International Journal of Pharmaceutics 422 (2012) 479–488

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Mathematical modelling of the transport of hydroxypropyl-β-cyclodextrin inclusion complexes of ranitidine hydrochloride and furosemide loaded chitosan nanoparticles across a Caco-2 cell monolayer

Armin Sadighi[a], S.N. Ostad[b], S.M. Rezayat[a,c], M. Foroutan[d], M.A. Faramarzi[e], F.A. Dorkoosh[f,*]

[a] Department of Medical Nanotechnology, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[c] Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
[d] Department of Physical Chemistry, Faculty of Chemistry, College of Science, University of Tehran, Tehran, Iran
[e] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran
[f] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 1439956131, Tehran 14174, Iran

ARTICLE INFO

Article history:
Received 23 July 2011
Received in revised form 14 October 2011
Accepted 6 November 2011
Available online 11 November 2011

Keywords:
Chitosan nanoparticles
Furosemide
Ranitidine hydrochloride
Hydroxypropyl-β-cyclodextrin
Caco-2 cell
Mathematical model

ABSTRACT

Chitosan nanoparticles (CS-NPs) have been used to enhance the permeability of furosemide and ranitidine hydrochloride (ranitidine HCl) which were selected as candidates for two different biopharmaceutical drug classes having low permeability across Caco-2 cell monolayers. Drugs loaded CS-NPs were prepared by ionic gelation of CS and pentasodium tripolyphosphate (TPP) which added to the drugs inclusion complexes with hydroxypropyl-β-cyclodextrin (HP-βCD). The stability constants for furosemide/HP-βCD and ranitidine HCl/HP-βCD were calculated as 335 M^{-1} and 410 M^{-1}, whereas the association efficiencies (AE%) of the drugs/HP-βCD inclusion complexes with CS-NPs were determined to be 23.0 and 19.5%, respectively. Zetasizer and scanning electron microscopy (SEM) were used to characterise drugs/HP-βCD-NPs size and morphology. Transport of both nano and non-nano formulations of drugs/HP-βCD complexes across a Caco-2 cell monolayer was assessed and fitted to mathematical models. Furosemide/HP-βCD-NPs demonstrated transport kinetics best suited for the Higuchi model, whereas other drug formulations demonstrated power law transportation behaviour. Permeability experiments revealed that furosemide/HP-βCD and ranitidine HCl/HP-βCD nano formulations greatly induce the opening of tight junctions and enhance drug transition through Caco-2 monolayers.

1. Introduction

Chitosan (CS), a partial deacetylation product of chitin that consists of glucosamine and N-acetylglucosamine, is a biocompatible, biodegradable, safe and low toxic polymer, particularly in the case of oral route of drug administration, with low immunogenicity possesses various applications in medical and pharmaceutical fields (Singla and Chawla, 2001; López-León et al., 2004; Wu et al., 2005). This polymer is widely used as a carrier for low molecular weight drugs, vaccines and DNA (Bowman and Leong, 2006). Nanoparticle (NP) formulations as excitement approaches to enhance the solubility and cellular permeability of drugs which result in improved clinical efficacy have been widely investigated in recent years (Xua et al., 2006; Li et al., 2009).

Statistics show that nearly 40% of the manufactured drugs through the world have unfavourable water solubility which limits the application of these medicines in physiological environments (Gao et al., 2008). Hydroxypropyl-β-cyclodextrin (HP-βCD) belongs to a large family of cyclic oligosaccharides which have been extensively studied are used to increase aqueous solubility and permeability of poorly soluble drugs (Loftsson and Brewster, 1996; Zhang et al., 2008).

Intracellular and paracellular pathways which believed as the most important pathways for drug absorption and permeation across cellular routes could be activated by CS-NPs which have a great potential to increasing the permeability of hydrophobic drugs (Huang et al., 2002; Dong et al., 2006; Gao et al., 2008; Sadeghi et al., 2008; Trapani et al., 2009). Paracellular drug transport is enhanced by CS-NPs transient and reversibly (Artursson et al., 1994) due to electrostatic interactions between positive charge of CS and negative charge of sialic acid involved in the cell tight junctions (Dodane et al., 1999; Lee et al., 2006; Wang et al., 2008). Depolymerisation of cellular F-actin as a consequence of the interactions between CS and ZO-1 (zonula occludin), a protein located in the cellular membrane, considered as another mechanism for raising drugs paracellular permeation using CS-NPs (Huang et al., 2002).

* Corresponding author. Tel.: +98 21 88009440; fax: +98 21 88009440.
E-mail address: Dorkoosh@tums.ac.ir (F.A. Dorkoosh).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.11.016

Ranitidine hydrochloride (ranitidine HCl) ($C_{13}H_{22}N_4OS_3$ HCl, Mw = 350.9, pK_a = 0.2 for ionised form and 1.2 for unionised form), which has high solubility and low permeability, is a class III compound (Mirmehrabi et al., 2004) and furosemide ($C_{12}H_{11}ClN_2O_5S$, Mw = 330.7, pK_a = 4), with low solubility and permeability, is a class IV substance according to the biopharmaceutical classification system (Beyers et al., 2000).

The human colorectal adenocarcinoma cell line Caco-2, is used as a standard model to assessing drugs absorption and transportation (Zuo et al., 2000).

Raising the solubility and permeability of drugs using a cosolvent like (HP-βCD), decreasing the size of formulations and enhancing their permeation by CS-NPs as a polymeric drug carrier have been investigated in this research. Main approach in this research was assessing only the effects of CS-NPs as a nanoparticulate drug carrier in order to increasing drugs permeability. So, high soluble ranitidine HCl was also complexed with HP-βCD to ignore the permeation enhancing effects of CD. On the other hand, presenting logic mathematical models to describe and distinguish between nano and non-nano drug/HP-βCD formulations and determine the effects of CS-NPs in the transport of two drug formulations across the biological monolayer were investigated.

2. Materials and methods

2.1. Materials

ChitoClear® CS base (viscosity 1%, w/v solution, 463 mPa s and 95% degree of deacetylation) was purchased from Primex (Siglufjörður, Iceland). Caco-2 cells were obtained from the Institute Pasteur (Iran). Ranitidine HCl was received as a generous gift from Shahre Daru Pharmaceutical Company (Tehran, Iran), and furosemide was a generous gift from Chemidarou Pharmaceutical Company (Tehran, Iran). Pentasodium tripolyphosphate (TPP) was obtained from Merck (Darmstadt, Germany). HP-βCD was purchased from Sigma–Aldrich (Saint Louise, USA). HPLC- (high performance liquid chromatography) grade acetonitrile was acquired from Merck. All other reagents used in this study were of analytical grade.

2.2. HPLC analysis

Furosemide and ranitidine HCl analyses were performed by a fully automated HPLC (Knauer, Germany) containing a UV detector. A LiChrospher 100 RP8 EC (250 mm × 4.6 mm, 5-μm particle size) stainless steel analytical column was used for both drugs. When measuring the levels of furosemide, the mobile phase consisted of water and acetonitrile in a ratio of 60:40 (v/v). The mobile phase pH was adjusted to 3.5 by adding 25 μl of orthophosphoric acid to 100 ml water. The flow rate was 2 ml/min, and UV detection was achieved at 228 nm. When assessing ranitidine HCl levels, the mobile phase was prepared by phosphate buffer (10 mM, pH = 7.1) and acetonitrile in a ratio of 80:20 (v/v). The flow rate was 1 ml/min, and UV detection was achieved at 230 nm. Furosemide and ranitidine HCl were detected at retention times of 5.5 and 11 min, respectively.

2.3. Phase solubility studies of drugs/HP-βCD inclusion complexes

Stability and phase solubility analyses for the HP-βCD inclusion complexes of furosemide and ranitidine HCl were performed according to the method depicted by Higuchi and Connors (1965). Briefly, excess amounts of drugs were added to different concentrations of HP-βCD (1–5 mM) in 0.02 N HCl solutions. Light exposure was avoided, and the mixtures were rotated end-over-end for 72 h at 25 °C. After achieving equilibrium, excess amounts of drugs were filtered through a cellulose nitrate Sartorius filter (0.45 μm). The filtered solutions were then diluted with methanol and analysed by HPLC-UV at 228 and 230 nm for furosemide/HP-βCD and ranitidine HCl/HP-βCD, respectively. Analyses were used to determine the amounts of soluble drugs in the presence of different concentrations of HP-βCD, which functioned as a cosolvent and stabilizer. The $K_{1:1}$ stability constant (K_C) for both drugs was calculated from an initial straight line portion of the phase solubility diagram using the following equation:

$$K_C = \frac{\text{Slope}}{S_0 \times (1 - \text{Slope})} \quad (1)$$

In this equation, S_0 and Slope represent the intrinsic solubility of the drug and the Slope of the phase solubility diagram, respectively.

2.4. CS-NPs synthesis

CS-NPs were produced by ionic gelation method first described by Calvo et al. (1997). Electrostatic forces between negative species generated from the dissociation of TPP in aqueous solution and positively charged CS result in the formation of CS-NPs. In the present work, the molar ratio of CS/TPP was changed to achieve CS-NPs with optimal physicochemical properties. The hydrodynamic size, polydispersity index (PDI) and zeta potential of CS-NPs were evaluated in order to select the optimal CS/TPP molar ratio for nanoparticle formation.

To produce the NPs, CS was dissolved in acetic acid at concentrations of 0.75, 1.5, 2.25, 3, 3.75, and 4.5 mg/ml to form a positive charge on the ammonium groups of CS, allowing for suitable water solubility. In all cases, NPs were prepared by adding 8 ml of TPP solution (0.5 mg/ml) and 1 ml of the drug inclusion complexes (drugs + 5 mM HP-βCD) to 20 ml of CS solution (pH 4.5) with various concentrations (0.5, 1, 1.5, 2, 2.5, and 3 mg/ml) under stirring conditions at room temperature. NPs were obtained after ultracentrifugation at 20,000 × *g* for 1 h. To separate NPs from the solution, the supernatant was discarded, and the pellet was resuspended with deionised water. Finally, the produced NPs were characterised by Zetasizer 3000 HS (Malvern instrument, Malvern, Worcestershire, UK) at 25 °C with a detection angle of 90° to perform NPs size, zeta potential and PDI. Scanning electron microscopy (SEM) images of the synthesized NPs were taken by a ZIESS DSM 960A, SEM (Carl Zeiss Inc., Oberkochen, Germany).

2.5. Association efficiency, loading capacity, and drugs release profiles

To determine association efficiency (AE%) and loading capacity (LC%)of the drugs nano formulations, NP solutions were first ultracentrifuged (20,000 × *g* at 4 °C) for 1 h, then the supernatants were discarded and pellets resuspended by deionised water. The amounts of non-associated and -loaded drugs in supernatants were calculated by measuring the difference between total amounts of drugs added to the NPs solution and the quantity of unbounded drugs using HPLC methods according to the following equations:

$$\text{AE\%} = \frac{\text{total amounts of drugs} - \text{amounts of free drugs}}{\text{total amounts of drugs}} \times 100 \quad (2)$$

$$\text{LC\%} = \frac{\text{total amounts of drugs} - \text{amounts of free drugs}}{\text{NPs weight}} \times 100 \quad (3)$$

To obtain drugs release profiles, 1 mg of each lyophilised drugs were dissolved in 1 ml of 0.1 M phosphate buffered saline solution (PBS) at pH of 7.4 and 4.0, representing the physiological environment (Apical side) and endolysosomal compartment (Intracellular space), respectively. 1 ml of the both drugs/HP-βCD-NPs were incubated in a dialysis bag and immersed in 19 ml of PBS (Boonsongrit