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ABSTRACT

Buccal mucosa has emerged as an attractive site for systemic administration of drug in paediatric patients. This route is simple and non-invasive, even if the saliva wash-out effect and the relative permeability of the mucosa can reduce drug absorption. Mucoadhesive polymers represent a common employed strategy to increase the contact time of the formulation at the application site and to improve drug absorption. Among the different mucoadhesive dosage forms, buccal films are particularly addressed for paediatric population since they are thin, adaptable to the mucosal surface and able to offer an exact and flexible dose. The objective of the present study was to develop bilayered buccal films for the release of propranolol hydrochloride. A primary polymeric layer was prepared by casting and drying of solutions of film-forming polymers, such as polyvinylpyrrolidone (PVP) or polyvinylalcohol (PVA), added with different weight ratios of gelatin (GEL) or chitosan (CH). In order to achieve unidirectional drug delivery towards buccal mucosa, a secondary ethylcellulose layer was applied onto the primary layer. Bilayered films were characterized for their physico-chemical (morphology, thickness, drug content and solid state) and functional (water uptake, mucoadhesion, drug release and permeation) properties. The inclusion of CH into PVP and PVA primary layer provided the best mucoadhesion ability. Films containing CH provided a lower drug release with respect to films containing GEL and increased the amount of permeated drug through buccal mucosa, thanks to its ability of interfering with the lipid organization. The secondary ethylcellulose layer did not interfere with drug permeation, but it could limit drug release in the buccal cavity.

Keywords: child-appropriate dosage forms, buccal administration, mucoadhesion, bilayered film, propranolol hydrochloride, permeation studies.

Chemical compounds studied in this article

Chitosan (PubChem CID: 71853); Polyvinylpyrrolidone (PubChem CID: 6017); Propranolol hydrochloride (PubChem CID: 62882); Ethylcellulose (PubChem CID: 24832091)

1. Introduction

Regulatory initiatives in the United States and Europe over the last decades have stimulated the development of suitable medicines for children taking into consideration appropriate route of administration, dosage form, excipients, taste/palatability and delivery device. In fact many drugs are routinely prescribed by physicians although they are not approved by registration agencies for use in children (Van Riet-Nales et al, 2017; Ernest et al., 2007; Strickley et al. 2008).

Buccal mucosa has emerged as an attractive site for systemic administration of drug in paediatrics by reason of advantages such as the direct passage of drug into the systemic circulation through the jugular vein, thus bypassing the stomach environment and first-pass liver metabolism, fast onset of action and rapid decline after removing the dosage form, ease access for self-medication. It allows a significant improvement in patient acceptance and compliance (Lam et al., 2014; Patel et al. 2011). Delivery of drugs via the buccal mucosa may be achieved by using mucoadhesive dosage forms able to maintain an intimate and prolonged contact with mucosa and favor the drug absorption to improve the bioavailability (Sudhakar et al. 2006). Among the different mucoadhesive formulations suitable for young children, buccal films ensure accurate dosing and compared to conventional buccal tablets they are thin, flexible, easily applicable and adequately strong to withstand breakage caused from mouth movements (Borges et al., 2015; Dixit and Puthli, 2009; Trastullo et al., 2016; Krampe et al., 2016).

To achieve an optimal mucoadhesion ability, but also a suitable drug release profile, it is important the selection of polymeric material (Salamat-Miller et al., 2005). Generally a single polymer may not possess adequate characteristics and polymeric blends prepared by physical mixing of two or more polymers often exhibit properties that are superior to any one of the component polymers (Munasur et al., 2006). Polyvinylalcohol (PVA) and polyvinylpyrrolidone (PVP) are synthetic and water soluble hydrophilic polymers available with different molecular weights, and have been widely used for decades in pharmaceutical and biomedical applications for their excellent mechanical, biocompatible, biodegradable, nontoxic properties. Moreover, they have been successfully employed as film-forming

materials (Falath et al., 2017; Hifumi et al., 2016; Kumar et al. 2014). Chitosan (CH) and gelatin (GEL) are naturally occurring polysaccharides that have been proposed as ideal carrier in oral mucosal drug delivery due to their good mucoadhesive properties (Salamat-Miller et al., 2005; Kumar et al., 2016; Abruzzo et al., 2015). Moreover, since CH has been shown to be capable to interfere with lipid micelle organization in the intestine, Şenel et al. (2000) explained that it could enhance the absorption of drugs across the buccal mucosa by interfering with the lipid organization in the buccal epithelium. In fact, the buccal mucosa (thickness of approximately 500-800 μm) shows the important function of protection of the underlying tissue and a variety of barrier mechanism are integrated in this part. The first is represented by saliva that contains high molecular weights glycoprotein (MG1) able to adhere to the surface of the oral epithelium, constituting the mucus layer. This is a viscoelastic layer of varying thickness (approximately 70 - 100 μm) that affects drug absorption. Nevertheless, it is known that the main penetration barrier for the drug molecules lies in the superficial layer of the epithelium. This layer shows cells with increased size and more flattened shape as compared with the basal layer and in the 200 μm outermost part it contents lipophilic intracellular material derived from the membrane coating granules (MCGs) (Teubl et al., 2013). In this work, the use of relatively low molecular weight polymers is justified by the hypothesis that the rate-limiting step in buccal drug transport is the biological barrier itself and that the use of high molecular weight polymers can represent an additional obstacle (del Consuelo et al., 2007).

As regard the choice of drug, some classes may benefit from buccal administration include antihypertensives. Propranolol is a β-blocker used in pediatric patients primarily for the treatment or prevention of cardiac arrhythmias, hypertension, outflow obstructions in congenital heart disease, and hypertrophic cardiomyopathy. It is commercially available in different dosage forms, including oral tablets, extended-release capsules, and liquid solutions. In this work propranolol was chosen as a model drug for incorporation into a buccal formulation because is a potent drug which has suitable physicochemical properties (MW 259.3 g/mol, logP = 3.48, log $D_{pH6.8}$ = 1.20), and extensive and highly variable first pass metabolism following oral administration with only ~ 25 % of oral drug

reaching the systemic circulation (Amores et al., 2014). Moreover as some of the oldest β-blocker, propranolol falls into the category of off-patent drugs used in paetiatrics (Chu et al., 2014).

In the present study we developed new bilayered buccal films using synthetic film-forming polymers in combination with natural mucoadhesive polymers for the release of propranolol hydrochloride in paediatric. Briefly the main steps were: (a) prepare a polymeric matrix (primary polymeric layer) based on PVA or PVP added with different weight ratios of CH or GEL; (b) demonstrate whether the addition of CH or GEL was able to maximize the mucoadhesion properties and the control of drug release of the primary polymeric layer; (c) after identification of an ideal polymeric blend, apply a non-dissolvable backing layer onto the first one to achieve unidirectional release towards the oral mucosa (bilayered film), avoiding drug release in the oral cavity. Particular attention was given to the drug permeation studies that until now have not been sufficiently described in literature.

2. Materials and methods

2.1. Materials

Type B gelatin from bovine skin (GEL; MW 50 kDa, 100-115 millimoles of free carboxyl groups per 100 g of protein, isoelectric point in the range of pH 4.7-5.2) was purchased from Sigma-Aldrich (Milan, Italy). Low-viscosity chitosan (CH; MW 150 kDa, deacetylation degree 97 %; pKa = 6.3), polyvinylpyrrolidone K30 (PVP; MW 40 kDa), polyvinylalcohol 49000 (PVA; MW 49 kDa) and propranolol hydrochloride were obtained from Fluka (Milan, Italy). Ethylcellulose (ETHOCEL® Standard E10 FP Premium, viscosity range 9-11 mPa×s) was obtained from Colorcon Ltd (Dartford, England). All other chemicals were of analytical grade and were purchased from Carlo Erba (Milan, Italy).

Water-uptake, residence time, release studies were carried out in aqueous buffer with the following composition (g/L): 4.61 KH_2PO_4 and 16.75 $Na_2HPO_4 \times 12H_2O$ adjusted with hydrochloric acid to pH 6.8 (healthy saliva pH = 6.7-7.4) (Marques et al., 2011; Gittings et al., 2015). Permeation studies were performed in buffer solution at pH 7.4 (g/L): 2.38 $Na_2HPO_4 \times 10H_2O$, 0.19 KH_2PO_4 and 8.0 NaCl.

2.2. Preparation of bilayered films

The primary polymeric layers were produced by a casting/solvent evaporation method. CH and PVP were dissolved in acetic acid solution (1 % w/w) and water, respectively. PVA and GEL were dissolved in water, previously heated at 70 and 50 °C, respectively. Different amounts of CH or GEL solutions were added to PVP or PVA solutions (3% w/w total polymeric concentration) thus obtaining different PVP:CH, PVP:GEL, PVA:CH and PVA:GEL weight ratios (10:0, 7:3, 5:5, 3:7 and 0:10). Propylene glycol (0.5 % w/w) was added to the final polymeric solutions as plasticizer. Propranolol hydrochloride was dissolved in water and added to the different polymeric solutions in a concentration (0.41 % w/w) able to guarantee a child-appropriate dose of drug in buccal films. The mixtures were stirred at room temperature for 60 minutes. Then, about 45 g of the mixture were placed in a Petri-dish (diameter of 9 cm and height of 1.5 cm), oven-dried at 50 °C for 15 h (heating oven FD series, Binder, Tuttlingen, Germania) and stored in a desiccator until use. The obtained primary polymeric layers were reported in Table 1. PVP/PVA:CH 0:10, PVA:CH/GEL 3:7, PVA:GEL 5:5 and PVA:GEL 7:3 polymeric solutions were not able to produce uniform films and were not considered for the next studies.

For bilayered film preparation, circles with a surface area of 1.33 cm^2 were cut from each primary polymeric films and an ethylcellulose solution (0.5 mL, 1% w/w in acetone) was sprayed onto their surface (oral spray bottle; RPC Plastiape, Osnago, Italy) and immediately oven-dried at 70 °C for 5 minutes. This procedure was repeatedly conducted in order to apply 75 mg of ethylcellulose. All primary polymeric layers were coated with ethylcellulose, except PVP:CH/GEL 10:0, that dissolved itself in presence of acetone.

2.3. Solution viscosity

The viscosity of solutions used for the preparation of the primary polymeric layers was measured with a falling ball viscometer at 25 °C (HAAKETM Falling Ball Viscometer Type C, Thermo electron corporation, Karlsruhe, Germany).

2.4. Differential scanning calorimetry (DSC)

DSC experiments were performed on primary polymeric layers to identify possible phase transitions (from crystalline to amorphous forms) of drug following manufacture. Calorimetric measurements were performed using a Netzsch DSC200 PC differential scanning calorimeter (Metzsch, Germany). The samples were placed in aluminum pans and then hermetically sealed with aluminum lids. Thermal analyses were performed from 25 °C to 300 °C under a dry nitrogen atmosphere with a flow rate of 25 ml/min and a heating rate of 10 °C/min.

2.5. Scanning electron microscopy (SEM)

SEM analysis were performed to evaluate the morphology of primary polymeric layers and bilayered films. Films were cut with a razor blade, 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., Cambridge, UK) using secondary electron imaging at 15 kV.

2.6. Film thickness and drug content

Primary polymeric layers and bilayered films were measured for thickness through a Mitutoyo pocket thickess gauge (Mitutoyo Mfc. Co. Ltd., Tokyo, Japan). Then each circle (surface area of 1.33 cm^2) was dissolved in 40 mL of phosphate buffer at pH 7.4 and the solutions obtained were analyzed by HPLC method in order to determine the amount of propranolol hydrochloride contained in the film. The results were expressed as mg/cm^2. The chromatographic system was composed of a Shimadzu (Milan, Italy) LC-10ATVP chromatographic pump and a Shimadzu SPD-10AVP UV-Vis detector

set at 290 nm. Separation was obtained on a Phenomenex (Torrance, CA, USA) Synergi 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 a mixture of 0.5 % (w/v) aqueous triethylamine adjusted with orthophosphoric acid to pH 3.0 and acetonitrile (30:70, v/v). The flow rate was 0.4 mL/min and manual injections were made using a Rheodyne 7125 injector with a 20 μL sample loop. Data processing was handled by means of a CromatoPlus computerized 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 and a good linearity was found (R^2 = 0.9998).

2.7. In vitro water uptake ability

Water uptake ability was studied to investigate the hydration properties of primary polymeric layers. Accurately weighted circle (surface area = 1.33 cm^2) was placed on filter paper (3 cm × 3 cm) soaked in phosphate buffer at pH 6.8 and positioned on top of a sponge (7 cm × 5 cm × 2 cm), previously soaked in the hydration medium. For bilayered films the primary polymeric layers was facing towards the sponge. The sponge was placed in a Petri dish filled with the same solution to a height of 0.5 cm (Bertram and Bodmeier, 2012). Water uptake (WU) was determined as weight increase of the film for 240 min, according to the following equation:

$$WU\ (\%) = (W_{Hff}\text{-}W_{Hf}\text{-}W_{Df}) \times 100 / W_{Df}$$

where W_{Hff} is the weight of hydrated film and wet paper filter, W_{Hf} is the weight of wet paper filter and W_{Df} is the weight of the dry film.

2.8. In vitro residence time and mucoadhesion ability

Residence time measurements allow assessment of the mucoadhesive strength as well as solubility or disintegration characteristics of the films by determining the time needed for completely remove the film from the mucosal surface. For this study, freshly isolated porcine oesophageal mucosa was

employed. As demonstrated (del Consuelo et al., 2005), the histology of pig oesophageal and buccal mucosa is comparable. Moreover, with respect to buccal mucosa, the oesophageal mucosa is easier to separate from the underlying tissue and its surface area is greater and generally undamaged by mastication. Pig oesophageal tissue used in these studies were obtained from a local slaughterhouse, immediately transported to the laboratory and used within 2 h. Oesophagus was cut longitudinally and rinsed with isotonic saline. The mucosa was separated from the muscular layer by cutting the loose connective fibers with a scalpel and cut into size of 4 cm^2. For the determination of *in vitro* residence time (Nair et al., 2013), the oesophageal mucosa was pasted on a glass slide using cyanoacrylate adhesive. Subsequently, primary polymeric layers and bilayered films (surface area = 0.20 cm^2) were attached to the mucosa with a sligth pressure (20 g standard calibration weight for 15 minutes) and immersed inside a beaker containing 40 mL of phosphate buffer at pH 6.8 (non biorelevant conditions). The time taken by the film to completely detach from the mucosa was considered as the residence time.

Mucoadhesive properties of the dosage forms were performed in order to evaluate their ability to establish an intimate and prolonged contact with mucosa and eventually favor the drug absorption. Mucoadhesion ability was measured in terms of the force needed to pull out a freshly oesophageal mucosa from primary polymeric layers with an adapted tensiometer (Krüss 132869; Hamburg, Germany). The mucosa was fixed to a support (surface area 1 mm^2) with cyanoacrylate adhesive and then suspended from the tensiometer spring. The mucosa was lowered until it just contacted the surface of the layer, 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 layer. This point represents the adhesive bond strength between mucosa and film and is expressed as a positive force in dyne.

2.9. In vitro release studies

In vitro release studies were performed in order to evaluate the drug amount released from primary polymeric layers and bilayered films over the time. These data are useful for the explanation of the permeation results and for the evaluation of the drug released in the buccal cavity from the bilayered films. For this study, primary polymeric layer (surface area of 1.33 cm^2) was attached on a glass slide using cyanoacrylate adhesive. For the evaluation of drug release from bilayered films, films (surface area of 1.33 cm^2) were attached on the glass side with the ethylcellulose layer facing towards the medium. The glass slide was immersed inside a beaker containing 40 mL of phosphate buffer at pH 6.8 and maintained under agitation with a magnetic bar. Aliquots of 1 mL were withdrawn at different time intervals and replaced with fresh medium. The studies were carried on for 4 h and samples were analyzed by the same HPLC method previously described. All experiments were performed under sink conditions (c_{max} in medium < 10% $c_{saturation}$). The results of release studies are shown as shown as cumulative drug amount released (expressed as fractional amount) plotted as a function of time.

2.10. In vitro permeation studies

In vitro permeation studies were performed in order to evaluate the amount of permeated drug from primary polymeric layers and bilayered films through buccal route. These studies were carried out introducing single circle (surface area = 0.20 cm^2) in the donor compartment of a Franz-type static glass diffusion cell (15 mm jacketed cell with a flatground joint and clear glass with a 12 mL receptor volume; diffusion surface area = 1.77 cm^2), equipped with a V6A Stirrer (PermeGearInc., Hellertown, PA, USA). For these experiments oesophageal epithelium was used as barrier as its structure and permeability characteristics are close to those of human tissue (del Consuelo et al., 2005) and positioned between the donor and receptor compartments. For epithelium isolation, the excised oesophageal mucosa (isolated as previously described) was immersed in saline at 60-65°C for 1 min, following which the epithelium was carefully peeled away from the connective tissue. The connective side of tissue was spread over a 0.45 µm cellulose acetate filter (MF-Millipore Membrane, Tullagreen, Carrigtwohill, Co. Cork, Ireland) and the ensemble was mounted in the diffusion cell.

Primary polymeric layers and bilayered films were placed on the oesophageal epithelium, previously hydrated with 200 µL of phosphate buffer at pH 6.8 and the system was closed with Parafilm® "M" sealing film (American National Can Company, Chicago, IL, USA) to avoid the evaporation of permeation medium an allow the establishment of a constant relative humidity around the insert. The receptor compartment was filled with phosphate buffer at pH 7.4 maintained at 37 ± 0.5 °C and continuously stirred at 100 rpm. Samples of the receptor solution were withdrawn at predetermined time intervals over 6 h and analyzed by HPLC system for the determination of permeated drug (choromatographic method previously described).

An aqueous solution (100 µL) of propranolol hydrochloride (6 mg/mL) was also prepared and its permeation ability was analyzed at the same conditions of films. The results of permeation studies are shown as cumulative drug amount perneated (expressed as fractional amount) plotted as a function of time.

2.11. Statistical Analysis

All experiments were done in triplicate, while transport experiments were done with five replicas. Results are expressed as mean ± SD. ANOVA and t-test were used to determine statistical significance of studies. The criterion for statistical significance was $p < 0.05$.

3. Results and discussion

3.1. Solution viscosity

Solvent cast evaporation method used for film preparation is based on the dissolution of the polymers in appropriate solvents, on the subsequent mixture of polymer solutions in order to obtain the desired polymer weight ratio and on the solvent evaporation allowing primary polymeric layer formation. Viscosity of polymeric solutions was measured because it affects final film properties, especially drug release ability. In fact, higher solution viscosity produces film that, after hydration on buccal mucosa,

provides higher hydrogel viscosity, thus limiting the drug diffusion and the fractional amount released over the time.

In this study, we measured the viscosity of all polymeric solutions except the solutions related to PVP/PVA:CH 0:10, PVA:CH/GEL 3:7, PVA:GEL 5:5 and PVA:GEL 7:3 that, as described before, were not able to produce uniform layers. As reported in Fig. 1, the addition of CH or GEL to PVP solution and of CH to PVA solution provided an increase of viscosity. Furthermore, the addition of increasing amount of CH or GEL proportionally increased the solution viscosity. Finally, CH greatly increased solution viscosity with respect to GEL, probably due to its higher molecular weight.

3.2. Differential scanning calorimetry (DSC)

In order to evaluate possible phase transitions of the active during the preparation process, differential scanning calorimetry was used. In fact, a metastable solid drug can change crystalline structure in response to changes in environmental conditions, processing, or over time. Polymorphs of a solid drug can have different chemical and physical properties, and these properties can have a direct impact on the quality/performance of drug products, such as stability, dissolution, and bioavailability. The most stable polymorphic form of a drug is often used because it has the lowest potential for conversion from one polymorphic form to another, while the metastable form may be used to enhance the bioavailability.

The DSC profiles of propranolol hydrochloride (Fig. 2) showed a single endothermic peak at 168 °C, due to the melting of drug. The thermograms of all samples did not show the melting peak of the active indicating that casting-solvent evaporation method induced the amorphization of propranolol hydrochloride. As previously described, the amorphous form of the drug should represent an advantage in terms of solubility and bioavailability.

3.3. Scanning electron microscopy (SEM)

SEM analysis (Fig. 3) showed that all primary polymeric layers showed an uniform and smooth surface and a dense and homogenous cross section. Furthermore, it is important to mention that the all primary polymeric layers, except PVP:CH/GEL 10:0, were uniformly coated by ethylcellulose, and that a perfect binding between hydrophilic layer and backing layer was achieved as is clearly demonstrated by SEM of bilayered film cross-section (Fig. 4).

3.4. Film thickness and drug content

The results related to the measurement of thickness and drug content are reported in Table 1. The bilayered films showed an higher thickness with respect to films without ethylcellulose layer. The very low standard deviations suggested that the preparation method provided no significant difference between the samples. Moreover, taking into account that thickness is directly related to the accuracy of dose, thickness in different point of the same film was measured and no significant difference was observed (data not shown). In addition, the experimental drug content was very close to the theoretical one (2.83 mg/cm^2; 10.48 % w/w) for each formulation, indicating that the preparative method is suitable to produce polymeric films containing propranolol hydrochloride.

3.5. Water uptake ability

In vitro water uptake values after 60 min of primary polymeric layers are reported in Table 1. PVP:CH/GEL 10:0 and PVA:CH 10:0 were completely solubilized within 5 min and therefore their water uptake values were not reported. The presence of CH or GEL led to the formation of primary polymeric layers able to hydrate over the time, producing a gelled matrix and the water uptake ability increased with the increase of CH or GEL content. In particular, primary polymeric layers containing CH showed the highest water uptake values between all formulations (e.g. PVP:CH 5:5> PVA:CH 5:5> PVP:GEL 5:5). This behaviour can be mostly related to the different polymer properties. In fact, CH is an hydrophilic molecule and in our operative conditions (phosphate buffer at pH = 6.8), it showed positive charge (143 mmoles of positively charged amino groups per 100 g of polymer; pKa

= 6.3). Also carboxylic groups of GEL (isoelectric point in the range of pH 4.7-5.2) were negatively charged at pH = 6.8, but the amount of charges was lower than CH (100-115 mmoles of free carboxyl groups per 100 g of protein, as reported in Product Information Sheet). Moreover, PVA:CH 7:3 and 5:5 showed a lower hydration ability than PVP:CH 7:3 and 5:5, respectively, probably due to the interaction between the hydroxyl groups of PVA with the amino and hydroxyl groups of CH (Berger et al., 2004).

3.6. In vitro residence time and mucoadhesion ability

Once administered into the oral cavity, firstly the films have to wet, hydrate and swell in order to establish an intimate contact with the mucosa. Then, there will be an interpenetration across the interface between polymeric chains of the film and the mucus gel network. The interpenetrate chains can therefore interact by means of entanglement and chemical bonds.

Results obtained from *in vitro* residence time studies on primary polymeric layers demonstrated that PVP:CH/GEL 10:0 and PVA:CH 10:0 completely solubilized within 5 min in phosphate buffer at pH 6.8 and for this reason, it was not possible to determine their residence times. PVP:CH 5:5 and PVA:CH 5:5 showed residence time of 36 ± 9 and 37 ± 10 minutes, respectively. In the experimental conditions of our study, these layers rapidly absorbed water and the consequent increase in weight and size led their detachment from porcine mucosa. In fact, the extent and rate of water uptake of mucoadhesive polymers exert a great influence on their adhesive properties (Mortazavi and Smart, 1993). Mucoadhesive polymers are supposed to take water from the underlying mucosal tissue by absorbing, swelling and capillary effects leading to considerably strong adhesion (Duchene and Ponchel, 1992). On the other hand, an excessive water uptake produce an over hydration of the dosage form that completely loses its adhesiveness (Lehr, 1996). Finally, PVP:GEL 5:5 and PVP:GEL 0:10 showed residence times of 29 ± 5 and 52 ± 11 minutes, respectively. Also this behaviour is probably connected with the hydration degree of these formulations. Bilayered films showed the same trend, but the presence of ethylcellulose layer allowed to prolong film residence time due to the decrease of

hydration rate. In particular, PVP:CH 5:5 and PVA:CH 5:5 showed residence times of 59 ± 6 minutes and 65 ± 7 minutes, while PVP:GEL 5:5 and PVP:GEL 0:10 detached from mucosa after 180 ± 14 minutes and 230 ± 21 minutes.

Mucoadhesion ability was measured with an adapted tensiometer and results showed that for PVP:CH/GEL 10:0 and PVA:CH 10:0 the detachment forces were 38 ± 5 and 36 ± 4 dyne, respectively. The addition of CH provided the best *in vitro* mucoadhesive properties among all the primary polymeric layers. In fact, the detachment forces for PVA:CH 5:5 and PVP:CH 5:5 were 60 ± 2 and 68 ± 3 dyne, respectively. This behaviour was probably due to their highest hydration among all the formulations. Moreover, the amino groups on chitosan chains were positively charged and could interact with negatively charged sialic acid (pKa 2.6) and sulphate residues of mucin glycoprotein. Finally, PVP:GEL 0:10 (48 ± 3 dyne) showed an higher detacment force than PVP:GEL 5:5 (40 ± 2 dyne), accordingly to hydration data.

3.7. In vitro release studies

Drug release behaviour is a crucial parameter in order to evaluate the drug amount able to permeate through buccal mucosa. The choice of an ideal composition in terms of polymers and weight ratio affects the drug permeation by affecting the drug release from buccal film.

Table 1 reports the fractional amounts of propranolol hydrochloride (Mt/M0) released from all the primary polymeric layers after 60 min, while release profiles (Fig. 5) are reported only for PVP:CH/GEL 10:0, PVA:CH 10:0, PVP:GEL 0:10, PVP:CH 5:5, PVP:GEL 5:5 and PVA:CH 5:5 as representative formulations of the different series. In fact, the drug release profiles for different weight ratios (7:3, 5:5, 3:7) of the same polymeric mixture were not significantly different. As can be seen, PVP:CH/GEL 10:0 and PVA:CH 10:0 provided a quick and complete release of propranolol hydrochloride within 50 minutes, due to their rapid dissolution. However, their rapid dissolution did not allow a prolonged drug release and it is possible to hypothize their use as solid dosage forms to be dispersed in the mouth and swallowed. The adding of CH or GEL produced a higher viscosity of

the polymeric network in the gelled state and consequently, provided a reduction of drug fractional amount released over the time. Moreover, drug release from PVP:GEL 0:10 was probably affected by the ionic interaction between positively charged drug (pKa 9.5) and the negative charges of acidic amino acids of GEL. The kinetic analysis of release was conducted according to the general Korsemeyer-Peppas equation:

$Mt / Mo = k \times t^{n}$

where *Mt/Mo* is the fractional drug release, *k* is a kinetic constant, *t* is the release time and *n* is the diffusional exponent that can be related to the drug transport mechanism. The release exponents for PVP:GEL 0:10, PVP:CH 5:5 and PVA:CH 5:5 were 0.58, 0.80 and 0.59, respectively, thus indicating an anomalous transport. In fact for a thin hydrogel film, when $n = 0.5$, the drug release mechanism is Fickian diffusion; when $n = 1$, Case II transport occurs, leading to zero-order release; when the value of n is between 0.5 and 1, anomalous transport is observed (Ritger and Peppas, 1987).

As propranolol is subject to significant hepatic metabolism, a drug delivery system designed for buccal absorption should minimize the extent to which the drug is swallowed. In this study, this need is achieved by the impermeable backing layer of ethylcellulose. Moreover, a limited release of drug inside buccal cavity could reduce the bitter taste of propranolol (Yuan et al., 2014), thus increasing children compliance. Bilayered films allowed the release of a lower amount of drug (fractional amount was lower than 35 % after 240 min), thus demonstrating that they could limit drug release inside buccal cavity.

3.8. In vitro permeation studies

In vitro permeation studies were performed in order to establish the absorption of the drug across the buccal epithelium to the systemic circulation. Even in this case PVP:CH/GEL 10:0, PVA:CH 10:0, PVP:GEL 0:10, PVP:CH 5:5, PVP:GEL 5:5 and PVA:CH 5:5 were chosen for the permeation studies as representative of the different series of the primary layers (Fig. 6) and compared with drug permeation from an aqueous solution. All the primary layers provided a lower permeation of the drug

within 6 hours with respect to drug solution. The presence of increasing amount of GEL led to a decrease of permeated drug (PVP:CH/GEL 10:0 > PVP:GEL 5:5 > PVP:GEL 0:10). Moreover, PVA:CH 10:0 showed an higher drug permeation with respect to PVP:GEL 5:5 and PVP:GEL 0:10 and a lower drug permeation with respect to PVP:CH/GEL 10:0. This behaviour can be correlated with drug release profiles from primary layers, which influenced drug availability at the absorption site. Furthermore, PVP:CH 5:5 provided the highest permeated drug amount at each time with respect to other samples. Senel et al. (2000) explained that a possible mechanism of action of chitosan in improving the transport of drug across the buccal mucosa is the ability of interfering with the lipid organization in the buccal epithelium. In the case of PVA:CH 5:5, the permeation enhancer effect of CH was probably limited by the interactions between CH and PVA.

Different studies demonstrated that occlusion is an effective method to enhance the permeation of a drug across the skin (Sparr et al., 2013). In the present study, permeation profiles obtained from bilayered films were no significantly different from films without ethylcellulose layer. In fact, the oesophageal epithelium is a nonkeratinized tissue, characterized by high water content (del Consuelo et al., 2005) and the backing layer did not increase epithelium hydration through its possible occlusion effect.

As concern the practical use of these formulations, the bilayered film surface area useful to obtain an effective plasmatic concentration of drug was calculated according to the following equation (children of 2 years of age; body weigth around 12 kg):

$$Css = J \times A / Cl$$

where *Css* is the concentration at the steady state (0.04 µg/ml), *Cl* is the propranolol clearance (9.2 mL/min/Kg) (Cilurzo et al., 2014) and *J* is the permeation flux of bilayered films. Permeation fluxes (J) and surface areas of PVP:CH/GEL 10:0, PVA:CH 10:0, PVP:GEL 0:10, PVP:CH 5:5, PVP:GEL 5:5 and PVA:CH 5:5 were reported in Table 2. Considering that patches administered to the buccal mucosa may have a size of up to about 1-2 cm^2 (Krampe et al., 2016), the obtained surface areas were

compatible with an easy application of drug delivery system and were suitable for potential use in pediatric population.

4. Conclusions

With orotransmucosal buccal films, a novel solid buccal dosage form was developed fulfill the current demand for child-appropriate dosage forms. They combine the convenience of solid dosage forms and the opportunity to avoid swallowing of a large unit. Moreover, the possibility to cut the film in different sizes during product manufacture allows to obtain different doses with only one production line. Finally, this study shows that the selection of suitable polymeric mixture allowed the modulation of the mucoadhesive ability, the release of the drug and its permeation through the buccal mucosa. The use of ethylcellulose based backing can limit the release of propranolol in the oral cavity, thus establishing a maximum drug activity gradient to the mucosa and reducing drug bitter taste in mouth.

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Table 1. Properties of primary polymeric layers and bilayered film: thickness, drug content, water-uptake ability and drug fractional amount released from the different formulations.

Polymeric mixtures	Polymer weight ratios	Thickness (mm)		Drug content (mg/cm^2)	WU after 60 min (%)	Mt/M0 released after 60 min (%)
		Primary layer	Bilayered film	Primary layer		
PVP:CH/GEL	10:0	0.25 ± 0.04	-	2.82 ± 0.08	-	100.22 ± 1.89
PVA:CH	10:0	0.22 ± 0.02	0.39 ± 0.03	2.63 ± 0.25	-	99.90 ± 2.25
PVP:GEL	0:10	0.23 ± 0.09	0.39 ± 0.01	2.89 ± 0.77	647.72 ± 31.49	51.06 ± 4.88
PVP:CH	7:3	0.23 ± 0.03	0.36 ± 0.05	2.80 ± 0.21	940.25 ± 45.68	34.57 ± 11.53
	5:5	0.24 ± 0.05	0.39 ± 0.08	2.53 ± 0.28	1335.91 ± 52.69	33.10 ± 14.80
	3:7	0.24 ± 0.05	0.35 ± 0.02	2.89 ± 0.53	1622.32 ± 78.48	32.98 ± 10.96
PVP:GEL	7:3	0.26 ± 0.04	0.37 ± 0.05	2.98 ± 0.51	193.77 ± 10.08	97.95 ± 1.12
	5:5	0.25 ± 0.04	0.36 ± 0.01	3.02 ± 0.12	429.52 ± 19.35	88.61 ± 3.23
	3:7	0.22 ± 0.04	0.34 ± 0.06	2.96 ± 0.26	602.21 ± 29.64	79.97 ± 4.87
PVA:CH	7:3	0.28 ± 0.05	0.39 ± 0.04	2.72 ± 0.13	697.36 ± 49.85	35.68 ± 10.25
	5:5	0.32 ± 0.07	0.43 ± 0.08	2.77 ± 0.32	997.91 ± 65.06	55.36 ± 12.36

Table 2. Properties of bilayered films: permeation flux and surface area useful to obtain an effective plasma concentration of drug.

	PVP:CH/GEL 10:0	PVA:CH 10:0	PVP:GEL 0:10	PVP:CH 5:5	PVP:GEL 5:5	PVA:CH 5:5
Flux ($\mu g/cm^2\ h$)	142.88 ± 10.35	165.10 ± 15.63	88.71 ± 3.12	273.66 ± 45.77	153.10 ± 8.52	118.37 ± 6.20
Area (cm^2)	1.85	1.60	2.99	0.97	1.73	2.24

Figure 1. Viscosity of polymeric solutions used for primary polymeric layer preparation.

Figure 2. DSC thermograms of drug and loaded primary polymeric layers: propranolol hydrochloride (a), PVP:CH/GEL 10:0 (b), PVA:CH 10:0 (c), PVP:GEL 0:10 (d), PVP:CH 5:5 (e), PVP:GEL 5:5 (f) and PVA:CH 5:5 (g).

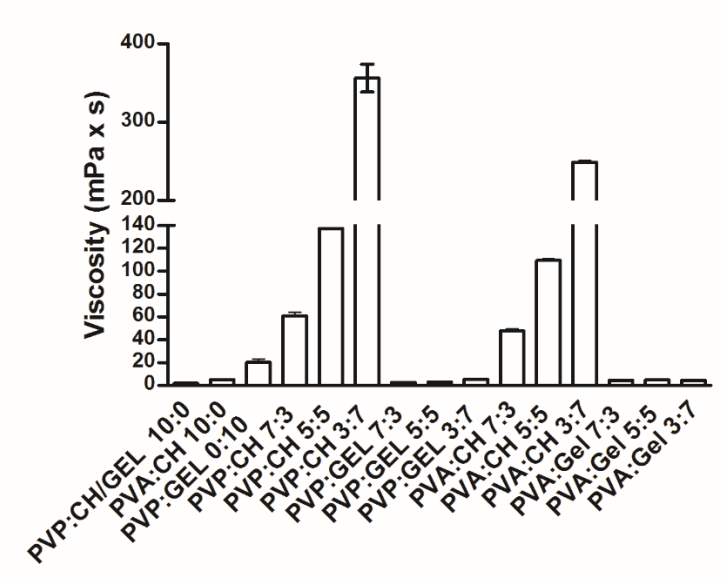
Figure 3. SEM images for the cross-section of the loaded primary polymeric layers: PVP:CH/GEL 10:0 (a), PVA:CH 10:0 (b), PVP:GEL 0:10 (c), PVP:CH 5:5 (d), PVP:GEL 5:5 (e) and PVA:CH 5:5 (f).

Figure 4. SEM image for the cross-section of the loaded PVA:CH 5:5 bilayered film: primary polymeric layer (a) and ethylcellulose backing layer (b).

Figure 5. In vitro release profiles of propranolol hydrochloride from primary polymeric layers.

Figure 6. Permeation profiles of propranolol hydrochloride through esophageal porcine epithelium from drug solution and primary polymeric layers.

Figure 1


Viscosity (mPa x s)
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300
200
140
120
100
80
60
40
20
0
PVP:CH/GEL 10:0
PVA:CH 10:0
PVP:GEL 0:10
PVP:CH 7:3
PVP:CH 5:5
PVP:CH 3:7
PVP:GEL 7:3
PVP:GEL 5:5
PVP:GEL 3:7
PVA:CH 7:3
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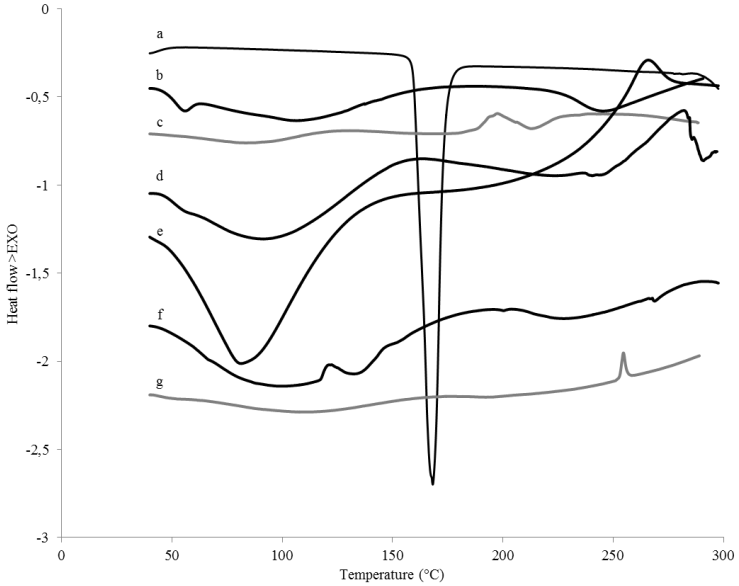
PVA:Gel 7:3
PVA:Gel 5:5
PVA:Gel 3:7

Figure 2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-1
-1,5
-2
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-3
Heat flow >EXO
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50
100
150
200
250
300
Temperature (°C)
a
b
c
d
e
f
g

Figure 3

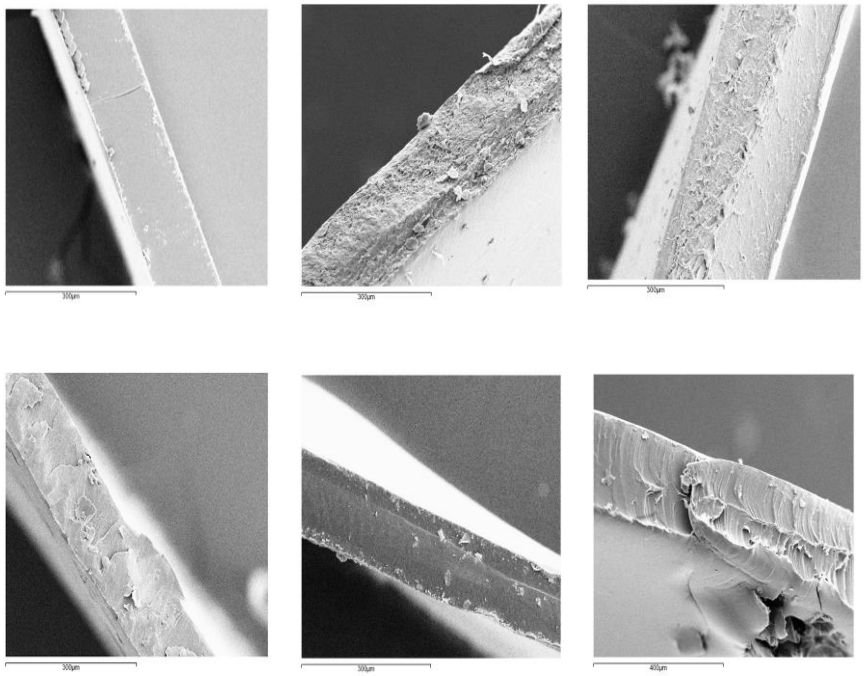


Figure 4

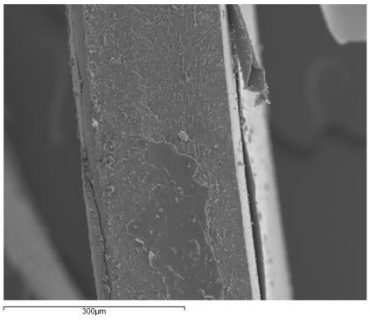


Figure 5

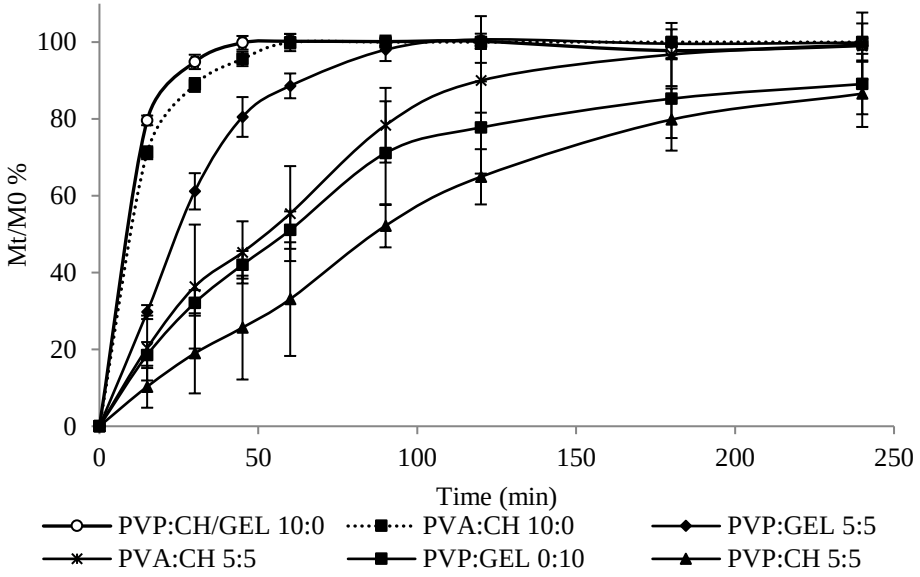
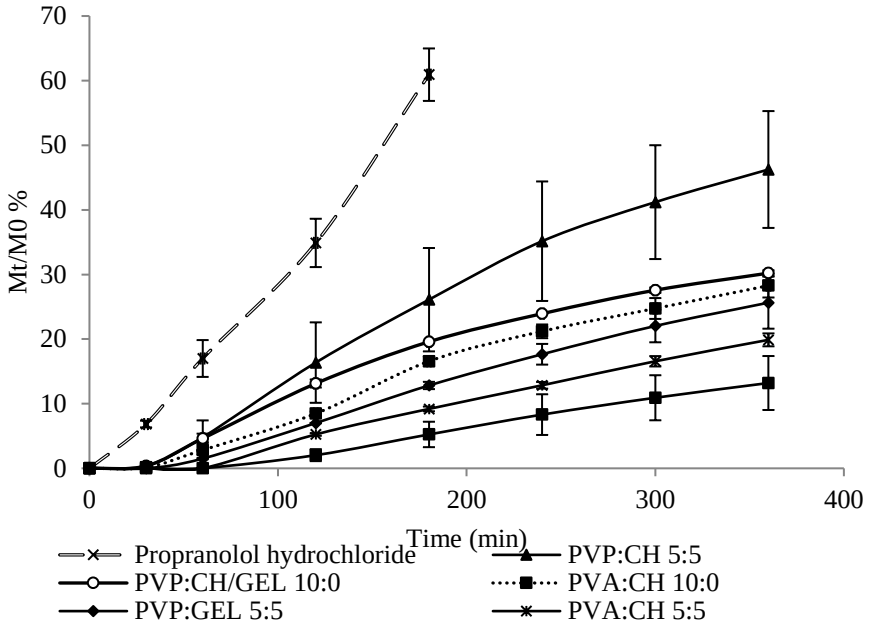

Mt/M0 %
0
20
40
60
80
100
0
50
100
150
200
250
Time (min)
PVP:CH/GEL 10:0
PVA:CH 10:0
PVP:GEL 5:5
PVA:CH 5:5
PVP:GEL 0:10
PVP:CH 5:5

Figure 6


70
60
50
40
30
20
10
0
Mt/M0 %
0
100
200
300
400
Time (min)
Propranolol hydrochloride
PVP:CH 5:5
PVP:CH/GEL 10:0
PVA:CH 10:0
PVP:GEL 5:5
PVA:CH 5:5