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pH-controlled sunitinib anticancer release from magnetic chitosan nanoparticles crosslinked with κ-carrageenan

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ABSTRACT

The main objective of this work was to develop κ-carrageenan-crosslinked magnetic chitosan with different molecular weights as pH-responsive carriers for controlled release of anticancer drug sunitinib. The characterization of magnetic carriers revealed that the size of magnetic nanoparticles is affected by the molecular weight of chitosan. Drug encapsulation efficiency and release performance influenced by the size of magnetic nanoparticles. Encapsulation efficiencies of sunitinib by low, medium and high molecular weights of magnetic chitosan carriers were found to be 62.38, 69.57 and 78.42%, respectively. The in vitro sunitinib release from magnetic chitosan/κ-carrageenan carriers was pH-dependent and followed a Fickian release mechanism. Sunitinib was efficiently released from magnetic carriers into environment under acidic pHs and the release rate was size- and molecular weight-dependent. The pH-dependent release of sunitinib with a minimal release content at pH = 7.4 makes the present magnetic carriers as promising candidate for anticancer drugs with reduced side effects.

1. Introduction

Because of threatening the human health by the malignant cells and tumors the cancer therapy using chemotherapy is mainly performed. Conventional chemotherapy to treat cancer cells is often associated with side effects to healthy tissues [1]. A suitable approach for reducing the side effects of cancer therapy through chemotherapy is the fabricating smart drug delivery system (DDS). The proposed systems should release the drug to a tumor region with minimizing release of drug to normal tissues [2]. In order to reach an optimum therapeutic threshold the designing a DDS able to respond the stimuli such as pH, temperature, magnetic, and light is demanded [3]. Among them, designing the pH-responsive DDSs with the ability to release the anticancer drug at the target sites has attracted a great research interest [4]. According to the chemical structure of drug and carriers, the pH-responsive DDS can release the encapsulated drug in a controlled manner in accordance to different pH condition [5]. Designing DDS with a pH-responsiveness feature is originated from the relatively acidic condition of interstitial of tumors regions (pH = ~6.5–7) as well as the pH inside the lysosomes of cells in inflamed regions (pH = ~5–5.5) [6]. In relation to the controlled release of various anticancer drugs, pH-responsive DDS using a variety of structural formulations and designing pathways have been investigated. Poly(ethylene glycol)-*graft*-Dextran [7], sodium alginate/polyvinyl alcohol/bovine serum albumin/Fe_3O_4 [8], β-Cyclodextrin Assemblied Fe_3O_4 Nanoparticles [9], calcium carbonate nanospheres [10], mesoporous MCM-41/polyvinyl pyrrolidone nanoparticles [11], and polyethylene glycolate-mesoporous silica nanoparticles [1] have been studied for controlled release of doxorobicine as popular anticancer drug. In addition to doxorobicine, the encapsulation and loading of methotrexate, cisplatin, and paclitaxel drugs have been investigated using pH-responsive carriers of hyaluronic acid/polyamidoamine nanoparticles, poly(N-isopropyl acrylamide/methacrylic acid nanogel, and cyclodextrin-derived pH-responsive nanoparticles, respectively [12–14]. Sometimes, an ideal result using an individual pH-responsive DDS which begins to release the drug by the variation of pH cannot achieve. Embedding other external stimuli such as magnetic results in a dual-sensitive DDS (pH- and magnetic-responsive DDS), receiving a favorable release performance [15]. In fact, the pH−/magnetic-responsive DDS not only deliver the drug under internal biological stimuli e.g. pH, but also the accelerating or diminishing drug release can be controlled by an external stimuli e.g. magnetic field [16]. In the last two decades, due to the biocompatibility and super-paramagnetic behavior, magnetic Fe_3O_4 nanoparticles have gained increasing interest as promising new systems such as carriers for anticancer drugs, contrast enhancement of magnetic resonance imaging, hyperthermia, and bioseparation [17, 18]. To improve their drug encapsulation efficiency and

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preventing their tendency to aggregate as well as targeted drug delivery the magnetic Fe_3O_4 nanoparticles have been modified using ligands, antibodies, receptors, biopolymers, and functional nanostructures [19]. Combining magnetic nanoparticles into supplementary polymers especially pH-responsive biopolymers systems such as chitosan, alginate, pectin, and proteins not only prevents the aggregation of Fe_3O_4 nanoparticles, but also a controlled and sustained release of toxic anticancer drug encapsulated in biopolymer/Fe_3O_4 system occurs [20–23]. Chitosan, a biocompatible and biodegradable polysaccharide with basic character due to the primary amine groups (-NH_2), has increasingly used for fabricating biomedical devices especially in drug and gene delivery [24]. Incorporating chitosan with magnetic nanoparticles not only can enhance the drug loading content and drug release efficiency, but also Fe_3O_4 nanoparticles can gain a pH-responsive property [25].

The loading efficiency and also the rate of drug release can be affected by the size of nanoparticles. One of the most important factors in the formation of nanoparticles with different size is the molecular weight of the used polymer. The loading and release of 5-fluorouracil and bovine serum albumin (BSA) using nanoparticles prepared with different molecular weight of chitosan showed that the release of drug and BSA decreases by increasing molecular weight of chitosan [26, 27]. Tripolyphosphate-crosslinked magnetic chitosan nanoparticles with different sizes (controlled by the amount of the used NH_4OH) have been investigated for doxorubicin release. The decrease in the magnetic chitosan nanoparticles sizes caused an increase in the rate of doxorubicin release [16]. In order to stabilize the chitosan nanoparticles with tripolyphosphate, some chemical crosslinkers such as epichlorohydrin and glutaraldehyde should be combined [28]. The toxic property of chemical crosslinkers limits the use of them in designing drug carriers. Among polyionic biopolymers, anionic κ-carrageenan (containing $-OSO_3^-$) can produce highly stable ionic crosslinked chitosan/κ-carrageenan hydrogels through the electrostatic interactions between positively charged amine groups on chitosan and negatively sulfate groups on κ-carrageenan [29].

To the best of our knowledge there is no report on formation of magnetic Fe_3O_4 nanoparticles through in situ method in the presence of chitosan with different molecular weights. Thus, we tried to investigate the effect of chitosan molecular weight on the formation of magnetic nanoparticles. The as-obtained magnetic chitosan nanoparticles were crosslinked with κ-carrageenan and their ability in loading and release of sunitinib anticancer drug was investigated.

2. Materials and methods

2.1. Materials

Low molecular weight of chitosan (LCS, 75–85% of degree of deacetylation, average M_w = 50–190 kDa), medium molecular weight of chitosan (MCS, 75–85% of degree of deacetylation, M_w = 190–310 kDa), and high molecular weight of chitosan (HCS, 75–85 of degree of deacetylation, M_w = 310–375 kDa) were purchased from Sigma–Aldrich Co., USA. Using potentiometric titration method, the degree of deacetylation of chitosan samples were determined [30]. κ-Carrageenan was obtained from Condinson Co. (Denmark, molecular M_w: 90 kDa; 96% of purity). Sunitinib malate was obtained from Sigma-Aldrich (USA). Iron salts, $FeCl_2{\cdot}4H_2O$ and $FeCl_3.6H_2O$, were obtained from Merck, Germany. All other chemicals were analytical grade and used as received.

2.2. Preparation of magnetic carriers

The magnetic carriers were generated through in situ co-precipitation of iron ions in the presence of chitosan with different molecular weights. Aqueous solution of κ-carrageenan was used for crosslinking the as-obtained magnetic chitosan nanoparticles. In overall, chitosan solutions were separately prepared by pouring 1 g of chitosan with different molecular weights in 100 mL acetic acid solution (1%wt) and allowed to dissolve entirely at 70 °C for 1 h. Then, 2 g of $FeCl_2{\cdot}4H_2O$ and 5.4 g of $FeCl_3{\cdot}6H_2O$ iron salts (nFe^{3+}/nFe^{2+} = 2) were dissolved in 20 mL of distilled water and added into chitosan solutions. The solutions purged with N_2 inert gas for 30 min. Ammonia solution (3 M) was slowly dropped into (Fe^{3+}/Fe^{2+})-loaded chitosan solutions until the pHs of the solutions reached to 11. When the pHs of solutions were adjusted at 11, dark solutions indicated the formation of Fe_3O_4 nanoparticles. The magnetic dispersions were allowed to stir at 70 °C for 1 h. The obtained magnetic chitosan dispersions were thoroughly washed with excess distilled water to remove unreacted reagents. The purification was continued until the pHs of solutions reached 7. To obtain homogeneous solutions, the purified magnetic chitosan samples were dispersed in 100 mL distilled water and sonicated at the frequency of 50 kHz for 30 min (Bandelin SONOPULS HD 2200). Then, κ-carrageenan solutions were distinctly prepared by dissolving 0.2 g of κ-carrageenan in 100 mL distilled water at 70 °C for 2 h. Eventually, magnetic chitosan dispersions were gradually added in the κ-carrageenan solution and stirred at 400 rpm at 70 °C for 45 min. By adding dilute HCl solution (0.1 M) the pHs of magnetic chitosan/κ-carrageenan mixtures was adjusted at 5.7. The coagulum-like particles were immediately formed due to the electrostatic interactions between magnetic chitosan and κ-carrageenan. The magnetic chitosan/κ-carrageenan carriers were separated by a magnet and the magnetic carries were further frozen by freeze-dryer (Freeze-dryer, Alfa 2-4LDplus, Christ Co., Germany). The protocol included flash freezing in liquid N_2, freezing at −80 °C under the vacuum pressure of 0.001 mBar and finally cooling at 4 °C for 48 h. The magnetic carriers were denoted as mLCSCar0.2, mMCSCar0.2, and mHCSCar0.2, where the “m” indicates “magnetic”, Car denotes to “κ-carrageenan”; and 0.2 indicates the amount of used κ-carrageenan (g).

2.3. Swelling measurements

The gravimetric analysis was used in order to study the swelling of magnetic carriers in distilled water. Approximately, 0.1 g of dried magnetic samples were immersed in 25 mL of distilled water and allowed to soak for 24 h to attain swelling equilibrium. The swollen magnetic carriers were filtered through a 400-mesh nylon bag and allowed to remove the un-absorbed water for 10 min. The degree of swelling (DS, g/g) of magnetic carriers was calculated by the Eq. (1):

$$DS\,(g/g) = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

where, the W_s and W_d are the weights of swollen and dried magnetic carriers, respectively. The all swelling experiments were done three times and the mean of data were reported.

2.4. Drug loading/release

The procedure used for preparing magnetic chitosan-based carriers for sunitinib malate was similar to the method mentioned above. The obtained magnetic chitosan dispersions were purified using distilled water until the washings were reached to neutral pH e.g. 7. The volume of magnetic chitosan dispersions were adjusted at 100 mL by adding distilled water. 10 mL of sunitinib solution (1 = mg/mL in phosphate buffer solution) was poured into magnetic chitosan dispersions and allowed to stir at ambient temperature for 2 h. The resultants were sonicated at the frequency of 25 kHz for 30 min. Magnetic chitosan dispersions containing sunitinib were slowly added in the κ-carrageenan solution and stirred at 400 rpm for 45 min. By adding dilute HCl solution (0.1 M) the pHs of sunitinib-loaded magnetic chitosan/κ-carrageenan was adjusted at 5.7. The drug-loaded magnetic carriers were separated by a magnet and were further frozen and dried by freeze-dryer. Using UV-spectrophotometer (Shimadzu UV-1800), the encapsulation efficiency (EE) of sunitinib was determined at