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Chitosan-based nanoparticles as drug delivery systems for doxorubicin: Optimization and modelling

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ABSTRACT

In the present work, two drug delivery systems were produced by encapsulating doxorubicin into chitosan and *O*-HTCC (ammonium-quaternary derivative of chitosan) nanoparticles. The results show that doxorubicin release is independent of the molecular weight and is higher at acidic pH (4.5) than at physiological pH. NPs with an average hydrodynamic diameter bellow 200 nm are able to encapsulate up to 70% and 50% of doxorubicin in the case of chitosan and *O*-HTCC nanoparticles, respectively. *O*-HTCC nanoparticles led to a higher amount of doxorubicin released than chitosan nanoparticles, for the same experimental conditions, although the release mechanism was not altered. A burst effect occurs within the first hours of release, reaching a plateau after 24 h. Fitting mathematical models to the experimental data led to a concordant release mechanism between most samples, indicating an anomalous or mixed release, which is in agreement with the swelling behavior of chitosan described in the literature.

1. Introduction

Drug delivery systems consist in a controlled release system, where the active substance is loaded into a carrier or device and then released at a predictable rate *in vivo* when administered through an injected or a non-injected route. The development of appropriate drug carriers requires previous knowledge on drug incorporation and release, formulation stability and shell life, biocompatibility, biodistribution and targeting, and functionality (De Jong & Borm, 2008; Doane & Burda, 2012; Parveen, Misra, & Sahoo, 2012).

Doxorubicin (DOX) is a member of the anthracyclines family, a family of chemotherapeutic drugs widely used clinically in the treatment of various solid tumors such as lung and breast cancer, as well as leukemia, and lymphomas. DOX is a perfect candidate for a drug delivery system due to its specific cytotoxicity and dose-dependent congestive heart failure (Soares, Dias, Novo, Ferreira, & Borges, 2012; Swain, Whaley, & Ewer, 2003). Liposomal doxorubicin (Caelyx® in Europe, Doxil® in USA) is currently approved for cancer treatment and its formulation has the advantage of enhancing the antitumor effect, although cardiotoxicity is still observed in patients treated with Caelyx® (Davies, 2004). Alternative formulations have been studied such as polymeric nanoparticles-based delivery systems that offer a significant advantage over other nanocarrier platforms. The versatility in the choice of polymeric matrices allows the tailoring of nanoparticles (NPs) properties to comply with the specific needs they are intended to meet (Baptista, Soares, Ferreira, & Borges, 2013).

Chitosan (CS) is a polysaccharide obtained from the deacetylation of chitin widely used as a drug delivery system for gene delivery, implants and nasal, oral, parental and transdermal administration (Rinaudo, 2006) and other biomedical applications such as tissue engineering (Depan & R.D. Misra, 2012; Depan, Pesacreta, & Misra, 2014; Depan, Pratheep Kumar, Singh, & Misra, 2014) Chitosan is often used in combination with other polymers (Echeverria et al., 2015) or inorganic materials such as magnetic nanoparticles (Zamora-Mora, Soares, Echeverria, Hernández, & Mijangos, 2015). This type of nanoparticles are widely used for biomedical applications (Soares, Alves et al., 2014; Soares, Ferreira, & Borges, 2014; Soares, Ferreira, Igreja, Novo, & Borges, 2012; Soares et al., 2015), including drug delivery systems (Depan and R.D.K. Misra, 2012; Misra, 2010). Ammonium quaternary chitosan derivatives such as *O*-HTCC have a permanent positive charge that enables its full solubility in a large range of pH and allows its interaction with cellular membranes (Sun & Wan, 2007).

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Drug release from chitosan-based nanoparticles is dependent upon its swelling behavior and drug-chitosan interactions, which are influenced by the pH of the medium (Yuan, Shah, Hein, & Misra, 2010). A controlled drug delivery from CS-NPs for cancer treatment can be achieved by controlling the pH of the medium. The tumor microenvironment is characterized by an acidic pH (around 6.5), while intracellular organelles such as liposomes and endosomes have a more acidic pH, between 3 and 5.5 (McDonald, Winum, Supuran, & Dedhar, 2012; Unsoy, Khodadust, Yalcin, Mutlu, & Gunduz, 2014). CS swelling depends upon pH, but is also influenced by other parameters such as the molecular weight (M_w) which has proven to reduce CS swelling and thus favor dissolution. Drug release still depends of solubility and drug M_w and is influenced by its concentration inside the polymeric network (Berger et al., 2004). Drug release from CS-NPs can occur through three different mechanisms: release from the surface of NPs; diffusion through the swollen rubbery matrix; and release due to polymer erosion. However, in most of the cases, the release occurs by combining more than one mechanism (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Janes, Fresneau, Marazuela, Fabra, & Alonso, 2001).

The present work includes the study of DOX release from chitosan and *O*-HTCC micro/nanoparticles, for different pH (4.5, 6.5 and 7.4) buffer solutions and different molecular weight of the polymers. Mathematical models were applied to the experimental data to understand the mechanisms of DOX release.

2. Experimental

All the chemical reagents used in this research work were of analytical grade and used without further purification.

2.1. Chitosan depolymerisation

Chitosan (DD 75.5%, 469 kDa, *Cognis*) was depolymerized by chemical reaction with sodium nitrite ($NaNO_2$, *Sigma-Aldrich*) based on a method adapted from Huang, Khor, and Lim, (2004). Briefly, 2.5 g of chitosan was dissolved in 250 mL of acetic acid 1% (V/V) (*Panreac)*, followed by addition of the desired amount of $NaNO_2$ dissolved in 10 mL of ultrapure water. The mixture was left to react for 1 h under mechanical agitation. Low molecular weight chitosan was precipitated with sodium hydroxide (NaOH, *Eka)* 1 M, followed by centrifugation and several washes with ultrapure water. The final obtained product was freeze-dried and stored in a dry place.

The M_w of depolymerized chitosan was measured by dilute solution viscosity using an Ubbelöhde capillary viscometer (No 0a) at 30 °C. The flow times of chitosan solutions with different concentrations in 0.2 M acetic acid/0.1 M sodium acetate (*Scharlau*) and solvent were recorded in quintuplet and the average value was calculated. The intrinsic viscosity [η] was calculated graphically by extrapolating the curve of reduced viscosity *versus* concentration to zero concentration. The Mark-Houwink-Sakurada equation was used to calculate the M_w of depolymerized chitosan ($K = 2.26 \times 10^{-5}$ dl g^{-1}, $\alpha = 0.95$) (Kassai, 2007).

2.2. O-HTCC synthesis

O-HTCC was synthetized using a method adapted from Wang et al. (2010) starting with chitosan of different M_w. Briefly, 5 g of chitosan was dissolved in 250 mL of acetic acid 2% and 125 mL of methanol (*Sigma-Aldrich*). After complete dissolution, 32 mL of benzaldehyde (*Fluka*) was added to the reaction vessel and let for 24 h with stirring, forming a white gel. The gel was neutralized with NaOH 1 M, filtered and washed in turns with acetone (*Fisher chemical*) and methanol. After freeze-drying, the product was mixed with 5 g of glycidil trimethyl ammonium chloride (GTMAC, *Sigma-Aldrich*) and 50 mL of isopropyl alcohol. The second reaction occurred at 70 °C for 16 h. The product obtained was filtered and washed with acetone and methanol. After freeze-drying, the product obtained was added to 100 mL of ethanolic HCl 0.25 M (*Fluka*) and left to react for 24 h at room temperature. At the end of the reaction, 25 mL of ultrapure water was added, followed by an excess of acetone to precipitate the final product. This product was filtered and washed several times with acetone and methanol. In order to purify *O*-HTCC, it was re-dissolved in water and precipitated with excess acetone. After filtering and washing, the obtained *O*-HTCC was freeze-dried and stored in a dry place.

2.3. DOX calibration curves

DOX calibration curves were constructed by measuring the absorbance at 480 nm, DOX's maximum absorbance peak (Fang et al., 2012; Unsoy et al., 2014), of a range of concentrations between 5 and 50 μg mL^{-1} of doxorubicin hydrochloride (*Fluka*) using an ultraviolet-visible spectrophotometer (UV–vis, *T90+ PG Instruments*).

DOX calibration curves were obtained for all solvents used in the experiments: water, PBS 7.4, PBS 6.5 and acetate buffer (pH 4.5). The measurements were made in quadruplicates.

2.4. Production of polymeric NPs

Chitosan and *O*-HTCC nanoparticles with four different M_w were produced through ionotropic gelation (Calvo, Remuñan-López, Vila-Jato, & Alonso, 1997) by rapidly adding to a known volume of the polymeric solution dissolved in acetic acid 1% (v/v), a known amount of tripolyphosphate (TPP, *Sigma-Aldrich)* and left under agitation for 24 h. The final product was freeze-dried and stored in a dry place for further characterization.

2.5. DOX encapsulation efficiency studies

DOX-loaded CS and *O*-HTCC-NPs were prepared by adding a given volume of different DOX solutions to chitosan or *O*-HTCC acidic solution (1.2% w/v in acetic acid at 1% v/v) followed by magnetic stirring for 10 min. Different CS:DOX and *O*-HTCC:DOX weight ratios were tested: 1 mL of CS solution was added to 2 mL of DOX with different concentrations (2 mg mL^{-1}, 1 mg mL^{-1}, 0.5 mg mL^{-1}, and 0.25 mg mL^{-1}). To 0.5 mL of each of the resultant solutions, 1.5 mL of TPP was added. Different CS:TPP and *O*-HTCC:TPP ratios were also tested: 1 mL of CS or *O*-HTCC solution was added to 1 mL of DOX solution at 2 mg mL^{-1}; 0.5 mL of the resultant solution was added to different amounts of TPP solution (0.5 mL, 1 mL, and 1.5 mL).

After adding TPP (0.25% w/v) to the DOX + CS or DOX + *O*-HTCC solution, the solution was centrifuged for 5 min at 10000 rpm. Supernatant was removed and 1 mL of distilled water was added to the pellet and centrifuged again under the same conditions. The absorbance of the supernatants obtained was measured by UV–vis spectroscopy at 480 nm.

2.6. DOX release studies

DOX release profiles were determined using three different buffers: PBS 7.4, PBS 6.5 and acetate buffer at pH 4.5, which represent the pH of blood, pH of the tumor microenvironment, and the pH of lysosomes and endosomes, respectively (Kievit et al., 2011; Tığlı Aydın & Pulat, 2012).

Release studies from polymeric NPs were prepared as following: 1 mL of polymer + DOX was added to 4.5 mL of TPP (0.25% w/v) and stirred in ULTRA-TURRAX for 5 min and freeze-dried for 24 h. The resultant powder was re-suspended in 1 mL of PBS 7.4 and dialyzed