European Journal of Pharmaceutics and Biopharmaceutics 105 (2016) 115–121

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Design and evaluation of buccal films as paediatric dosage form for transmucosal delivery of ondansetron

Ramona Trastullo [a], Angela Abruzzo [a], Bruno Saladini [b], Maria Caterina Gallucci [c], Teresa Cerchiara [a], Barbara Luppi [a], Federica Bigucci [a,*]

[a] Department of Pharmacy and Biotechnology, University of Bologna, Via San Donato 19/2, 40127 Bologna, Italy
[b] PolyCrystalLine srl, Via F.S. Fabri 127/1, 40059 Medicina, Bologna, Italy
[c] Department of Chemistry and Chemical Technology, University of Calabria, Via P. Bucci, Cubo 15D, 87036 Arcavacata di Rende, Cosenza, Italy

ARTICLE INFO

Article history:
Received 31 March 2016
Revised 25 May 2016
Accepted in revised form 31 May 2016
Available online 4 June 2016

Chemical compounds studied in this article:
Hydroxypropylmethylcellulose (PubChem CID: 57503849)
Chitosan (PubChem CID: 71853)
Sodium hyaluronate (PubChem CID: 3084049)
Ondansetron hydrochloride dihydrate (PubChem CID: 59774)

Keywords:
Paediatric formulation
Buccal film
Polymeric mixture
Ondansetron hydrochloride
Permeation study

ABSTRACT

In the process of implementation and innovation of paediatric dosage forms, buccal films for transmucosal administration of drug represent one of the most interesting approach. In fact, films are able to provide an extended duration of activity allowing minimal dosage and frequency and offer an exact and flexible dose, associated with ease of handling. The objective of the present study was to develop polymeric films for the sustained release of ondansetron hydrochloride, a selective inhibitor of 5-HT_3 receptors indicated in paediatrics for the prevention and treatment of nausea and vomiting caused by cytotoxic chemotherapy or radiotherapy and postoperatively. Films were prepared by casting and drying of aqueous solutions containing different weight ratios of hydroxypropylmethylcellulose (HPMC) with chitosan (CH) or sodium hyaluronate (HA) or gelatin (GEL) and characterized for their physico-chemical and functional properties. The presence of HA, GEL and CH did not improve the mucoadhesive properties of HPMC film. The inclusion of GEL and CH in HPMC film increased in vitro drug release with respect to the inclusion of HA, although films containing HA showed the highest water uptake. Moreover in agreement with the release behaviour, the inclusion of CH and GEL provided higher drug permeation through porcine buccal mucosa with respect to HPMC film and ensured linear permeation profiles of drug.

1. Introduction

The extensive changes into the regulatory environment for paediatric medicines, designed to better protect the health of children, have stimulated the research into child-appropriate dosage forms. These dosage forms should satisfy important requisites: easy administration, possibility of weight-based dosing and dose titration, acceptability and palatability, and finally minimum dosing frequency. Moreover, excipients should be safe in the target age group [1–4].

One approach in the process of implementation and innovation of paediatric dosage forms for young children is represented by the use of buccal films for transmucosal administration of drug [5]. Buccal films are relatively new dosage form intended to deliver drug substances through the oral mucosa directly onto the systemic circulation, avoiding the hepatic first pass metabolism and similarly, the drug degradation along the gastrointestinal tract, thus allowing the reduction of the dose necessary to achieve the therapeutic action. Compared to conventional buccal tablet formulation, they are thin, flexible and better adaptable to the mucosal surface, and therefore more acceptable to younger patients. Moreover, buccal films are safe and convenient unit dosage systems since they can be easily applied or removed from the application site, even during a state of patient unconsciousness or when swallowing is impaired [6–8].

From the technological point of view, buccal films are matrices fabricated using mucoadhesive and film forming polymers and

* Corresponding author.
E-mail addresses: ramona.trastullo2@unibo.it (R. Trastullo), angela.abruzzo2@unibo.it (A. Abruzzo), bruno.saladini@polycrystalline.it (B. Saladini), cate75@gmail.com (M.C. Gallucci), teresa.cerchiara2@unibo.it (T. Cerchiara), barbara.luppi@unibo.it (B. Luppi), federica.bigucci@unibo.it (F. Bigucci).

http://dx.doi.org/10.1016/j.ejpb.2016.05.026
0939-6411/

loaded with the active ingredient(s). The use of mucoadhesive polymers is essential to maintain an intimate and prolonged contact of the formulation with the oral mucosa allowing a longer duration of absorption [9]. Polymers that are commonly used in the development of buccal films include cellulose derivatives, chitosan, gelatin, hyaluronic acid, carrageenan, pectin, sodium alginate and acrylic polymers [10].

Effective design of such delivery system, requires careful consideration of other relevant parameters, including the choice of the active substance [11,12]. These involve good lipophilicity and water solubility at physiological pH, as well as high potency. Ondansetron (ODS), a selective inhibitor of serotonin (5-hydroxytryptamine) subtype 3 (5-HT_3) receptors indicated in paediatrics for the prevention and treatment of nausea and vomiting caused by cytotoxic chemotherapy or radiotherapy and postoperatively, represents a suitable candidate for buccal delivery (octanol/water log P at pH 7.4: 2.4, water solubility at pH 7.4: 2.42 mg/mL, small molecular size) [7,8,13]. ODS is commercially available as injection, oral liquid and solid oral dosage form. All these formulations are indicated for administration in multiple daily dosing, potentially for a series of days (recommended oral maintenance dose for children of 4–11 years: 4 mg every 4–8 h). This is due to the pharmacokinetic profile of ondansetron, which has a half-life of approximately 3–6 h and a time to peak plasma levels of approximately 2 h. This profile is often associated with alternating periods of increased side effects and lacking efficacy and therefore, there is a need to develop sustained release formulations able to maintain a constant drug concentration for a specific period of time with minimum side effects [14–17].

The objective of this study was to: (1) implement paediatric dosage forms for young children with buccal films intended for ODS systemic absorption through the buccal mucosa over a prolonged period of time; (2) prepare mucoadhesive films based on non-toxic, biocompatible and hydrophilic polymers as hydroxypropylmethylcellulose (HPMC), chitosan (CH), sodium hyaluronate (HA) and gelatin (GEL), and by using an easy and economic method as solvent casting method; (3) investigate the influence of preparative parameters on the physico-chemical properties of drug; and (4) study the influence of polymeric composition (different polymer blends and different weight ratio) on the drug loading, mucoadhesion potential, water uptake properties, and drug release and permeation ability.

2. Materials and methods

2.1. Materials

Hydroxypropylmethylcellulose (MW 250 kDa, methoxyl content 19–24%, hydroxypropyl content 7–12%) was purchased from Eigenmann & Veronelli (Milan, Italy); chitosan (MW 150 kDa, deacetylation degree 97%) was commercially obtained from Fluka (Milan, Italy); sodium hyaluronate (MW 1800–2300 kDa, D-glucuronic acid > 42%) was provided by ACEF (Piacenza, Italy); type B Gelatin from bovine skin (MW 50 kDa, 100–115 mmol of free carboxyl groups per 100 g of protein, isoelectric point in the range of pH = 4.7–5.2) and ondansetron hydrochloride dihydrate (MW 365.85 g/mol) were commercially obtained from Sigma-Aldrich (Milan, Italy). All other chemicals and solvents were of analytical grade and supplied by Carlo Erba (Milan, Italy). Release and permeation studies were conducted in NaCl solution (0.9% w/v); mucoadhesion studies were carried out in aqueous buffer with the following composition: 33.9 mM KH_2PO_4, 46.8 mM $Na_2HPO_4 \cdot \times 12H_2O$ adjusted with hydrochloric acid to pH = 6.8 [18] (healthy saliva pH = 6.7–7.4 [19]); buccal tissue was stored after excision in Krebs Ringer bicarbonate buffer with the following composition: 115.5 mM NaCl, 4.2 mM KCl, 2.5 mM $CaCl_2$, 1.6 mM NaH_2PO_4, 0.8 mM $MgSO_4$, 4.0 mM HEPES, 17.3 mM Na_2CO_3, and 12.2 mM glucose [20].

2.2. Preparation of buccal films

Buccal films were prepared by casting-solvent evaporation method. An aqueous solution of GEL, an aqueous solution of HA and an acid solution (acetic acid 1% v/v) of CH were separately added to an aqueous solution of HPMC at different weight ratios (10:0, 9:1, 7:3, 5:5, 0:10 HPMC:GEL or HPMC:HA or HPMC:CH), in order to obtain 1% w/w polymeric mixtures. All mixtures were stirred at room temperature for 2 h and allowed to stand overnight to eliminate the air bubbles. 15 g of each polymeric solution was spread on a Petri dish (diameter = 5 cm) and oven-dried at 50 °C for 6 h (heating oven FD series; Binder, Tuttlingen, Germany). Loaded films were prepared by the same procedure, adding to each mixture 17.45 mg of ODS. Circles of 1.3 cm in diameter (surface area = 1.33 cm^2) were cut to obtain a child-appropriate dosage form and were used for the studies described below. Each circle contains theoretically 1.18 mg of drug.

Different films were named in this work as follows: HPMC:CH 10:0, HPMC:HA 10:0, HPMC:GEL 10:0, films based on HPMC (they are also reported as HPMC:CH(GEL,HA) 10:0); HPMC:CH 0:10, HPMC:HA 0:10, HPMC:GEL 0:10, films based on CH, HA and GEL, respectively; HPMC:CH (or HPMC:HA or HPMC:GEL) 9:1 (or 7:3 or 5:5), films based on HPMC mixtures with CH or HA or GEL at different weight ratios.

2.3. Solution viscosity

The viscosity of the polymeric solutions used for the preparation of loaded and unloaded buccal films was measured at room temperature with an Ubbelohde capillary viscometer equipped with an electronic time-measuring unit ViscoClock (capillary tubes I and II; Schott, Mainz, Germany) for CH and GEL solutions (1% w/w) and with a rotational viscometer (spindle TR8-TR9, RPM 60-200; Visco Star, Fungilab S.A., Barcelona, Spain) for all the others.

2.4. Characterization of buccal films

2.4.1. Scanning electron microscopy (SEM)

SEM analysis was performed to evaluate the morphologic characteristics. 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 in order to examine their surface morphology and their internal structure.

2.4.2. Thickness and drug content

Each film obtained from a Petri dish (diameter = 5 cm) was accepted as a single batch in these studies and for each formulation three batches were prepared. Thickness of loaded film was measured as mean of three batches. The thickness of films was determined by means of a Mitutoyo pocket thickness gauge (Mitutoyo Mfc. Co. Ltd., Tokyo, Japan). Drug content was assessed by dissolving one circle (diameter = 1.3 cm) from each batch in 20 ml of 0.9% (w/v) NaCl solution. The system was stirred for 2 h until complete release and the amount of drug in solution was evaluated. The results were expressed as milligrams of drug for square centimetre (mg/cm^2).

In these tests as well as in subsequent experiments the ODS concentration was determined by HPLC equipped with a UV detector. The HPLC system consisted of Shimadzu (Milan, Italy) LC-10ATVP chromatographic pump and a Shimadzu SPD-10AVP

UV–vis detector set at 310 nm. Separation was obtained at room temperature on a Phenomenex (Torrance, CA, USA) Sinergy Fusion-RP 80A (150 mm × 4.6 mm I.D., 5 μm) coupled with a Phenomenex SecurityGuard C18 guard cartridge (4 mm × 3.0 mm I.D., 5 μm). The mobile phase was prepared by mixing acetonitrile (33% v/v) and 20 mM sodium hydrogen phosphate buffer pH = 4.0 (67% 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 computerised integration system (Shimadzu Italia, Milan, Italy). The calibration curve of concentration versus peak area was plotted at concentration range of 0.24–24 μg/mL; good linearity was found (r^2 = 0.9997).

2.4.3. Surface pH

The surface pH of loaded buccal films was determined in order to evaluate their compatibility with the pH of buccal mucosa. The films were left to swell on a sponge soaked with phosphate buffer (pH = 6.8) and the pH was measured after 3 h by placing universal pH paper (pH scale from 6.0 to 8.1; Carlo Erba, Milan, Italy) on the film surface.

2.4.4. Physicochemical properties

Differential scanning calorimetry (DSC) and X-ray Powder Diffraction (XRPD) experiments were performed on loaded polymeric films to identify the solid-state properties of the drug in the formulation and possible phase transitions during the film preparation process.

The DSC analysis was performed using a Perkin-Elmer DSC 6 (Waltham, USA). The experiments were conducted in non-hermetically sealed aluminum pans using nitrogen as purge gas at a flow rate of 20 ml/min. Samples of 8.0 ± 1.0 mg were heated from 30 to 220 °C at the heating rate of 10 °C/min.

The XRPD analysis was performed using a Panalytical X'Pert PRO Diffractometer (Almelo, the Netherlands). The voltage and current were 40 kV and 40 mA, respectively and the measurements were carried out in the angular scan rage from 3° to 40° (2θ).

2.5. In vitro water-uptake studies

Water uptake studies were performed to investigate the hydration ability of films. A sponge (5 cm × 5 cm × 2 cm) fully soaked in the hydration medium (0.9% NaCl solution) was placed in a glass container filled with the same solution to a height of 0.5 cm [21]. Filter paper was also soaked in the hydration medium and positioned on the top of the sponge. The experimental set-up was equilibrated for 30 min. Accurately weighted films (unloaded samples) were then placed on the filter paper and the water-uptake ability was determined as weight increase of the film after 3 h, according to the following equation: % Water Uptake (WU) = $(W_2 - W_1) \times 100/W_1$, where W_1 was the initial weight of dried film and W_2 is the weight of hydrated film.

2.6. In vitro residence time

Mucoadhesion properties of unloaded buccal films were determined in terms of residence time of films on a freshly excised mucosa. The porcine buccal tissue was obtained from a local slaughterhouse and used due to its similarity to the human buccal tissue. After removal, it was immediately transferred into cold Krebs Ringer bicarbonate buffer, placed in sealed ice box filled with dry ice, immediately transported to the laboratory and used within 2 h [20,22]. The buccal mucosa was separated from the connective tissue using a sharp scalpel and then it was cut to an appropriate size (surface area = 1.54 cm^2), wetted with few drops of aqueous mucin solution (0.05% w/v) and fixed on a microscope slide with cyanoacrylate adhesive. The films were attached to the porcine buccal mucosa by applying a light pressure for 2 min. The microscope slide was then placed in a beaker filled with 40 ml of phosphate buffer pH = 6.8 and slowly stirred using a magnetic bar. The time taken by the films to completely detach from the mucosa was considered as the residence time [23].

2.7. In vitro release studies

In vitro release studies were performed in order to evaluate the drug amount released from films over the time and use these data to better understand the permeation results. Loaded films were attached on the internal side of a beaker containing 40 ml of 0.9% (w/v) NaCl solution. The medium was stirred at 50 rpm using a magnetic bar and maintained at 37 °C by immersion of the beaker in a thermostated water bath**.** Samples of 500 μl were withdrawn at predetermined time intervals and replaced by fresh medium. The experiments were conducted for 5 h and all samples were analyzed by HPLC analysis. The results of the release experiments are shown as cumulative drug amount released (expressed as fractional amount) plotted as a function of time.

2.8. In vitro permeation studies

In vitro permeation studies were performed in order to evaluate transmucosal absorption of drug from buccal films. These studies were made through a porcine buccal mucosa using Franz-type static glass diffusion cells (15 mm jacketed cell with a flat ground joint and clear glass with a 12 mL receptor volume, diffusion surface area: 1.77 cm^2) and equipped with a VSA stirrer (PermeGear Inc., Hellertown, Pennsylvania, USA). Buccal mucosa was obtained as previously described and mounted between the donor and the receiver compartments of cells. Loaded films were placed on the top of the porcine mucosa, while the receptor compartment was filled with 12 ml of 0.9% (w/v) NaCl solution maintained at 37 °C by means of a surrounding jacket and continuously stirred. Samples of 100 μl were withdrawn from the receptor compartment at predetermined time intervals and replaced by fresh medium. Sink conditions were maintained at any time. The experiment was conducted for 6 h and all samples were analyzed by HPLC analysis. An aqueous solution (500 μL) of ondansetron hydrochloride (2.36 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 permeated (expressed as fractional amount) versus time.

2.9. 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

The development of a suitable dosage form for paediatric patients still remains a challenge. An ideal paediatric formulation must allow accurate dose administration and be in a dosage form that can be safely handled by the target age group. Polymeric buccal films offer an exact and flexible dose and ease of handling; they also allow the direct access of the active into the systemic circulation avoiding the first-pass metabolism and thus reducing the dose needed.

3.1. Solution viscosity

Casting-solvent evaporation method was employed to prepare buccal films, using non-toxic and non-irritant polymers, such as HPMC, CH, HA and GEL, thus suitable for the administration in children. This method is based on the dissolution of the polymers in appropriate solvents (distilled water or acetic acid 1% v/v), and on the subsequent mixture of polymer solutions in order to obtain the desired polymer weight ratio. All the final solutions had the same total polymeric concentration (1% w/w), but they showed different viscosities. As reported in Fig. 1, unloaded polymer solutions had viscosities of 250 ± 18, 38.77 ± 1.60, 1150 ± 101 and 3.20 ± 0.08 mPa × sec for HPMC, CH, HA and GEL, respectively. Furthermore, as regards the mixtures, the addition of increasing amount of HA to the HPMC solution, proportionally increased the solution viscosity, while increasing amount of CH and GEL decreased the viscosity of the HPMC solution. This behaviour is chiefly related to the different molecular weight of the polymers used for the preparation of the films. In fact, HA shows the highest molecular weight with respect to HPMC, CH and GEL. The presence of the drug into the polymeric solutions did not affect their viscosity (Fig. 1).

3.2. Characterization of buccal films

SEM analysis (Fig. 2) showed that HPMC:CH 5:5 and HPMC:GEL 5:5 exhibited a dense and compact cross-section, while HPMC:HA 5:5 had a heterogeneous structure characterized by flakes.

The thickness of the films ranged from 44 ± 6 μm for HMPC:HA 0:10 to 107 ± 6 μm for HPMC:CH(GEL,HA) 10:0 (Table 1). The low standard deviations suggested that the preparative method provided no significant difference in terms of thickness between different batches. In addition, the measurement of ondansetron hydrochloride content in the dosage form showed that the experimental drug content was very close to the theoretical one (0.9 mg/cm^2) for each formulation (Table 1), indicating that casting-solvent evaporation method is a suitable technique to produce polymeric buccal films containing ondansetron.

The film surface pH was measured to avoid damages of the buccal mucosa leading to patient discomfort [19]. The pH of all prepared films was found near the neutral pH indicating its compatibility with buccal pH, causing no irritation to the mucosa and achieving patient compliance.

3.3. Physicochemical properties

In order to evaluate possible phase transitions of the active during the film formulation process, differential scanning calorimetry and X-ray powder diffraction were used. This data is an important factor to consider because the amorphous form of the drug represents its most energetic solid state and thus it should produce big advantages in terms of solubility and bioavailability.

The DSC profiles (Fig. 3a) showed a single endothermic peak at 187.54 °C, in agreement with the melting point of ondansetron hydrochloride raw material and a large dehydration process between 50 and 120 °C. The thermograms of all films presented a large endothermic profile around 60–120 °C related to the dehydration of polymers. Conversely, the melting peak of the active was absent in the DSC profiles of all loaded films, except for HPMC:CH 0:10 and HPMC:CH 5:5. This means that in almost all the cases casting-solvent evaporation method induced the amorphization of the active, while in HPMC:CH 0:10 and HPMC:CH 5:5 part of it remained as crystalline material.

The same results were confirmed by the XRPD analysis (Fig. 3b). The diffractograms of the loaded films did not report the characteristic peaks of ondansetron hydrochloride raw material (2θ values of 8.26°, 13.28°, 16.84°, 20.20°, 23.96°, 24.36°, 25.72°, 27.88°, 30.84°) [24], indicating an amorphous profile of all the films, except for HPMC:CH 0:10 and HPMC:CH 5:5. In fact, these films exhibited XRPD patterns characterized by a peak of low intensity at about 7° 2θ, probably related to a crystalline form of the active.

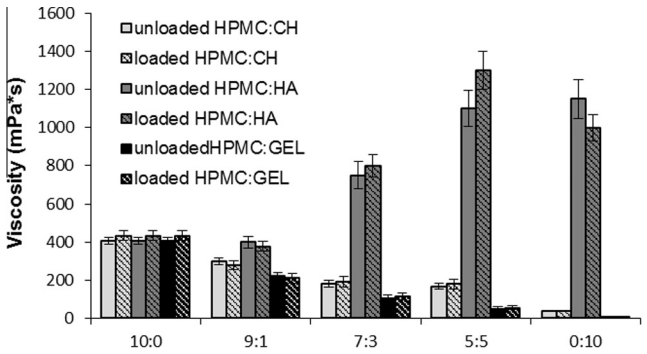

Fig. 1. Viscosity of the solutions used for the preparation of loaded and unloaded buccal films.

3.4. In vitro water uptake studies

In vitro water uptake values after 80 min are reported in Table 1. The presence of HA and CH in the polymeric mixtures increased the water-uptake ability of HPMC:CH(GEL,HA) 10:0. In particular, the increase of the hydration capacity was more evident for HPMC:HA, with respect to HPMC:CH. When GEL was introduced in the polymeric mixtures, instead, it did not affect the hydration ability of HPMC:CH(GEL,HA) 10:0. This behaviour can be mostly related to the different polymeric charge density. In fact, in our operative conditions (0.9% w/v sodium chloride solution at pH = 6.3) HPMC resulted completely neutral, HA (pKa = 2.9) resulted negatively charged with all its carboxylic group deprotonated, CH (pKa = 6.3) showed positive charge with 50% of neutral amine groups and 50% of protonated amine groups, while GEL was slightly negatively charged. In particular, the highest charge density allows the highest entrance of water in the system and the highest hydration of the film, thus permitting the formation of gels with different viscosities.

3.5. In vitro residence time

Once administered into the oral cavity, the films have to hydrate, adhering to the buccal mucosa, and forming a gel in order to allow drug delivery. In vitro residence time value is useful to evaluate whether the drug delivery system remains at the site of administration for a sufficient time to ensure drug permeation for an extended period of time. It has been reported in the literature that the maximum duration for buccal drug delivery systems is approximately 4–6 h, since meal intake and drinking may require dosage form removal [25]. Moreover, mucoadhesive systems guarantee an intimate contact with the mucosa, which may result in high drug concentration in a local area and hence high flux.

A variety of factors affect the mucoadhesive properties of polymers, such as molecular weight, chain flexibility, charge, hydrogen bonding capacity, cross-linking density, and hydration ability [10]. As shown in Fig. 4, HPMC:CH(GEL,HA) 10:0 demonstrated the highest residence time (1320 min). HPMC is a long chained, non-ionic polymer and its mucoadhesion ability is chiefly attributable to the interpenetration and entanglement of polymer chains into the mucus layer. Furthermore, it possesses a large number of

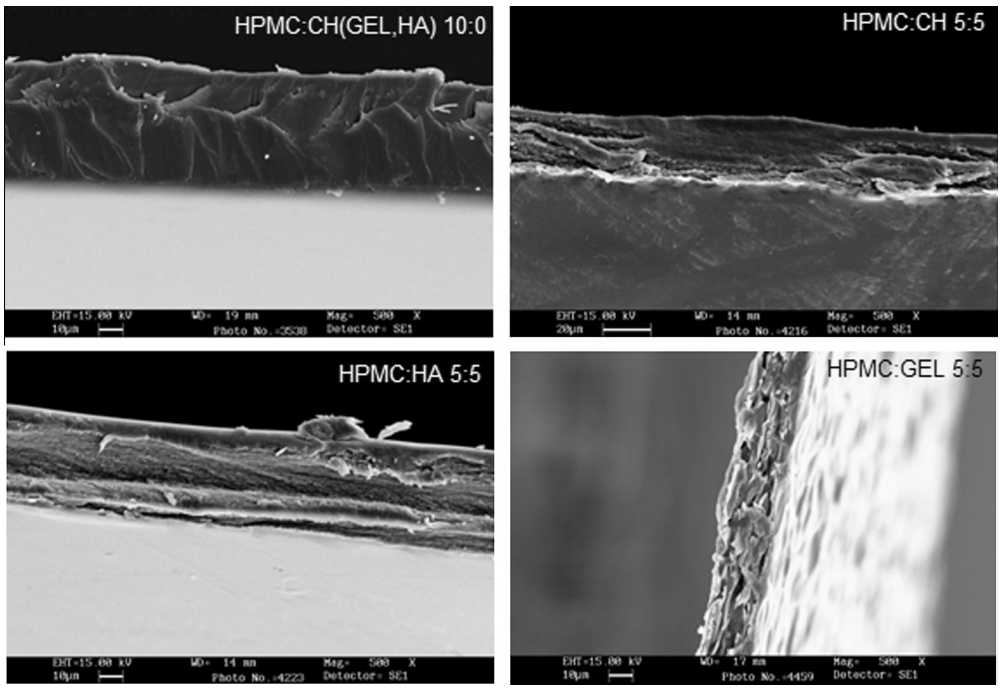

Fig. 2. Scanning electron micrographs of HPMC:CH(GEL,HA) 10:0, HPMC:CH 5:5, HPMC:HA 5:5 and HPMC:GEL 5:5 (scale bars: 10 μm for HPMC:CH(GEL,HA) 10:0, HPMC:HA 5:5, HPMC:GEL 5:5; 20 μm for HPMC:CH 5:5).

Table 1
Characterisation of buccal films: film thickness, drug content and water uptake.

Formulation	Film thickness (μm)	Drug content (mg/cm^2)	WU after 80 min (%)
HMPC:CH(HA,GEL) 10:0	107 ± 6	1.03 ± 0.21	1246.46 ± 38.23
HMPC:CH 9:1	63 ± 6	1.05 ± 0.13	1862.66 ± 60.50
HMPC:CH 7:3	57 ± 6	1.02 ± 0.11	2000.02 ± 110.55
HMPC:CH 5:5	53 ± 12	1.05 ± 0.14	1934.25 ± 60.20
HMPC:CH 0:10	63 ± 1	1.15 ± 0.20	2767.78 ± 90.54
HMPC:HA 9:1	73 ± 12	0.98 ± 0.10	1713.68 ± 88.57
HMPC:HA 7:3	67 ± 6	0.91 ± 0.15	5001.29 ± 210.43
HMPC:HA 5:5	69 ± 6	0.95 ± 0.08	4933.55 ± 180.40
HMPC:HA 0:10	44 ± 6	0.82 ± 0.15	5208.63 ± 225.34
HMPC:GEL 9:1	93 ± 2	1.03 ± 0.01	1305.12 ± 42.20
HMPC:GEL 7:3	96 ± 3	0.87 ± 0.15	1249.03 ± 79.92
HMPC:GEL 5:5	101 ± 3	0.90 ± 0.01	1182.97 ± 67.91
HMPC:GEL 0:10	76 ± 2	0.98 ± 0.05	1371.65 ± 84.86

hydrophilic groups that are able to form hydrogen bonds between the hydrophilic groups of mucus [26].

In our studies HPMC:GEL 5:5 and HPMC:GEL 0:10 demonstrated the lowest residence time. This behaviour could be related to GEL molecular weight (50 kDa) since it has been reported that a minimum polymer molecular weight of 100 kDa is required for mucoadhesion [27]. The addition of CH and HA to HPMC:CH(GEL, HA) 10:0 did not increase its mucoadhesion properties. Moreover, the addition of CH produced a lower residence time than the addition of HA, although CH has positively charged amino groups that can electrostatically interact with the negatively charged sialic acid of mucin. This behaviour could be attributed to the higher hydration ability of HPMC:HA 5:5 with respect to HPMC:CH 5:5 at pH = 6.3.

3.6. In vitro release studies

Drug release from a gelled matrix is a complex phenomenon of water penetration, relaxation of the polymer chains, swelling and spreading of the matrix, interactions between drug and polymeric material, and drug dissolution and diffusion through the rehydrated matrix. The release of ODS from HPMC:CH, HPMC:GEL and HPMC:HA films were investigated and Fig. 5 shows the release profiles of HPMC:CH(GEL,HA) 10:0, HPMC:CH 5:5, HPMC:GEL 5:5 and HPMC:HA 5:5, as representative formulations of the three different series. All the formulations exhibited a prolonged release of the drug. Moreover, HPMC:CH(GEL,HA) 10:0 and HPMC:CH 5:5 released the maximum amount of the drug within 45 min, while HPMC:HA 5:5 and HPMC:GEL 5:5 showed the maximum release of ODS after 120 min.

The inclusion of CH and GEL in the formulation allowed a higher cumulative amounts of ODS released from the dosage form, rather than the inclusion of HA. As described above, HPMC:HA 5:5 showed the highest molecular weight and the greatest hydration ability due to the high charge density at pH = 6.3; this permitted higher viscosity of the polymeric network in the gelled state, thus limiting the drug diffusion. HPMC:CH 5:5 and HPMC:GEL 5:5, once hydrated, created a less viscous gelled state, allowing a greater release of ODS from the dosage form [28].

3.7. 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 HPMC:CH(GEL,HA) 10:0, HPMC:CH 5:5, HPMC:GEL 5:5 and HPMC:HA 5:5 were chosen for the permeation studies as representative of the three different series (Fig. 6). All the formulations demonstrated a sustained permeation of the drug within 6 h. In particular the presence of HA in HPMC:HA 5:5 did not improve the permeation ability of HPMC:CH (GEL,HA) 10:0, while both HPMC:CH 5:5 and HPMC:GEL 5:5 provided higher permeated drug amount at each time with respect to HPMC:CH(GEL,HA) 10:0. This behaviour is in agreement with the release profiles: the more the amount of drug released from the dosage form, the more absorption inside the buccal mucosa. Moreover, since chitosan is believed to interfere with lipid micelle

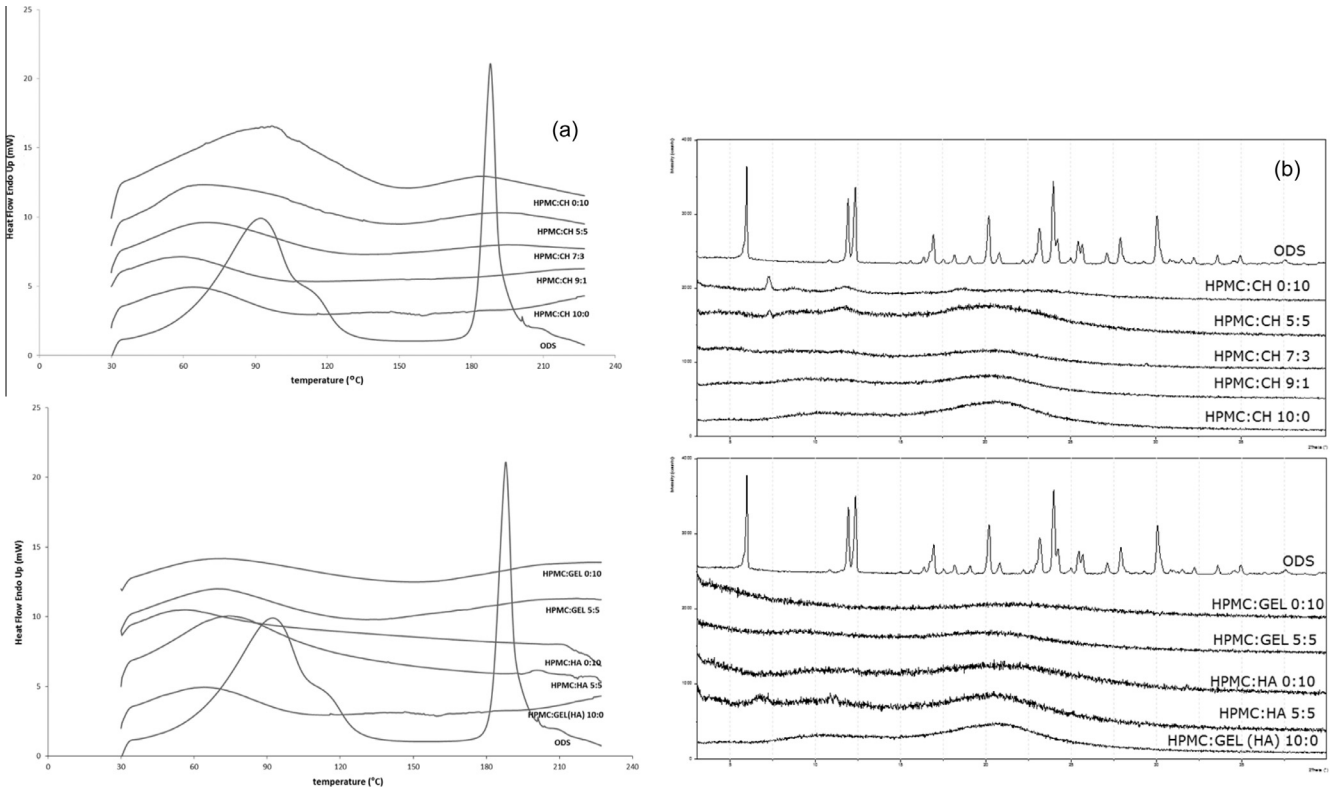

Fig. 3. Physicochemical characterization of buccal films: (a) DSC profiles of HPMC:CH (all the mixtures) and HPMC:HA(GEL) (all the most significant mixtures), with respect to pure ODS; (b) XRPD patterns of HPMC:CH (all the mixtures) and HPMC:HA(GEL) (all the most significant mixtures), with respect to pure ODS.

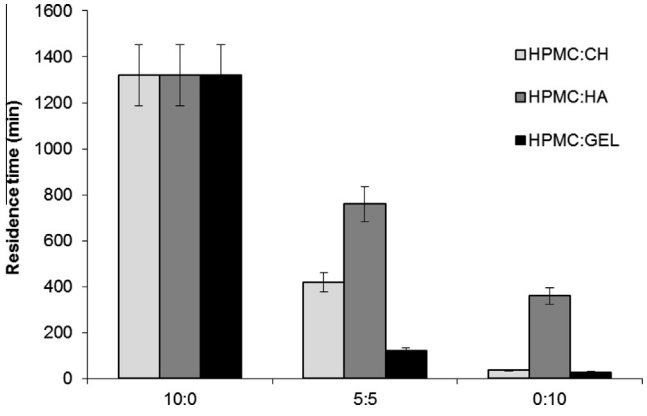

Fig. 4. Residence time of buccal films on porcine buccal mucosa.

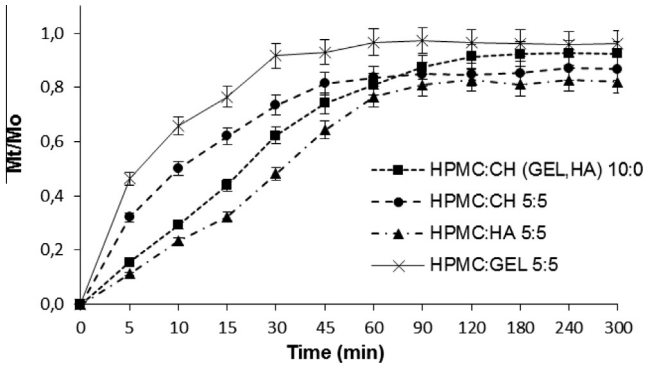

Fig. 5. *In vitro* release profile of ondansetron hydrochloride from HPMC:CH(GEL, HA) 10:0, HPMC:CH 5:5, HPMC:GEL 5:5 and HPMC:HA 5:5.

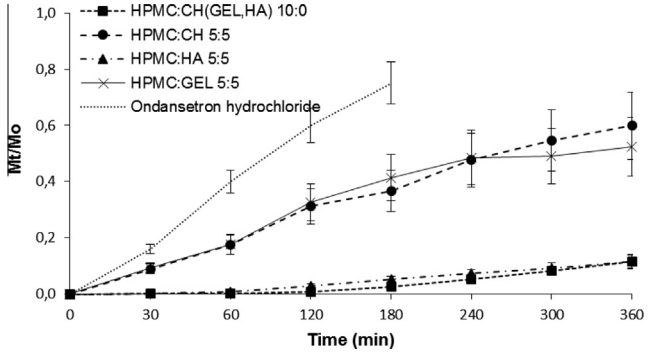

Fig. 6. *In vitro* permeation profiles of ondansetron hydrochloride from drug solution, HPMC:CH(GEL,HA) 10:0, HPMC:CH 5:5, HPMC:GEL 5:5 and HPMC:HA 5:5.

organization in the intestine, Şenel et al. [29] 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.

As concern the practical use of these formulations, the recommended oral maintenance dose for children of 4–11 years is 4 mg every 4–8 h. This dosage can be achieved by use of film with a surface area of 7.7 cm^2, 9.9 cm^2, 1.9 cm^2 and 2.1 cm^2 for HPMC:CH (GEL,HA) 10:0, HPMC:HA 5:5, HPMC:GEL 5:5 and HPMC:CH 5:5, respectively. The surface area of the film was calculated according to the following equation: Css = J·A/Cl, where Css is the concentration at the steady state (39.5 ng/ml) [30], Cl is the ondansetron clearance (0.39 L/h/kg) [31] and J is the permeation flux of film ($J_{HPMC:CH(GEL,HA)\ 10:0}$ = 23.9 ± 3.3 μg/cm^2 h, $J_{HPMC:HA\ 5:5}$ = 18.7 ± 2.5 μg/cm^2 h, $J_{HPMC:GEL\ 5:5}$ = 99.6 ± 18.1 μg/cm^2 h, $J_{HPMC:CH\ 5:5}$ = 87.6 ± 14.4 μg/cm^2 h). Whereas buccal adhesive drug delivery systems with a size of 1–3 cm^2 are

preferable [32], the most promising candidates for therapeutical use are represented by HPMC:GEL 5:5 and HPMC:CH 5:5.

4. Conclusions

With polymeric buccal films, a novel solid oral dosage form was developed, fulfilling all current demands for child-appropriate dosage forms. HPMC mixtures with HA, GEL and CH can be used as materials to develop sustained release films able to allow minimal dosage and frequency, and characterized by minimal impact on lifestyle, and easy and reliable administration. The selection of suitable polymeric mixture and appropriate weight ratio allowed the modulation of the residence time of the dosage form on the application site, the release of the drug and its permeation through the buccal mucosa.

Further studies are in progress to optimize ODS release from buccal films and to improve organoleptic characteristics of the dosage form. In particular, we are applying a second film layer onto a first one to achieve unidirectional release towards the oral mucosa, avoiding drug release in the oral cavity and covering the ODS bitter taste.

Acknowledgments

The authors would like to thank Valentina Ragazzini for her contribution to this work.

References

[1] EMA (European medicine Agency), Guideline on Pharmaceutical Development of Medicines for Paediatric Use, EMA/CHMP/QWP/805880/2012 Rev. 2, 2013 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/07/WC500147002.pdfd (accessed 18.02.16).
[2] T.B. Ernest, D.P. Elder, L.G. Martini, M. Roberts, J.L. Ford, Developing paediatric medicines: identifying the needs and recognizing the challenges, J. Pharm. Pharmacol. 59 (2007) 1043–1055.
[3] R.G. Strickley, Q. Iwata, S. Wu, T.C. Dahl, Pediatric drugs – a review of commercially available oral formulations, J. Pharm. Sci. 97 (2008) 1731–1774.
[4] WHO (World Health Organization), Expert Committee on Specifications for Pharmaceutical Preparations, WHO Technical Report Series, No. 970, Annex 5 http://www.who.int/medicines/areas/quality_safety/quality_assurance/expert_committee/trs_970/en/2012 (accessed 18.02.16).
[5] A.F. Borges, C. Silva, J.F. Coelho, S. Simões, Oral films: current status and future perspectives. I – Galenical development and quality attributes, J. Control. Release 206 (2015) 1–19.
[6] R.P. Dixit, S.P. Puthli, Oral strip technology: overview and future potential, J. Control. Release 139 (2009) 94–107.
[7] J.K. Lam, Y. Xu, A. Worsley, I.C. Wong, Oral transmucosal drug delivery for pediatric use, Adv. Drug Deliv. Rev. 73 (2014) 50–62.
[8] V.F. Patel, F. Liu, M.B. Brown, Advances in oral transmucosal drug delivery, J. Control. Release 153 (2011) 106–116.
[9] Y. Sudhakar, K. Kuotsu, A.K. Bandyopadhyay, Buccal bioadhesive drug delivery – a promising option for orally less efficient drugs, J. Control. Release 114 (2006) 15–40.
[10] N. Salamat-Miller, M. Chittchang, T.P. Johnston, The use of mucoadhesive polymers in buccal drug delivery, Adv. Drug Deliv. Rev. 57 (2005) 1666–1691.
[11] WHO (World Health Organization), Model Formulary for Children http://www.who.int/childmedicines/publications/en/2010 (accessed 18.02.16).
[12] WHO (World Health Organization), Model List of Essential Medicines for Children (5th List) http://www.who.int/medicines/publications/essentialmedicines/en/2015 (accessed 18.02.16).
[13] R.C. Mashru, V.B. Sutariya, M.G. Sankalia, J.M. Sankalia, Effect of pH on *in vitro* permeation of ondansetron hydrochloride across porcine buccal mucosa, Pharm. Dev. Technol. 10 (2005) 241–247.
[14] M. Koland, R.N. Charyulu, K. Vijayanarayana, P. Probhu, *In vitro* and *in vivo* evaluation of chitosan buccal films of ondansetron hydrochloride, Int. J. Pharm. Invest. 1 (2011) 164–171.
[15] R. Kumria, V. Gupta, S. Bansal, J. Wadhw, A.B. Nair, Oral buccoadhesive films of ondansetron: development and evaluation, Int. J. Pharm. Invest. 3 (2013) 112–118.
[16] H. Patil, R.V. Tiwari, S.B. Upadhye, R.S. Vladyka, M.A. Repka, Formulation and development of pH-independent/dependent sustained release matrix tablets of ondansetron HCl by a continuous twin-screw melt granulation process, Int. J. Pharm. 496 (2015) 33–41.
[17] D.M. Park, Y.K. Song, J.P. Jee, H.T. Kim, C.K. Kim, Development of chitosan-based ondansetron buccal delivery system for the treatment of emesis, Drug Dev. Ind. Pharm. 38 (2012) 1077–1083.
[18] A. Abruzzo, T. Cerchiara, F. Bigucci, M.C. Gallucci, B. Luppi, Mucoadhesive buccal tablets based on chitosan/gelatin microparticles for delivery of propranolol hydrochloride, J. Pharm. Sci. 104 (2015) 4365–4372.
[19] M.R.C. Marques, R. Loebenberg, M. Almukainzi, Simulated biological fluids with possible application in dissolution testing, Dissolut. Technol. 18 (2011) 15–28.
[20] D. Imbert, C. Cullander, Buccal mucosa in vitro experiments: I. Confocal imaging of vital staining and MTT assays for the determination of tissue viability, J. Control. Release 58 (1999) 39–50.
[21] U. Bertram, R. Bodmeier, Effect of polymer molecular weight and of polymer blends on the properties of rapidly gelling nasal inserts, Drug Dev. Ind. Pharm. 38 (2012) 659–669.
[22] U. Kulkarni, R. Mahalingam, I. Pather, X. Li, B. Jasti, Porcine buccal mucosa as *in vitro* model: effect of biological and experimental variables, J. Pharm. Sci. 99 (2010) 1265–1277.
[23] A.B. Nair, R. Kumria, S. Harsha, M. Attimarad, B.E. Al-Dhubiab, I.A. Alhaider, *In vitro* techniques to evaluate buccal films, J. Control. Release 166 (2013) 10–21.
[24] S. Pattnaik, K. Swain, S. Mallick, Z. Lin, Effect of casting solvent on crystallinity of ondansetron in transdermal films, Int. J. Pharm. 406 (2011) 106–110.
[25] A.K. Mitra, H.H. Alur, T.P. Johnston, Peptides and Proteins—Buccal Absorption, in: J. Swarbrick, J.C. Boylan (Eds.), Encyclopedia of Pharmaceutical Technology, second ed., Marcel Dekker Inc, New York, 2002, pp. 2081–2095.
[26] U. Bertram, R. Bodmeier, In situ gelling, bioadhesive nasal inserts for extended drug delivery: *in vitro* characterization of a new nasal dosage form, Eur. J. Pharm. Sci. 27 (2006) 62–71.
[27] J.W. Lee, J.H. Park, J.R. Robinson, Bioadhesive-based dosage forms: the next generation, J. Pharm. Sci. 89 (2000) 850–866.
[28] F. Bigucci, A. Abruzzo, B. Saladini, M.C. Gallucci, T. Cerchiara, B. Luppi, Development and characterization of chitosan/hyaluronan film for transdermal delivery of thiocolchicoside, Carbohydr. Polym. 130 (2015) 32–40.
[29] S. Şenel, M.J. Kremer, S. Kaş, P.W. Wertz, A.A. Hıncal, C.A. Squier, Enhancing effect of chitosan on peptide drug delivery across buccal mucosa, Biomaterials 21 (2000) 2067–2071.
[30] K.H. Simpson, P. Murphy, P.V. Colthup, P. Whelan, Concentration of ondansetron in cerebrospinal fluid following oral dosing in volunteers, Psychopharmacology 109 (1992) 497–498.
[31] I.A. Spahr-Schopfer, J. Lerman, N. Sikich, J. Palmer, U. Jorch, Pharmacokinetics of intravenous ondansetron in healthy children undergoing ear, nose, and throat surgery, Clin. Pharmacol. Ther. 58 (1995) 316–321.
[32] H.H. Alur, T.P. Johnston, A.K. Mitra, Peptides and Proteins—Buccal Absorption, in: J. Superbrick, J.C. Boylan (Eds.), Encyclopedia of Pharmaceutical Technology, second ed., Marcel Dekker Inc, New York, 2001, pp. 193–218.