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Chitosan-graft-β-cyclodextrin nanoparticles as a carrier for controlled drug release

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ABSTRACT

Chitosan (CS) grafted with β-cyclodextrin (CD-g-CS) nanoparticles as a new carrier for poorly water-soluble drugs has been developed. The CD-g-CS polymer is readily synthesized from chitosan and mono-6-deoxy-6-(*p*-toluenesulfonyl)-β-cyclodextrin. Three different degrees of substitution (DS) of β-cyclodextrin (β-CD) on CD-g-CS (9.6, 14.0 and 20.0%) are designed and evaluated by controlling the mole ratio of β-CD to chitosan. Then CD-g-CS nanoparticles are prepared by an ionic gelation method, with the controlled size of 202.0–589.0 nm. Stable colloidal dispersion of the nanoparticles has been formed with the zeta potential of +23.0 to +43.0 mV. In vitro stability test indicates that CD-g-CS nanoparticles are more stable in phosphate-buffered saline compared with CS nanoparticles. Finally, the poorly water-soluble drug, ketoprofen (KTP), is used as a model drug to evaluate the efficiency of the new drug delivery carrier. It is found that the encapsulation efficiency of KTP in the nanoparticles with 20% DS of CD is as high as 1.36-fold than that of CS nanoparticles. Moreover, notably KTP is released from the nanoparticles in a controlled-release manner and is pH-responsive on DS of CD. In summary, these results suggest that the CD-g-CS nanoparticles, as a general promising drug delivery system, can be used as a potential biodegradable nano-drug delivery system for controlled release of poorly water-soluble drugs with pH-responsive capability.

1. Introduction

Nanoparticles (NP) have been widely investigated as new drug delivery systems in the pharmaceutical research. In general, they display capacity of solubilizing drugs, protecting them from degradation, enhancing their absorption by facilitating diffusion through epithelium and subsequently bioavailability, and modifying their tissue distribution profile (Ahmed et al., 2012; Rajesh and James, 2009). Chitosan (CS), as an attractive biodegradable and biocompatible polymer, has been extensively employed in the preparation of nanoparticles. The polymer features the controlled ability in a drug release system, mucoadhesive character of increasing residual time at the site of absorption, and good solubility in aqueous acidic solution which eliminates the use of hazardous organic solvents while allowing for fabricating particles. Furthermore, it can be readily functionalized and chemically cross-linked via its ionic amino groups with multivalent anions (Sunil et al., 2004). Several research groups have reported the use of CS nanoparticles for the delivery of poorly water-soluble drugs (Jameela et al., 1996; Mitra et al., 2001) as well as water-soluble protein (Gan and Wang, 2007; Lubben et al., 2001). Recently, they are also intensively explored in gene delivery (Mao et al., 2001; Susan et al., 2011).

Cyclodextrin (CD) contains a hydrophilic outer surface and a lipophilic central cavity. It has been used as host units to construct host–guest delivery carriers. Due to hydrophobic interactions, a variety of lipophilic drugs which are able to fit inside the hydrophobic cavity can be accommodated, thus improving the solubility of hydrophobic drugs and the stability of labile drugs (Liu and Zhu, 2007; Marques et al., 1990). Grafting CD molecules onto CS backbone can result in a mucoadhesive delivery systems, having cumulative effects of inclusion, bioavailability improvement, and specific mucosal targeting (Prabaharan and Mano, 2006). Different types of chitosan-graft-β-cyclodextrin (CD-g-CS) derivatives have been synthesized (Devassine et al., 2001; Khaled et al., 2006), which were designed as adsorbent matrices for the delivery of organic molecules (Warayuth et al., 2012) and scaffolds for tissue engineering applications (Prabaharan and Jayakumar, 2009). Insulin/CD-g-CS nanocomplexes have also been prepared, based on the electrostatic interaction between insulin and CD-g-CS (Zhang et al., 2009). Nevertheless, there is no report for preparing CD-g-CS

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nanoparticles through an ionic gelation method, which may afford a new approach for the construction of drug carrier/delivery systems.

With a molecular weight of 254.28 g/mol and a low aqueous solubility of 250 μg/ml for the undissociated molecule (Soheila et al., 2010), ketoprofen (KTP) is a potent non-steroidal anti-inflammatory drug commonly used for the treatment of acute and chronic rheumatoid arthritis. It is easily absorbed from the gastrointestinal tract, where a high concentration often generates side effects (Meastrelli et al., 2006). Due to its short plasma half-life (2–4 h), it must be frequently administered. The short half-life, poor solubility in water, low bioavailability and side effects in the gastrointestinal tract make KTP a promising choice for formulation of a controlled-release dosage form (Sheng et al., 2006).

Toward this end, we wish to report a new drug carrier made of CD-g-CS for a poorly water-soluble drug (e.g., KTP). The CD-g-CS polymers with three different degrees of substitution (DS) of β-cyclodextrin (β-CD) are designed, synthesized and evaluated. CD-g-CS nanoparticles are prepared via ionic gelation using sodium tripolyphosphate (TPP) as cross-linking agent for the first time. The effects of forming process on the physicochemical properties of those nanoparticles and in vitro stability are investigated. Finally, KTP is selected as a model drug to investigate the properties of the new delivery system. The KTP-loaded CD-g-CS nanoparticles are characterized and KTP release in vitro at different pH conditions is further determined. The studies reveal that the encapsulation efficiency of KTP in the nanoparticles with 20% DS of CD is as high as 1.36-fold than that of CS nanoparticles. Furthermore, notably KTP is released from the nanoparticles in a controlled-release manner and is pH-responsive on DS of CD.

2. Materials and methods

2.1. Materials

Chitosan (CS, Mw = 110 kDa, 90.0% deacetylation degree) was purchased from Yuhuan Ocean Biochemical Co. Ltd. (Zhejiang China). β-Cyclodextrin (β-CD) and sodium tripolyphosphate (TPP) were purchased from Shanghai Chemical Reagent Company (China). *p*-Toluenesulfonyl chloride was purchased from Shanghai Ling-Feng Chemical Reagent Co. Ltd. (China). Ketoprofen (KTP) was provided by Shanghai Aidie Chemical Reagent Co. Ltd. (China). All other reagents used were commercially available and were of analytical grade. The above materials were used from the indicated sources without further purification procedures.

2.2. Preparation of mono-6-deoxy-6-(p-toluenesulfonyl)-β-cyclodextrin (6-OTs-β-CD)

6-OTs-β-CD was synthesized as described by the previously reported method (Petter et al., 1990). Briefly, *β*-CD (30.0 g, 26.4 mmol) was suspended in 250 ml of water, and NaOH (82.1 mmol) in 10 ml of water was added dropwise over 6 min. The suspension became homogeneous and slightly yellow before the addition was complete. *p*-Toluenesulfonyl chloride (5.04 g, 26.4 mmol) in 15 ml of acetonitrile was added dropwise over 8 min, causing immediate formation of a white precipitate. After 2 h of stirring at 23 °C, the precipitate was removed by filtration and the filtrate was refrigerated overnight at 4 °C. The resulting white precipitate was recovered by filtration and dried for 12 h.

2.3. Preparation of chitosan grafted with β-cyclodextrin (CD-g-CS)

CD-g-CS was synthesized using Gonil's method (Gonil and Sajomsang, 2011) with slight modification. Briefly, CS (1.0 g) was dissolved in 1% (v/v) acetic acid (pH 4, 80 ml). The solution of 6-OTs-β-CD (1.0–5.0 g) in *N*,*N*-dimethylformamide (DMF, 40 ml) was added into the CS solution. The reaction mixture was refluxed at 100 °C for 16 h and dialyzed with deionized water for 3 days. The solution was then freeze-dried to give a cotton like powder of CD-g-CS. The CD-g-CS with different degrees of substitution (DS) could be obtained by adjusting mass ratio of 6-OTs-β-CD to CS.

2.4. Characterization of CD-g-CS

The Fourier transform infrared (FT-IR) spectra of the products was recorded on a double-beam Mattson Galaxy Series FTIR-3000 spectrometer in the range of 4000–400 cm^{-1} using KBr pellets (Zhu et al., 2006).

The ^1H NMR spectrum of the samples was recorded on a Bruker DPX300 spectrometer using tetramethylsilane as an internal standard and D_2O as a solvent at 25 °C (Gonil and Sajomsang, 2011; Zhu et al., 2006).

The X-ray diffraction (XRD) experiments were performed in a XDS-2000 (Scintag Inc., USA) diffractometer using CuKα radiation source (Samuels, 1981).

2.5. Preparation of CD-g-CS nanoparticles

Nanoparticles were prepared using the mild ionic gelation method according to the procedure previously developed by Aktas et al. (2005). Nanoparticles were spontaneously obtained via ionic gelation between the positively charged amino groups of CD-g-CS and negatively charged TPP. Briefly, CD-g-CS (DS = 9.6, 14.0 and 20.0%) solutions (0.05–0.25%, w/w) were prepared with glacial acetic acid, 1.75 times that of CD-g-CS weight. Then the CD-g-CS solution was adjusted to pH = 3.5–6.0 with 0.1 N NaOH. Cross-linking agent TPP (1 ml, 0.05–0.20%, w/w) was added dropwise to CD-g-CS solution (5 ml) with magnetic stirring at 800 rpm and continued stirring for 30 min. The resultant suspension was subjected to particle size analysis. The purified nanoparticles were freeze dried to obtain dry nanoparticles with 3% mannitol as lyophilized protection agent. Every sample was prepared in triplicate and the results represented the average value.

2.6. Preparation of ketoprofen (KTP) loaded CD-g-CS nanoparticles

KTP loaded CD-g-CS nanoparticles were prepared by incorporating KTP in 0.2% (w/w) CD-g-CS (DS = 0, 9.6, 14.0, and 20.0%) by magnetic stirring at room temperature for 24 h followed by similar process explained in Section 2.5. The weight ratio of KTP to CD-g-CS was 1:2. Four independent batches of nanoparticles with KTP were prepared for physicochemical characterization and results presented as mean ± SD.

2.7. Physicochemical and morphological characterization of CD-g-CS nanoparticles

The particle size and zeta potential of the assemblies in aqueous solutions were performed using dynamic light scattering (DLS, NanoZS4700 nanoseries, Malvern Instruments, UK) equipped with 4 mW He Ne laser at a wave length of 633 nm at 25 °C.

Morphological evaluation of CD-g-CS nanoparticles was performed with transmission electron microscope (TEM). For TEM images, the particle morphology of polymer was observed on JEM-2010 JEOL from Japan. The sample was stained with 2% (w/v) phosphotungstic acid and then dropped on a copper grid.