Carbohydrate Polymers 87 (2012) 581–588

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride

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ARTICLE INFO

Article history:
Received 23 May 2011
Received in revised form 20 June 2011
Accepted 10 August 2011
Available online 17 August 2011

Keywords:
Chitosan/gelatin complexes
Buccal delivery
Mucoadhesive films
Propranolol hydrochloride

ABSTRACT

The aim of this work was to develop and characterize chitosan/gelatin films as innovative mucoadhesive system for buccal delivery of propranolol hydrochloride. FT-IR and TGA analysis confirmed the interaction between chitosan and gelatin. The presence of higher chitosan amounts in chitosan/gelatin films allowed the lowest percent water-uptake ability (235.1 ± 5.3%) and the highest *in vivo* residence time in the buccal cavity (240 ± 13 min). Moreover, the presence of mannitol in the formulation allowed 80% drug permeation through porcine buccal mucosa in 5 h. This behaviour suggests that the application of four and two films containing 5 mg of propranolol hydrochloride could be suitable for achieving the proposed daily dose for hypertension and atrial fibrillation treatment, respectively. Another interesting aspect of chitosan/gelatin films was their compatibility with buccal microflora in the absence of drug and their ability to determine growth inhibition for pathogen bacteria, but not for probiotic species, when loaded with drug.

1. Introduction

Buccal route offers several advantages than oral route (Harris & Robinson, 1992) due to the high total blood flow which ensures systemic bioavailability, avoiding first-pass hepatic metabolism and gastrointestinal drug degradation (Junginger, Hoogstraate, & Verhoef, 1999; Salamat-Miller, Chittchang, & Johnston, 2005). Moreover, it is easily accessible for self-medication and suitable for dosage forms administration and removal. However, the accidental swallowing of delivery systems and the continuous dilution of the released drug by saliva could determine a low residence time of formulation in buccal cavity and, consequently, a low drug bioavailability (Shojaei Amir, 1998). For this reason, various bioadhesive buccal formulations (Sudhakar, Kuotsu, & Bandyopadhyay, 2006), such as tablets (Llabot, Manzo, & Allemandi, 2002), gels (Mortazavi, 2002; Pelin et al., 2004), patches (Burgalassi, Panichi, Saettone, Jacobsen, & Rassing, 1996; Cheng, Padmanbh, & Thomas, 1997; Reinhold & Hans, 1989; Wong, Yuen, & Peh, 1999), and films (Kohda et al., 1997; Remuñán-López, Portero, Vila-Jato, & Alonso, 1998), have been developed using mucoadhesive polymers which can establish a strong adhesive contact with the buccal mucosa, allowing to increase residence time of delivery systems and to optimize drug bioavailability. In particular, mucoadhesive buccal films can ensure an accurate drug dosing with respect to liquid formulations and gels, which can be easily washed away by saliva, and can be more comfortable with respect to conventional solid formulations. In fact, films are flexible and elastic, so that patient compliance is increased and also adequately strong to withstand breakage, caused from mouth movements (Peh & Wong, 1999).

In this study the properties of films based on chitosan/gelatin polyelectrolyte complexes were investigated. Chitosan, a N-deacetylated product of the polysaccharide chitin, shows interesting biological properties, including biocompatibility, non-toxicity, biodegradability and mucoadhesivity (He, Davis, & Illum, 1998; Koga, 1998; Luppi, Bigucci, Cerchiara, & Zecchi, 2010a; Muzzarelli, 1997). Chitosan is also a promising matrix carrier for sustained drug release and it possesses excellent film-forming properties (Remuñán-López & Bodmeier, 1996). At pH below its pKa, chitosan is a polycation and has been used extensively to prepare ionically crosslinked hydrogels with anionic polymers (Hamman, 2010, Berger et al., 2004; Meshali & Gabr, 1993). In this study, type B gelatin was used as anionic polymer. Type B gelatin is a heterogeneous mixture of protein fractions consisting of single or multi-stranded polypeptides and it is derived from alkaline hydrolysis of cattle hides and bones (Hamman, 2010).

Propranolol hydrochloride is a β-blocker almost completely absorbed although it shows a low bioavailability due to extensive first-pass metabolism, so that only 25% approximately reaches systemic circulation (Reiter, 2004). It is used clinically for hypertension, angina, postinfarction, sinus tachycardia, arrhythmias, and obstructive cardiomyopathy. Because of differences in clearance and variation in drug binding there is a wide range of effective oral dosage. In particular, for hypertension treatment, the initial average daily dose of propranolol hydrochloride is 40 mg twice daily, while

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0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.08.024

for atrial fibrillation, the initial usual dose is 10 mg three or four times daily. Considering drug oral bioavailability of approximately 25%, for hypertension treatment and for atrial fibrillation, the anticipated buccal doses of drug are 10 mg twice daily and 7.5–10 mg daily, respectively.

The aim of this work was to develop mucoadhesive chitosan/gelatin films able to easily administer propranolol hydrochloride by buccal route, allowing suitable drug permeation. In particular, their use for chronic treatment can be suggested due to their tolerability and compatibility with buccal mucosa.

2. Materials and methods

2.1. Materials

Type B gelatin from bovine skin (~225 Bloom, isoelectric point in the range of pH 4.5–5.5) was obtained commercially from Sigma–Aldrich (USA); chitosan (Mr. 150,000; deacetylation degree 84%; pKa 6.3) and propranolol hydrochloride were obtained commercially from Fluka (Milan, Italy). All other chemicals and solvents were of analytical grade and purchased from Carlo Erba (Milan, Italy). Water-uptake, mucoadhesion, release and permeation studies were carried out in aqueous buffers with the following compositions (g) per liter of distilled water: 2.38 $Na_2HPO_4{\cdot}10H_2O$, 0.19 KH_2PO_4, 8.0 NaCl for buffer solution pH 7.4; 4.609 KH_2PO_4, 16.748 $Na_2HPO_4{\cdot}12H_2O$ adjusted with hydrochloric acid to pH 6.8.

2.2. Preparation of chitosan/gelatin complex buccal films

As described in Cheng et al. (2003), known amounts of chitosan and gelatin were dissolved separately in 1% w/v acetic acid and water, respectively. Then chitosan solution and gelatin solution were mixed obtaining two final polymeric concentrations, F1 (1% w/v) and F2 (2% w/v) and different weight mixing ratios. The mixing ratio r (i.e. the percentage of gelatin in the mixture) was defined as:

$$r = \frac{W_g}{(W_c + W_g)};$$

where W_c and W_g were the weights of chitosan and gelatin, respectively.

50 mL of the final mixture were cast into a petri dish (11 cm in diameter) and dried at 50 °C for 24 h through casting-solvent evaporation method. Loaded films were prepared by the same procedure, adding a known amount of propranolol hydrochloride into the polymeric solutions, in order to obtain films containing 1.67 mg/cm^2.

Mannitol, a hydrophilic absorbing material, was added to F1 polymeric solutions obtaining Fm films (1.55 mg/cm^2 of mannitol).

Films were washed with 80% ethanol until neutrality (pH = 7), cut into appropriate sizes, packed in aluminium foil and stored at 4 °C for further studies.

2.3. FT-IR spectroscopy, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC)

To verify interactions between chitosan and gelatin, FT-IR spectroscopy (FT-IR-4100 spectrophotometer recorded with a Jasco, 650–4000 cm^{-1}) and TGA (Mettler TA 4000 apparatus equipped with a TG 50 cell on 8–10 mg samples; $\beta = 10$ K min^{-1}, static air atmosphere, 30–400 °C temperature range) of unloaded films, chitosan and gelatin powders and their physical mixture were performed. Measurements were carried out at least in triplicate (relative standard deviation ± 5%). To verify the absence of crystal drug in films, thermal analysis were performed using a thermocryostat (Mettler 821e/800/847) connected to the thermal analyzer (Mettler-Toledo S.p.a., Novate Milanese, Italy). Samples of loaded films and propranolol hydrochloride powder (about 5 mg) were sealed in a 30 μL aluminium pan and were scanned between 30 °C and 340 °C at a heating rate of 10 °C/min.

2.4. Characterization of buccal films

In order to determine film thickness, three circles of 3 cm^2 were cut from each film. The average thickness of the buccal films was determined using a Mitutoyo pocket thickness gauge; Mitutoyo Mfc. Co. Ltd., Tokyo, Japan.

For determination of weight uniformity, circles of 3 cm^2 of each film were randomly selected and accurately weighted using an electronic balance. The results are expressed as the mean values of three determinations.

Drug content was calculated as follows: three circles of 3 cm^2 were dissolved in 10 mL of phosphate buffer (pH 7.4) containing 2 mL of HCl 0.1 M solution, in order to determine the amount of propranolol hydrochloride in the films. The amount of drug was determined with chromatographic system, composed of a Shimadzu (Milan, Italy) LC-10ATVP chromatographic pump and a Shimadzu SPD-10AVP UV–Vis detector set at 254 nm. Separation was obtained on a Phenomenex (Torrance, CA, USA) Sinergy Fusion-RP 80A (150 mm × 4.6 mm I.D., 5 μm) coupled to a Phenomenex (Torrance, CA, USA) SecurityGuard C18 guard cartridge (4 mm × 3.0 mm I.D., 5 μm). The mobile phase was composed of a mixture of acetonitrile-pH 3.0 solution of triethylamine (0.5%) 30:70 (v/v). The flow rate was 0.4 mL/min and manual injections were made using a Rheodyne 7125 injector with a 50 μL sample loop. Data processing was handled by means of a CromatoPlus computerised integration system (Shimadzu Italia, Milan, Italy). Calibration curve of concentration versus peak area ratio was plotted at concentration range of 0.1–10 μg/mL; good linearity was found ($r^2 = 0.9998$). Repeatability assays were carried out on propranolol hydrochloride standard solutions, at concentrations corresponding to the lower and upper limit and the middle point of the calibration curve. Method precision was satisfactory: RSD% values of 3.1, 3.0 and 1.3 were obtained for propanolol hydrochloride concentrations of 0.1, 1.0 and 10.0 μg/mL, respectively.

The results were expressed as milligrams of drug for square centimetre (mg/cm^2). All determinations were carried out in triplicate.

2.5. Scanning electron microscopy (SEM)

The morphological structure of buccal films was studied by SEM analysis. Buccal films were fixed on supports and coated with gold–palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with LEO 420 (LEO Electron Microscopy Ltd., England) using secondary electron imaging at 15 kV in order to examine the structure of the films.

2.6. In vitro water-uptake studies

In vitro water-uptake studies were performed in phosphate buffer at pH 6.8 that simulated human saliva and measuring the increase of weight for predetermined periods of time. Circles of 3 cm^2 of each films were weighted (W_1) and dipped in simulated saliva fluid for predetermined periods of time. Then, the circles were wiped off from the excess surface water using filter paper and weighted (W_2). Water-uptake (WU) ability was determined as a weight increase of the films after 5 h, according to the follow equation:

WU (%) = $[(W_2 - W_1) \times 100/W_1]$, where W_1 was the initial weight of dry film and W_2 is the weight of hydrated films.