Carbohydrate Polymers 137 (2016) 65–74

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Fabrication, characterization and cytotoxicity studies of ionically cross-linked docetaxel loaded chitosan nanoparticles

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ARTICLE INFO

Article history:
Received 21 July 2015
Received in revised form 1 October 2015
Accepted 4 October 2015
Available online 23 October 2015

Keywords:
Chitosan
Docetaxel
Tripolyphosphate
Nanoparticles
Cross-linking

ABSTRACT

The present investigation aimed at the fabrication and characterization of ionically cross-linked docetaxel (DTX) loaded chitosan nanoparticles (DTX-CH-NP) using ionic gelation technique with sodium tripolyphosphate (TPP) as the cross-linking agent. The formulated nanoparticles were characterized in terms of particle size, drug entrapment efficiency (EE), scanning electron microscopy (SEM), *in vitro* release and cytotoxicity studies. Formulation factors (chitosan, TPP and drug concentration) were examined systematically for their effects on size of the nanoparticles. The average size of the nanoparticles was observed to be in the range of 159.2 ± 3.31 to 220.7 ± 2.23 nm with 78–92% encapsulation efficiency (EE). The *in vitro* cytotoxicity studies on breast cancer cell lines (MDA-MB-231) revealed the advantages of DTX-CH-NP over pure DTX with approximately 85% cell viability reduction. The results indicate that systematic modulation of the surface charge and particle size of ionically cross-linked nanoparticles can be readily achieved with the right control of critical processing parameters. Thus, DTX-CH-NP presents a promising delivery alternative for breast cancer treatment.

1. Introduction

Cancer is a growing menace, with the number of patients increasing at an alarming rate. Chemotherapy as a treatment option has been successful, but to some extent in cancer treatment. The main drawbacks of chemotherapy include the limited accessibility of drugs to the tumor tissues, high toxicity, development of multiple drug resistance and non-specific targeting (Parveen & Sahoo, 2008). Nanoparticles (NPs), an evolution of nanotechnology, have demonstrated great potential in successfully addressing the problems related to chemotherapeutic drug delivery by providing high drug payload, improvement in the biodistribution of drugs and ability to target tumors with an enhanced accumulation (Bertrand, Wu, Xu, Kamaly, & Farokhzad, 2014; Danhier, Feron, & Preat, 2010; Ferrari, 2005; Naahidi et al., 2013).

In the recent decades, polymeric nanoparticles have witnessed an exceptional growth and usage in anti-cancer drug delivery. The high surface-to-volume ratio of polymeric nanoparticles improves the loading capacity of the selected molecule, while providing it the required protection (Peer et al., 2007; Prabhu, Patravale, & Joshi, 2015; Rodrigues, Rosa da Costa, & Grenha, 2012). These particles also help in enhancement of the therapeutic efficacy of anticancer drugs by regulating their release, improvement in stability and prolonged circulation time. A number of studies have reported that nano-sized drug carriers composed of natural and synthetic polymers sustain in the body for prolonged periods by evading the reticulo-endothelial system (RES) (Hwang, Kim, Kwon, & Kim, 2008). Polymeric nanoparticles accumulate in the tumor tissue, resulting in a disorganized vascular architecture, referred to as the enhanced permeability and retention (EPR) effect (Garcia-Fuentes & Alonso, 2012; Sultana, Khan, Kumar, Kumar, & Ali, 2013).

Chitosan-based polymeric nanoparticles have received great attention in the recent times due to their biodegradability, biocompatibility, non-toxicity and low immunogenicity (Dash, Chiellini, Ottenbrite, & Chiellini, 2011). Chitosan, the N-deacetylated form of chitin, mostly found in the exoskeleton of crustaceans, insects, and fungi, is a natural polysaccharide (Dutta, Dutta, & Tripathi, 2004; Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli, 2004). It has been recognized as a promising polymer for drug delivery, possessing high density of positive charge, attributed to the presence of glucosamine group on its backbone (Cooney, Petermann, Lau, & Minteer, 2009; Jee et al., 2012). Chitosan acts on tumor cells to interfere with the cell metabolism by inhibiting cell growth or inducing cell apoptosis (Cao & Zhou, 2005). Previous studies have reported

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http://dx.doi.org/10.1016/j.carbpol.2015.10.012
0144-8617/

that low-molecular weight and modified chitosan could inhibit tumor growth leading to good prospects for their application in cancer therapy (Maeda & Kimura, 2004). The strong mucoadhesive interactions of chitosan with the mucous membranes associated with tumors, makes it capable for efficient anticancer drug delivery (Zhou, Hong, & Fang, 2007). Chitosan contains abundant amino and hydroxyl groups, thus enabling nanoparticulate formulation *via* both physical and chemical cross-linking (Makhlof, Tozuka, & Takeuchi, 2011). Ionic cross-linking of chitosan with negatively charged multivalent ions such as tripolyphosphate (TPP) is a typical non-covalent interaction (Tsai, Chen, Bai, & Chen, 2011). Under acidic conditions, the amino group of chitosan molecule protonizes to $-NH_3^+$ which ultimately, interacts with an anion such as tripolyphosphate (TPP) to form nanoparticles (Tsai, Bai, & Chen, 2008). The reversible physical cross-linking by electrostatic interaction is a very simple method and prevents possible toxicity of reagents and other undesirable side effects (Ringel & Horwtiz, 1991).

Docetaxel (DTX), a second-generation semi-synthetic taxane derivative is effective against a variety of solid tumors including breast, ovarian, prostate and non-small cell lung cancer (Bissery, Nohynek, Sanderink, & Lavelle, 1995; Lyseng-Williamson & Fenton, 2005). The clinical applications of DTX are limited due to its poor aqueous solubility, rapid phagocytic activity, renal clearance and non-selective distribution (Zhao et al., 2010). Since, DTX is highly lipophilic and practically insoluble in water, the main marketed product of DTX (Taxotere®) used clinically is formulated using Tween 80 (Zhang & Zhang, 2013). Tween 80 tends to alter the membrane fluidity, resulting in an increase of membrane permeability associated with serious hypersensitivity reactions and cumulative-fluid retention (Dou, Zhang, Liu, Zhang, & Zhai, 2014). A number of drug delivery systems have been reported for DTX such as micelles, liposomes, self-emulsified DTX formulations (Yang, Li, Wang, Dong, & Qi, 2014), PEGylated immunoliposomes (Zhao et al., 2009) and PEG-liposomes-folic acid bioconjugates (Song et al., 2011), lipid emulsified nanoparticles (Zhang et al., 2015), folate decorated human serum albumin nanoparticles (Jiang, Gong, Zhag, & Zu, 2015), hypersensitive and biodegradable nanoparticles (Liu et al., 2012; Wu et al., 2015), and diblock copolymer nanoparticles (Tao et al., 2013). All these delivery systems are limited by the complicated preparative procedures, high cost and low stability of the formulations.

In the present study, ionically cross-linked docetaxel loaded chitosan nanoparticles (DTX-CH-NP) were formulated by ionotropic gelation method. Chitosan was cross-linked with tripolyphosphate (TPP) and the influence of a number of formulation parameters like chitosan and TPP concentration, chitosan–TPP volume ratio, pH and temperature of chitosan solution on the fabrication process were investigated systematically. The nanoparticles were characterized in terms of particle size, zeta potential, polydispersity index, drug entrapment efficiency (EE), loading capacity (LC), transmission electron microscopy (TEM), scanning electron microscopy (SEM), *in vitro* release and cytotoxicity screening, stability studies and drug release kinetics.

2. Materials and methods

2.1. Materials

Docetaxel was purchased from Sigma–Aldrich (USA). Chitosan (degree of deacetylation – 80%, molecular weight 40–80 kDa) was procured from Himedia Pvt. Ltd. (Mumbai, India). Sodium tripolyphosphate (TPP) and glacial acetic acid were procured from Loba chemie Pvt. Ltd. (Mumbai, India). All other chemicals and reagents used in the study were of analytical grade.

2.2. Preparation of ionically cross-linked docetaxel loaded Chitosan nanoparticles (DTX-CH-NP)

Blank ionically cross-linked chitosan nanoparticles were prepared by the ionic gelation of chitosan with TPP anions as described by Calvo et al. (Calvo, Remuñán-López, Vila-Jato, & Alonso, 1997; Vila et al., 2004). Briefly, chitosan was dissolved in 1% acetic acid solution (0.2–1%, w/v) at room temperature under sonication. The pH of the resulting solution was adjusted to 3–6 using 20% (w/v) sodium hydroxide solution and passed through a syringe filter (pore size 0.45 μm, Millipore, USA) to remove insoluble particles. Sodium TPP was dissolved in double distilled water at a concentration of (0.25–1.25%, w/v) and also passed through a syringe filter (pore size 0.22 μm, Millipore, USA). Blank nanoparticles were prepared upon the dropwise addition of TPP solution (0.25–1.25%, w/v) to chitosan solution, kept under stirring at room temperature. Fig. 1 depicts the procedure for the preparation of DTX-CH-NP by ionotropic gelation method. For the preparation of DTX-CH-NP, various concentrations of DTX (0.2, 0.4, 0.6, 0.8 and 1 mg/ml) in TPP solution (firstly drug was dissolved in 1 ml of methanol) were prepared. Nanoparticles were formed by adding this solution dropwise into chitosan solution. The nanoparticle suspension was continuously stirred for 1 h and centrifuged at 16,000 rpm for 30 min (Cooling centrifuge C-24BL, Remi Instruments, Mumbai, India). The pellet obtained was further redispersed in 10 ml of Phosphate buffer saline (pH 7.4). Mannitol (2%, w/v) was added as a cryoprotectant and freezed at −80 °C for 4 h followed by lyophilization in laboratory model freeze dryer (Alpha 2–4 LD Plus, Martin Christ, Germany) for 24 h at −48 °C and 0.0010 mbar.

2.3. Characterization of ionically cross-linked docetaxel loaded chitosan nanoparticles (DTX-CH-NP)

2.3.1. Micromeritics and zeta potential

The mean particle size, size distribution and zeta potential of DTX-CH-NP was analyzed by photon correlation spectroscopy using the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). All the measurements were carried out in triplicates at 25 °C. The nanoparticle suspension was diluted ten times with deionized water and the analysis was performed at a scattering angle of 90°.

2.3.2. Entrapment efficiency and loading capacity

The entrapment efficiency (%) of docetaxel in DTX-CH-NP was determined by separating DTX containing supernatant from nanoparticles by centrifugation at 15,000 rpm for 40 min (C-24BL, Remi Instruments, Mumbai, India). The clear supernatant was analyzed for the contents of docetaxel by measuring the absorbance in a UV–Visible spectrophotometer (Shimadzu UV spectrophotometer, Japan) at 230 nm. All the samples were measured in triplicates. The percentage entrapment efficiency and loading capacity were calculated as follows:

$$EE(\%) = \frac{DTX_t - DTX_f}{DTX_t} \times 100 \quad (1)$$

$$LC(\%) = \frac{DTX_t - DTX_f}{\text{weight of lyophilized nanoparticles}} \times 100 \quad (2)$$

where DTX_t is the total amount of docetaxel used in the preparation of DTX-CH-NP and DTX_f is the free docetaxel present in the supernatant.

2.3.3. Morphological characterization of nanoparticles

The shape and surface morphology of DTX-CH-NP was determined by scanning electron microscopy (Jeol, JSM-6100, Japan). The lyophilized samples were mounted on an aluminium stub using a