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Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride

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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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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.

2.7. In vitro and in vivo mucoadhesion properties

For these studies porcine buccal mucosa was used as biological membrane due to the similarity to the human buccal tissue (Hoogstraate et al., 1992; Shojaei Amir, 1998). Porcine buccal mucosa was removed from a freshly killed male pig obtained from a local slaughter house. The buccal cavity was placed in phosphate buffer at pH 7.4; then buccal mucosa was surgically removed from the oral cavity using fine-point forceps and surgical scissors to turn away the connective tissue. Finally, buccal mucosa was cleaned in phosphate buffer at pH 7.4 and immediately used for tests.

The *in vitro* mucoadhesion was measured in terms of the force needed to pull out a freshly excised buccal mucosa (surface area 1 mm^2) from a film with an adapted tensiometer (Krüss 132869; Hamburg, Germany). As reported in Luppi et al. (2010b), the mucosa was fixed to a support with cyanoacrylate adhesive and then suspended from the tensiometer spring. The mucosa was lowered until it just contacted the surface of the film, previously hydrated with phosphate buffer at pH 6.8 for 5 min. A 20 dyne force, measured by the torsion balance of the instrument as a negative force, was applied to the films for 60 s. Then the mucosa was raised until it was separated from the film. The assay was performed for three different circles from each film and it was calculated the average.

The *in vivo* mucoadhesion properties of buccal unloaded films were tested in five healthy volunteers aged 25–40 years. The volunteers were instructed to press the films against the gingival mucosa above the canine tooth for 60 s (Perioli et al., 2004; Yehia, El-Gazayerly, & Basalious, 2008). The films were observed for 5 h. The volunteers were refrained from food and drinks during the test and were asked to monitor for irritation and to record the residence time which was taken as the time for the film to dislodge completely.

2.8. In vitro release of propranolol hydrochloride from buccal films

The release studies of propranolol hydrochloride were performed in 10 mL of phosphate buffer at pH 7.4 at 37 ± 0.5 °C under magnetic stirring. Aliquots of 0.2 mL were withdrawn at different time intervals, filtered through cellulose acetate membrane (0.45 μm), and replaced by fresh medium. The studies were carried on for 5 h. The release studies were conducted in triplicates and the mean values were plotted versus time.

2.9. In vitro permeation studies

In vitro permeation studies through buccal porcine mucosa were conducted in a Franz-type permeation cell with a diffusional area of 1.76 cm^2. At time zero, films were placed in the donor compartment and 20 μL of phosphate buffer at pH 6.8 simulating human saliva were placed on mucosa. The receiver phase (6.0 mL of a phosphate buffer solution, pH 7.4, maintained at 37 °C by means of a surrounding jacket) was stirred constantly and, at predetermined time intervals, samples of 100 μL were taken and replaced by fresh medium, in order to assess the amount of drug permeated. As control formulation, the permeation study of propranolol hydrochloride from 50 μL of solution containing 5 mg of drug was performed. The studies were carried on for 6 h. The permeability coefficient (P) was calculated using the following equation: $P = (dM/dt)/(M_0 A)$, where dM/dt represents the permeability rate and M_0 stands for the initial concentration in the donor chamber, while A is the effective surface area of the mucosa.

2.10. Antimicrobial activity assay

The antimicrobial activity was evaluated against Gram-positive bacteria (*Lactobacillus acidophilus* LA14, *Bifidobacterium infantis* BI07, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 29213), Gram-negative bacteria (*Escherichia coli* ATCC 11105, *Pseudomonas aeruginosa* ATCC 9027) and yeasts (*Candida albicans* ATCC 10231). *L. acidophilus* LA14 and *B. infantis* BI07 are probiotic strains purchased by Danisco Inc. (Madison, WI). *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* were grown aerobically in LB medium (Difco, Detroit, MI) at 37 °C for 24 h. *L. acidophilus* and *B. infantis* were cultured in MRS medium (Difco) supplemented with 0.05% L-cysteine at 37 °C for 18–36 h under an anaerobic atmosphere by using Anaerocult A (Merck, Darmstadt, Germany). *C. albicans* was grown aerobically in SD medium (Difco) at 30 °C for 48 h. The disc-agar diffusion method was used to test the antimicrobial activities of unloaded and loaded films (6 mm diameter) containing different concentrations of chitosan and gelatin ($r = 0.2$, $r = 0.4$, $r = 0.6$). In parallel, the antimicrobial activity was evaluated for: (i) chitosan solutions (1%, w/v), without and with propranolol hydrochloride (25 mg/mL) in order to simulate polymer and drug amounts in the films; (ii) chitosan/gelatin (1%, w/v) solutions, without and with drug. Suspensions of the test microorganisms (10^8 colony-forming units [CFU]/mL) were spread on the agar plates containing the appropriate culture media (LB, MRS or SD). Sterile paper discs of 6 mm diameter (Schleicher and Schuell, Dassel, Germany) were impregnated with 20 μL of each solution. These paper discs and the circular films with the same diameter were placed on the surface of the agar plates. Plates were incubated at the appropriate conditions and the diameter of the inhibition zone around the paper discs and films were measured. The experiments were performed in triplicate.

The minimal inhibitory concentration (MIC) of propranolol hydrochloride was determined by the agar dilution method. A stock solution of 20 mg/mL of drug in water was used to prepare agar plates containing scalar concentrations of the drug (3.75–120 μg/mL). Pure propranolol hydrochloride was used to obtain agar plates containing 25 mg/mL of the drug. Microbial suspensions of 10^5–10^6 CFU/mL, prepared from broth cultures in log phase growth, were used to inoculate plates containing propranolol hydrochloride. Plates were made in triplicate and incubated at the appropriate conditions.

2.11. Statistical analysis

All the experiments were done in triplicate. Results are expressed as mean ± SD. Kruskal–Wallis and Anova tests were used to determine statistical significance of permeation studies and of all other studies, respectively. Differences were considered to be significant for values of $P < 0.05$.

3. Results and discussion

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

Fig. 1 shows the FT-IR spectra of chitosan (a), gelatin (b), chitosan/gelatin physical mixture (c) and F1 film, $r = 0.6$ (d).

The FT-IR spectra of chitosan showed bands at 1654 cm^{-1} relative to the vibration of the carbonyl group of acetylated amide and at 1580 cm^{-1} relative to stretching of the free amino group. Gelatin showed the bands at 1654 cm^{-1} and 1535 cm^{-1} relative to the vibration of the amide carbonyl and stretching of the free amino groups, respectively; it also showed band at 1704 cm^{-1} relative to undissociated carboxyl group. These characteristics bands

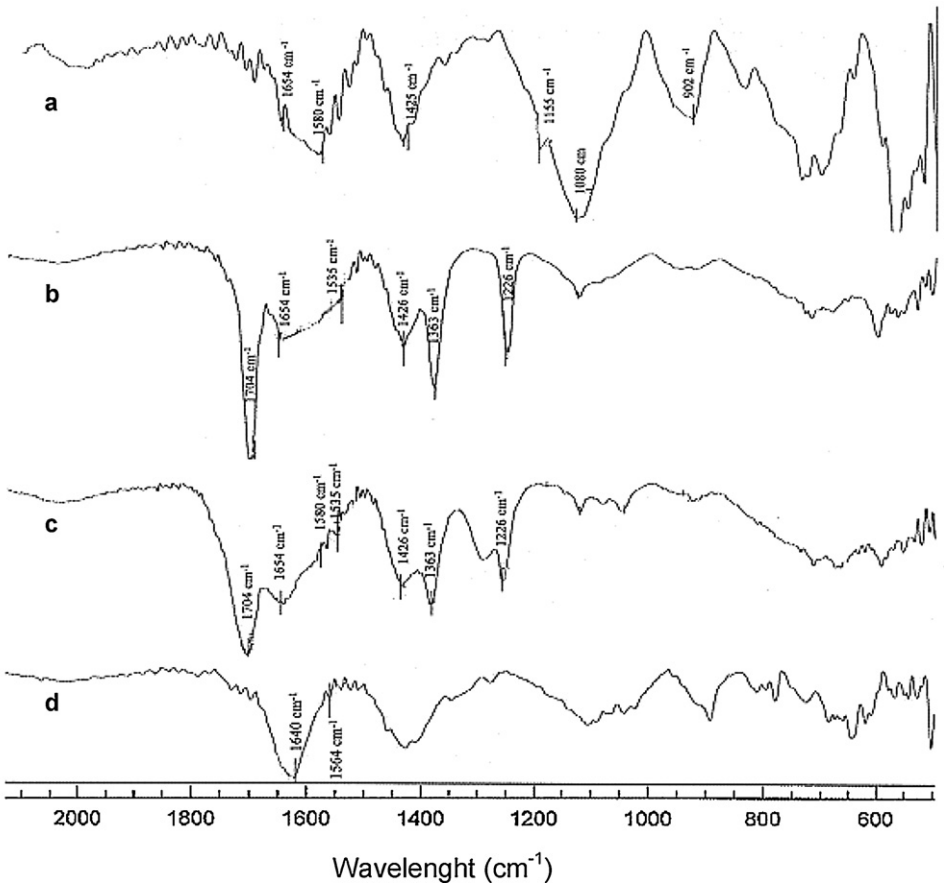

Fig. 1. FT-IR of chitosan (a), gelatin (b), chitosan/gelatin physical mixture (c), F1 film $r = 0.6$ (d).

were also in the FT-IR spectra of physical mixture of chitosan and gelatin. The FT-IR of film showed the shift in amide carbonyl group to 1640 cm^{-1} and the shift in amino group of chitosan to 1564 cm^{-1}, confirming the interaction between chitosan and gelatin, also reported in Yin, Yao, Cheng, and Ma (1999).

Fig. 2 shows the thermograms of chitosan (a), gelatin (b), chitosan/gelatin physical mixture (c) and F1 film, $r = 0.6$ (d).

Chitosan and gelatin degraded at about 285 °C (inflection point temperatures). The degradation of polyelectrolyte complex film showed one event at 250 °C that can be considered as a proof of chitosan and gelatin complexation. The shift to a lower temperature in the thermal degradation of the complex indicated a loss of organization, probably due to the formation of ionic bonds between chitosan and gelatin.

As can be seen from Fig. 3, propranolol hydrochloride showed a melting point at 165.6 ± 0.2 °C. DSC analysis of all films showed the absence of exothermic melting peak of propranolol hydrochloride and consequently the absence of crystal drug in the films.

3.2. Characterization of buccal films

Table 1 reports drug content, thickness and weight of loaded and unloaded films.

All loaded films consisting exclusively of chitosan did not show uniformity of drug content, weight and thickness (data not reported) and they were not considered for the subsequent tests. The others films showed weight uniformity and different thickness that can be related to the different polymeric concentration (for F1 and F2) and to the presence of mannitol in the Fm formulations.

Moreover, the experimental drug content of loaded films was close to the theoretical one (1.67 mg/cm^2 for all films) with low standard deviation, suggesting that the method employed for their preparation was capable of giving an uniform drug distribution.

3.3. Scanning electron microscopy (SEM)

Fig. 4 shows the morphology of unloaded F1 film ($r = 0.6$, a) and all loaded F1 films ($r = 0.2$, b; $r = 0.4$, c; $r = 0.6$, d; $r = 0.8$, e; $r = 1$, f) which showed a homogeneous structure and devoid of crystals.

Moreover, loaded films containing an excess of gelatin (d–f) did not show a continue structure but an increasingly evident convex pattern on the top surface.

3.4. In vitro water-uptake studies

In vitro water-uptake studies, performed at pH 6.8 on unloaded F1 films, showed the highest water-uptake ability % at 30 min.

In particular, film consisting exclusively of gelatin completely solubilised in the medium in 10 min, while in the complex based films, the presence of a greater amount of gelatin provided a higher water-uptake ability % than films with an excess of chitosan ($P < 0.05$). In fact, for films with $r = 0$, $r = 0.2$, $r = 0.4$, $r = 0.6$ and $r = 0.8$ the water-uptake ability % values were 190.0 ± 7.9, 235.1 ± 5.3, 254.1 ± 4.3, 286.5 ± 6.4, 352.7 ± 8.7, respectively. This behaviour can be correlated to the presence of a great number of ionized amino acids in gelatin structure and consequently to the presence of free charges favouring the entry of water.

This trend was also observed for loaded F1 films. However, the presence of propranolol hydrochloride provided the formation of loaded films characterized by a lower water-uptake ability % than unloaded films ($P < 0.05$). In particular, water-uptake ability % was as follows: 193.8 ± 8.4 for film $r = 0.2$; 209.3 ± 7.0 for film $r = 0.4$;

Table 1
Characteristics of the different films (mean ± SD, $n = 3$).

Formulation type	Drug content (mg/cm^2)	Film thickness (μm)	Weight films (mg/cm^2)
F1 unloaded	–	50 ± 3	7.08 ± 0.06
F2 unloaded	–	70 ± 5	10.12 ± 0.13
Fm unloaded	–	62 ± 3	10.01 ± 0.02
F1 loaded	1.67 ± 0.05	70 ± 8	8.53 ± 0.32
F2 loaded	1.68 ± 0.03	100 ± 9	11.73 ± 0.36
Fm loaded	1.70 ± 0.04	82 ± 7	11.48 ± 0.33

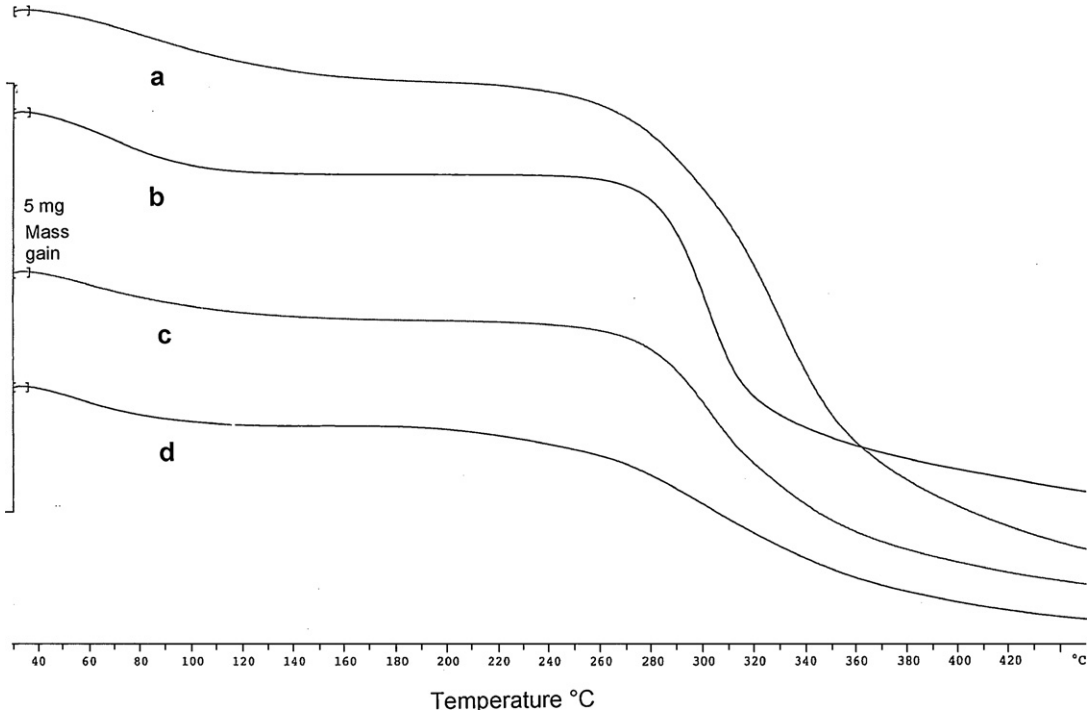

Fig. 2. Thermogravimetric analysis of gelatin (a), chitosan (b), chitosan/gelatin physical mixture (c), F1 film $r = 0.6$ (d).

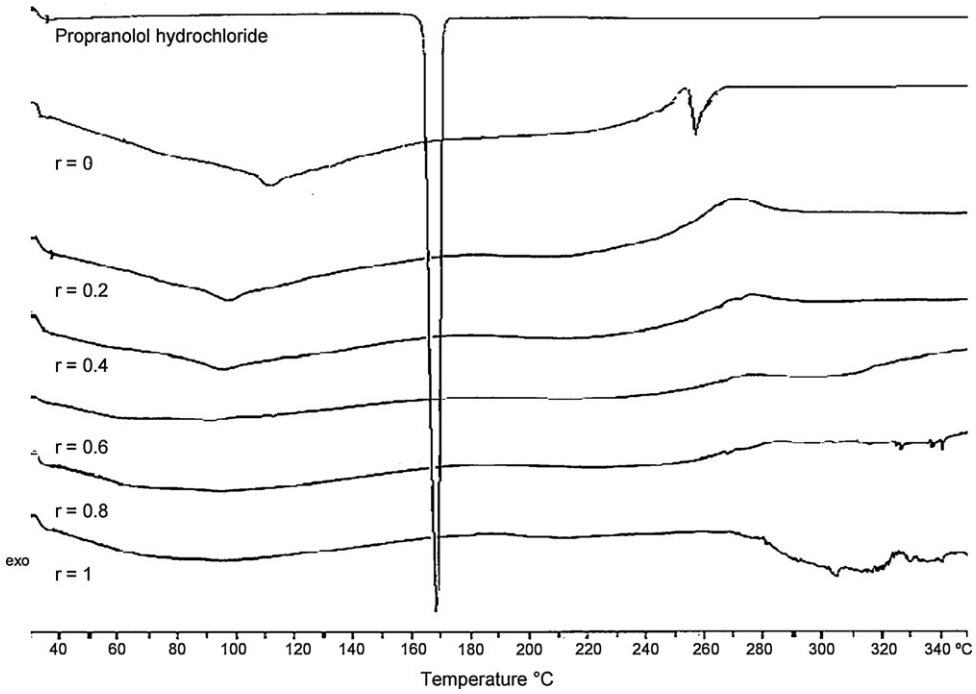

Fig. 3. DSC of propanolol hydrochloride and F1 films.

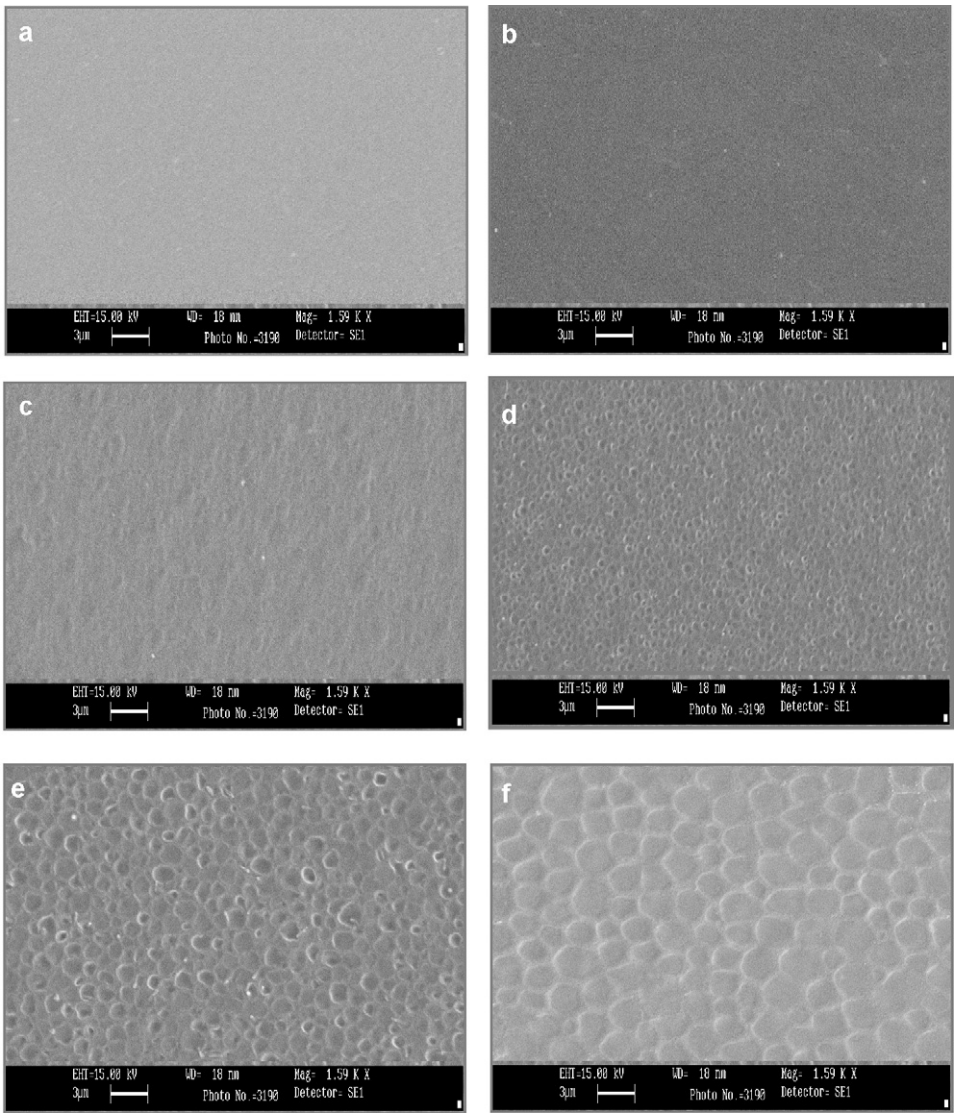

Fig. 4. Scanning electron micrographs of unloaded F1 film $r = 0.6$ (a) and of loaded F1 films: $r = 0.2$ (b), $r = 0.4$ (c), $r = 0.6$ (d), $r = 0.8$ (e), $r = 1$ (f).

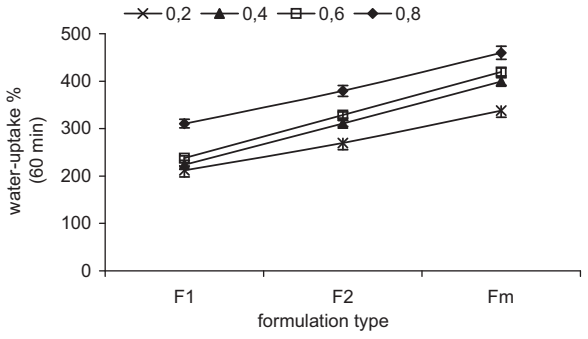

Fig. 5. Water-uptake ability after 60 min of different film formulation (F1, F2 and Fm) at pH 6.8 ($n = 5$, the SD did not exceed the 5%).

237.5 ± 8.3 for film $r = 0.6$; 295.3 ± 4.5 for film $r = 0.8$. We must consider that at pH 6.8 propranolol hydrochloride is present in its ionized form (pKa 9.5) and its positive charge can interact with free negative charges of acidic amino acids of gelatin. These possible drug/protein ionic interactions can reduce free charges on gelatin structure thus determining the lower tendency of film hydration.

Fig. 5 showed the water-uptake ability after 60 min for all loaded films (F1, F2 and Fm). As can be seen, the presence of a higher amount of polymer (F2 films) increased water-uptake ability % respect to F1 films. As previously described the presence of free charges in the formulations is extremely important for film hydration and greater amount of polymers in the films can improve water-uptake ability % ($P < 0.05$).

As can be expected, films with the addition of mannitol (Fm) showed a greater water-uptake ability % than F1 and F2 films ($P < 0.05$), due to the presence of a hydrophilic molecule able to favour a major entry of water in the system.

3.5. *In vitro and in vivo mucoadhesion properties*

Table 2 reports the results of *in vitro* and *in vivo* mucoadhesion tests for unloaded and loaded F1 films.

As can be seen, films with an excess amount of chitosan showed the best *in vitro* mucoadhesive properties among all films. In fact, amino groups of chitosan chains were positively charged and could interact with sialic acid (pKa 2.6) and sulphate residues of mucin glycoprotein, that, at pH 6.8, were negatively charged (Peppas & Sahlin, 1996).

Table 2
In vitro mucoadhesive capacity (expressed as detachment force, mean ± SD, $n = 3$) and *in vivo* residence time in buccal cavity (mean ± SD, $n = 3$) of unloaded films (F1).

r	0	0.2	0.4	0.6	0.8	1
In vitro force detachment (dyne)	18.7 ± 0.4	15.8 ± 0.3	14.6 ± 0.5	13.8 ± 0.3	11.4 ± 0.5	10.3 ± 0.2
In vivo residence time (min)	270 ± 15	240 ± 13	230 ± 12	220 ± 11	150 ± 7	50 ± 4

F2 films showed higher mucoadhesion values than F1 films; in fact, for film with $r = 0$, for example, the detachment force increased until a value of 22.8 ± 0.5 (other data were not reported). This behaviour can be explained with the presence of a greater amount of polymer respect F1 film and consequently, with a greater presence of positively charged amino groups.

Moreover, Fm films, containing mannitol, showed the best mucoadhesion properties (30.7 ± 0.9 for film with $r = 0$); in fact, in addiction to ionic interaction, mannitol promoted the entry of water, a more efficient chain mobility and physical entanglement with mucus. Despite loaded films showed a lower significant water-uptake ability than unloaded films, as a consequence of interaction between drug and gelatin (see Section 3.5), the ionic interaction between chitosan and mucus provided not significantly different mucoadhesion properties respect mucoadhesion of unloaded films.

In vivo mucoadhesive tests were performed to assess the ability of films, without drug, to adhere to the gingivae and to study the potential irritant effect. Films did not have irritating effects on the buccal mucosa; in fact, after the removal of the film, buccal tissue revealed no signs of damage to the mucosa. Volunteers reported no irritation during or after the study.

Moreover, films with an excess of chitosan showed the best *in vivo* mucoadhesive properties, confirming *in vitro* mucoadhesion studies, while films with an excess of gelatin showed a lower residence time respect to films with an excess of chitosan ($P < 0.05$). For F1 and Fm films ($r = 0$) the residence times were 300 ± 12 min and 320 ± 5 min, respectively (other data were not reported).

3.6. In vitro release of propranolol hydrochloride from buccal films

In vitro release studies showed that propranolol hydrochloride release stopped in the first 30 min for all films analysed.

In particular, only film based on gelatin alone ($r = 1$) provided complete drug release due to its dissolution. Films with an excess of chitosan ($r = 0.2$ and $r = 0.4$) showed a higher release of drug with respect to films with a greater amount of gelatin ($P < 0.05$) allowing 83% and 66% of drug released in 30 min, respectively. On the contrary, films containing an excess of gelatin, $r = 0.6$ and $r = 0.8$, provided a percentage drug release of 54% and 48% respectively in 30 min.

Propranolol hydrochloride was not completely released from all the formulations containing chitosan/gelatin complexes; this behaviour can be related to the presence of possible interactions between drug and gelatin, which, proportionally to gelatin increase in the films, limit drug diffusion through the chitosan/gelatin polymeric network.

Differently, all films containing mannitol provided a complete drug release (100%) in 30 min, due to a greater entry of water favouring polymeric chain mobility and thus drug diffusion through the hydrated films.

3.7. In vitro permeation studies

Fig. 6 showed the permeation profiles and permeability coefficients P (cm/min) of propranolol hydrochloride across porcine buccal mucosa, after application of loaded F1 films. As can be seen from the figure, control formulation provided the complete permeation of the drug in 90 min. Films consisting exclusively of gelatin

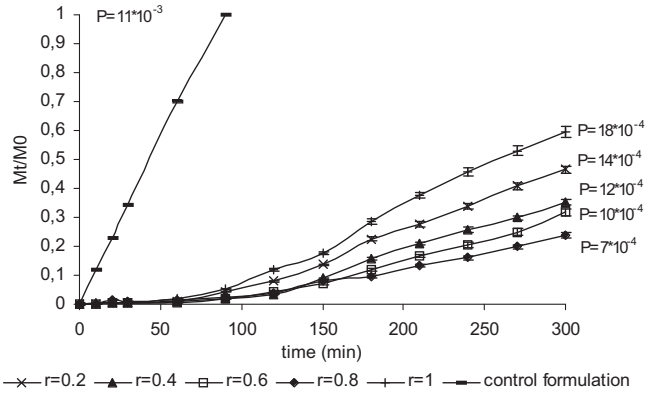

Fig. 6. Permeation profiles (mean ± SD, $n = 3$) and permeability coefficients P (cm/min) of propranolol hydrochloride across the porcine buccal mucosa from F1 films.

provided the greater amount of permeated drug, due to its rapid dissolution. Instead, films containing chitosan/gelatin complexes provided a lower amount of permeated drug; in particular, films with a higher content of gelatin provided the lower amount of permeated drug.

This behaviour can be correlated with drug release profiles from buccal films, which influenced drug availability at the absorption site.

While control formulation was rapidly removed from buccal cavity by saliva and swallowing, mucoadhesive films, in particular films containing an excess of chitosan ($r = 0.2$ and $r = 0.4$), provided a higher buccal residence times, optimizing drug bioavailability.

As can be seen from Fig. 6, these films provided the permeation of only 46% and 35% ($P = 14 \times 10^{-4}$ cm/min and $P = 12 \times 10^{-4}$ cm/min) of drug, respectively. Differently, permeation studies relative to films ($r = 0.2$ and $r = 0.4$) containing mannitol showed an amount of permeated drug around 80% and 68% ($P = 28 \times 10^{-4}$ cm/min), respectively.

As underlined in Section 1, considering drug oral bioavailability of approximately 25%, for hypertension treatment and for atrial fibrillation the anticipated buccal doses of propranolol hydrochloride are 10 mg twice daily and 7.5–10 mg daily, respectively. The application of four and two films containing mannitol and an excess of chitosan could allow achieving the proposed daily dose for hypertension treatment and for atrial fibrillation, respectively.

3.8. Antimicrobial activity assay

Buccal preparations intended for chronic treatment should guarantee the adequate dosing regimen avoiding any potential undesirable side effects related to their prolonged residence at the administration site. An interesting characteristic of chitosan/gelatin films was their compatibility with buccal microflora.

The disc-agar diffusion test showed an antibacterial activity associated with propranolol hydrochloride. Loaded films ($r = 0.2$, $r = 0.4$, $r = 0.6$), chitosan/drug and chitosan/gelatin/drug solutions determined growth inhibition zones for the pathogen bacteria *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* with diameters ranging from 8 to 28 mm. No growth inhibitory effect was observed against

the probiotic species *L. acidophilus* and *B. infantis* and against the yeast *C. albicans*.

Differently, unloaded films, chitosan and chitosan/gelatin solutions did not show any noticeable inhibition zone for the microorganisms tested, indicating the absence of antimicrobial activity for chitosan and gelatin at the concentrations used in the preparation of the films.

The MIC of propranolol hydrochloride was evaluated by the agar dilution method. MIC resulted > 120 μg/mL, indicating that propranolol hydrochloride can not be considered a strong antimicrobial agent. Since drug concentration in the films was 25 mg/mL, the antimicrobial activity of this concentration was also evaluated. The antimicrobial effects of propranolol hydrochloride at 25 mg/mL was demonstrated for all the microorganisms tested, confirming the data related to the inhibitory zones. Notably, the formulation of propranolol hydrochloride in the polymeric films counteracted the antibacterial effects of this drug against the probiotic species *L. acidophilus* and *B. infantis*, suggesting a selective action against pathogen bacteria.

4. Conclusion

Polyelectrolyte complexes based on chitosan and gelatin can be successfully employed for the formulation of buccal films.

The selection of the appropriate chitosan/gelatin ratio and polymer concentration in the film, as well as the addition of mannitol, supports the goal of ensuring the necessary dose for treatment of hypertension and atrial fibrillation.

Moreover, along with adequate drug release and permeation, desirable film characteristics such as suitable hydration and mucoadhesion, were obtained. Finally, film tolerability and compatibility with buccal mucosa suggests their possible use as formulations intended for treatment of chronic diseases.

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