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Mucoadhesive buccal films based on a graft co-polymer – A mucin-retentive hydrogel scaffold

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ABSTRACT

From a patient-centric perspective, oromucosal drug delivery is highly attractive due to the ease of administration without the need of swallowing, and improved patient safety. The aim of the presented work was to prepare a buccal film using a self-forming micellar drug solubiliser as the film matrix, combining it with a mucoadhesive polymer for an enhanced retention on the buccal mucosa. Specifically, we propose the use of a graft co-polymer (Soluplus®), as a solubiliser and film former, supplemented with polymers with more hydrophilic properties and known mucoadhesive properties; hydroxypropyl methylcellulose (HPMC) or modified hydroxypropyl pea starch (Lycoat®). The film was manufactured by the solvent casting method. The resulting dual polymer film containing HPMC exhibited resistance to erosion and mucoadhesive properties superior to the control films of single polymers. In an *in vitro* oral cavity model, these properties were shown to correlate with increased residence time on simulated oral mucosa. Furthermore, all films containing the graft co-polymer showed similar permeability characteristics of furosemide towards buccal TR146 epithelial cells. This work illustrated that it is possible to manufacture dry, solid, dual polymer films containing an advanced drug delivery system with a cheap and simple method. The combination of a graft co-polymer with a mucoadhesive polymer transform into drug solubilising micelles in a mucin-retentive hydrogel scaffold with longer retention time on buccal mucosa for safe and enhanced advanced formulation.

1. Introduction

The oral route is the most preferred drug administration route; however, many patients find it difficult to swallow tablets and capsules. Many drug formulations have been developed in order to overcome the swallowing problem, including oral gels, buccal tablets, patches and various kind of fast dissolving drug delivery systems, just to name a few. Even with rapidly dissolving systems, a fear of choking may persist in some patients. Mucoadhesive buccal films offer many advantages over other oral formulations; the film is designed to attach to the buccal mucosa and release the drug in a controlled manner, for either trans-mucosal or local therapy. The buccal trans-mucosal administration of drugs is a non-invasive route for systemic administration that has many advantages over oral administration, such as a more rapid onset of action due to rich vascularisation of the mucosa, bypassing the enzymatic degradation of the gastrointestinal tract, avoiding the first pass metabolism and possibly improving bioavailability (Fonseca-Santos and Chorilli, 2018; Hoffmann et al., 2011; Smart, 2005). Another advantage is the easy access to the oral cavity and the buccal mucosa, which makes application as well as removal of a drug delivery system simple for the patient or the care giver (Pather et al., 2008). However, there are also disadvantages associated with the natural functions of the oral cavity in swallowing, speaking, eating and drinking. The oral mucosa is constantly being rinsed by saliva, and the movements of the tongue and jaw can further limit the usefulness of a buccally administered drug delivery system (Laffleur, 2014). In addition, it is known that the drug permeability of the buccal mucosa is lower as compared to the small intestine, although low permeability may be compensated by longer residence

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time (Pather et al., 2008; Rathbone et al., 2015; Rathbone and Hadgraft, 1991). Increased retention time on the buccal mucosa can be achieved by selecting a mucoadhesive formulation.

Oral films can be either orodispersible, i.e. fast dissolving and intended for swallowing, or mucoadhesive, intended for application on the oral mucosa (Borges et al., 2015). Absorption of drug released from the film occurs either trans-mucosal or in the GI tract, governed by the properties of the film. The mucoadhesive buccal film is classified as a prolonged release formulation, which can be single-layer or multi-layer in action (Borges et al., 2015; Preis et al., 2013). Multi-layer films are often designed as oral patches with a non-dissolvable layer promoting uni-directional drug release, either for transmucosal absorption or for local effect in the oral cavity where absorption is undesired (Smart, 2005). Multi-layer films often need to be removed manually (Preis et al., 2014c). Single-layer buccal films can be manufactured to erode with time, and can thus be left in place and may be seen as an enhancer of a traditional orodispersible fast-dissolving drug delivery vehicle.

An oromucosal buccal film is expected to have a longer residence time on the oral mucosa than a fast dissolving oral film, properties that can be controlled by the hydration, swelling, and dissolution processes of the matrix polymers (Smart, 2005). Crucial points for buccal films are their wetting and disintegration properties, effect of mucoadhesion, and for enhancement of a drug delivery platform, also, solubilising of a poorly soluble drug (Fonseca-Santos and Chorilli, 2018; Smart, 2005). In this study, we propose the use of a graft copolymer, Soluplus®, known for its capacity to solubilise poorly soluble drugs and form amorphous solid dispersions (BASF, 2010), as a film former and a novel drug delivery formulation as basis for mucoadhesive buccal film. Soluplus® was originally developed for hot-melt extrusion and to form amorphous solid dispersions (Hardung et al., 2010). It has a polyethylene backbone with one or two grafted sidechains consisting of vinyl acetate randomly copolymerised with vinyl caprolactam (Fig. 1). The overall composition being 57% vinyl caprolactam, 13% polyethylene glycol 6000 and 30% vinyl acetate, and the molecular weight ranging from 90 to 140,000 g/mol (BASF, 2010). The CMC of Soluplus® is very low (7.6 mg/L), according to the producer (BASF, 2010), and in aqueous media it readily forms micelles, which can be used to solubilise poorly soluble drugs. The intrinsic behaviour of Soluplus®-micelles was recently investigated under conditions relevant for oral drug delivery (Alopaeus et al., 2019) and CMC at 37 °C, determined through isothermal titration calorimetry, was found to be 0.5 mg/mL in water. To supplement the mucoadhesive properties, two different potentially mucoadhesive polymers were combined with Soluplus® as well as evaluated individually. Hydroxypropyl methylcellulose (HPMC), also known as hypromellose, is a commonly used film former (Li et al., 2005; Timur et al., 2019; Zulfakar et al., 2016). HPMC is known for its mucoadhesive properties and often referred to as one of the first generation mucoadhesive polymers (Fonseca-Santos and Chorilli, 2018; Hiorth et al., 2014; Smart, 2005). Lycoat® is a modified hydroxypropyl pea starch, which was originally developed as a coating agent for tablets and capsules, but the aqueous film properties designed for immediate release might have interesting applications as film modifier in mixed multi-polymer oral films (Nagar et al., 2011; Parissaux et al., 2007). Starches are known adhesive polymers and different starches, such as hydroxyethyl starch, are listed among mucoadhesive non-ionic polymers (Fonseca-Santos and Chorilli, 2018); hence, Lycoat® should be an interesting reference with the purpose of obtaining mucoadhesive properties of oral films.

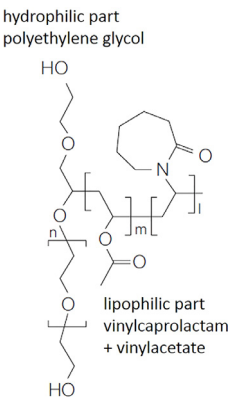

Fig. 1. The structural formula of Soluplus®.

The overall aim of the study was to design a new mucoadhesive oral film formulation utilising the solubilisation capabilities of Soluplus® micelles. The novel formulation should provide the stability and user-friendliness of a dry oral film, assuring the mucoadhesive properties and enabling an increased residence time on the buccal mucosa. Our hypothesis is that Soluplus® in the film will disperse and form micelles upon contact with water. In the amount of saliva accessible, the concentration is expected to be above CMC. A rapid hydration of the film and disintegration of the Soluplus® film is desirable in order to release the micelles containing drug into the formed hydrogel scaffold, thereby providing the drug in a solubilised form that can produce a concentration gradient over the buccal epithelium ensuring passive diffusion over the barrier. At the same time, increased mucoadhesion is necessary for the micelles to remain in close proximity to the epithelium for a prolonged period to increase the total amount of drug that can permeate. The film undergoes a transformation from a dry, solid and stable formulation into a functionalised advanced delivery system upon application, i.e. contact with water or saliva. To the best of our knowledge, this is the first time the graft co-polymer, Soluplus®, has been combined with a mucoadhesive polymer to form a self-dispersible functionalised advanced buccal drug delivery system. This combination of a solubilising agent as the film matrix with a polymer that aids in forming a hydrogel scaffold and hinders too fast erosion, gives a novel formulation that acts like a combination of a fast dissolving orodispersible film and buccal formulation intended for extended release, combining the best qualities of both formulation types. In order to gain a mechanistic understanding of the film formulations and their interactions with moisture and liquid, their mechanical and mucoadhesive properties as well as the permeability of a BCS class IV drug across buccal cell layers, films based on individual and polymer combinations were evaluated. Furosemide was selected as a BCS class IV model drug (Granero et al., 2010).

2. Materials and methods

2.1. Materials

Furosemide was purchased from Fagron (Copenhagen, Denmark). Soluplus® was kindly donated by BASF (Ludwigshafen, Germany). HPMC, with a viscosity grade of 5 cPs, was purchased from Norsk Medisinaldepot AS (Oslo, Norway) and glycerol from Apoteksproduksjon AS (Oslo, Norway). Modified hydroxypropyl starch: Lycoat® RS720, was kindly gifted from Roquette Pharma (Lestrem, France) and is referred to as Lycoat®. Methanol (MeOH) of high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany) was used. The water was purified by the Milli-Q® integrated water purification system for ultrapure water (Merck Millipore, Darmstadt, Germany) and is referred to as Milli-Q water. All salts for buffer preparation were purchased from Sigma-Aldrich (St. Louis, MO, USA). Medium for cell growth Dulbecco's Modified Eagle's Medium with high glucose (DMEM), inactivated foetal bovine serum, non-essential amino acids, penicillin and streptomycin (Pen-Strep) were purchased from Sigma-Aldrich (St. Louis, MO, USA) in the culture of HT29-MTX cells. For the culture of TR146 cells all medium for cell growth was purchased from Invitrogen Corporation (Life Technologies, S.A., Madrid, Spain) as was Hanks Balanced Salt solution (HBSS). All the

other chemicals and solvents were of reagent grade or HPLC grade.

0.01 M phosphate-buffered saline (PBS), pH 7.4 was prepared from tablets acquired from Sigma-Aldrich (St. Louis, MO, USA) and Milli-Q water. For analyses where larger quantities of PBS were needed, the medium was prepared according to European Pharmacopoeia (Ph.Eur.) Chapter 4.1.3. In addition, phosphate buffer with a pH of 6.8 for the mobile phase was prepared according to Ph.Eur. Chapter 4.1.3. Saliva substitute (pH 6.8) was prepared according to the Documenta Geigy Scientific Tables (Diem and Lentner, 1970) of natural saliva contents, and was prepared as a solution of 0.21 g/L of $NaHCO_2$, 0.43 g/L NaCl, 0.75 g/L KCl, 0.22 g/L $CaCl_2 \cdot 2H_2O$, 0.91 g/L $NaH_2PO_4 \cdot H_2O$, and was prepared both with and without 3% (w/w) porcine mucin (type II, Sigma Aldrich, St.Louis, USA).

2.2. Film preparation and compositions

2.2.1. Solvent casting and evaporation

In short, the film forming polymer was dissolved in Milli-Q water and films prepared by the solvent casting method. In the films containing furosemide, the drug was solubilised in Soluplus® overnight, before the rest of the ingredients were added and the film cast. Solutions were cast on a levelled glass plate of the film casting apparatus (Coatmaster 510 ERICHSEN GmbH & Co. KG, Hemer, Germany), using a casting knife with a gap height of 1000 µm. To allow easy removal of the dry film, cellophane (Panduro AS, Gressvik, Norway) was used on top of the glass plate as release liner. The films were allowed to dry in ambient conditions for 24 h before cutting into square pieces, where 2 × 2 cm was defined as a single-unit dose. The film pieces were then stored in a desiccator at RH of 33.2–33.6% (oversaturated $MgCl_2 \cdot 6H_2O$ solution) and room temperature, for a minimum of two weeks before being used in experiments to ensure constant conditions and homogeneous humidity throughout the batch parallels.

2.2.2. Film formulation optimisation

In addition to films containing Soluplus® as single polymer, films containing an additional polymer were prepared. Based on their potentially bioadhesive properties, HPMC and Lycoat® were chosen as the additional polymers. Optimal formulations were developed by testing different ratios of Soluplus® paired with either of the mucoadhesive polymers, HPMC and Lycoat®. Glycerol was added as plasticiser. Separate films of each of the bioadhesive polymers were prepared as controls, and a commercially available over the counter (OTC), fast dissolving oral film was also included. Table 1 shows the composition overview of the optimised film formulations chosen for further experiments, before solvent evaporation as well as the theoretically estimated contents after drying was complete. The commercial reference (F6 reference) was Melatonin Ratiopharm, a pullulan-based rapidly dissolving oral film (Ratiopharm, 2019).

Table 1
Composition of wet film formulations before casting and drying, and estimated composition of the dry films; all amounts in% (w/w).

Component	F1 Soluplus	F2 Soluplus-HPMC	F3 Soluplus-Lycoat	F4 HPMC	F5 Lycoat
Composition of wet film formulation					
Furosemide	0.1	0.1	0.1	–	–
Soluplus®	25.0	16.0	16.0	–	–
HPMC	–	0.5	–	8.0	–
Lycoat®	–	–	0.5	–	17.0
Glycerol	3.5	3.5	3.5	2.0	3.5
Milli-Q water	71.4	79.9	79.9	90.0	79.5
Estimated composition of dry film					
Furosemide	0.3	0.4	0.5	–	–
Soluplus®	80.6	67.7	70.6	–	–
HPMC	–	2.1	–	65.5	–
Lycoat®	–	–	2.4	–	74.9
Glycerol	12.9	17.2	17.9	18.7	16.9
Rest moisture content*	6.3	12.5	8.7	15.9	8.2

* Determined by IR-moisture analyser.

2.3. Film characterisations

2.3.1. Mass, thickness, uniformity and morphology

Basic film characterisations were performed on single-unit doses. The thickness of the film samples was measured using a micrometer screw (Mikrometer Cocraft, Clas Ohlson, Sweden) with a measuring range of 0 – 25 mm and resolution of 0.01 mm ($n = 6$). Residual moisture content for dried films that had been kept in desiccator for a minimum of two weeks was measured with an IR moisture analyser (MA30 Sartorius, Goettingen, Germany). The samples were heated for 30 min at 120 °C and all samples were tested in triplicate. The mass was measured using an Sartorius Research R160P balance (Richmond Scientific Ltd., England) and uniformity of mass evaluated according to the monograph for tablets (uncoated or film coated) of 80 mg or less (Ph.Eur. Chapter 2.9.5), since there are no official monographs specifically for the test of uniformity of mass for oral films. Briefly, 20 randomly selected single-unit doses were weighed and the average and standard deviations calculated, then the percentage deviation of each individual mass from the average mass was calculated. According to the monograph, not more than two of the individual masses should deviate from the average mass by more than 10% and none should deviate by more than twice that percentage, i.e. 20%.

The surface and morphology of the films were visualised with Scanning Electron Microscopy (SEM). Films were fixed on aluminium stubs with double-sided carbon tapes and then covered with a thin conductive gold layer using a BAL-Tec SCP 050 Sputter Coater (Leica Instruments, Wetzlar, Germany). Coated samples were investigated with a Phenom World XL (Phenom-World B. V., Eindhoven, The Netherlands) using the Backscatter detector and a working voltage of 10 kV.

2.3.2. Quantification of content and content uniformity

The quantification of furosemide was done by HPLC-UV/VIS as previously published (Alopaeus et al., 2019). Briefly, a reversed-phase column (Nova-Pak®, C18, 4 µm, 3.9 × 150 mm, Waters, Wexford, Ireland) was used, and the mobile phase consisted of filtered (0.45 µm) phosphate buffer with a pH of 6.8 (see Section 2.1): MeOH (70:30 v/v). The injection volume was 10 µL, flow rate 1 mL/min, column temperature 30 °C, and detection wavelength 276 nm. The retention time for furosemide was approximately 8 min, and the calibration curve was in the range of 0.1–5.0 µg/mL ($R^2 \geq 0.99$). For the quantification of samples containing Soluplus®, it was essential to make sure that the polymer was sufficiently washed off the column between injections by regularly running a washing program.

Drug content and content uniformity was evaluated with a slight modification to the uniformity of content monograph for tablets (Ph.Eur. Chapter 2.9.6). Film pieces were accurately weighed and dissolved separately in 100 mL PBS pH 7.4. Films were allowed to dissolve under stirring and protected from light for a suitable amount of time and contents quantified by HPLC. Film formulations F1-F3 were assessed ($n = 10$), the average and standard deviations for all formulations were calculated and the results were interpreted so that no more than a 15% deviation between samples was deemed acceptable.

2.3.3. Mechanical studies

Mechanical properties of the films were evaluated by a puncture test using Texture Analyser Ta-XT2i (Stable Micro Systems, Godalming, UK), equipped with a flat-faced cylindrical probe with a diameter of 7.03 mm. The software supplied by the manufacturer was Exponent version 6.1. The sensitivity of the Texture Analyser 5 kg load cell is 0.001 N. Film pieces of 2 × 2 cm were fixed by screws between two

plates, with a cylindrical hole with an area of 38.82 mm^2.

The Texture Analyser was adjusted to move the probe with a pre-test velocity of 2.0 mm/s. Measurement started when the probe obtained contact to the sample surface, defined by trigger force, which was set at 0.049 N. After that, the system started recording force and displacement of the probe. The test speed was constant 0.1 mm/s until the film ruptured. The maximum force to break (N) and distance (mm) of probe movement until break was measured, and tensile strength (N/mm^2) and elongation at break (%) were calculated (Preis et al., 2014b). All experiments were conducted at room conditions and all samples were tested in triplicate.

Tensile strength was calculated using the following equation:

$$Tensile\ strength = \frac{force}{area} \quad (1)$$

where the force is the measured maximum force at film rupture (N) and area is the probe contact area with the film (mm^2).

Elongation to break was calculated using the following equation:

$$Elongation\ to\ break\ \% = \left(\frac{\sqrt{a'^2 + b^2} + r}{a} - 1\right)*100 \quad (2)$$

where a represents the radius of the film in the sample holder opening called initial length (a = 6.985 mm), a' represents the initial length of the film sample that is not punctured by the probe (a' = 3.47 mm), b represents the penetration depth of the probe (i.e. distance or displacement) and r represents the radius of the probe (r = 3.52).

2.3.4. Wettability

The wettability was estimated by measuring the contact angle of a droplet (1 μL) of bidistilled water towards each of the film formulations (F1-F6) at ambient conditions using a manual contact angle microscope Type G1 from Krüss GmbH (Hamburg, Germany). Since the films were relatively hydrophilic and started swelling very quickly and the reading was performed manually, the experiment had to be conducted fast and in a standardised manner, i.e. reading 3 s after application of the droplet. To obtain a robust observation, twenty measurements were performed for each film formulation. The mean and standard deviation were calculated.

2.3.5. Dynamic vapour sorption (DVS)

The behaviour of the films at defined relative humidities (RH) was investigated with a DVS-Resolution from Surface Measurement System Ltd (London, UK), which measures humidity-dependent mass change. The respective moisture sorption isotherms were studied at 25 ± 1 °C and 37 ± 1 °C for an increase of p/p_0 from 0.0 p/p_0 to 0.9 p/p_0 in steps of *0.1* p/p_0 followed by a decrease to 0.0 p/p_0 *as* described by Mönckedieck et al. (2017). The moisture content was kept stable for up to 360 min to allow equilibration, until the change in mass was less than 0.005%/min. Finally, the cycle was repeated to enable statements on water uptake and possible crystallisation event occurring because of the applied stress. Film samples of approximately 5 × 5 mm were placed in the microbalance in a position allowing water vapours to access the film from both sides. The moisture sorption and desorption isotherms were plotted for each temperature. All formulations (F1-F6) were evaluated at both temperatures.

2.3.6. Swelling and erosion

A film piece (2 × 2 cm) was weighed (initial weight W_0) and placed in a dry beaker. 1 mL simulated saliva (pH 6.8) was added on the film with a pipette to allow the film to swell and/or erode. At regular time intervals, the excess of water not absorbed by the film was carefully removed, and the wet film and beaker was weighed (W_t). Then more simulated saliva was added to continue the analysis, the added amount varied by film and time, as enough was added to saturate the film surface but not more that it would run over and wet the beaker. From the weight of the swelling film at different time points, the swelling and erosion could be estimated and W_t/W_0 was plotted as a function of time. All formulations were tested in triplicate.

2.3.7. Disintegration

Two different methods of determining disintegration were used. The first method, petri dish method was as follows; films were placed in a petri dish and 3 mL of simulated saliva (pH 6.8) was added. The petri dish was shaken at a constant speed (200 rpm) to allow the irrigation media to rinse over the film. The endpoint was set when disintegration of the film matrix was observed.

The second method, the TA-XT2i Texture Analyser method, was executed using a flat-faced cylindrical probe and the film mounted as described for the puncture test (see 2.3.3). Briefly explained, 200 μL of simulated saliva (pH 6.8) was pipetted onto the film; the lag time of 5 s after test start before probe started moving was used to allow the liquid placement. The probe was programmed to stop at target distance (5 mm) and monitor the force throughout the test. The typical force vs. time profile showed that initially the force would increase before the wetting of the film resulted in reduction of the force as the film disintegrated and finally come down to the baseline when film disintegration was complete. Endpoint of disintegration was defined at the time when the probe returned to a force of 0.03 N. This specific force was chosen, because according to studies, 0.03 N is the minimal force exerted by the human tongue when licking over a probe (Preis et al., 2014a).

2.3.8. Dissolution and re-micellisation

A simple dissolution study was performed on films F1-F3. A film piece (2 × 2 cm) was weighed and added in a 100 mL beaker with 50 mL pre-warmed PBS at 37 °C. The films were allowed to dissolve freely in the media under constant shaking (150 rpm) in a temperature-controlled environment (Environmental Shaker-Incubator ES-20, BioSan, Latvia). The samples in the beakers were not shaken or stirred in any other way than the natural movement by the shaker. Samples of 1 mL were taken out at set time points and aliquots diluted suitably for HPLC content determination as described above.

The rest of the sample was used to determine Z-average, which was interpreted as estimated micelle size (nm) and polydispersity index (PDI) using dynamic light scattering (Zetasizer Nano Series, Malvern Instruments Ltd., Malvern, UK). Values were derived from average of three subsequent runs with 10 measurements each. Samples were run in triplicate at 25 °C with 173° backscatter angle.

2.4. Mucoadhesion studies

2.4.1. Interaction with mucin-dispersion

A simple mucin-interaction test was conducted as described by Hagesaether et al. (2009). Briefly, 50 μL simulated saliva with 3% (w/w) porcine mucin was evenly spread on the top of two different pieces of filter papers with an inert backing layer (WhatmanVR Benchkote, Chicago, USA). The pieces of filter paper had dimensions of 1.5 × 1.5 cm. Both pieces were attached with double sided adhesive tape; one of them was placed on a lower stationary part of a TA-XT2i Texture Analyser), and the other was attached to a flat, upper, movable probe. A film piece of 1 × 1 cm was placed on the lower paper. Based on previous work, a preload-force of 200 g for 100 s was applied before the upper probe was lifted off at a speed of 0.01 mm/s at which the force of detachment was documented. The same was done for simulated saliva, without the addition of mucin, to distinguish between the unspecific adhesion (no mucin interaction) and general adhesion (with mucin interaction). Measurements were repeated 10 times for each film sample (F1-F6), both with and without mucin interaction. The displacement and force of detachment were recorded. Based on the force vs. time curve obtained, the peak force (F_{max}, g) and work of adhesion, i.e. the area under the peak (AUC, g/s) were obtained.

2.4.2. Mucus-producing HT29-MTX cells as mucosal surface

Instead of buccal mucosal tissue from slaughtered animals, we utilised living mucus-producing cells from the HT29-MTX cell line, kindly provided by Dr. Thécla Lesuffleur (INSERM UMR S 938, Paris, France). These mucus-secreting cells were originally adapted and cultured for several passages in a medium containing 10^{-6} M methotrexate (MTX) and reversed for several passages in a drug-free medium (Lesuffleur et al., 1990, 1993). They do not need to be maintained in media containing MTX in order to differentiate into a mixed population of mucus-secreting goblet cells and enterocytes after confluency. The cells used in this study were from passages 27–28. The medium for cell growth was Dulbecco's Modified Eagle's Medium with high glucose (DMEM), containing L-glutamine, sodium pyruvate and phenol red with a pH in the range of 6.8–7.2 (sodium bicarbonate buffer), which was further supplemented with 10% inactivated foetal bovine serum, 1% non-essential amino acids, penicillin (100 units/mL) and streptomycin (100 µg/mL).

The HT29-MTX cells were seeded at a density of $2.4 \times 10^4/cm^2$ in petri dishes with a growth surface of 55 cm^2 and grown for 21 days to allow the cells to differentiate into mucus producing goblet cells and a distinct mucus layer to form on top of the cell monolayer. The cells were incubated at 37 °C under an atmosphere of 5% CO_2. For the preservation, the cells were passaged before reaching 80% of confluency with a solution of trypsin-EDTA. The medium was changed 3 times weekly. The cell monolayer integrity as well as the mucus layer were inspected with a microscope before use in the retention experiments (see 2.4.3).

2.4.3. Retention model using mucus-producing cells

The retention of the formulation to a mucosal surface was evaluated in a modified version of an oral cavity model previously described by Madsen et al. (2013). Mucus-producing cells grown in a petri dish were used as the mucosal surface and a film piece (2 × 2 cm) was placed on the mucus on top of the cell monolayer. To simulate the flow of saliva the formulation was exposed to a constant flow of PBS (pH 7.4) rinsing over the film. The PBS was collected at the outlet, and samples were withdrawn at predetermined time points and the drug content quantified. The model was used to estimate the retention of the drug to the mucosa and was taken as an indication of mucoadhesiveness of the film formulation.

The retention model was set up using a water-bath GD100 (Grant Instruments, Cambridge, UK) to warm PBS, which was transported through a high-precision multichannel dispenser pump (ISMATEC ISM931C, Wertheim, Germany) through pipette tips fitted onto a platform situated in a closed humidity chamber. The PBS rinsing over the mucosa was 37 ± 1 °C and the humidity and temperature in the chamber was kept at > 80% and 37 ± 1 °C, respectively. The PBS rinsing over films came from 4 individual nozzles to spread the media equally over the whole formulation. Each nozzle had a flow rate of 0.4 mL/min equalling a total flow rate of 1.6 mL/min for the set-up. Film formulations F1, F2 and F3 as well as free drug (furosemide dissolved in PBS) were tested in triplicate.

2.5. Transepithelial drug diffusion study using TR146 cells

2.5.1. Cultivation and maintenance of the cells

Permeability across buccal membrane was assessed using TR146 human buccal epithelium cell line culture (ATCC; American Type Culture Collection, Barcelona, Spain). TR146 cells were chosen to mimic stratified epithelium of human buccal mucosa (Castro et al., 2018; Jacobsen et al., 1995; Nielsen and Rassing, 2000). TR146 is a cell line originating from a neck node metastasis of a human buccal carcinoma (Rupniak et al., 1985) and are known to express characteristics of human buccal epithelium, such as no tight junctions and absence of complete keratinisation (Jacobsen et al., 1995). The permeability of furosemide from formulations F1-F3, as well as free drug as control, was assessed in Falcon® Transwell inserts (PET, pore size 3.0 µm), using 6-well plates. TR146 cells (passage P19) were seeded with a density of 2×10^5 cells/well on the inserts and medium was changed three times weekly for 24 days of culture before using the cells in the experiment. The growth medium used was DMEM, containing L-glutamine, sodium pyruvate and phenol red with a pH in the range of 6.8–7.2 (sodium bicarbonate buffer), which was further supplemented with 10% inactivated foetal bovine serum, 1% non-essential amino acids, penicillin (100 units/mL) and streptomycin (100 µg/mL). Cells were maintained in an incubator (CellCulture® Incubator, ESCO GB Ltd., UK) at 37 °C and 5% CO_2. Trans-epithelial electric resistance was monitored using an EVOM epithelial voltohmmeter equipped with chopstick electrodes (World Precision Instruments, Sarasota, FL, USA), starting from day 7 and throughout the growth period, as well as during and after the permeability study. The TEER values of the cell layers were measured before cell medium was changed to monitor the evolution of confluence. Only wells with sufficient and stable values after 24 days of culture were used in the experiment.

2.5.2. Permeability studies

For the permeability study, film pieces equivalent to 200, 210 and 220 µg of furosemide content for films F1, F2 and F3, respectively, were added to the apical side, where 1.5 mL of HBSS had been added replacing the growth media. HBSS is a buffered salt solution designed to maintain the solution pH at a physiological interval (7.1–7.4). Free drug was in concentration equivalent of 500 µg per well, also dissolved in HBSS. The medium from the basolateral side was replaced with pre-warmed HBSS (2.5 mL). The plates were incubated under stirring (100 rpm) at 37 ± 1 °C. Samples of 200 µL were withdrawn at 15, 30, 45 and 60 min from the basolateral side, with pre-heated fresh media added every time to replace the withdrawn volume maintaining sink-conditions. After finished experiment, the samples withdrawn from the basolateral side, and a sample from the apical side, were suitably diluted and analysed using HPLC-UV/VIS. Moreover, the cells were subjected to lysis using 1% Triton-X in order to quantify the furosemide adsorbed to the cell surface or internalised by TR146 cells. Briefly, the furosemide quantification was performed in a Merck Hitachi LaChrom® system (Merck Millipore, NJ, USA) equipped with a D-7000 Interface, a L-7455 Diode Array Detector, a L-7200 Autosampler, and a L-7100 Pump. Furosemide samples were injected (20 µL) on a LiChrospher® 100 RP-18 (125 × 4 mm, 5 µm, Merck Millipore, NJ, USA) with a LiChrospher® 100 RP-18 guard column (4 × 4 mm, 5 µm, Merck Millipore, NJ, USA) and mobile phase consisting of acidified water (pH 5.5) and 2-propanol (70:30, v/v) at a flow rate of 1.0 mL/min. Furosemide elution was monitored at 238 nm.

The flux (J) and the apparent permeability coefficient (P_{app}) was calculated for each permeability experiment and each film formulation was tested in triplicate. The reported values are the average of the individually calculated P_{app} for each parallel. The flux was calculated as (Di Cagno et al., 2015) the slope of the linear regression of the cumulative permeated drug plot, normalised by the surface area (A), as according to Eq. (3):

$$J = \frac{dQ}{A\ x\ dt} \tag{3}$$

where dQ is the fractional amount of permeated drug expressed as moles, and dt is the time interval expressed in seconds. The P_{app} was calculated by normalising the flux (J) over the total concentration of drug in the apical side, as described in Eq. (4)

$$P_{app} = \frac{J}{C_0} \tag{4}$$

2.6. Statistical analysis

All the values are shown as mean ± standard deviations. Where

applicable, a one-way ANOVA with Tukey's post hoc test was applied to determine statistical significance. All the analyses were performed using the software program GraphPad Prism 8® (Graphpad Software San Diego, CA, USA) with the statistical significance set to $p \leq 0.05$.

3. Results and discussion

3.1. Optimisation of Soluplus®-based films

Soluplus® in water produced thin-flowing micellar suspensions of low viscosity up to concentrations between 15–20% w/w (Alopaeus et al., 2019). The viscosity or flow properties of the film formulation is an essential parameter allowing the preparation of films with the solvent casting method using the Erichson film applicator, i.e. casting on a glass plate with no limiting walls (Krampe et al., 2016). Formulations with too low viscosity would flow off the plate whereas too high viscosity limits even spreading with the knife. Aqueous Soluplus®-dispersions with a concentration of 25% w/w were found empirically to have suitable properties as a single polymer. However, Soluplus® in combination with another polymer formed dispersions with high viscosity. Therefore, various concentrations of Soluplus® in combination with each of the hydrophilic polymers HPMC or Lycoat® were screened. Addition of concentrations above 1% of the mucoadhesive polymers led to phase separation as determined by visual inspection. The combination of Soluplus® 16% w/w with 0.5% w/w of either of the additional polymer showed suitable casting properties and no phase separation occurred either in wet or in dried condition.

Glycerol was added to the film formulations as plasticiser, which are typically added in films up to 20% (Arya et al., 2010). The glycerol content was selected based on the texture and mechanical properties to allow easy handling. Concentrations over 6% for single polymers and over 3.5% for Soluplus®-based formulations resulted in sticky film surface and highly plastic films, whereas too low concentrations gave brittle films that were difficult to cut or handle. Finding the right glycerol level was more challenging for Soluplus®-containing films than for the HPMC (F4) or Lycoat® (F5) reference films. It should be mentioned that residual moisture content also acts as plasticiser in the films, and the Soluplus®-based films dried more slowly than their single-polymer references. To avoid adding too much glycerol to the film formulations and obtaining highly plastic films, the films were cut into single dose-units while still in the drying process. This means that they were cut after drying overnight and transferred to a humidity-controlled desiccator for the stabilising of the moisture content.

Furosemide was selected as a model drug because it is a BCS class IV drug with poor solubility and permeability (Granero et al., 2010), and it is on WHO Model List of Essential Medicines for Children (WHO, 2017). The amount of drug that could be solubilised into the Soluplus®-containing films was selected based on the solubilising capacity of Soluplus® dispersion, determined in an earlier study (Alopaeus et al., 2019). Considering that the solvent loss on drying can trigger re-crystallisation of the drug, the solubilisation of dehydrated micelle-dispersions would be limited. It was decided to add the same amount of drug to all film formulations since it was challenging to predict the final concentration in the film prior to preparation. The optimised formulations and the estimated composition of the resulting dry films are found in Table 1.

3.2. Film characterisations

All films were cast with the same gap height; therefore, the wet film thickness was the same (1000 μm) for all formulations. After drying, it could be noted that the edges of the large film sheet had a larger variation in the film thickness as compared to the central part. Therefore, a minimum of 2 cm of the outer edges was removed before single units of 2 × 2 cm, defined as single dose, were cut. Basic film characterisations showed that all film formulations differed relative to each other in the mass, film thickness and drug content per single unit-dose (Table 2).

Table 2
Overview of film thickness ($n = 10$), mass of single unit-dose ($n = 20$) and furosemide content per single unit-dose ($n = 10$) of all film formulations (mean ± SD).

Film formulation	Film thickness (μm)	Mass of 2 × 2 cm (mg)	Drug content (μg/2 × 2 cm)
F1 Soluplus	235 ± 3	134.3 ± 10.1	404 ± 28
F2 Soluplus-HPMC	175 ± 10	124.1 ± 13.0	602 ± 124
F3 Soluplus-Lycoat	213 ± 6	104.7 ± 8.1	499 ± 64
F4 HPMC	71 ± 7	54.8 ± 4.0	n.d.
F5 Lycoat	130 ± 8	73.3 ± 4.6	n.d.
F6 reference	126 ± 8	45.7 ± 2.5	n.a.

n.d.: contained no drug, n.a.: not available (commercial reference with different drug).

The thickness spanned from around 70 μm to around 235 μm. Films with Soluplus® as the single polymer gave the thickest final films. Soluplus® also had great influence on the mixed polymer films; they were considerably thicker than single-polymer films of HPMC or Lycoat®. Weight and thickness were mostly correlated, except for F2 and the reference.

All films except F2 passed the Ph.Eur. requirements for uniformity of mass, when applying the criteria for the test intended for small tablets (Ph.Eur. Chapter 2.9.5), in the lack of recognised criteria for oral films. The drug content correlated well with the estimated theoretical content (Table 2); however, it was different per dose for the three Soluplus®-based films due to the differences in the film composition and weight/thickness ratio. The drug content was within the requirements for uniformity of dosage units (Ph.Eur. Chapter 2.9.6.) for films F1 and F3, but for F2 the variance was again too large to pass the requirements for tablets with low dose. Likely, this is related to the fact that the weight and thickness were not completely homogeneous across the whole film for F2 and thus these variations naturally occur. To reduce the variation, an even larger outer part could have been removed and discharged.

All films were transparent with a non-sticky surface and smooth appearance (Fig. 2A-F). The scanning electron micrographs in Fig. 2 showed that they had a flat and smooth, none-porous surface, only the commercial reference (Fig. 2F) had a slightly different morphology. These films were white and appeared more fibrous or phase separated when examined with the naked eye, and the fibrous structure could also be recognised in SEM.

3.3. Mechanical properties

A buccal film formulation should have mechanical properties that allow easy handling and placing on the buccal mucosa. Suitable mechanical properties would be intermediate strength and certain flexibility to promote and facilitate interaction with the mucosa. Since it is difficult to quantify these expectations, a commercially available reference was included in the test set (F6). The Soluplus® film (F1) was among the strongest with the second highest tensile strength, but it exhibited relatively low flexibility determined as elongation at break (Table 3), at least compared to the films containing HPMC. The reference films (F4-F6) were further compared to the films containing furosemide, as the drug is not expected to have an effect on the mechanical properties as the amount is so low (less than 0.45 w%). Formulations containing HPMC, both the reference HPMC film (F4) and Soluplus®-HPMC film (F2) were the most flexible and showed the highest elongation at break. The Lycoat® film (F5) was hard to break (i.e. high tensile strength) but elongation at break was very low, and even lower when paired with Soluplus® (F3). Soluplus®-HPMC showed relatively low tensile strength but elongation at break was even higher than the pure HPMC. Compared to the commercial reference film (F6)

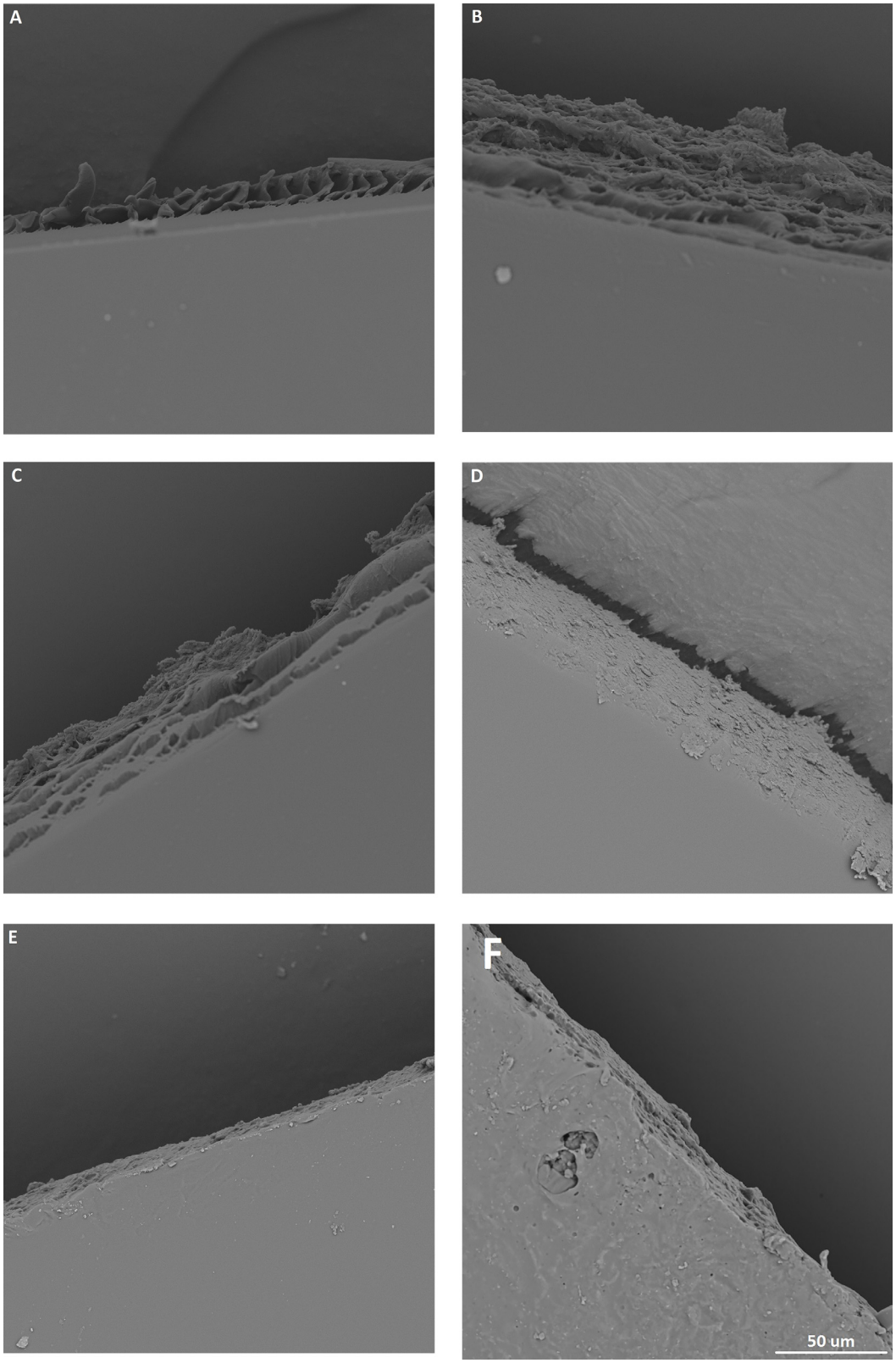

Fig. 2. SEM images of the surface of film formulations A:F1, B:F2, C:F3, D:F4, E:F5 and F:F6, the magnification is 1500x and the bar indicates 50 μm.

the mechanical properties of all Soluplus® containing films were in comparable range, except that the elongation to break of the combination with HPMC (F2) was significantly higher. However, the cutting and handling properties of the Soluplus®-HPMC film (F2) was not impaired by the higher elasticity. Preis et al. (2014b) investigated the mechanical properties of commercial films and found tensile strength values ranging between 0.08 and 0.49 N/mm^2, and slightly higher (up to 1.02 N/mm^2) for the films made in their lab. Elongation was not found to be a crucial parameter, which correlates well with our findings of elongation at break in Soluplus®-based film formulations; where

Table 3
Mechanical properties obtained by Texture Analyser (n = 3) (mean ± SD).

Film formulation	F_{max} (N)	Displacement (mm)	Tensile strength (N/mm^2)	Elongation at break (%)
F1 Soluplus	26.00 ± 11.25	1.68 ± 0.46	0.67 ± 0.29	5.75 ± 2.86
F2 Soluplus-HPMC	13.40 ± 1.23	4.39 ± 0.78	0.35 ± 0.03	30.69 ± 8.58
F3 Soluplus-Lycoat	8.29 ± 1.44	1.0 ± 0.13	0.21 ± 0.04	2.12 ± 0.51
F4 HPMC	44.11 ± 9.47	4.01 ± 0.59	1.14 ± 0.24	26.41 ± 6.41
F5 Lycoat	34.22 ± 9.73	1.52 ± 0.25	0.88 ± 0.25	4.62 ± 1.43
F6 reference	21.19 ± 0.78	1.42 ± 0.09	0.55 ± 0.02	4.03 ± 0.51

values ranged from ca. 2% for F3 to over 30% for F2 as this could not be correlated with any of the other film properties. The amount of glycerol is known to play a role on the mechanical properties results as this excipient acts as a plasticiser (Aulton et al., 1981), and is added to the formulation to ease handling. Residual moisture content will also act as a plasticiser, therefore, the total amount of plasticiser can be more challenging to estimate, and it is of crucial importance that the films are equilibrated under similar RH prior to tests on mechanical properties. Adding a hygroscopic polymer, such as HPMC, will result in increased flexibility as could be seen with film F2. All Soluplus® containing films were concluded to have acceptable mechanical properties for the use as a buccal delivery system, however, the higher flexibility of the films containing HPMC (F2 and F4), are likely to be better received from a patient-centric view, as this enhances comfort of the formulation and might even affect the mucoadhesion through improved interaction with mucosa.

3.4. Interactions with moisture vapour and liquid

Dynamic vapour sorption analyses were conducted to determine the moisture sorption and desorption capacities of the various formulations. The films were exposed to two subsequent cycles of changing RH from 0% to 90% to 0% RH in steps of 10% RH during DVS analysis. Fig. 3 shows the entire cycle of water sorption and desorption taking place during DVS measurements for a Soluplus®-HPMC film. The sorption capacity (% mass change) was dependent on the composition of the formulation, the relative humidity and the time. The time it took to complete the entire measuring cycle was different for the different film compositions. The sorption isotherms of all film formulations at 25 °C can be seen in Fig. 3. Water sorption isotherms of all films initially showed a moderate increase in moisture content with a progressive increase in relative humidity up to around 40%. This was followed by a rapid increase in water absorption for higher RH. For hygroscopic films, this is expected behaviour. The HPMC film showed the highest sorption of water, likely due to an increased number of hydrogen binding sites for water on the hydrophilic polymer chain (Li et al., 2005). In addition, in the mixed films of Soluplus®-HPMC films, the difference is more pronounced than with Lycoat® (Akhtar et al., 2013).

The results obtained at 80 ± 2% RH (indicated with a red line in Fig. 4) and 25 ± 1 °C were used to compare the hygroscopicity of the different film formulations. Table 4 summarises the percentage mass increase observed at 80% RH for the two temperatures 25 ± 1 °C and 37 ± 1 °C. All tested oral films showed a mass increase of more than 15% after equilibration in 80% RH at 25 °C, and would therefore be classified as very hygroscopic according to the Ph.Eur. (Chapter 5.11.). Film formulations F1 and F6 showed very similar values for the two

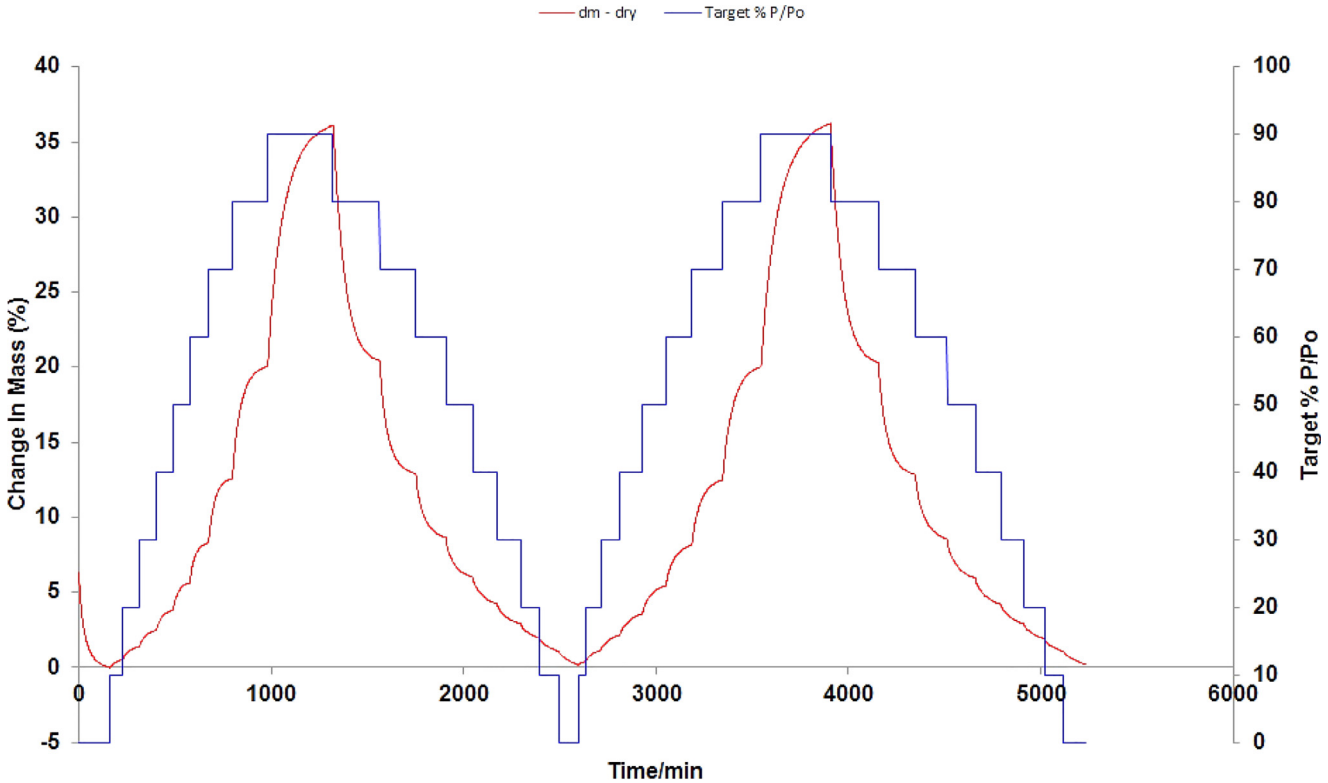

Fig. 3. DVS analysis of film sample of Soluplus®-HPMC film. Two cycles from 0% to 90% RH in steps of 10% RH at 25 °C. The blue line denotes the target RH profile (secondary axis), whereas the red line shows the% changes in the film mass as a result of change in% RH (primary axis).

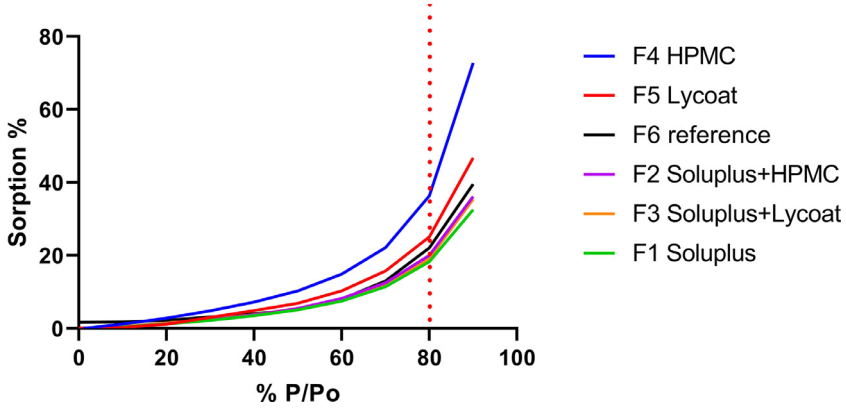

Fig. 4. Sorption isotherms for the investigated film formulations. Red line indicate the% mass change after equilibration at 80 ± 2% RH and 25 ± 1 °C, i.e. the conditions for determining hygroscopicity according to Ph.Eur. (Chapter 5.11).

Table 4
Percentage mass increase after equilibration at 80% RH for all film formulations (values are the arithmetic mean of the two cycles).

Film formulation	dm (%) at 25 °C	dm (%) at 37 °C
F1 Soluplus	18.27	18.47
F2 Soluplus-HPMC	20.03	17.25
F3 Soluplus-Lycoat	19.25	16.58
F4 HPMC	36.37	18.78
F5 Lycoat	25.08	19.98
F6 reference	22.10	22.20

tested temperatures, whereas film formulations F2 - F5 showed decreasing mass increase-values with increasing temperature. The largest change can be seen in the pure HPMC film, which is also the most hygroscopic of the tested materials. Since HPMC is a derivative of cellulose, obtained by substituting hydroxypropyl and methyl groups to primary and secondary hydroxyl groups, three factors, namely, methyl content, hydroxypropyl content, and molecular weight control the final properties and behaviour of HPMC (Li et al., 2005). The molecular weight determines the viscosity in aqueous solution, with low molecular weight also correlating to good water solubility and good film-forming properties. HPMC and Lycoat® are thermoresponsive polymers with low gelation temperatures (and low critical solution temperature) and at higher temperatures, the sol-gel transformation has a role in the number of free binding points available for water molecules to bind, hence lower absorption at higher temperatures as the moisture sorption is temperature dependent in thermoresponsive gels (Joshi, 2011). For Soluplus®, the temperature has less of an effect as the mechanism is different. Gelation mechanism and sol-gel transformation in Soluplus® is an effect of increased entanglements of Soluplus® micelles at increasing temperatures, which does not correlate to increased moisture absorption (Alopaeus et al., 2019; Tanida et al., 2016; Taylor et al., 2017).

Another way to investigate the moisture interaction with the film is to determine the contact angle of a droplet of water on the dry film surface. Contact angle measurements indicate the degree of wetting when a solid and a liquid interact. Theoretically, small contact angles < 90° correspond to high wettability and large contact angles > 90° correspond to low wettability. Generally, all tested film formulations exhibited contact angles of far less than 90°, indicating good wettability properties (Fig. 5). The highest contact angle was found for the single-polymer Soluplus® film (F1) at 52.0° ± 2.9, which was in the same order of magnitude as reported for the Soluplus®-indomethacin solid dispersion at 54.9° ± 8.0 (Semjonov et al., 2018). The authors further showed that formation of solid dispersions of Soluplus® increased the wettability of the polymer, compared to the dry solid in powder form. The contact angle of the hydrophilic polymer films, HPMC (F4), Lycoat® (F5) and pullulan (F6), were significantly lower than the Soluplus® film ($p < 0.05$). This is in agreement with the DVS findings. The water sorption and the wettability of hydrophilic materials are linked to the hydrogen bonding ability of these biopolymers (Dahlberg et al., 2010). The lowest contact angle was found for Soluplus®-HPMC (F2) film at 24.5° ± 6.9. It is notable that the Soluplus®-polymer combination films (F2 and F3) displayed a synergistic effect where mixing Soluplus® with a hydrophilic polymer resulted in better wetting capacity (reduced contact angle) then either component had on its own. It is also known that surface topology might have an effect on the contact angle, where two phenomena: spreading and absorption, will interplay (Farris et al., 2011). However, according to the SEM images and morphological inspection of the films, all films had a smooth surface with similar topology; thereof this is not likely to be an issue here.

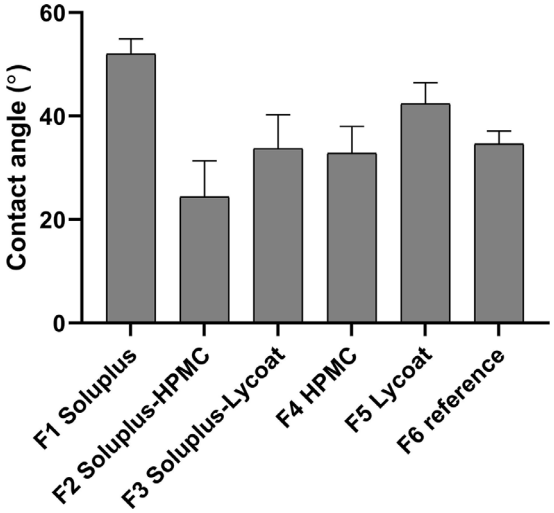

Fig. 5. Contact angle determined with a droplet of bidistilled water on the film at ambient temperature. Mean ± SD (n = 20).

The next step was to look at the behaviour of the films in contact with aqueous liquid. The swelling-erosion test gives an indication of the degree of moisture uptake in the film before erosion starts (Adrover et al., 2018). A fast disintegrating film formulation would be expected to have a high and fast uptake but erosion starting very rapidly. A film meant for buccal administration, on the other hand, will have desirable properties of relatively good wetting behaviour and moisture uptake, but erosion should be slow, so the film matrix retains its shape and can stay in place for longer (Preis et al., 2013). Fig. 6 shows the results from the swelling-erosion test in simulated saliva. Typically, the weight of the film initially increased until dissolution occurred and the film weight decreased because of the eroded material

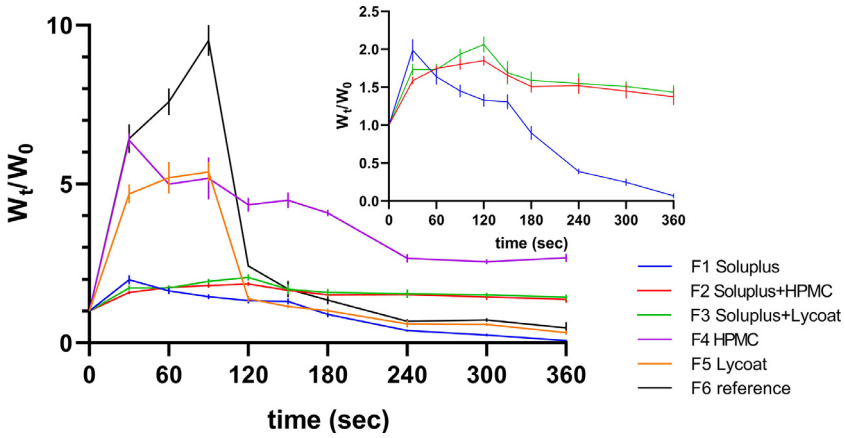

Fig. 6. The results from swelling and erosion studies conducted in simulated saliva for all film formulations at ambient temperature. Films F1-F3 are also showed as a magnified insert.

was removed together with the excess of liquid. The commercial reference (F6), which is a rapidly dissolving orodispersible film, showed typical properties with rapid increase in weight and then quickly eroding until completely disintegrating within minutes. The Lycoat® reference film (F5) had the same profile but the weight gain ratio was not as high, whereas HPMC reference film (F4) had similar swelling capacities as F5, but the erosion did not happen as fast, and by visual inspection, the film was transformed into a swollen hydrogel (Li et al., 2005). The three formulations based on Soluplus® (F1-F3) did not swell and gain weight as much as the other film formulations, the erosion was also a very slow process and the films retained their shape much longer throughout the test without dissolving visibly. The good structural integrity is especially evident for the combination films, F2 and F3, which presented very stable profiles with minimal erosion after 6 min (insert in Fig. 6). The single Soluplus® film (F1), lacked the support structure of the combination film-forming polymer, thus dispersed in the final stage, and was removed with the excess of liquid.

Two different types of disintegration tests were performed on all films (F1-F6), a petri dish method and a drop method using a Texture Analyser, respectively. The petri dish method was described in literature earlier (Preis et al., 2014c). However, since the method is highly subjective and, similarly to the swelling erosion-test, it does not subject the films to any mechanical stress, a more objective method was developed to evaluate how the different films disintegrated. The texture analyser method introduces a mechanical punch and might therefore provide information that is more relevant for the disintegration properties of a buccal dosage form. Table 5 provides an overview of the results from the two disintegration tests. In the petri dish method the commercial reference (F6) and the Lycoat® reference (F5) both disintegrated within 30 s, which is the FDA definition of an orally disintegrating tablet (FDA Guideline, 2019). The criteria is often also applied for orally disintegrating films (Borges et al., 2015; Dixit and Puthli, 2009; Speer et al., 2018). All Soluplus®-containing films showed significantly longer disintegration times, from around 2.5 min up to around 5 min, depending on which of the other polymers Soluplus® was mixed with; HPMC prolonged the disintegration time whereas Lycoat® shortened it. This correlates well with the results from the swelling-erosion studies where Lycoat® (F5) and the commercial reference (F6) showed properties of rapid erosion and HPMC was observed to form a type of hydrogel, which would show a very slow erosion pattern. In the texture analyser method, we could determine some correlations to the swelling/erosion and petri dish disintegration studies where the addition of Lycoat® considerably shortened the time for a film piece to disintegrate. Soluplus® micelle formation leading to disintegration of film is concentration-dependent; in this test-setup, there is not a lot of liquid, which explains why the single polymer film had the longest disintegration time. The addition of small amounts of liquid resulted in plasticisation of the polymer rather than disintegration of the film, which would be expected with excessive liquid. The over-plasticised polymer could be elongated much more before the force limit was reached; hence the observed long disintegration time for the Soluplus® single polymer film. In this specific type of test, the addition of a hydrophilic polymer led to faster disintegration (F2 and F3). Some of the films could not be measured by this method due to their rapid dissolving or formation of a soft hydrogel, which was the case for the reference films with single hydrophilic polymer and rapidly dissolving commercial reference (F4-F6).

Table 5
Film disintegration studies (mean ± SD; $n = 3$).

Film formulation	Disintegration time (sec) petri dish method	Disintegration time (sec) drop method – Texture Analyser
F1 Soluplus	210 ± 9	248 ± 3
F2 Soluplus-HPMC	277 ± 8	211 ± 8
F3 Soluplus-Lycoat	156 ± 5	135 ± 12
F4 HPMC	43 ± 5	n.a.
F5 Lycoat	27 ± 5	n.a.
F6 reference	28 ± 2	n.a.

n.a.: no available data. The method used was not suitable for this kind of film.

A simple dissolution study was conducted on all mixed-polymer films (F1-F3) (Table 6). After 15 min F1 had released half of the drug content, whereas F2 only 16% and F3 close to 65%. F1, containing only Soluplus® showed extreme variation between the samples tested (very high standard deviation). This can be explained by Soluplus® dispersing rapidly into micelles, and being influenced largely from the behaviour of the film during the test. It was observed that films that were stuck on the beaker showed a very different release rate of the micelles as compared to floating film that had access to water from two sides. For formulations with HPMC added (F2) a hydrogel scaffold was formed and a delayed dissolution occurred. Addition of Lycoat® (F3) gave a release that was similar to F1 in the beginning, but after some minutes the film fell completely apart and fast release was observed. The Lycoat® polymer did not retain the micelles in a hydrogel scaffold in the same way as the HPMC. On the contrary, Lycoat® mixed in between the graft co-polymer seemed to accelerate the hydration and disintegration rate, which corresponds with the fast dissolving properties of this polymer (Parissaux et al., 2007).

After finalising the dissolution test, all samples (solutions containing the released micelles) were also concurrently tested to estimated micelle size and polydispersity index (PDI). These results in Table 6 showed that the values corresponded well with the micelle size for Soluplus® micelles in aqueous solution (Alopaeus et al., 2019)

Table 6
Film dissolution expressed in% dissolved furosemide in solution and micelle size (z-average) and polydispersity index (PDI) (mean ± SD, $n = 3$).

Film formulation	F1 Soluplus			F2 Soluplus-HPMC			F3 Soluplus-Lycoat		
Sample time point (min)	Dissolved furosemide (%)	PDI	Z-average (nm)	Dissolved furosemide (%)	PDI	Z-average (nm)	Dissolved furosemide (%)	PDI	Z-average (nm)
5	8.94 ± 8.01	0.045 ± 0.019	65.36 ± 0.36	0.32 n.a.	0.102 ± 0.017	61.29 ± 0.22	9.49 ± 0.48	0.075 ± 0.02	63.02 ± 0.56
7	10.46 ± 7.43	0.036 ± 0.017	64.87 ± 0.19	1.62 n.a.	0.136 ± 0.010	62.40 ± 1.07	10.97 ± 2.77	0.033 ± 0.01	64.88 ± 0.57
10	31.22 ± 31.43	0.036 ± 0.009	62.98 ± 0.09	5.01 ± 4.45	0.075 ± 0.018	62.19 ± 0.26	55.30 ± 25.88	0.044 ± 0.023	62.44 ± 0.58
15	48.82 ± 19.42	0.023 ± 0.019	63.01 ± 0.31	16.26 ± 12.51	0.015 ± 0.008	62.14 ± 0.47	63.49 ± 20.38	0.031 ± 0.014	62.93 ± 0.56
30	102.40 ± 6.50	0.026 ± 0.008	62.57 ± 0.09	24.50 ± 7.21	0.022 ± 0.014	61.93 ± 0.54	78.42 ± 13.13	0.02 ± 0.005	63.02 ± 0.12

confirming that as the films dissolve the micelles do not disassemble in the process. The PDI and its standard deviation both had a trend of reducing for samples taken after longer time, indicating that micelles were becoming more homogeneous with a monophasic size distribution with increasing time. Only the formulation containing HPMC showed higher PDI in samples taken after short dissolution times, indicating that the HPMC might connect the micelles to a certain degree, even though the size was in the same size range. After 10 min also this formulation showed a size distribution below 0.1, suggesting that the HPMC was dissolved.

Based on the behaviour in the vapour sorption, wetting, swelling, erosion and disintegration studies it can be summarised that single Soluplus® films, have a more hydrophobic surface with high contact angle and lower vapour sorption isotherms as compared to mixed films of Soluplus® with either of the hydrophilic polymers HPMC or Lycoat®. The swelling of all Soluplus® films (F1-F3) was in the same order of magnitude, and much lower than the more hydrophilic reference films (F4-F6), however, the erosion and disintegration results revealed an interesting difference; Soluplus® films (when single polymer) disintegrated into micelles that after a while was dispersed into the liquid. The mixed Soluplus® films, on the other hand, had a supportive hydrogel network holding the Soluplus® micelles in an intact structure. For the Soluplus®-HPMC formulation, this structure was more stable and not as easily ruptured by mechanical stress as observed for the Soluplus®-Lycoat® (F3) formulation. These findings suggest that the mixed film of Soluplus® with HPMC (F2) have properties that are highly attractive for a buccal drug delivery system; Soluplus® micelles can be utilised as a solubiliser and nanocarrier, contained in the scaffold created by the hydrogel, formed from a relatively low content of HPMC (2.1% w/w of the dry film; Table 1).

3.5. Mucoadhesive capacity

Mucoadhesion was investigated in various *in vitro* assays. The simple mucin interaction studies were conducted with a Texture Analyser to observe the interaction of the various film samples with mucin dispersions. Fig. 7 shows the determined work of adhesion. The ratio between general and unspecific adhesion is the estimated mucin interaction. Formulations F3, F5 and F6 showed no mucin interaction, which can be seen from the insignificant difference between the general and the unspecific adhesion ($p^{>}0.05$). HPMC films (F4), Soluplus®-HPMC mixed films (F2) as well as Soluplus® alone films (F1) showed significantly higher general adhesion as compared to unspecific adhesion ($p < 0.05$), which indicates that these films showed high mucin interaction. However, for the Soluplus® containing film (F1), it was observed that the moistened film acted as a "glue" also towards the filter when moistened with buffer, without mucin, which contributed to a higher value for the unspecific adhesion and might be a confounder in the determined mucin interaction. This phenomenon is illustrated in Fig. 8, where the over-plasticising effect of the added liquid can be observed. For the mixed Soluplus® films (F2-F3), this phenomenon was less pronounced, but elevated unspecific binding was also observed also for these films. The similar behaviour was not seen for films that did not contain Soluplus®. In films with HPMC (F2 and F4), the general adhesion was clearly higher than the unspecific adhesion, suggesting that there is an interaction between the mucin and polymeric film. Generally, good wetting and swelling properties should correlate to good mucoadhesion but only up to a certain point; polymer swelling is essential for the exposure of the bioadhesive sites for hydrogen bonding to be able to happen (Peppas and Buri, 1985). However, there is a critical degree of hydration and too much can simply lead to disintegration of the polymer network. Film formulations F5 and F6 are made up of mostly fast disintegrating hydrophilic polymers, and as a result, the mucoadhesive capacity in these formulations is poor and the difference between specific and unspecific adhesion is not significant. For mucoadhesion to happen there has to be enough contact time at the interface of the polymer and the mucosal surface. Over-wetting and disintegration will only lead to a formation of a slippery surface without mucoadhesion (Smart, 2005). In the mixed film with Soluplus® and Lycoat® (F3), the effect of Lycoat® is dominant, the same effect can be observed here also.

More biorelevant information on the mucoadhesive capacity of the various Soluplus® films was obtained from the oral cavity model, where the drug retention from the film formulations was tested on a mucosal surface under constant rinsing, simulating salivation (Madsen et al., 2013). In our setup, mucus-producing cells, grown for 3 weeks, were used as the mucosal surface and PBS pH 7.4 used as simulated saliva. The flow rate of 1.4 mL/min was taken from literature (Dawes, 1996), and resembled an average of stimulated saliva flow in humans. Fig. 9 indicated that the combination film Soluplus® with HPMC as the mucoadhesive polymer (F2), showed the highest retention of the drug to the mucosal surface, especially for longer times. Films F1 and F3 showed similar profiles, and even though the variation was large between parallels for film formulation F1, both these formulations were washed off more rapidly than the Soluplus®-HPMC film (F2). Free drug, dissolved in PBS, showed no retention on the mucosal surface in the setup, and > 92% of the API was found rinsed off the mucosa 10 min after application (data not shown). The behaviour of the various films in the oral cavity model supports the suggested structural characteristics of the Soluplus® containing films; to avoid rinsing off the mucosa by salivation, Soluplus® micelles need to be contained in a hydrogel scaffold of a mucoadhesive polymer. To obtain buccal absorption of drug the prolonged contact time on the mucosa is an important parameter. Increased retention time on the buccal mucosa was achieved for the mixed HPMC film. HPMC was found to be more successful as a mucoadhesive scaffold, which might be related to the higher mucin interaction of this polymer as compared to Lycoat®, but also a contribution of the overall better wetting properties of HPMC recognised in the lower contact angle (Fig. 5) and higher hygroscopicity (Table 4) discussed above. As comparison, Roque et al. showed drug retention between 10–27% after 60 min in a similar oral cavity retention model, with buccal formulations based mainly on HPMC (Roque et al., 2018). Comparing this to approximately 70% drug retention after 120 min exhibited by the Soluplus®-HPMC films that were tested here, the advantage is evident. Importantly, the mucoadhesive capacity of the formulations was ranked in the same order by the simple mucin

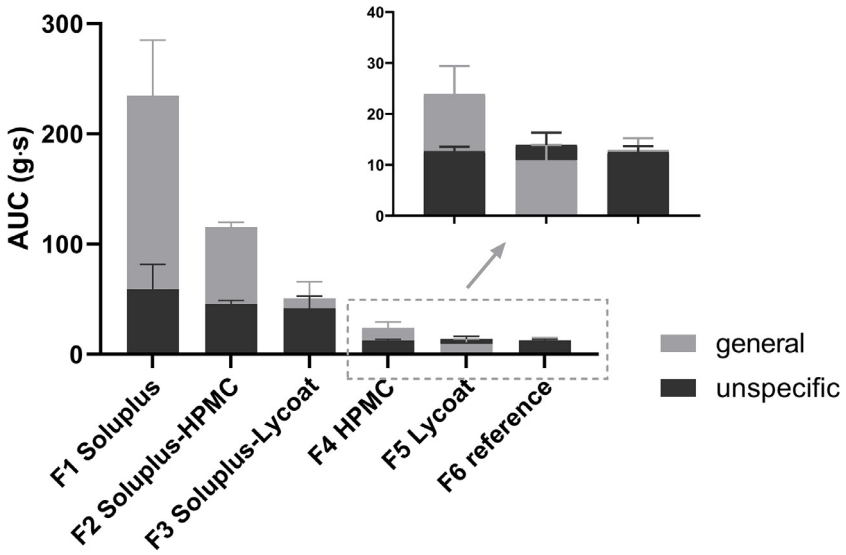

Fig. 7. Interactions with mucin and the film formulations, results for F4-F6 are magnified in the insert for clarity (mean ± SD, n = 10) (unspecific adhesion: no mucin interaction, general adhesion: with mucin interaction).

Fig. 8. Picture illustrating the over-plasticised effect of wetting a Soluplus® single polymer film.

interaction test and the retention to the mucosal surface in the oral cavity model.

3.6. Buccal transepithelial permeability of film-associated furosemide

Transepithelial permeability of the different Soluplus®-based film formulations (F1-3) was assessed in a TR146 buccal cell culture model used to mimic the buccal epithelia (Nielsen and Rassing, 2000). Transepithelial electrical resistance (TEER) was measured before and during the incubation with the formulations. The TEER values were around 270 Ω/cm^2 and did not decrease significantly during the measurement period or directly after (supplementary material).

The permeability of furosemide from the three Soluplus®-based formulations (F1-F3) was similar and no statistical differences were determined in the apparent permeability coefficients (P_{app}) or in the flux (J_{flux}) between the tested formulations (Table 7). The slight difference in% cumulative permeability of furosemide at earlier time points was due to the variations in film formulations F1-F3, (Fig. 10). It was following the same order as in the retention model, where film containing HPMC showed lower cumulative permeability in% than a film containing Soluplus® as single polymer, and a higher retention to the mucin surface in the retention model. This is likely due to the hydrogel scaffold structure of the film being retained for longer, as discussed earlier, which leads to furosemide releasing more slowly from

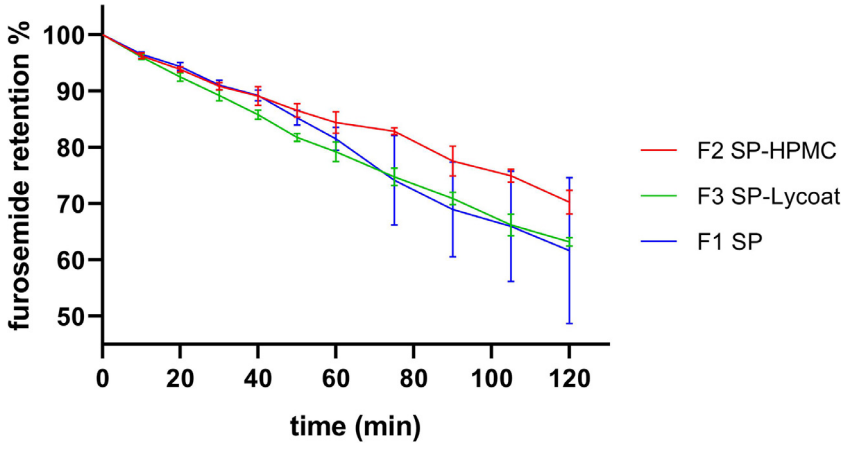

Fig. 9. Comparison of furosemide retention on the mucosal surface in films (F1-F3) presented in% (mean ± SD, n = 3).

Table 7
The calculated flux (J_{flux}) and apparent permeability constants (P_{app}) of furosemide from the three tested films (F1-F3) and free drug dissolved in HBSS (mean ± SD, $n = 3$).

Film formulation	J_{flux} (10^{-4} µm/cm^2•s^{-1})	P_{app} (10^{-6} cm/s)
F1 Soluplus	5.6 ± 0.1	0.9 ± 0.01
F2 Soluplus-HPMC	5.6 ± 0.8	0.9 ± 0.13
F3 Soluplus-Lycoat	7.0 ± 1.8	1.0 ± 0.27
Free drug	31 ± 1.6*	2.1 ± 0.10*

* Significantly different from the other tested formulations ($p = < 0.05$).

the film matrix. At later time points (60 min) the difference is no longer significant, and all film formulations have similar% cumulative permeability. The permeability of free furosemide dissolved in HBSS was considerately higher and faster than from the formulations. The higher flux obtained for the free drug was expected as the concentration of the drug was higher (> 2x higher than for the formulations), which creates a higher concentration gradient for passive diffusion across the membrane following Fick's first law of diffusion. The lower P_{app} values of the film formulations F1-F3, are likely caused by the slower release of furosemide from Soluplus® micelles (Alopaeus et al., 2019), before drug is in free solute form and transepithelial permeation can happen. Furthermore, it has been shown that solubilising lipophilic drugs may result in lowering permeability, where increasing apparent solubility will result in decreased membrane permeability (Beig et al., 2013; Dahan et al., 2016). The P_{app} values obtained for free drug across the buccal multilayer reflect those values found in literature for permeability across Caco-2 monolayers (Granero et al., 2010; Jung et al., 2006; Pade and Stavchansky, 1997), but were found to be somewhat higher in the buccal cells. This might be explained with that furosemide permeates across cell epithelia through both the paracellular as well as the transcellular route, governed mostly by the state of ionisation (i.e. pH of the surrounding liquid) (Pade and Stavchansky, 1997). The physiological pH of HBSS indicates a high degree of ionisation and in Caco-2 cells this has been shown to result in a roughly 50:50% distribution of permeation through paracellular and transcellular routes. When the permeation was pushed to favour the transcellular pathway the P_{app} value increased as well (Pade and Stavchansky, 1997). The TR146 buccal cells represent stratified epithelia and lack tight junctions; therefore for lipophilic compounds, transepithelial permeation (i.e. the transcellular path) is usually more favourable compared to paracellular passive diffusion (Smart, 2005). Furosemide was chosen as a model drug demonstrating poor solubility and poor permeability (BCS class IV) (Granero et al., 2010). It is a lipophilic small molecular drug with a pKa of 3.8 and a logP of approximately 2, and will permeate through the cell epithelia through passive diffusion when given the opportunity. Drugs are known to permeate along the route that provides the least hindrance of passage. This might explain why the permeability constant is slightly higher in buccal cells as compared to intestinal cell models. The fact that the drug from film formulations did not permeate as fast as the free drug in solutions is not necessarily a disadvantage, as this analysis did not account for the mucoadhesive properties of the formulations and to a lesser degree for disintegration kinetics. For buccal permeation, residence time is an important factor, and the amount of permeated drug will therefore be a sum of permeation kinetics and mucoadhesion capacity (Shojaei, 1998).

3.7. Overall discussion

Our working hypothesis was that a rapid disintegration of Soluplus® was desirable in order to release the micelles containing solubilised drug, thereby providing the drug in a form that produces a concentration gradient over the buccal epithelium ensuring passive diffusion over the barrier. At the same time, increased bioadhesion is necessary for the micelles to remain in close proximity to the epithelium for a prolonged period of time to increase the total amount of drug that permeates. Based on the results obtained in this study there is one formulation that stands out as the best choice, namely F2, the Soluplus®-HPMC combination formulation. The results from DVS and contact angle measurements indicated that these combination films are hygroscopic and would easily interact and attract moisture. The disintegration test (petri dish method) showed that the disintegration of F2 was complete after 4.5–5 min (Table 5), whereas without the hydrogel scaffold the Soluplus® film disintegrated more rapidly. The erosion test showed that the mass of F2 was still intact after 6 min (Table 5), and the oral cavity model suggested that the same formulation could remain on the mucosa for more than an hour under the simulated saliva flow employed (Fig. 9). Considered together these findings suggests that the mechanical stress as well as amount of accessible liquid will strongly influence the removal of the film from the mucosa. Nevertheless, the results showed that the hydrogel scaffold, contributed by the added HPMC, provide support as well as mucoadhesive properties. The dissolution test indicated that around 25% of the drug load would be released after 30 min (Table 7) and the permeability studies showed that available furosemide would permeate the buccal epithelium at a constant rate already from the first time point after 15 min (Fig. 10). Sufficient retention time to increase the permeability of the drug is difficult to estimate without doing *in vivo* studies. However, based on the various characteristics of the formulations in this study, it might be realistic to estimate retention on the buccal mucosa as somewhere between 10 min and 30 min, maximally 1 h. Naturally, the erosion and dissolution of the buccal formulation will be greatly influenced by picking of the tongue, drinking, stimulated salivation and so on. Therefore, the contribution of the swallowed drug fraction to the absorbed dose may be regarded as an advantage.

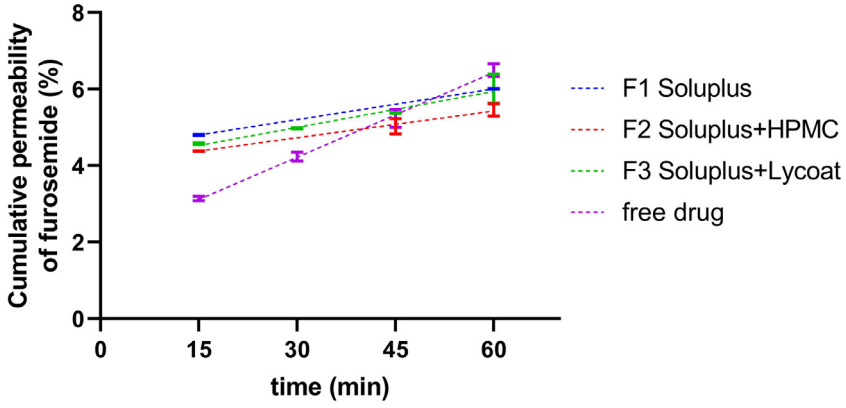

Fig. 10. The cumulative permeability of furosemide in three different film formulations (F1-F3) and free drug, represented in% permeated across the cell monolayer. Values represent mean ± SD, $n = 3$.

The concept as such is not restricted to the model drug used in these studies, but could work equally well for other poorly soluble, small molecular APIs, and also small biological drugs, such as peptides should be investigated. The concept may also be used for local treatment in the oral cavity.

Even though, *in vivo* studies would be required to obtain the final proof of the hypothesis that the mucin-retentive scaffold provide the mucoadhesion that result in increased permeability, the current results strongly suggest that the F2 formulation is promising in this respect. The logical next step in the product development is to test these systems in an animal model.

4. Conclusions

The novel buccal delivery system investigated in this study combines the solubilisation and nanocarrier capabilities of Soluplus®, with the mucoadhesive properties of an additive polymer, that allow prolonged contact with the mucosal surface, in a dry and user-friendly oral film. Mixed films of Soluplus® with HPMC or Lycoat®, disintegrated into a hydrogel structure where the micelles were maintained in a supportive scaffold of the added hydrophilic polymer. The combination film with Lycoat® dispersed upon agitation and did not show increased mucoadhesive properties, when tested towards a mucosal surface under constant simulated salivation. The combination film with HPMC, on the other hand, showed higher retention even after longer rinsing times. All Soluplus® containing films showed similar permeability in buccal TR146 epithelial cells and the permeability was superior to reported permeability in intestinal cell models. The combination film Soluplus®-HPMC showed the most promise as a novel buccal formulation, especially in terms of mucoadhesion properties and resistance to erosion, which resulted in increased drug retention on the mucosal surface.

Declaration of Competing Interest

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Julia F. Alopaeus: Conceptualization, Formal analysis, Visualization, Writing - original draft, Investigation. **Marie Hellfritzsch:** Writing - review & editing, Investigation. **Tobias Gutowski:** Writing - review & editing, Investigation. **Regina Scherließ:** Supervision, Writing - review & editing. **Andreia Almeida:** Writing - review & editing. **Bruno Sarmento:** Supervision, Writing - review & editing. **Nataša Škalko-Basnet:** Conceptualization, Supervision, Writing - review & editing. **Ingunn Tho:** Conceptualization, Writing - original draft, Supervision.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2019.105142.

References

Adrover, A., Varani, G., Paolicelli, P., Petralito, S., Di Muzio, L., Casadei, M., Tho, I., 2018. Experimental and modeling study of drug release from HPMC-based erodible oral thin films. Pharmaceutics 10, 222.

Akhtar, M.-J., Jacquot, M., Jamshidian, M., Imran, M., Arab-Tehrany, E., Desobry, S., 2013. Fabrication and physicochemical characterization of HPMC films with commercial plant extract: influence of light and film composition. Food Hydrocoll 31, 420–427.

Alopaeus, J.F., Hagesæther, E., Tho, I., 2019. Micellisation mechanism and behaviour of Soluplus®–furosemide micelles: preformulation studies of an oral nanocarrier-based system. Pharmaceuticals 12, 15.

Arya, A., Chandra, A., Sharma, V., Pathak, K., 2010. Fast dissolving oral films: an innovative drug delivery system and dosage form. Int. J. ChemTech. Res. 2, 576–583.

Aulton, M., Abdul-Razzak, M., Hogan, J., 1981. The mechanical properties of hydroxypropylmethylcellulose films derived from aqueous systems part 1: the influence of plasticisers. Drug. Dev. Ind. Pharm. 7, 649–668.

BASF, 2010. Soluplus-Technical Information. The BASF Chemical Company-Pharma ingredients and services, Limburgerhof, Germany.

Beig, A., Miller, J.M., Dahan, A., 2013. The interaction of nifedipine with selected cyclodextrins and the subsequent solubility–permeability trade-off. Eur. J. Pharm. Biopharm. 85, 1293–1299.

Borges, A.F., Silva, C., Coelho, J.F., Simões, S., 2015. Oral films: current status and future perspectives: i—galenical development and quality attributes. J. Controlled Release 206, 1–19.

Castro, P.M., Baptista, P., Madureira, A.R., Sarmento, B., Pintado, M.E., 2018. Combination of PLGA nanoparticles with mucoadhesive guar-gum films for buccal delivery of antihypertensive peptide. Int. J. Pharm. 547, 593–601.

Dahan, A., Beig, A., Lindley, D., Miller, J.M., 2016. The solubility–permeability interplay and oral drug formulation design: two heads are better than one. Adv. Drug. Deliv. Rev. 101, 99–107.

Dahlberg, C., Millqvist-Fureby, A., Schuleit, M., Furó, I., 2010. Polymer–drug interactions and wetting of solid dispersions. Eur. J. Pharm. Sci. 39, 125–133.

Dawes, C., 1996. Factors influencing salivary flow rate and composition. Saliva Oral Health 2, 27.

Di Cagno, M., Bibi, H.A., Bauer-Brandl, A., 2015. New biomimetic barrier Permeapad™ for efficient investigation of passive permeability of drugs. Eur. J. Pharm. Sci. 73, 29–34.

Diem, K., Lentner, C., 1970. Documenta geigy. Scientific tables 6. pp. 85–103.

Dixit, R., Puthli, S., 2009. Oral strip technology: overview and future potential. J. Controlled Release 139, 94–107.

Farris, S., Introzzi, L., Biagioni, P., Holz, T., Schiraldi, A., Piergiovanni, L., 2011. Wetting of biopolymer coatings: contact angle kinetics and image analysis investigation. Langmuir 27, 7563–7574.

FDA Guideline, 2019. FDA guidance orally disintegrating tablets. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/orally-disintegrating-tablets web page accessed on 26.05.2019.

Fonseca-Santos, B., Chorilli, M., 2018. An overview of polymeric dosage forms in buccal drug delivery: state of art, design of formulations and their in vivo performance evaluation. Mater. Sci. Eng. C 86, 129–143.

Granero, G., Longhi, M., Mora, M., Junginger, H., Midha, K., Shah, V., Stavchansky, S., Dressman, J., Barends, D., 2010. Biowaiver monographs for immediate release solid oral dosage forms: furosemide. J. Pharm. Sci. 99, 2544–2556.

Hagesaether, E., Hiorth, M., Sande, S.A., 2009. Mucoadhesion and drug permeability of free mixed films of pectin and chitosan: an in vitro and ex vivo study. Eur. J. Pharm. Biopharm. 71, 325–331.

Hardung, H., Djuric, D., Ali, S., 2010. Combining HME & solubilization: Soluplus®—the solid solution. Drug. Deliv. Technol. 10, 20–27.

Hiorth, M., Nilsen, S., Tho, I., 2014. Bioadhesive mini-tablets for vaginal drug delivery. Pharmaceutics 6, 494–511.

Hoffmann, E.M., Breitenbach, A., Breitkreutz, J., 2011. Advances in orodispersible films for drug delivery. Expert Opin. Drug. Deliv. 8, 299–316.

Jacobsen, J., van Deurs, B., Pedersen, M., Rassing, M.R., 1995. TR146 cells grown on filters as a model for human buccal epithelium: I. Morphology, growth, barrier properties, and permeability. Int. J. Pharm. 125, 165–184.

Joshi, S.C., 2011. Sol-gel behavior of hydroxypropyl methylcellulose (HPMC) in ionic media including drug release. Materials (Basel) 4, 1861–1905.

Jung, S.J., Choi, S.O., Um, S.Y., Kim, J.I., Choo, H.Y.P., Choi, S.Y., Chung, S.Y., 2006. Prediction of the permeability of drugs through study on quantitative structure–permeability relationship. J. Pharm. Biomed. Anal. 41, 469–475.

Krampe, R., Visser, J.C., Frijlink, H.W., Breitkreutz, J., Woerdenbag, H.J., Preis, M., 2016. Oromucosal film preparations: points to consider for patient centricity and

manufacturing processes. Expert Opin. Drug. Deliv. 13, 493–506.
Laffleur, F., 2014. Mucoadhesive polymers for buccal drug delivery. Drug. Dev. Ind. Pharm. 40, 591–598.
Lesuffleur, T., Barbat, A., Dussaulx, E., Zweibaum, A., 1990. Growth adaptation to methotrexate of HT-29 human colon carcinoma cells is associated with their ability to differentiate into columnar absorptive and mucus-secreting cells. Cancer Res. 50, 6334–6343.
Lesuffleur, T., Porchet, N., Aubert, J.-.P., Swallow, D., Gum, J.R., Kim, Y.S., Real, F.X., Zweibaum, A., 1993. Differential expression of the human mucin genes MUC1 to MUC5 in relation to growth and differentiation of different mucus-secreting HT-29 cell subpopulations. J. Cell. Sci. 106, 771–783.
Li, C.L., Martini, L.G., Ford, J.L., Roberts, M., 2005. The use of hypromellose in oral drug delivery. J. Pharm. Pharmacol. 57, 533–546.
Madsen, K.D., Sander, C., Baldursdottir, S., Pedersen, A.M.L., Jacobsen, J., 2013. Development of an ex vivo retention model simulating bioadhesion in the oral cavity using human saliva and physiologically relevant irrigation media. Int. J. Pharm. 448, 373–381.
Mönckedieck, M., Kamplade, J., Fakner, P., Urbanetz, N., Walzel, P., Steckel, H., Scherließ, R., 2017. Dry powder inhaler performance of spray dried mannitol with tailored surface morphologies as carrier and salbutamol sulphate. Int. J. Pharm. 524, 351–363.
Nagar, P., Chauhan, I., Yasir, M., 2011. Insights into polymers: film formers in mouth dissolving films. Drug Invent. Today 3 (12), 280–289.
Nielsen, H.M., Rassing, M.R., 2000. TR146 cells grown on filters as a model of human buccal epithelium: IV. Permeability of water, mannitol, testosterone and β-adrenoceptor antagonists. Comparison to human, monkey and porcine buccal mucosa. Int. J. Pharm. 194, 155–167.
Pade, V., Stavchansky, S., 1997. Estimation of the relative contribution of the transcellular and paracellular pathway to the transport of passively absorbed drugs in the Caco-2 cell culture model. Pharm. Res. 14, 1210–1215.
Parissaux, X., Joshi, A.A., Francois, A., Lefevre, P., 2007. Evaluation of a novel modified starch polymer in an easy to formulate thin-film drug delivery system and comparison with some marketed formulations. Young 1070, 323.
Pather, S.I., Rathbone, M.J., Şenel, S., 2008. Current status and the future of buccal drug delivery systems. Expert Opin. Drug. Deliv. 5, 531–542.
Peppas, N.A., Buri, P.A., 1985. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. J. Controlled Release 2, 257–275.
Preis, M., Gronkowsky, D., Grytzan, D., Breitkreutz, J., 2014a. Comparative study on novel test systems to determine disintegration time of orodispersible films. J. Pharm. Pharmacol. 66, 1102–1111.
Preis, M., Knop, K., Breitkreutz, J., 2014b. Mechanical strength test for orodispersible and buccal films. Int. J. Pharm. 461, 22–29.
Preis, M., Woertz, C., Kleinebudde, P., Breitkreutz, J., 2013. Oromucosal film preparations: classification and characterization methods. Expert Opin. Drug. Deliv. 10, 1303–1317.
Preis, M., Woertz, C., Schneider, K., Kukawka, J., Broscheit, J., Roewer, N., Breitkreutz, J., 2014c. Design and evaluation of bilayered buccal film preparations for local administration of lidocaine hydrochloride. Eur. J. Pharm. Biopharm. 86, 552–561.
Rathbone, M., Senel, S., Pather, I., 2015. Oral Mucosal Drug Delivery and Therapy. Springer.
Rathbone, M.J., Hadgraft, J., 1991. Absorption of drugs from the human oral cavity. Int. J. Pharm. 74, 9–24.
Ratiopharm, 2019. http://ratiopharm.fi/tuote/melatoniini-travel-ratiopharm-19-mg/, p. web page accessed on 30.04.2019.
Roque, L., Alopaeus, J., Reis, C., Rijo, P., Molpeceres, J., Hagesaether, E., Tho, I., Reis, C., 2018. Mucoadhesive assessment of different antifungal nanoformulations. Bioinspir Biomim 13, 055001.
Rupniak, H.T., Rowlatt, C., Lane, E.B., Steele, J.G., Trejdosiewicz, L.K., Laskiewicz, B., Povey, S., Hill, B.T., 1985. Characteristics of four new human cell lines derived from squamous cell carcinomas of the head and neck. J. Natl. Cancer Inst. 75, 621–635.
Semjonov, K., Salm, M., Lipiäinen, T., Kogermann, K., Lust, A., Laidmäe, I., Antikainen, O., Strachan, C.J., Ehlers, H., Yliruusi, J., 2018. Interdependence of particle properties and bulk powder behavior of indomethacin in quench-cooled molten two-phase solid dispersions. Int. J. Pharm. 541, 188–197.
Shojaei, A.H., 1998. Buccal mucosa as a route for systemic drug delivery: a review. J. Pharm. Pharm. Sci. 1, 15–30.
Smart, J.D., 2005. Buccal drug delivery. Expert. Opin. Drug. Deliv. 2, 507–517.
Speer, I., Steiner, D., Thabet, Y., Breitkreutz, J., Kwade, A., 2018. Comparative study on disintegration methods for oral film preparations. Eur. J. Pharm. Biopharm.
Tanida, S., Kurokawa, T., Sato, H., Kadota, K., Tozuka, Y., 2016. Evaluation of the micellization mechanism of an amphipathic graft copolymer with enhanced solubility of ipriflavone. Chem. Pharm. Bull. 64, 68–72.
Taylor, M., Tomlins, P., Sahota, T., 2017. Thermoresponsive gels. Gels 3, 4.
Timur, S.S., Yüksel, S., Akca, G., Şenel, S., 2019. Localized drug delivery with mono and bilayered mucoadhesive films and wafers for oral mucosal infections. Int. J. Pharm. 559, 102–112.
World Health Organization, et al. WHO model list of essential medicines for children: 6th list, March 2017. 2017.
Zulfakar, M.H., Goh, J.Y., Rehman, K., 2016. Development and mechanical characterization of eugenol–cetalkonium chloride sustained release mucoadhesive oral film. Polym. Compos. 37, 3200–3209.