engineering as well as drug and gene delivery [6–8]. Chitosan [9], alginate [10], dextran [11], cyclodextrins [12], hyaluronic acid [13] as well as starch [14] are the main polysaccharides which are applied for drug delivery purpose.

Among them, starch is a biodegradable, inexpensive, renewable, and abundantly available macromolecule, which consists of two polysaccharides known as amylose (AM; typically 18–33%) and amylopectin (AP; typically 67–82%). These polysaccharides contains α-D-glucose units connected through α-1,4 and α-1,6 linkages. Starch can be produced from wide variety botanical sources such as cereal, legume, root and tuber, and unripe fruit [15–19]. Starch can be incorporated in numerous applications such as, packaging industries, food technology, cushioning and insulating materials, foam panels, building materials, mulch films, dermatological and cosmetic, cigarette smoke filter, shoe component, wood adhesives, as well as biomedical applications (*e.g.,* drug delivery, biocatalysts, and tissue engineering) [20–23]. Among the mentioned applications, the drug delivery application is of particular interest due to its some inherent advantages, such as improving drug stability and solubility, excellent biocompatibility, decreasing drug toxicity and side effects, and storage stability [24–26].

However, some drawbacks of native starches such as, water insolubility, low resistance to shear, low stability under various temperatures and pHs, highly resistant to enzymatic hydrolysis, and their tendency to easily retrograde and undergo syneresis and therefore form unstable pastes and gels restrict their application range [17,27,28]. Therefore, the modification of this natural polymer is an important step for extending its application range. Several

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strategies such as, chemical (*e.g.,* functionalization or polymer grafting through its hydroxyl groups, oxidation, and crosslinking) [29,30], physical (*e.g.,* treatment of native starch under different temperature/moisture combinations, pressure, shear, and irradiation) [31,32], enzymatic [33], and biotechnological modifications [34], as well as preparation of starch-based composites [35,36] have been developed for starch modification. Among these, the polymer grafting method with tailored surface properties is of particular interest due to their potential biomedical applications such as, drug and gene delivery. In this context, grafting of thermo and/or pH-responsive polymers onto starch can be considered as an efficient approach toward smart hydrogels, which can applied as *de novo* drug delivery systems [37–40].

Since the introduction of theranostic term by Funkhouser in 1998 [41], as a materials that combines therapy with diagnostic, more and more research efforts have been devoted to design and development of navel multi-functional (nano-)biomaterials as theranostic (nano-)systems [42–44]. In theranostic nanosystems passive and/or active targeting nanoparticles can target the disease site and deliver therapeutic agents in response to external stimuli, which lead to improve therapeutic outcomes with minimum side effects [45–47]. In the last decade, a wide verity of nanomaterials such as, superparamagnetic iron oxide nanoparticles (SPIONPs), [48,49], carbon nanotubes (CNTs) [50], gold nanoparticles [51], graphene and its derivatives [52], and quantum dots (QDs) [50] have been developed for theranostic applications. In this context, SPIONPs is approved by FDA, and has been received much attention due to its excellent physicochemical characteristics such as, superparamagnetism, high field irreversibility, high saturation field, extra anisotropy contributions or shifted loops after field cooling [53–55]. These nanosystems can be targeted to the disease site through external magnets, and the nanoparticles no longer show magnetic interaction after removing of the external magnetic field. However, it is worth noting that the non-degradable feature of SPIONPs restrict their repeated applications for therapeutic efficacy due to their accumulation [56,57].

In this investigation, the design and development of a novel stimuli-responsive polymeric nanosystem for theranostic applications is reported. To aim this purpose, the itaconat-functionalized starch macromonomer (starch-IA) with carboxylic functional groups was adsorbed onto the surface of Fe_3O_4 NPs, and then copolymerized with *N*-isopropylacrylamide monomer *via* a 'free' radical initiated polymerization technique to afford a temperature-responsive magnetic nanohydrogel (MNHG). Some physicochemical characteristics such as, LCST, thermal responsibility, morphology, elemental composition, thermal stability, and magnetic properties of the synthesized MNHG were investigated. In addition, the MTX-loading capacity and stimuli-responsive drug release ability of the nanosystem were also examined.

2. Experimental

2.1. Materials

N-Isopropylacrylamide (NIPAAm, 97%, Sigma-Aldrich, USA) was purified through recrystallization from *n*-hexane/toluene mixture (90/10 v/v) before use. Corn starch (27% amylose), ammonium hydroxide (NH_4OH, 25 wt.% of ammonia), itaconic anhydride (IA), ammonium peroxydisulfate (APS), sodium hydroxide (NaOH), ferrous chloride tetrahydrate ($FeCl_2.4H_2O$, 99%), and ferric chloride hexahydrate ($FeCl_3.6H_2O$, 98%) were purchased from Merck (Darmstadt, Germany), and were used as received. All other reagents were purchased from Merck and purified according to the standard methods.

2.2. Synthesis of itaconat-functionalized starch macromonomer (starch-IA)

The starch-IA was synthesized by esterification of starch in an aqueous medium as described in literature [18]. In brief, a 100 mL two-neck round-bottom flask equipped with a condenser, and a magnetic stirrer, was charged with starch (4.00 g; 0.05 mol anhydroglucose unit or AGU), and deionized water (50 mL). The dispersion mixture was stirred, then added NaOH solution (2 $molL^{-1}$; 50 mL) and the reaction mixture was stirred for about 1 h at room temperature. At the end of this period, itaconic anhydride (5.6 g; 0.05 mol) was added and the reaction mixture was stirred continuously for about 4 h at 100 °C. The starch-IA macromonomer was separated by precipitation the contents of the flask into a large amount of ethanol, filtrated, washed several times with ethanol and dried in reduced pressure at room temperature (Scheme 1). The degree of functionalization (DF) corresponding to the carboxylic acid value of the polymer was calculated to be 0.27 through the chemical titration with HCl solution (0.1 mol L^{-1}).

2.3. Synthesis of Fe_3O_4 nanoparticles

The Fe_3O_4 NPs were synthesized by coprecipitation method as described in our previous works [53,54]. Briefly, a 250 mL three-neck round-bottom flask equipped with a condenser, gas inlet/outlet, and a magnetic stirrer was charged with deionized water (150 mL), and then de-aerated by bubbling highly pure argon gas for 30 min. At the end of this time, ferric chloride (40 mL; 0.1 $molL^{-1}$), and ferrous chloride (20 mL; 0.1 $molL^{-1}$) were added, and then flask was heated to 80 °C followed by addition of NH_4OH (10 mL, 25 wt.%) under vigorous stirring. The resultant suspension was maintained at 80 °C for about 1 h while being stirred under argon protection, and then cooled to room temperature. The precipitated black NPs were washed five times with water, and ethanol until its pH becomes completely neutral and dried under reduced pressure at room temperature.

2.4. Synthesis of starch-IA/Fe_3O_4

A 50 mL round-bottom flask was charged with starch-IA (200 mg), Fe_3O_4 NPs (200 mg), and 20 mL deionized water. The mixture was irradiated with ultrasonic vibrations for 10 min, and then stirred for about 24 h at room temperature. At the end of this period, the un-modified Fe_3O_4 NPs were precipitated by centrifugation at 7000 rpm for about 15 min. The starch-IA/Fe_3O_4 was further purified by external magnetic field and decantation process for several times, in order to remove un-adsorbed polymeric chains (Scheme 2).

2.5. Synthesis of starch-g-PNIPAAm/Fe_3O_4 MNHG

The starch-g-PNIPAAm/Fe_3O_4 magnetic nanohydrogel (MNHG) was synthesized *via* precipitation polymerization method. In brief, a 100 mL three-neck round-bottom flask equipped with a condenser, gas inlet/outlet, and a magnetic stirrer was charged with starch-IA/Fe_3O_4 ferrofluid (20 mL; 12.6 mg mL^{-1}), and NIPAAm monomer (0.50 g, 4.4 mmol). The reaction mixture was de-aerated by bubbling highly pure argon for 10 min. In a separate container, 65 mg (0.28 mmol) of APS was dissolved in distilled water (30 mL). This solution was de-aerated by bubbling highly pure argon for about 15 min, and then added to the above mentioned reaction mixture as radical initiator. The flask was placed in a silicon oil bath at 70 ± 3 °C, and the reaction mixture was refluxed for about 24 h under argon atmosphere. The crud product was purified through external magnetic field and decantation process for several