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Functional physico-chemical, *ex vivo* permeation and cell viability characterization of omeprazole loaded buccal films for paediatric drug delivery

Sajjad Khan, Vivek Trivedi, Joshua Boateng*

Department of Pharmaceutical, Chemical and Environmental Science, Faculty of Engineering and Science, University of Greenwich at Medway, Central Avenue, Chatham Maritime, ME4 4TB, Kent, UK

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ABSTRACT

Buccal films were prepared from aqueous and ethanolic Metolose gels using the solvent casting approach (40 °C). The hydration (PBS and simulated saliva), mucoadhesion, physical stability (20 °C, 40 °C), *in vitro* drug (omeprazole) dissolution (PBS and simulated saliva), *ex vivo* permeation (pig buccal mucosa) in the presence of simulated saliva, *ex vivo* bioadhesion and cell viability using MTT of films were investigated. Hydration and mucoadhesion results showed that swelling capacity and adhesion was higher in the presence of PBS than simulated saliva (SS) due to differences in ionic strength. Omeprazole was more stable at 20 °C than 40 °C whilst omeprazole release reached a plateau within 1 h and faster in PBS than in SS. Fitting release data to kinetic models showed that Korsmeyer–Peppas equation best fit the dissolution data. Drug release in PBS was best described by zero order via non-Fickian diffusion but followed super case II transport in SS attributed to drug diffusion and polymer erosion. The amount of omeprazole permeating over 2 h was 275 ug/cm^2 whilst the formulations and starting materials showed cell viability values greater than 95%, confirming their safety for potential use in paediatric buccal delivery.

1. Introduction

The development of age appropriate formulations for paediatric populations is of current topical interest and buccal films have been proposed as suitable alternatives to traditional dosage forms such as tablets and liquids (Lui et al., 2014; Khan et al., 2015). An ideal and effective buccal dosage form is required to possess certain functional properties including bioadhesion (mucoadhesion), hydration and swelling upon imbibing saliva, drug release from the swollen gel and eventual permeation through the buccal membrane (Boateng et al., 2014).

Formulations prepared using mucoadhesive polymers have gained significant interest because of the well-established advantages including prolonging the residence time of the dosage form at the site of application (Tiloo et al., 2011). The process of mucoadhesion involves wetting and swelling of polymer, interpenetration between the polymer chains and the mucosal membrane and formation of chemical bonds between the entangled chains and mucin (Palacio et al., 2012). There are several approaches used to assess the mucoadhesive performance of polymeric dosage forms including texture analyser (Thirawong et al., 2007; Ayensu et al., 2012), rheometry (Tamburic and Craig, 1997) and chemometrics (Boateng et al., 2015). The texture analyser technique (TA) assesses the stickiness, the total work of adhesion (TWA) and the cohesiveness of the dosage forms. Stickiness is described as the maximum force (peak adhesive force—PAF) required to separate the probe attached to the formulation from the mucosal substrate whereas, the total amount of work exerted in detaching the probe from the mucosal substrate is referred to as work of adhesion and is calculated from the area under the force versus distance curve. Cohesiveness is defined as the intermolecular attraction between the mucosal substrate and formulation, and determined by the travel distance in mm on the force versus distance plot (Thirawong et al., 2007).

Hydration (swelling) is the process that occurs when hydrophilic polymers spread over the surface of a mucosal membrane in order to produce direct contact with the membrane. Hydration and eventual swelling occurs because the individual component chains situated within the polymer network have an affinity for water and this forms an important stage in mucoadhesion as well as affecting

* Corresponding author. Fax: +44 208 331 9805.
E-mail addresses: J.S.Boateng@gre.ac.uk, joshboat40@gmail.com, joshboat@hotmail.com (J. Boateng).

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other functional characteristics such as drug release (Boateng and Ayensu, 2014).

Drug release is affected by several factors such as physico-chemical properties of the drug, dissolution environment, structural characteristics of the polymeric system and the possible interactions between these factors as described by Fu and Kao (2010). In the case of swelling controlled drug release systems such as polymer films, a drug is molecularly dispersed within the formulation matrix. Penetration of water (or dissolution medium such as saliva) into the polymer matrix causes the formulation to swell to form a gel and drug diffusion through the swollen polymer matrix is the main driving force controlling the release of drug from the system (Longer and Robison, 1986). However, to understand the mechanism of drug release, various mathematical models are used to study and evaluate the overall kinetics of drug release from polymeric dosage forms such as films (Dash et al., 2010).

The main barrier to a drug intended for systemic activity following release from a given buccal formulation, is the buccal mucosa and epithelial membrane, which the drug must cross to reach the systemic circulation. Various *in vivo and ex vivo* models for investigating drug permeation through the buccal mucosa have been reported for different animals such as hamster (Eggerth et al., 1987), rabbit (Nair and Chien, 1993; Dowty et al., 1992), dog (Galey et al., 1976), pig (Chen et al., 1999; Artusi et al., 2003; Sandri et al., 2004) and sheep (Giovino et al., 2013; Boateng and Ayensu, 2014). However, the buccal epithelium of rodents such as hamsters is thick and keratinised and the surface area is small (Shojaei, 1998), which limits the extent of drug permeation. Though the dog's buccal mucosa is non keratinised and similar to human buccal epithelium, it is expensive for routine use in *in vivo* permeation experiments (Shojaei, 1998), whilst their use as the most common household pet, makes their availability for *ex vivo* experiments, very expensive.

However, pig buccal mucosa is also non-keratinised and closest to human tissue in terms of structure and permeability (Franz-Montan et al., 2015). It is smooth and intact and consists of stratified, squamous epithelium supported on a connective-tissue layer (Squier and Kremer, 2001). In addition, its low cost for *in vivo* studies and ready availability in local butcheries for *ex vivo* experiments, makes the porcine buccal mucosa an ideal model for drug permeation studies. Various studies have reported on permeation through pig buccal mucosa for different drugs including fentanyl citrate (Del Consuelo et al., 2005), beta blockers (Amores et al., 2014), propofol (Tsagogiorgas et al., 2013) and galantamine (De Caro et al., 2008).

In addition to the above functional characteristics, buccal formulations for paediatric patients are required to be non-toxic, for example, they should not irritate or cause permanent damage to the buccal mucosa membrane, with continuous application (Liu et al., 2014). Cell viability assays are used for drug screening and cytotoxicity tests for chemicals, and pharmaceutical formulations. Specific cell cultures can be used to screen for toxicity by estimation of the basal function of the cell and such testing using specialised cells have proven most useful when the *in vivo* toxicity of a chemical is already well established (Ekwall et al., 1990).

Omeprazole (OME) is an effective short-term treatment for gastric and duodenal ulcers and used in combination with antibiotics for eradication of *Helicobacter pylori* (Stroyer et al., 2006). An initial short course of OME is the treatment of choice in gastro-oesophageal reflux disease with severe symptoms; children with endoscopically confirmed erosive, ulcerative, or stricturing (narrowing or tightening) of oesophagus (Fass et al., 1998). OME is effective in the treatment of Zollinger–Ellison syndrome and is used to reduce the degradation of pancreatic enzyme supplements in children with cystic fibrosis (Nishioka et al., 1999). In aqueous solution its stability is entirely dependent on the initial pH and in acidic and neutral conditions, it is rapidly degraded. To prevent degradation of the drug in the acid medium of the stomach, the drug is formulated as enteric-coated granules in capsule form (Lind et al., 1983). Although OME is well absorbed from the gastrointestinal tract, its oral bioavailability in humans is about 40–50% suggesting pronounced first pass metabolism for this drug. This makes OME a good candidate for buccal drug delivery where it can avoid both first pass metabolism and gastric acid degradation and was therefore chosen as the model drug in this study.

Metolose (MET) is a non-ionic cellulose ether comprising methylcellulose and three substitution types of HPMC each available in several grades with varying viscosities. Key properties of MET include solubility in cold water, formation of transparent solutions and forming reversible gels during heating due to its viscoelastic properties, with the formed gel maintaining its shape during the heating. MET can produce transparent films by casting from their gel solutions (Roy et al., 2009).

In this study, the functional characteristics (swelling, mucoadhesion and stability) of optimised films prepared using metolose (MET), intended for paediatric drug delivery, have been investigated. Further, *in vitro* drug dissolution properties (and release mechanisms), the *ex vivo* permeation of omeprazole (OME) released from the MET films across pig buccal tissue, *in vitro* bio-adhesion of the films on the buccal membrane and cell toxicity using MTT assay have been characterised.

2. Materials and methods

2.1. Materials

Metolose (MET) was obtained from Shin Etsu (Stevenage, Hertfordshire, UK), polyethylene glycol (PEG 400), L-arginine (L-arg), gelatine and mucin from bovine submaxillary gland, Type I-S, Krebs–Ringer bicarbonate buffer, thiazolyl blue tetrazolium bromide, MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich (Gillingham, UK). Omeprazole (OME) was obtained from TCI (Tokyo, Japan). Ethanol, potassium di-hydrogen phosphate, sodium hydroxide, sodium chloride, sodium phosphate di-basic were all obtained from Fisher Scientific (UK). Dulbecco's Modified Eagles Medium (DMEM), foetal bovine serum (FBS), penicillin, streptomycin and glutamine were all obtained from Gibco (Paisley, UK).

Table 1
Composition of the ethanolic (20% v/v) gels (weight in 50 mL of solvent) used to prepare the film formulations. (* This formulation was brittle and therefore not used for further tests except in MTT assay).

Polymer (MET) (g)	Drug (OME) (g)	L-arginine (L-arg) (g)	Plasticiser (PEG 400) (g)	formulation code	Description
0.5	0.0	0.0	0.0	BLK-MET film*	Unplasticised
0.5	0.0	0.0	0.5	BLK-MET film	Plasticised
0.5	0.1	0.2	0.0	DL-MET film	Unplasticised
0.5	0.1	0.2	0.5	DL-MET-PEG film	Plasticised