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

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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 presence of simulated saliva, ex vivo bioadhesion and cell viability using MTT of drug loaded (DL) 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 hour 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 hours was 275ug/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.

Key Words: Permeation, Cell toxicity, Omeprazole, Metolose, Buccal films, Pediatric.

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 other functional characteristics such as drug release. (Boateng & 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 (Langer & 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 & Chien, 1993; Dowty et al., 1992), dog (Galey et al., 1976), pig (Chen et al., 1999; Artusia et al., 2003; Sandri et al., 2004) and sheep (Giovino et al., 2013, Boateng & 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-Motan et al., 2015). It is smooth and intact and consists of stratified, squamous, epithelium supported on a connective-tissue layer (Squier & 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. The porcine oesophageal mucosa is smooth and intact and consists of stratified, squamous, non-keratinised epithelium supported on a connective-tissue layer (Squier & Kremer., 2001). 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 to 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).

2.2 Formulation development

The films were prepared from metolose (MET) gels containing omeprazole (OME) (0.2% w/w), L-arginine (L-arg) (0.4% w/w) with or without PEG 400 (0 and 0.5% w/w). The formulations prepared are summarised in table 1. Blank films were prepared by dissolving the required amount of PEG in 20% v/v ethanol to yield 0.00 or 0.5% w/w PEG solution and heated to 40°C). The required amount of MET (0.5g) was added to 50mL PEG solution to yield 1% w/w MET gel. For the DL films, OME (0.1g) and L-arg (0.2g) as stabiliser were added to 50mL of 20% v/v ethanol to yield homogeneous OME-L-arg solution. The required weight (0.5g) of polymer to achieve 1% w/v gel was added to the OME, L-arg solution (22°C) and continuously stirred till complete hydration and then heated to 40°C. Based on the total weight of polymers, different amounts of PEG were added to obtain different concentrations (0.00%, 0.50%) in the final gels prepared. Stirring was continued for 30 min to achieve a homogeneous dispersion and the gels were left to stand overnight to remove entrapped air bubbles. 20 g was poured into Petri dishes (86 mm diameter) and dried at 40°C to obtain the final films. The films obtained

were visually examined and it was observed that the unplasticised films were brittle and difficult to cut into strips for further testing and therefore all subsequent tests were performed using only the plasticised films (with the exception of MTT assay).

2.3 Hydration (swelling) capacities

The hydration (swelling) capacities of the plasticised blank (BLK) and drug loaded (DL) films with the model drug (OME) and stabiliser (L-arg) were determined in two different media [(0.01M PBS solution (pH 6.8 ± 0.1) and simulated saliva (SS) (pH 6.8 ± 0.1)] and both set at a temperature of 37 ± 0.1°C. The buffer solution was prepared by dissolving 6.80g of potassium dihydrogen phosphate in 1L of deionised water and adjusting the pH to 6.8 using sodium hydroxide. SS solution was prepared by dissolving potassium dihydrogen phosphate (0.19g), sodium chloride (8.00g) and sodium phosphate dibasic (2.38g) in 1L of deionised water and adjusting the pH to 6.8 using phosphoric acid. The films were cut into 2 × 2 cm^2 strips and placed into small Petri-dishes containing 10mL of the media (PBS or SS) and initially weighed. It should be noted that the DL films used were similar to samples used for drug dissolution studies (below) with drug loading determined to ensure homogeneity. At predetermined time intervals (5 minutes) the liquid media was removed using a syringe, excess media blotted off with tissue paper and weighed again. After the weight of hydrated film had been recorded, 10mL of fresh medium (PBS or SS) was placed back in the Petri dish using a syringe and the process continued. These studies were performed in triplicate (n = 3) for each set of formulated samples and average values were calculated for data analyses. The % swelling capacity (swelling index) was calculated using the equation below:

$$Swelling\ Index(\%) = \frac{Ws-W}{W} \times 100 \qquad \text{Equation 1}$$

Where Ws is the weight of the film before hydration and Ws the initial weight of the film after hydration.

200

2.4 In vitro mucoadhesion using gelatin gel substrate with texture analyser

The in vitro mucoadhesion experiments were performed on BLK and DL films with a TA HD plus Texture Analyser (Stable Micro Systems, Surrey, UK) fitted with a 5kg load cell. The film was attached to an adhesive rig probe (75mm diameter) with the help of double sided adhesive
205 tape. An 88mm diameter Petri dish was filled with 20g of gelatine solution (6.67% w/w) and allowed to set as a solid gel and the surface of the gel was equilibrated with 0.5 ml of SS (pH 6.8) or PBS (pH 6.8) and both used to represent the buccal mucosa (Boateng et al., 2014). The film was positioned in contact with the equilibrated gelatine gel for 60 seconds to provide optimal contact and then detached to determine adhesive properties using the following
210 settings: pre-test speed 0.5mm/s; test speed 0.5 mm/s; post-test speed 1.0mm/s; applied force 1N; contact time 60.0s; trigger type auto; trigger force 0.05N and return distance of 10.0mm. Texture Exponent 32 software was used to record and process the data. The stickiness or peak adhesive force (PAF) required to separate the film from the mucosal surface, total work of adhesion (TWA) and cohesiveness of the samples were determined from the force distance
215 plots.

2.5 Drug stability

OME stability of DL MET films were determined using two different storage conditions according to ICH (2003) guidelines. Samples were placed in humidity controlled desiccators
220 (using activated silica gel) and placed in an oven (40°C) and at room temperature (ambient) and the stability studied over 3 months. The films were wrapped with aluminium foil due to the light sensitivity of OME. An Agilent1200 HPLC equipped with auto sampler (Agilent Technology, Cheshire, UK) with Chemstation® software program was used to determine the amount of drug present in the films after storing under the two sets of conditions.

For HPLC analysis, the samples stored under the various stability conditions above, were weighed (5mg) and dissolved using 0.01M PBS solution (pH 6.8 ± 0.1) in volumetric flasks (10mL). The 5mg strips were cut from a given section of each film prior to stability testing for each time point 1mL aliquots from each flask was sampled and placed into HPLC vials for analysis. The stationary phase used for analysis was a Hypersil™ ODS C18 reversed phased column, 5µm particle size (250 x 4.6 mm) (Thermo Scientific, Hampshire UK). The mobile phase consisted of a mixture of ammonium acetate and acetonitrile in the ratio of 60:40 v/v and a flow rate of 2mL/min and diode array UV detector wavelength for OME was set at 302nm. 20µL volumes were injected during each run, respectively. The concentration of OME in each film sample was calculated from the linearized calibration curve ($R^2 > 0.99$).

2.6 In vitro drug dissolution studies

2.6.1 In vitro release of OME using Franz-type diffusion cell

The release profiles of drug from films prepared from gels containing 20% v/v ethanol, 0.5% w/w PEG 400, OME and L-arg (OME:L-arg ratio was 1:2) were determined. The ratio of 1:2 for OME:L-arg was chosen because it was previously determined to be the optimum concentration of the amino acid to prevent OME degradation whilst maintaining appropriate tensile properties (Khan et al., 2015). Before the dissolution studies, the content (assay) and uniformity of OME within the film was determined. This was measured by weighing the film accurately to 5mg (n = 3) and hydrated in 8mL each of two dissolution media (0.01M PBS pH 6.8 and SS pH 6.8 at 37°C). The hydrated film was gently stirred at 37 ± 0.5°C until completely dissolved. The concentration of OME was analysed using HPLC as described in section 2.5 above.

In vitro drug dissolution studies were carried out using Franz-type diffusion cells. 5mg of optimised (plasticised) DL MET film were placed in the donor compartment on stainless

steel wire mesh (0.5 mm x 0.5 mm) which separated the donor and receiver compartments. The wire mesh acted both as a support for the film whilst allowing contact with the dissolution medium (Boateng et al., 2009). The mucoadhesive surface was in contact with the wire mesh and facing the receiver compartment of the Franz diffusion cell (Cui et al., 2008). Each receiver compartment was filled separately with 8mL of 0.01M PBS pH 6.8 or SS pH 6.8 with magnetic stirring at a speed of 250 rev/min. The two compartments were held together by a cell clamp and sealed with parafilm, in order to limit evaporation and the temperature of the diffusion cell was maintained at 37 ± 0.5°C and stirred throughout the experiment. 1mL of the dissolution medium was sampled at predetermined time intervals and replaced with the same amount of fresh medium to maintain a constant volume for 2 hours. The sampled dissolution medium was measured at 302nm using HPLC. The concentration of OME released from the film was determined by interpolation from the linearized calibration curve ($R^2 > 0.99$) and cumulative percentage drug release profiles plotted against time in minutes.

2.6.2 Drug release mechanisms

Based on the drug dissolution data, four kinetic models with their corresponding relationships were constructed as shown in Table 2. The mathematical equations corresponding to the kinetic models are shown in equations 2 to 5 below.

Zero order

$$Q_t = Q_0 + k_0 t \qquad \text{Equation 2}$$

Where

Q_0 = the initial amount of drugs

Q_t = cumulative amount of drug release time t

K_0 = zero order rate constant

t = time in minutes

First order

$$\text{Log } Q_t = \text{Log } Q_0 + kt/2.303 \qquad \text{Equation 3}$$

Where

Q_0 = initial amount of drugs

Q_t = cumulative amount of drug release in time t

K_1 = first order rate constant

t = time in minutes

Higuchi

$$Q = K_H t^{½} \qquad \text{Equation 4}$$

Q = cumulative amount of drug release in time (t)

K_H = Higuchi release rate constant

t = time in minutes

Korsmeyer-Peppas

$$F = (M_t/M) = K_p t^n \qquad \text{Equation 5}$$

F = fraction of drug release in time (t)

M_t = Amount of drug released at time (t)

M = total amount of drug in dosage from

K_p = release rate constant

n = diffusion or release exponent

t = time in minutes

'n' is estimated from linear regression of log (M_t/M) versus log t.

2.6.3 Comparison of release profiles

Release parameters from the dissolution profiles for variables under investigation (PBS and SS media) were used to characterise the drug release data and compare the results in PBS and SS.

The parameters used were $t_{x\%}$, and sampling time. The $t_{x\%}$ corresponds to the time necessary for the release of a determined percentage of drug (e.g., $t_{20\%}$, $t_{50\%}$, $t_{80\%}$). Sampling time corresponds to the amount of drug dissolved in that time (e.g., $t_{20min,}$). In this study, the time to release 20% of the drug originally loaded ($t_{20\%}$) and the percentage cumulative release at 60 minutes (t_{60min}) were used to compare dissolution profiles in PBS and SS (Costa et al., 2001).

2.7 Tissue preparation

Buccal tissues (cheek) from pigs were obtained from a local slaughterhouse (Tunbridge Wells, Kent, UK). After removal, the tissues were immediately transferred into cold Krebs buffer (pH 6.8) modified with sodium carbonate, placed in sealed ice box filled with dry ice and immediately transported to the laboratory. The buccal mucosa, with part of sub mucosa, was carefully separated from the fat and muscles using a sharp scalpel and the epithelium isolated from the underlying tissue. The thickness of the sample was approximately 500µm and the buccal mucosa was used within 2 hours (Patel et al., 2012).

2.8 Ex vivo buccal permeation studies

The mucosal membrane prepared above was washed with SS at 37°C. The obtained buccal mucosa membrane was mounted between the donor and receiver compartments of the Franz-type diffusion cell, with the epithelial side facing the donor compartment to allow contact with the film (Attia et al., 2004). The receiver chamber was filled with 8mL of SS at 37°C and uniform mixing was provided by magnetic stirring at 250 rev/min. After an equilibration period of 30 minutes, 0.5mL SS was placed in the donor compartment and 5mg of the OME loaded film was placed in the donor chamber with the mucoadhesion layer in contact with the epithelial surface. The compartments were held together by a cell clamp accessory and sealed with parafilm to limit evaporation. At predetermined time intervals, aliquots (1mL) were withdrawn

from the sampling port of the receiver compartment and replaced with the same amount of SS to maintain a constant volume for 2 hours. The sampled aliquots were analysed using HPLC as described above (n=3) and the % cumulative permeation plotted against time.

2.9 Ex-vivo mucoadhesion using porcine buccal tissue with texture analyser

The ex vivo mucoadhesion experiments were performed on DL MET films (n = 3) to estimate the effect of PBS and SS on adhesion of the films on porcine buccal tissue. The samples were tested using a TA HD plus Texture Analyser (Stable Micro Systems, Surrey, UK) fitted with a 5kg load cell following the procedure described in section 2.4 above. The peak adhesive force (PAF), TWA and cohesiveness of the sample were determined from the force-distance plots.

2.10 MTT assay

MTT assay on Vero cells was used to evaluate the cytotoxicity of pure MET, pure L-arg, pure OME, optimised BLK and DL films. Vero cells (ATCC®CCL-81™) are adherent cells derived from the kidney of the African Green monkey (Cercopithecus aethiops) and are one of the commonly used mammalian cell lines in cell, microbiology and molecular biology (Ammerman et al., 2008). These cells were obtained from cell and tissue culture labs within the Faculty of Engineering and Science, (Richardson Lab, University of Greenwich, Medway) and stored at -80°C. The cells (Vero, $1x10^4$cells/well) were used to seed a sterile, flat-bottomed 96 well tissue culture plate with Dulbecco's modified eagles medium (DMEM), Foetal bovine serum (FBS) 10% (v/v), penicillin (100units/mL), streptomycin (100µg/mL) and glutamine 0.292mg/mL. Two cultures (treated and control) were kept under sterile conditions in a laminar hood) and incubated at 37°C in 5 % (v/v) CO_2 for 24 hours.

After 24 hours, the cells (except those in the control wells) were exposed to either pure MET, pure L-arg, pure OME, blank (BLK) or DL films in cell culture medium)] and incubated

for 68 hours. This was used to replace the existing media covering the cells after the designated incubation period. MTT stock solution (5mg/mL) was prepared by dissolving 250mg of MTT reagent in 50mL of PBS (1x) buffer, and sterilized by passing it through a 0.22μm filter (Corning®, Germany). 10μL (equivalent to 50μg) of the MTT stock solution was added to each well and the plate incubated for a further 4 hours using the same incubator conditions as above bringing the total incubation time to 72 hours. The contents of the plate were decanted and 100μL of DMSO was added to each well, incubated at room temperature for 30 minutes and the absorbance read on a Multi-scan EX Micro-plate photometer (Thermo Scientific, Essex, UK) at optical density (OD) of 540nm. Data obtained was expressed as percentage cell viability (mean ± standard error of the mean) for all the samples tested.

2.11 Statistical analysis

Statistical data analysis was performed to compare hydration and mucoadhesion results using two tailed student t-test with 95 % confidence interval (p-value < 0.05) as the minimal level of significance. All the results were performed in triplicates for all experiments with mean and standard deviation.

3. RESULTS AND DISCUSSION

3.1 Hydration (swelling) capacity

Figure 1 shows the swelling profiles of BLK (Figure 1a) and DL (Figure 1b) MET films in PBS and SS with the DL films both showing a linear swelling versus time profiles whilst the BLK films were less linear. The swelling index in SS pH 6.8 was significantly ($p < 0.05$) lower at each time point compared to PBS at pH 6.8 for all the films (both BLK and DL). This may be due to the higher ionic strength of the SS media which plays an important role in affecting the swelling of MET films due to its effect on the osmotic gradient with ions and

the polymer chains competing for diffusing water molecules. The calculated ionic strengths for PBS and SS were 0.275M and 0.286M respectively. Though the difference appears small, the SS contained many more types of ions (K^+, H^+, PO_4^{3-}, Na^+ and Cl^-), than PBS (K^+ and PO_4^{3-}) and therefore more likelihood of osmotic competition for the water molecules. The effect of ionic strength and pH on the swelling of polymers has been described by Peh and Wong, (1999) and the results obtained in the current study for PBS and SS showed similarities to their reported results. Generally, the BLK films showed higher swelling index compared to the DL films in the corresponding respective media (PBS and SS). This suggests that the presence of drug slowed down the swelling of the films.

3.2 In vitro mucoadhesion on gelatine gel

The mucoadhesive data for the plasticised BLK and DL films obtained from equilibrating gelatine with the two different media (PBS and SS) simulating the buccal mucosa are shown in figure 2. The texture analysis results when the gelatine was equilibrated with SS at pH 6.8 showed lower stickiness (PAF), WOA and cohesiveness compared to when gelatine was equilibrated with PBS and the differences were statistically significant ($p < 0.05$). Once again this may be due to the difference in ionic strength of the media affecting the hydration of the films as observed during the swelling study. In general, the initial stages of mucoadhesion involves the hydration of the polymer which is essential to allow the polymer chains to diffuse into the chains of the mucosal substrate to allow formation of physical entanglement between the chains and establishing of the adhesive forces between the two interacting surfaces (Bodupalli et al., 2010).

Hydration and presence of salts have been reported among factors that affect the mucoadhesion of polymer based systems for mucosal applications (Salamat-Miller et al., 2005; Asane et al., 2008; Roy et al., 2009). A slower rate of hydration and swelling of the

film, in SS due to presence of extra salts, will therefore slow down the formation of such physical entanglements between the polymer chains and the chains of the gelatine, with resultant decrease in adhesive properties, whilst the reverse was true for gelatine equilibrated with PBS. However, since the SS is more representative of the buccal environment, the mucoadhesive results using this media is a better indication of the formulation performance following buccal administration. Further, the presence of drug slightly increased the mucoadhesive values (PAF, TWA and cohesiveness), however, this effect did not appear significant.

3.3 Drug stability

Short-term stability studies were performed for DL MET film obtained from ethanolic (20% v/v) gels containing OME: L-arg 1:2, 0.5% w/w PEG 400, and exposed to two temperature conditions 40ºC (± 0.5ºC) and 20ºC ± 0.5ºC) (ICH 2003 guidelines) for a period of three months and the results are shown in figure 3. The results of the stability study reveal that there was statistically significant ($p < 0.05$) difference in the drug loss (%) between the films kept in the oven (40ºC) and ambient conditions. After 14 days, the percentage of OME remaining at 40ºC and room temperature were 87% and 80% respectively and whilst the percentage remaining after 28 days was 82% and 62% respectively. This might relate to the lower humidity at the higher temperature of 40ºC given that OME is highly sensitive to moist conditions due to easy hydrolysis. However, the % drug content after 28 days at room temperature remained constant at 62% up to 84 days whilst the % content decreased to 47% at 40 ºC, implying that the accelerated temperature conditions had a greater effect after 4 weeks, and speeded up the degradation of the drug in the longer term.

These findings suggest that overall, films are relatively more stable at room temperature conditions over 3 months, though the % loss after 3 months was still quite high in terms of long

term storage. Iuna and Bojita (2010) investigated the degradation kinetics of OME at 25ºC and 40.8ºC, confirming the degradation followed zero order kinetics, which was faster at higher temperature and relative humidity whilst relatively more stable at 25ºC. The most likely implication is that such films in addition to protection from light, will need to be stored in airtight blisters or peel packs to avoid contact with atmospheric moisture and must be stored at ambient temperatures. This however, requires further investigation to confirm such proposition.

3.4 In vitro drug dissolution

3.4.1 Drug release profiles

The dissolution profile for DL MET films in PBS solution is shown in figure 4. During the early stage of dissolution, percentage cumulative release versus time plot showed an almost linear fit with 55.8 % release within the first 15 minutes and 66.15% after 30 minutes, after which the % release was fairly constant. The release data show that the presence of L-arg may have the ability to enhance drug solubility; therefore, this facilitates the process of hydration as previously reported (Figueiras et al, 2010). This hydration occurs via water penetration through the process of diffusion and eventual dissolution of the drug within the film matrix. After 60 minutes the release was observed to be sustained and might represent complete swelling of the hydrated polymer.

The dissolution profile for DL MET films containing OME were also determined using SS to more accurately mimic the environment within the oral cavity in terms of both pH and ionic strength and shown in figure 4. During the initial stages of dissolution, the % release into SS was observed to be lower with 1.08% released in the first 15 minutes after which it gradually increased to 18.21% and 21.77% at 45 and 60 minutes respectively, and then the release remained largely steady till 120 minutes.

The films appeared to show more sustained release in the SS dissolution media. The release of OME from the MET films could be attributed to the swelling of the polymeric network and releasing the drug progressively into the dissolution medium. The results show that drug release rates were faster in PBS and the L-arg had little solubilizing effect on the in SS due to difference in osmotic pressure and ionic strength as SS contains more salts (sodium, chloride and potassium) than PBS. This also confirmed the hydration (swelling data) suggesting that the ionic strength significantly affects water penetration into the film matrix, drug dissolution and subsequent drug release into the dissolution medium. The results further suggest that it is important to simulate as closely as possible, the natural environment within which a drug is expected to be administered during in vitro experiments to allow more accurate in vivo correlations.

Overall, drug release in both media seems to hit a plateau after about 60 minutes without achieving 100% release of the drug and this may suggest possible interactions between formulation components. In our previous publication (Khan et al., 2015) we showed that the drug which was added originally in crystalline form was present in amorphous form or molecularly dispersed within the film matrix. However, the dissolution profiles seem to correlate with the swelling profiles suggesting that most of the release occurring in the first hour occurs via drug diffusion through the swollen gel. For buccal administration, drug release is usually measured over only 2 hours to simulate real life application since patients cannot hold a dosage form in the cheek for longer than 2 hours without dislodging by tongue and teeth movements.

3.4.2 Evaluation of drug release mechanisms

The dissolution data were fitted to different kinetic equations to explain the release kinetics of OME from the buccal films. The release parameters obtained from fitting experimental

dissolution release data to the different kinetic equation evaluated has been summarised in table 3 (Shoaib et al., 2006). Interpretation of the data was based on the value of the resulting regression coefficient. The release kinetics of OME in PBS and in SS both followed Korsmeyer-Peppas model as the R^2 values were the highest compared to other models. The 'n' values from the Korsmeyer-Peppas equation, describe the diffusion state or release exponent used for elucidation of the drug release mechanism. The 'n' value is estimated from linear regression of log (M_t/M) versus log t plot. Analysis of the experimental data using kinetic equations and interpretations of the release exponents (n) gives a better understanding of the controlling release mechanism. OME release in PBS showed an 'n' value of 0.2 which is less than 0.45 indicating that the drug release mechanism followed non-Fickian diffusion. However, for SS an 'n' value of 2.2 was obtained which is greater than 0.89 therefore follows super case II transport mechanism of drug release. This indicates controlled drug release with zero-order kinetics attributed to the erosion of the polymeric chain matrix.

3.4.3 Comparison of release profiles

The time to 20% release ($t_{20\%}$) of initial amount of OME present, for each media can be seen in table 4. The results showed that in SS media, 20% of drug was released in 50 minutes whereas in PBS media 20% was released in 5.5 minutes indicating a statistically significant ($p < 0.05$) difference. The t_{60min} in PBS and SS was 70% and 22% respectively which was again statistically significant ($p = 0.05$). These results are attributed to the swelling of the DL films within the two media due to difference ionic strength as SS contains more salts (sodium chloride and sulphate) than PBS as noted previously.

3.5 Ex vivo studies with pig buccal tissue

This study investigated the ex vivo permeation of OME released from the DL films and ex vivo mucoadhesion properties of these films using a pig buccal tissue which resembles human buccal mucosa in structure and permeability (Werts, 1991) as well as cell viability of the DL films.

3.5.1 Ex vivo buccal permeation studies

Maintaining buccal tissue integrity and viability during isolation and storage before experimental testing is crucial to obtaining reliable permeation results (Patel et al., 2012). The storage of porcine buccal mucosa in Krebs bicarbonate Ringer solution helped to maintain its integrity whiles storage in other solutions such as PBS at 4°C for 24 hours has been reported to result in the loss of epithelial integrity (Kulkarni et al., 2010).

Before a buccal drug delivery system can be optimised, the permeation characteristics must be investigated to determine the feasibility of this route of administration for the delivery of the drug (OME) of interest. The permeability profile of OME released from the DL MET films in the presence of L-arg is shown in figure 5. There was a lag period of about 20 minutes and then near linearity was observed up to 60 minutes of permeation which followed a first order kinetic mechanism. The total cumulative amount of OME permeating over 2 hrs was 275ug/cm^2. This suggests that porcine buccal membrane is generally quite permeable to OME and also confirms that the OME is released from the MET films. However, there are generally other factors which affect the extent of permeability in vivo. One barrier to drug permeability in the presence of SS for example, is the enzymatic degradation as it contains moderate level of esterases, phosphatases and amylases. Further, due to poor physical contact with the pig buccal epithelial surface and/or possibly absence or very minimal volume of mucosal fluid (mucin) on the membrane, no significant covalent interaction could be established and this could affect the extent of permeation. This is possible as the membrane was initially washed in

physiological fluid during preparation before mounting on the donor compartment of the Franz-type diffusion cell.

3.5.2 Ex-vivo mucoadhesion evaluation using porcine buccal tissue with texture analyser

Films are considered one of the most suitable dosage forms for buccal administration in children (Nunn and Williams, 2005). Figure 6 illustrates a comparison of the adhesive properties of the films on three model mucosal surfaces i.e. gelatine surface equilibrated with PBS, and gelatine surface with equilibrated with SS and porcine buccal epithelium. SS equilibrated gelatine showed the lowest adhesive (PAF, TWA) values compared to PBS equilibrated gelatine and porcine epithelium while the lowest cohesiveness was obtained on the porcine tissue. Because the mucous was washed with Krebs buffer, the polymer adheres directly to the tissue surface rather than to mucous which will have made it travel a shorter distance before detachment. However, the film on porcine buccal tissue had the highest TWA which indicates the possibility of strong hydrogen bond interaction between the entangled polymer chains of the hydrated films (due to the presence of hydrogen bond forming groups such as OH and COOH) and the buccal mucosa membrane.

3.6 MTT assay

Tissue viability was assessed using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) cytotoxicity study for pure OME, L-arg, BLK film (MET, EtOH 20% v/v), BLK film (MET, PEG 400 (0.5% w/w, EtOH 20% v/v), and DL film (MET, PEG 400 (0.5% w/w, EtOH 20% v/v, and OME: L-arg 1:2). This is a reduction assay where yellow MTT was reduced to purple formazan primarily by the action of enzymes which are located inside the mitochondria of the viable cells (Koschier et al., 2011). Figure 7 displays the respective cell viability data for the samples described above when exposed to Vero cells as measured by MTT

assay. The results show a clear profile of the cytotoxity of the pure materials and DL films on adherent mammalian cells (Vero cells) with almost 100% cell viability confirming that the pure drug (OME), starting materials and the drug released from the DL films were non-toxic and can be employed for paediatric drug delivery. There are several factors involved in determining the successful and safe application of polymers as drugs carriers in humans, with toxicity being an important factor. Kendall (2003) revealed that OME is generally regarded as a non-toxic drug however, none of the literature have shown a clear profile on the complete absence of toxicity of OME on endothelial cells. Therefore this study confirms that OME poses no physical threats to endothelial cells when used as a drug carrier for potential buccal application in paediatric patients.

4. CONCLUSION

The functional properties of OME loaded MET buccal films for paediatric delivery, have been characterised. The stability studies suggested that films remained more stable at room temperature (ambient ± 0.5ºC) conditions when compared to 40ºC, over a three month period though the drug content in both cases was lower than the initial drug loaded and therefore require specialised packaging to avoid contact with moisture and should be protected from light. The swelling, mucoadhesion and in vitro drug release in the presence of PBS was higher than when SS was present, attributed to the higher ionic strength of the SS which affected the initial rate of polymer hydration. Though most of the literature simulates the buccal environment on the basis of the pH alone, our results suggest that this is not sufficient and the ionic environment within which drug release and permeation occurs needs to be replicated as closely as possible.

The Korsmeyer-Peppas equation best fit the dissolution data in both PBS and SS media. The drug release in PBS followed zero order release kinetics via non-Fickian diffusion whilst

release in SS followed super case II transport, attributed to both drug diffusion and polymer erosion. The films showed enough drug permeability after release of OME which is expected to ensure therapeutic bioavailability and therefore a potentially useful alternative to oral administration via the GI tract. The ex vivo adhesive properties suggest it will provide a long enough residence time in the cheek to allow drug permeation, while MTT assay showed that all the starting materials and the films were relatively safe for continuous attachment in the cheek region, suitable for pediatric patients.

Figure Legends

Figure 1 Swelling profiles of plasticised MET DL films cast from ethanolic (20% /v/v) gels containing 0.5% w/w PEG 400 and OME: L-Arg 1:2 in two different media [(PBS and SS at pH 6.8)] (mean ± SD, (n=3) and MET BLK films cast from ethanolic gels containing 0.5% w/w PEG 400 in SS at pH 6.8 (mean ± SD, (n=3)**.**

Figure 2 In-vitro mucoadhesion properties (PAF, TWA and cohesiveness) of plasticised BLK and DL MET film cast from ethanolic (20% v/v) gels containing OME:L-arg 1:2 using mucosal substrate equilibrated with PBS and SS (pH 6.8) (mean ± SD, (n=3)).

Figure 3 Plots showing the % OME content for MET DL film during storage at oven temperature 40ºC (± 0.5ºC) and room temperature (ambient ± 0.5ºC) up to three months (mean ± SD, (n=3)).

Figure 4 Drug dissolution profile of MET DL films prepared from ethanolic (20% v/v) gel containing 0.5% w/w PEG 400 and OME: L-Arg 1:2 ratio in PBS at pH 6.8 and SS pH 6.8 (mean ± SD, (n=3)).

Figure 5 Cumulative permeation curve of OME released from MET film through pig buccal tissue (mean ± SD, (n=3)).

Figure 6 In-vitro mucoadhesion measurements of MET DL film obtained from ethanolic (20% v/v) gels containing 0.5% w/w PEG 400, OME: L-Arg 1:2 ratio and in PBS pH 6.8, SS pH 6.8 (gelatine surface) and epithelium of porcine buccal tissue (mean ± SD, (n=3)).

Figure 7 MTT assay results, showing cell viability showing pure OME, L-arg, BLK and DL films (mean ± SD, (n=3)). (BF = blank film)

REFERENCES

Ammerman, N.C., Beier-sexton, M. Azad, A.F. 2008. Growth and maintenance of vero cell lines. Curr Protoc Microbiol. DOI: 10.1002/9780471729259.mca04es11.

Amores, S., Lauroba, J., Calpena, A., Colom, H., Gimeno, A., Domenech, J. 2014. A comparative ex vivo drug permeation study of beta-blockers through porcine buccal mucosa. Int J Pharm. 468(1–2), 50–54.

Artusia, M, S. P. 2003. Buccal delivery of thiocolchicoside: in vitro and in vivo permeation studies. Int J of Pharm. 250(1), 203-213.

Asane, G.S., Nirmal, S.A., Rasal, K.B., Naik, A.A., Mahadik, M.S. 2008 Polymers for mucoadhesive drug delivery system: a current status, Drug Dev Ind Pharm. 34, 1246–1266.

Attia, M.A., El-Gibaly, I., Shaltout, S.E., Fetih, G.N. 2004. Transbuccal permeation, anti-inflammatory activity and clinical efficacy of piroxicam formulated in different gels. Int J Pharm. 276(1-2),11-28.

Ayensu, I., Mitchell, J. C. and Boateng, J. S. 2012. In vitro characterisation of chitosan based xerogels for potential buccal delivery of proteins, Carb Polym. 89(3), 935-941.

Boateng, J.S. Ayensu, I. 2014. Preparation and characterisation of laminated thiolated chitosan-based freeze-dried wafers for potential buccal delivery of macromolecules. Drug Dev Ind Pharm. 40(5), 611-618.

Boateng, J.S., Mitchell, J.C., Pawar, H.V., Ayensu, I. 2014. Functional characterisation and permeation studies of lyophilized thiolated chitosan xerogels for buccal delivery of insulin. Pept Prot Lett. 21(11) 1163-1175.

Boateng, J.S., Matthews, K.H., Auffret, A.D., Humphrey, M.J., Eccleston, G.M., Stevens, H.N.E. 2012. Comparison of the in vitro release characteristics of mucosal freeze-dried wafers and solvent cast films containing an insoluble drug. Drug Dev Ind Pharm. 38(1), 47-54.

Boateng, J.S., Pawar, H.V., Tetteh J. 2015. Evaluation of in vitro wound adhesion characteristics of composite film and wafer based dressings using texture analysis and FTIR spectroscopy: A chemometrics factor analysis approach. RSC Adv. 5, 107064-107075.

Boddupalli, B. M., Mohammed, Z. N. K., Nath, R. A. and Banji, D. 2010. Mucoadhesive drug delivery system: An overview. J Adv Pharm Techno Res. 1(4), 381.

Chen, L.L., Chetty, D.J., Chien, Y.W. 1999. A mechanistic analysis to characterize oramucosal permeation properties. Int J Pharm. 184(1), 63-72.

Cotsa, P., Lobo, J.M.S. 2001. Modelling and comparison of dissolution profiles. Eur J Pharm Sci. 3(2)123-133.

Cui, F., He, C., He, M., Cui, T., Yin, L., Qian, F., Yin, C. 2008. Preparation and evaluation of chitosan-ethylenediaminetetraacetic acid hydrogel films for the mucoadhesive transbuccal delivery of insulin. J Biomed Mater Res A. 89 (4), 1064-1071.

Dash, S., Murthy, P. N., Nath, L., Chowdhury, P. 2010. Kinetic modelling on drug release from controlled drug delivery systems. Acta Pol Pharm. 67(3) 217 – 223.

De Caro, V., Giandalia, G., Siragusa, M.G., Paderni, C., Campisi, G., Giannola, L.I. 2008. Evaluation of galantamine transbuccal absorption by reconstituted human oral epithelium and porcine tissue as buccal mucosa models: Part I. Eur J Pharm Biopharm. 70 (3), 869–873.

Del Consuelo, D.I., Falson, F., Guy, R.H., Jacques, Y. 2005. Transport of fentanyl through pig buccal and esophageal epithelia in vitro: influence of concentration and vehicle pH. Pharm Res. 22(9)1525-1529.

Dowty, M.E., Knuth, K.E., Irons, B.K., Robinson, J.R. (1992) Transport of thyrotropin releasing hormone in rabbit buccal mucosa in vitro. Pharm Res. 1113-1122.

Eggerth, R.M., Rashidbaigi, Z.A., M. M. (1987). Evaluation of hamster cheek pouch as a model for buccal absorption. Proceed Int Symp Control Rel Bioact Mater. 180-181.

Ekwall, B., Silano, V., Stammati, P., Zucco, F. (1990) Toxicity test with mammalian cell cultures In Short-term Toxicity test for non-genotoxic effects, Ed Bourdeau P et al., chapter 7, pp75-93, John Wiley and Sons Ltd, US.

Fass, R.1., Fennerty, M.B., Ofman, J.J., Gralnek, I.M, Johnson, C., Camargo, E., Sampliner, R.E., 1998. The Clinical and Economic Value of a Short Course of Omeprazole in Patients With Noncardiac Chest Pain. Gastroenterol. 115(1) 42-49.

Figueiras A, Sarraguça J, Pais A, Carvalho R, and Veiga F. 2010. The Role of L-arginine in Inclusion Complexes of Omeprazole with cyclodextrins. AAPS PharmSciTech. 11(1) 233-240.

Franz-Montan, M., Serpe, L., Martinelli, C.C., da Silva, C.B., Santos, C.P., Novaes, P.D., Volpato, M.C., de Paula, E., Lopez, R.F., Groppo, F.C. 2015. Evaluation of different pig oral mucosa sites as permeability barrier models for drug permeation studies. Eur J Pharm Sci. (In Press) doi:10.1016/j.ejps.2015.09.021.

Fu, Y., Kao, W.J. 2010. Drug release Kinetics and transport mechanisms of non-degradable polymeric delivery system. Expert Opin Drug Deliv. 7(4) 429-444.

Galey, W.R., Lonsdale, H.K., Nacht, S. 1976. The in vitro permeability of skin and buccal mucosa to selected drugs and tritiated water, J. Invest. Demat. 67(6), 713-717.

Giovino, C., Ayensu, I., Tetteh, J., Boateng, J.S. 2013. An integrated buccal delivery system combining chitosan films impregnated with peptide loaded PEG-b-PLA nanoparticles. Coll Surf B: Biointerf. 112, 9-15.

International Conference on Harmonization. 2003. Guidelines on stability conditions.

Iuga, C., Bojita, M. 2010. Stability study of omeprazole. Famacia, 52(2), 203 – 210.

Kendall, M.J. 2003. Review article: esomeprazole the first proton pump inhibitor to be developed as an isomer. Alment Pharmacol Ther. 1, 1-4.

Khan, S., Boateng, J.S., Mitchell, J., Trivedi, V. 2015. Formulation, Characterisation and Stabilisation of Buccal Films for Paediatric Drug Delivery of Omeprazole. AAPS PharmSciTech DOI 10.1208/s12249-014-0268-7.

Koschier, F., Kostrubsky, V., Toole, C., Gallo, M.A. 2011. In vitro effect of ethanol and mouthrinse on permeability in an oral buccal mucosal tissue contruct. Food Chem. 49(10) 2524-2529.

Kulkarni, U., Mahalingam, R., Pather, I., Li, X., Jasti, B. 2010. Porcine buccal mucosa as in vitro model: effect of biological and experimental variables. J Pharm Sci. 99(3), 1265-1277.

Lind, T, Cederberg, C, Ekenved, G, Haglund, U., Olbe, L. 1983. Effect of omeprazole-a gastric proton pump inhibitor-on pentagastrin stimulated acid secretion in man. Gut. 24,270-276.

Longer M, Robison J. 1986. Fundamental aspects of bioadhesion, Pharm. Int. 7, 114-117.

Lui, F., Ranmal, S., Batchelor, H.K., Orlu-Gul, M., Ernest, T.B., Thomas, I.W., Flanagan, T., Tuleu, C. 2014. Patient-Centred Pharmaceutical Design to Improve Acceptability of Medicines: Similarities and Differences in Paediatric and Geriatric Populations. Drugs. 74(16), 1871–1889.

Nair, M., Chien, Y.W. 1993. Buccal delivery of progestational steroids: l. Characterization of barrier properties and effect of penetrant hydrophilicity. Int J Pharm. 89(1), 41-49.

Nishioka, K., Nagao, T., Urushidani, T., 1999. Correlation between acid secretion and proton pump activity during inhibition by the proton pump inhibitors omeprazole and pantoprazole. Biochem Pharmacol. 58, 1349-1359.

Nunn, T., Williams, J. 2005. Formulation of medicines for children. Br J Clin Pharmacol. 59(6), 674-676.

Palacio, M. L., Schricker, S. R., Bhushan, B. 2012. Bio-adhesion of various proteins on random, diblock and triblock copolymers surface and the effect of pH condition. J R Soc Interf. 8(58), 630-640.

Patel, V.F., Liu, F., Brown, M.B. 2012. Modelling the oral cavity: In vitro and in vivo evaluation of buccal delivery systems. J control release. 161(3), 746-756.

Peh, K.K., Wong, C.F. 1999. Polymer films as vehicle for buccal delivery: Swelling, Mechanical, and Bio-adhesive properties. J Pharm Pharma Sci. 2(2)53-61.

Roy, S., Pal, K., Anis, A., Pramanik, K., Prabhakar, B. 2009. Polymers in mucoadhesive drug-delivery systems: A Brief Note', Design Monom Polym. 12(6), 483-495.

Salamat-Miller, N., Chittchang, M., Johnston, T.P. 2005. The use of mucoadhesive polymers in buccal drug delivery, Adv Drug Deliv Rev. 57, 1666–1691.

Sandri, G., Rossi, S., Ferrari, F., Bonferoni, M.C., Muzzarelli, C., Caramella, C. 2004. Assessment of chitosan derivatives as buccal and vaginal penetration enhancers. Eur J Pharm Sci. 21(2-3), 351-359.

Shoaib, M, H., Tazeen, J., Merchant, H. A. and Yousuf, R. I. 2006. Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC, Pak J Pharma Sci. 19(2), 119-24.

Shojaei, A.H. 1998. Buccal mucosa as a route for systemic drug delivery: a review. J Pharm Sci, 1(1), 15-30.

Squier, C.A., Kremer, M. 2001. Biology of oral mucosa and esophagus. J Natl Cancer Inst Monogr. (29), 7-15.

Stroyer, A., McGinity, J., Leopold, C., 2006. Solid State Interactions between the Proton Pump Inhibitor Omeprazole and Various Enteric Coating Polymers. J. Pharm Sci. 95(6), 1343-1353.

Tamburic, S., Craig, D.Q.M. 1997. A comparison of different in vitro methods for measuring mucoadhesive performance. Eur J Pharm Biopharm. 44, 159 – 167.

Thirawong, N., Nunthanid, J., Puttipipatkhachorn, S. & Sriamornsak, P. 2007. Mucoadhesive properties of various pectins on gastrointestinal mucosa: An in vitro evaluation using texture analyzer. Eur J Pharm Biopharm. 67:(1), 132-140.

Tiloo, S.K., Rasala, T.M., Kale, V.V. 2011. Mucoadhesive Microparticulate Drug Delivery System. Int J Pharm Rev Res , 9(1), 52.

Tsagogiorgas, C., Theisinger, S., Holm, P., Thiel, M., Quintel, M., Holm, R. 2013. Buccal absorption of propofol when dosed in 1-perfluorobutylpentane to anaesthetised and conscious Wistar rats and Göttingen mini-pigs. Eur J Pharm Biopharm. 85: (3), 1310–1316.