In vitro, ex vivo and in vivo evaluation of taste masked low dose acetylsalicylic acid loaded composite wafers as platforms for buccal administration in geriatric patients with dysphagia

Smirna Farias[1], Joshua S. Boateng[*1]

School of Science, Faculty of Engineering and Science, University of Greenwich at Medway, Chatham Maritime, Kent, UK, ME4 4TB

**Correspondence: j.s.boateng@gre.ac.uk, joshboat40@gmail.com (Dr Joshua Boateng)*

Abstract

This study reports the development and characterization of taste masked, freeze-dried composite wafers for potential oral and buccal delivery of low dose aspirin (acetylsalicylic acid) to prevent thrombosis in elderly patients with dysphagia. The wafers were formulated by combining metolose (MET) with carrageenan (CAR), MET with chitosan (CS) at low molecular weight or CAR with CS using 45 % v/v ethanol as solvent for complete solubilization of acetylsalicylic acid. Each wafer contained 75 mg of acetylsalicylic acid and sweetener (sucralose, stevia or aspartame) with a drug: sweetener ratio of 1:1 w/w. The formulations were characterized for physical properties using texture analyzer (hardness and mucoadhesion), scanning electron microscopy (SEM), X-ray diffractometry (XRD), Fourier transform infrared (FTIR) spectroscopy, swelling capacity, and *in vitro* drug dissolution. Further, permeation studies with three different models (Permeapad™ artificial barrier, EpiOral™ and porcine buccal mucosa) using HPLC, cell viability using MTT assay and *in vivo* taste masking evaluation using human volunteers were undertaken. The sweeteners increased the hardness and adhesion of the wafers, XRD showed the crystalline nature of the samples attributed to acetylsalicylic acid, SEM confirmed a compacted polymer matrix due to recrystallized acetylsalicylic acid and sweeteners dispersed over the surface. Drug dissolution studies showed that acetylsalicylic acid was rapidly released in the first 20 minutes and then continuously over 1 hour. EpiOral™ had a higher cumulative permeation than porcine buccal tissue and Permeapad™ artificial barrier, while MTT assay using Vero cells (ATCC® CCL-81) showed that the acetylsalicylic acid loaded formulations were non-toxic. *In vivo* taste masking study showed the ability of sucralose and aspartame to mask the bitter taste of acetylsalicylic acid and confirm that acetylsalicylic acid loaded MET:CAR, CAR:CS and MET:CS composite wafers containing sucralose or aspartame have potential for buccal delivery of acetylsalicylic acid in geriatric patients with dysphagia.

Key words*: Acetylsalicylic acid; aspartame; buccal mucosa; drug permeation; geriatric patients; sucralose; taste masking*

1. Introduction

The buccal region of the oral mucosa cavity offers an attractive route of administration for systemic drug delivery. The oral cavity is highly acceptable by patient as the mucosa is relatively permeable with a rich blood supply, it is robust and shows short recovery times after stress or damage (Rathabone & Hadgraft , 1991). In addition, the oral mucosa route bypasses first pass metabolism by delivering the drug directly into the bloodstream. These factors make the oral mucosa a very attractive and feasible site for systemic drug delivery (Shojaei, 1998). Further, there has been an increased interest in novel drug delivery systems, over the past few decades, to improve safety, efficacy and patient compliance and increase the product patent life cycle (Panda, et al., 2012). Fast dissolving and sustained release lyophilized wafers and films are examples of formulations for oral and buccal mucosa drug delivery and can be used for various classes of drugs (Peh & Wong , 1999).

Acetylsalicylic acid (commonly referred to as aspirin) has anti-thrombin action, which inhibits clot formation, thus reducing the rate of heart attacks and strokes. Such administration for the purpose of reducing the clotting action of platelets, is referred to as 'low-dose aspirin' (usually administered as a single tablet with 75mg of the drug). Acetylsalicylic acid acts as an acetylating agent and causes an irreversible inhibition of cyclooxygenase (COX)-1 which is an essential enzyme for the production of thromboxane A_2 (TxA_2) in the platelets and supresses the generation of prostaglandin H_2, which is a precursor of TxA_2. TxA_2 is a powerful stimulant of platelet aggregation and use of acetylsalicylic acid inactivates these platelets (Hovens, et al., 2006). Advantages of acetylsalicylic acid over other 'blood thinners' such as warfarin, include low cost, once-daily administration and no need for dose monitoring (Mekaj, et al., 2015).

Low dose acetylsalicylic acid is recommended for people with heart or vascular disease and patients who have had heart bypass surgery (British Medical Association, 2014) and most people who suffer from these problems are older (geriatric) patients, who usually also present with other chronic conditions. The impact of demographic ageing is likely to be of major significance in the coming decades, due to low birth rates and higher life expectancy. Older people generally require more prescribed medicines due to the presence of multiple conditions such as dysphagia (difficulty in swallowing). This occurs when the swallowing physiology changes with advancing age due to reduction in the muscle mass and connective tissue elasticity, resulting in the loss of strength and motion. These changes

reduce the effective and efficient flow of materials, such as food and medications through the upper aero digestive tract (Sura, et al., 2012).

Freeze-dried wafers are usually prepared by freeze-drying a polymeric solution or gel in an appropriate solvent (usually water). Freeze drying of water-soluble polymers produces shaped materials of highly porous nature that can be turned back to gels and solutions when they come into contact with fluids such as saliva. Lyophilized wafers can easily be applied to mucosa surfaces and they offer advantages over solid polymer gels and solvent cast films (Boateng, et al., 2010). Semi solid polymer gels flow easily after application, while wafers can maintain their swollen gel structure for a longer period and therefore longer residence times (Matthews, et al., 2005) to allow for effective drug absorption.

Freeze dried wafers are preferred over chewable acetylsalicylic acid tablets because the latter contains sorbitol which causes diarrhoea and flatulence. In addition the flavouring agents present in chewable tablets may cause ulcers in the oral cavity and the prolonged chewing may cause pain in the facial muscles which may increase the risk of poor adherence, medication errors or reduced patient quality of life. This is because of a loss of muscle strength in the mouth and throat regions, which makes it difficult for geriatric patients to chew. Further, these chewable tablets also show fragile (poor mechanical strength) and effervescent granular characteristics and therefore careful handling is required (Renu, et al., 2015; (Farias & Boateng., 2018)).

This paper reports the formulation design and development of composite polymer based lyophilized wafers, taste masked with sweeteners, for potential buccal delivery of acetylsalicylic acid to geriatric patients and improved compliance from masking the bitter taste of the drug. The formulations were initially characterized for their physico-chemical properties (resistance to compression – 'hardness' and mucoadhesion), crystallinity, internal and surface morphology and chemical interactions. Drug dissolution and permeation studies using three different models (EpiOral^{TM}, porcine buccal tissue and Permeapad^{TM} an artificial buccal membrane) were performed for the optimized acetylsalicylic acid loaded and taste masked wafers using HPLC and finally the *in vivo* taste masking of acetylsalicylic acid by sucralose and aspartame was investigated using healthy adult human volunteers.

2. Materials and methods

2.1 Materials

Metolose (MET) grade type (60SH), viscosity (4000 cP) and MW (1261.4 g/mol) was obtained as a gift from Shin Etsu (Stevenage, Hertfordshire, UK), gelatin from porcine skin, MW (10000 g/mol), mucin from bovine submaxillary glands, MW (4000 kDa), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide] reagent, dimethyl sulfoxide (DMSO) and acetyl salicylic acid were purchased from Sigma-Aldrich (Gillingham, UK). Kappa carrageenan [(CAR) (low viscosity grade NF 911, MW < 100,000Da, 25% sulfate esters, stable at pH values > 3.8)] was obtained as a gift from IMCD Ltd (Sutton, UK), low molecular weight chitosan (CS) with 95% degree of deacetylation and MW of 3000Da was purchased from Qingdao Yuda Century Economy and Trade CO, Ltd (China), calcium chloride, sodium chloride, sodium phosphate dibasic, magnesium chloride hexahydrate, potassium carbonate hemihydrate and sodium phosphate monobasic monohydrate were purchased from Fisher Scientific (Loughborough, UK). Sucralose, stevia and aspartame were obtained from a local ASDA Supermarket (London, UK). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin and glutamine were all obtained from Gibco (Paisley, UK). Pig cheeks were obtained from a local slaughterhouse (Tunbridge Wells, Kent, UK). Permeapad™ was a gift from InnoME GmbH (Espelkamp, Germany) and EpiOral™ ORL-200 buccal tissue kit was purchased from MatTek Corporation (Ashland MA, USA).

2.2 Formulation optimization

The drug loaded (DL) wafers were prepared by freeze-drying solutions combining MET with CAR and MET with CS in different weight ratios with each final wafer containing 75 mg of acetylsalicylic acid as previously reported (Farias & Boateng, 2018) and subsequently taste masked using different ratios of sucralose (Suc), aspartame (Asp) and stevia (Stev) as summarized in (Table 1). Then 1 g was poured into each well of a 24 multi-well plate (diameter 15.5 mm) with 75 mg of acetylsalicylic acid per well. The freeze-drying process was conducted using an automated lyophilization cycle on a Virtis Advantage XL 70 freeze-dryer (Biopharma Process Systems, Winchester, UK). In the freezing step the samples were frozen to produce a required condition for low temperature drying (Nireesha, 2013). The sample was cooled from room temperature to 5 ℃ for 40 minutes, 5 ℃ to -10 ℃ for 40 minutes, -10 ℃ to -55 ℃ for 120 minutes. An annealing process was integrated into the

freezing cycle to boost pore size distribution by increasing the temperature from -55 ℃ to -35 ℃ (2 hours), cooled back to – 55 ℃ (3 hours) and maintained for 1 hour, at a pressure of 200mTorr to assure uniformity. For the primary drying phase, the pressure was reduced to 50 mTorr, and temperature was increased from -55 ℃ to -20 ℃ (8 hours) and further increased from -20 ℃ to -15 ℃ (10 hours). The secondary drying occurred at the same pressure as primary drying with temperature raised from -15 ℃ to 25 ℃ over 12 hours 30 minutes to remove the amount of water molecules that remained during primary drying (Okeke & Boateng, 2016).

142 *Table 1. Polymeric solutions for preparing taste masked DL freeze-dried formulations in 100 ml aqueous ethanol (45 % v/v). The acetylsalicylic*
143 *acid loading was such that each final wafer contained 75 mg of the drug.*
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Sample name	**MET (% w/v)**	**CAR (% w/v)**	**CS (%w/v)**	**Polymer ratio**	Acetylsalicylic acid **(g)**	**drug: sweetener ratio**	**Total polymer excipient content in the solution (% w/v)**
DL MET:CAR Suc 1	1.87	0.63	0.00	3:1	7.5	1:1	2.50
DL MET:CAR Asp 1	1.87	0.63	0.00	3:1	7.5	1:1	2.50
DL MET:CAR Stev 1	1.87	0.63	0.00	3:1	7.5	1:1	2.50
DL MET:CS Suc 2	3.00	0.00	1.00	1:3	7.5	1:1	4.00
DL MET:CS Asp 2	3.00	0.00	1.00	1:3	7.5	1:1	4.00
DL MET:CS Stev 2	3.00	0.00	1.00	1:3	7.5	1:1	4.00
DL CAR:CS Suc 3	0.00	1.00	3.00	1:3	7.5	1:1	4.00
DL CAR:CS Asp 3	0.00	1.00	3.00	1:3	7.5	1:1	4.00
DL CAR:CS Stev 3	0.00	1.00	3.00	1:3	7.5	1:1	4.00

2.3 *In vivo* taste masking evaluation

Aspartame and sucralose were used at a sweetener to drug ratio of 1:1 to mask the bitter taste of acetylsalicylic acid in the optimized DL wafers, as outlined in Table 1. Twelve healthy adult volunteers were recruited to take part in the taste masking assessments and were provided detailed written information about the study, and they subsequently gave signed informed consent with approval from the Ethics Committee of the University of Greenwich (12 December 2017). The volunteers were expected to make a suitable judgment on the taste of the wafers and give a written score. The wafers were placed on the tongue for 1 minute, the volunteers recorded their score and the sample was spat out and the mouth washed immediately with fresh drinking water. They subsequently responded to a questionnaire and scored each wafer from (1-10), using the following criteria: 1 (bitter), 5 (bland or no taste) and 10 (sweet). After collecting the results from the questionnaire, it was possible to identify which sweetener (sucralose or aspartame) was able to better mask the bitter taste of acetylsalicylic acid. As a control, commercially available chewable acetylsalicylic acid tablets (Bayer 81 mg), available in orange flavor was also judged by the volunteers in order to obtain a comparison of the formulated acetylsalicylic acid with a currently marketed chewable acetylsalicylic acid tablet.

2.4 Physico-chemical characterization

2.4.1 Texture analysis

Texture analyzer (HD plus, Stable Micro System, Surrey, UK) fitted with a 5 kg load cell, was used to analyze the mechanical hardness (resistance to compression) and mucoadhesion properties of the taste masked DL wafers as previously reported (Boateng & Ayensu, 2014). Briefly, wafers (n = 3) were compressed in 3 or more places using a 2mm diameter probe and the resistance to compression determined for each formulation. To analyze the *in vitro* mucoadhesion behavior, each formulation was attached to an adhesive probe (35 mm diameter). Gelatin solution [6.67% (w/v)], was allowed to set to a gel, and 500 µl of simulated saliva (SS) at pH 6.8 ± 0.1 spread over the surface of the set gelatin.

The *ex vivo* mucoadhesion experiment was performed on taste masked DL wafers (n = 3) to estimate the effect of SS on their adhesion profiles on porcine buccal tissue. The samples were tested using the TA HD plus Texture Analyzer described above and the wafers were attached to an adhesive probe (75 mm diameter) with double sided adhesive tape. An 88 mm diameter Petri dish containing buccal epithelium membrane of porcine tissue was used.

The wafers were positioned in contact with the epithelium for 60 seconds to provide optimal contact before being detached (Khan et al., 2015).

2.4.2 Swelling capacity

The swelling capacity of the taste masked DL wafers was determined by immersing each formulation into 5 ml of SS pH 6.8 ± 0.1 set at a temperature of 37 ± 0.1°C and weighing the swollen wafer at predetermined time intervals. The swelling capacity was determined for three replicates (n = 3) and calculated using equation 1.

$$Swelling\ index = \frac{Ws - Wd}{Wd} \times 100 \quad \text{(Equation 1)}$$

where; Wd = dry weight of wafers; Ws = weight of wafers after swelling

The composition of various salts in 1L of SS was: 0.228 g of calcium chloride dihydrate, 1.017 g of sodium chloride, 0.204 of sodium phosphate dibasic, 0.061 g of magnesium chloride hexahydrate, 0.603 g of potassium carbonate hemihydrate, 0.273 g of sodium phosphate monobasic monohydrate and 1.000 g of submaxillary mucin. The pH was adjusted to 6.8 ± 0.1 with phosphoric acid (Marques, et al., 2011).

2.4.3 Scanning electron microscopy (SEM)

The surface morphology of the gold coated taste masked DL wafers was analyzed using a Hitachi SU8030 (Hitachi High-Technologies, Krefeld, Germany at an accelerating voltage of 1 kV.

2.4.4 Pore analysis

The porosity of the wafers was measured using the solvent displacement method. Ethanol was used as it fills the pores and wets the wafers without hydrating them, compared to water which hydrates and eventually dissolves them. The taste masked DL wafers were weighed, completely immersed in 10 ml ethanol, covered, and left to stand for 2 hours for complete saturation. The saturated wafers were degassed to remove all air bubbles and the wafers subsequently removed very quickly from the solvent and immediately weighed. The porosity (%) was calculated using equation 2 (Okeke & Boateng, 2016).

$$P = \frac{Vp}{Vg} \times 100 = \frac{Wf - Wi}{\rho e\, Vg} \quad \text{(Equation 2)}$$

where; V_p = pore volume

V_g = wafers geometrical volume

W_f = final weight of wafer

W_i = initial weight of wafer

ρ_e = ethanol density (0.789 g/cm^3)

2.4.5 X-ray diffraction (XRD)

X-ray diffractograms of taste masked DL wafers were obtained using a D8 Advantage X-ray diffractometer, by pressing the formulations before placing on the holder, mounting on the sample cell and analyzed in transmission mode at diffraction angle range of 5° to 50° 2θ, step size 0.04°, and scan speed of 0.4 s/step.

2.4.6 Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra were obtained with a Perkin Elmer Spectrum instrument equipped with a diamond universal ATR unit. The taste masked DL wafers were placed on the ATR diamond crystal and force applied with a pressure clamp to allow suitable contact between the samples and the diamond crystal. Spectra were recorded at a resolution of 4 cm^{-1} within the range of 500-4000 cm^{-1} with subtraction of background spectra before plotting, to allow consistent absorbance of each sample. In addition, pure acetylsalicylic acid, aspartame, sucralose and physical mixtures of the drug with each sweetener, were analyzed by placing a small amount of the powder on the diamond crystal and the same process used for analyzing wafers was followed.

2.4.7 In vitro drug release

Drug dissolution of taste masked DL wafers was performed using a Franz-diffusion cell apparatus with its receptor compartment filled with 8 ml of SS pH 6.8 ± 0.1. The system was placed on a water bath at 37 °C and magnetically stirred (200 rpm). At predetermined time intervals, 0.5 ml aliquots (n = 3) of the SS were withdrawn, filtered through a 0.45 µm cellulose acetate membrane, and analyzed using HPLC by following the method previously reported (Farias & Boateng., 2018).

2.5 Permeation studies

Permeation studies were undertaken for DL wafers using three different model buccal membranes; (i) *in vitro* EpiOral™ tissue culture membrane, (ii) *ex vivo* buccal tissue from pig cheek, (iii) *in vitro* artificial membrane barriers (*Permeapad™*).

2.5.1 EpiOral™ permeation studies

EpiOral™ assay medium (MatTek, Ashland MA, USA) was pre warmed to 37 ± 0.1 °C for 30 minutes. Then, using a sterile technique, 0.3 mL/well of EpiOral™ assay medium were pipetted into 4 wells of a 24 well plate and labelled 1hour equilibrium. The remaining wells were labelled 30 minutes, 1, 2, 3 and 4 hours. The EpiOral™ samples were transferred into the 30 minute labelled well, treated with 0.5 mL donor solution (SS pH 6.8 ± 0.1) into which 15 mg of wafers was added with the mucoadhesive layer in contact with the apical surface of the EpiOral™ buccal tissue and returned to the incubator. After 30 minutes, the tissue was moved to the next time point until the total elapsed time (4 hours). 50 µL of the receiver fluid was collected at predetermined time intervals and transferred to a vial for HPLC analysis.

2.5.2 Ex vivo permeation studies using pig cheek membrane

The *ex vivo* permeation studies was performed using Franz diffusion cell. The Franz diffusion cell is a simple, reproducible test for measuring the *in vitro* drug release from formulations. The Franz cells consists of two primary chambers separated by a membrane of defined diameter, which determines the transportation area. The formulation is applied to the membrane via the top chamber (donor compartment). The bottom chamber (receptor compartment) contains the fluid from which samples are taken at regular intervals for analysis, which determines the amount of active drug per unit area that has permeated the membrane at each time point.

Ex vivo permeation was performed by following previously reported method (Okeke & Boateng, 2016) (Ayensu, et al., 2012). Briefly, buccal tissues from the cheek of 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 ± 0.1) modified with sodium carbonate, placed in sealed box filled with dry ice and quickly transported to the laboratory. The buccal mucosa, with part of the sub mucosa, was immediately 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 500 µm and the buccal mucosa was used within 2 hours (Patel, et al., 2012).

The prepared mucosal membrane was washed with SS at 37 °C and mounted between the donor and receiver compartments of a Franz-type diffusion cell, with the epithelial side facing the donor compartment to permit contact with the DL wafer (Attia, et al., 2004). 8 ml of SS at 37 ± 0.1 °C was placed in the receiver chamber with magnetic stirring at 250 rev/min to provide uniform mixing. After an equilibration period of 30 min, 0.5 ml of SS was placed in the donor compartment and 5 mg of the acetylsalicylic acid wafers 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 and sealed with parafilm to avoid evaporation. At predetermined time intervals, aliquots (1 ml) were withdrawn from the sampling port of the receiver compartment and replaced with the same amount of SS pH 6.8 ± 0.1 also at 37 ± 0.1 °C to maintain a constant volume for 2 hours. The sampled aliquots were analyzed using HPLC (*n* = 3) and the % cumulative permeation plotted against time (Khan, et al., 2015).

2.5.3 Permeapad™ permeation studies

Permeapad™ barriers were placed between the donor and receiver chambers of the Franz-diffusion cells as described in previous studies (Bibi et al., 2015, 2016). The receiver compartment contained (8 mL) of SS at 37 ± 0.1 °C with magnetic stirring at 250 rev/min and the donor compartment was filled with (1.5 mL) of SS and 5 mg of DL wafers. The compartments were held together by a cell clamp and sealed with parafilm to avoid evaporation. At predetermined time intervals, aliquots (1 ml) were withdrawn from the sampling port of the receiver compartment and replaced with the same amount of SS pH 6.8 ± 0.1 to maintain a constant volume for 4 hours. The sampled aliquots were analyzed using HPLC (*n* = 3) and the % cumulative permeation plotted against time (Bibi, et al., 2015) (Bibi, et al., 2016).

The permeation flux (J) across the EpiOral™, pig cheek membrane and Permapad™ was determined using equation 3.

$$J = \frac{dQ}{dt} \cdot \frac{1}{A} \qquad \text{(Equation 3)}$$

where; J = steady state flux

d_Q/dt = amount of drug permeated

306 A = effective diffusion area

307

308 **2.6 Cell viability (MTT assay)**

309 MTT assay on Vero cells was used to determine the cytotoxicity of pure MET, CAR,
310 CS, acetylsalicylic acid, and the various formulated wafers. Vero cells (ATCC® CCL-81™)
311 are adherent cells derived from the kidney of the African Green monkey (*Cercopithecus*
312 *aethiops*) and are one of the commonly used mammalian cell lines in cell biology,
313 microbiology and molecular biology (Ammerman, et al., 2008). The Vero cells were obtained
314 from the cell and tissue culture labs within the School of Science (Richardson Lab,
315 University of Greenwich, Medway) and stored at -80 °C. The cells were used to seed a
316 sterile, flat-bottomed 96 well tissue culture plate with Dulbecco's modified Eagle's medium
317 (DMEM), fetal bovine serum (FBS) 10% (v/v), penicillin (100 units/mL), streptomycin (100
318 µg/mL) and glutamine 0.292 mg/mL. Two cultures (treated and control) were kept under
319 sterile conditions in a laminar hood and incubated at 37 °C in 5% (v/v) CO_2 for 24 hours
320 (Khan, et al., 2015). The controls only contained cells in growth media. The wafers and pure
321 compounds were initially weighed (~ 175mg) and placed in flow cabinet under UV light for
322 24 hours to sterilize. For the treated groups, the weighed sterilized samples were placed in 2.5
323 mL of growth medium and left in the incubator for 24 hours, and the extract was filtered
324 through 0.2 µm filter and collected into Eppendorf tubes.

325 The cells in culture medium were exposed to the collected sample extracts and
326 incubated for 24 and 72 hours. For the former time period (24 hours), the cells were initially
327 incubated for 20 hours, then 10 µL of MTT stock solution was added to each well and
328 incubated for a further 4 hours. For the latter incubation period (72 hours), the cells were
329 initially exposed to new set of samples for 68 hours, 10 µL of the MTT stock solution was
330 added to each well and the plate incubated for a further 4 hours, bringing the total incubation
331 time to 72 hours. The contents of the plates (24 and 72 hours) were decanted and 100 µL of
332 DMSO was added to each well, incubated at room temperature for 30 minutes and the
333 absorbance read on a Multiscan EX Micro-plate photometer (Thermo Scientific, Essex, UK)
334 at optical density (OD) of 540 nm. Data obtained was expressed as percentage cell viability
335 (n = 3) for all the samples tested (Khan, et al., 2015).

336

2.7 Statistical analysis

Statistical analysis was carried out to compare the results using two tailed student t-test with 95% confidence interval (p-value < 0.05) as the minimum level of significance. All the experiments were carried out in triplicates with mean and standard deviation.

3. Results and discussion

Table 2 shows the porosity (%) of taste masked DL wafers relative to total polymer content in the original gels and polymer ratios. The results demonstrated that taste masked DL formulations containing sucralose were more porous than those with aspartame. However, there were no significant differences (p > 0.05) between the same formulation containing sucralose and aspartame. Formulations containing CS (DL CAR:CS Asp 3 and DL MET:CS Asp 2) showed lower porosity of 55 ± 5 % and 49 ± 4 % respectively, compared with the DL MET:CAR Suc 1 formulation, which showed a porosity of 65 ± 3 %. The porosity results are confirmed by the SEM images in section 3.6, which showed that taste masked DL MET:CAR Suc 1 appeared more porous than the DL MET:CS Suc 2 and DL CAR:CS Suc 3 wafers, which were more compact. The porosity of the taste masked DL wafers was lower when the hardness results from section 3.3.1 were higher. This is due to the addition of the sweeteners which formed a compressed solid resulting in smaller pores and therefore, slowed down the penetration of solvent within the taste masked drug loaded wafer. This also affected swelling capacity as the DL MET:CAR taste masked formulations were able to swell more while the DL MET:CS and DL CAR:CS formulations which were less porous showed a lower swelling capacity in section 3.5, due to the compacted polymer structure, decreasing the water ingress (hydration) and subsequently % swelling capacity.

360 *Table 2: Mechanical properties, porosity (%) and in vitro and ex vivo mucoadhesive profiles of taste masked DL wafers in simulated saliva (SS)*

361 *Three replicates were performed for each sample (mean ± SD, n = 3).*

			In vitro mucoadhesion			Ex vivo mucoadhesion		
Taste masked formulations	**Hardness (N)**	**Porosity (%)**	**PAF (N)**	**TWA (mJ)**	**Cohesiveness (mm)**	**PAF (N)**	**TWA (mJ)**	**Cohesiveness (mm)**
DL MET:CAR Suc 1	26.16 ± 3.04	65 ± 3	0.21 ± 0.11	0.12 ± 0.10	0.96 ± 0.09	0.37 ± 0.15	0.42 ± 0.08	6.62 ± 1.54
DL MET:CAR Asp 1	22.96 ± 4.45	63 ± 4	0.12 ± 0.05	0.05 ± 0.03	0.81 ± 0.12	-	-	-
DL MET:CS Suc 2	20.00 ± 3.09	59 ± 2	0.12 ± 0.11	0.03 ± 0.03	0.83 ± 0.19	-	-	-
DL MET:CS Asp 2	18.38 ± 1.02	49 ± 4	0.05 ± 0.04	0.02 ± 0.01	1.00 ± 0.14	0.25 ± 0.13	0.42 ± 0.12	5.14 ± 0.81
DL CAR:CS Suc 3	18.79 ± 6.22	56 ± 2	0.17 ± 0.10	0.03 ± 0.00	1.18 ± 0.07	-	-	-
DL CAR:CS Asp 3	10.35 ± 3.07	55 ± 5	0.03 ± 0.01	0.01 ± 0.00	1.09 ± 0.21	0.15 ± 0.10	0.93 ± 0.18	11.53 ± 0.81

362

Compared with the blank DL (non-taste masked) wafers (BDL), previously reported (Farias & Boateng, 2018), it can be observed that the BDL MET:CAR and BDL MET:CS showed higher porosity of 82 ± 12 and 75 ± 7 % respectively. This confirms both the hardness and SEM results which showed that the pores originally present before loading of sweeteners were largely filled with excess sweetener as well as recrystallized acetylsalicylic acid after freeze-drying.

3.4 *In vivo* taste masking evaluation

To improve patient adherence to medication, proven methods for reduction and inhibition of bitter taste have resulted in improved palatability of these formulations (Kleinert, et al., 1993). Taste is a function of sensation by the taste buds in the mouth and for formulations intended for geriatric, non-cooperative and bed ridden patients, the main challenge is to mask the taste of bitter drugs, to enhance patient acceptability and to ensure they will receive the optimal therapeutic dose of their medication (Momin, et al., 2012). Some of the methods employed in taste masking include coating of bitter drug particles with coating agents such as starch, polyvinyl pyrrolidone, gelatin, ethyl cellulose (Gowthamarajan, et al., 2004). Microencapsulation is a process of applying thin coating to small particles of solids, droplets of liquids and dispersions using coating agents such as gelatin and povidone.

Another commonly used method is to add sweeteners, as they impart a sweet taste that is highly preferred by geriatric and paediatric patients (Mennella, et al., 2011) and was the method of choice in this study. Taste-masked formulations can be challenging to develop, and the best method is often dictated by the physicochemical properties and taste profile of the drug (Vesey, 2018). Taste masking by amino acids, sweeteners and flavours is the most simple and oldest technique for improving taste characteristic of active ingredients within formulations. The sweeteners and flavours overcome the unpleasant taste by occupying the taste buds and therefore preventing direct sensation of the bitter taste of the drug of interest, long enough to allow effective therapeutic dosing (Karolewicz, 2016). The aim of the taste masking study was to solicit judgement of human volunteers about the taste of the optimized DL wafers (CAR:CS, MET:CS and MET:CAR) loaded with sucralose or aspartame, to help in the selection of the most suitable formulations for the target (geriatric) patient group. The participants were required to choose a number between 1 and 10, in which 1 was (bitter) 5 (bland or no taste) and 10 (sweet) and scores for each formulation by the 12 volunteers are summarised in Table 3.

Table 3. Results of in vivo taste masking study showing the selected optimized samples and the scores per taste category (i.e. bitter, bland, and sweet) for each formulation as judged by the volunteers. Scores from 5 to 10 (bland – sweet) were considered as having masked the bitter taste.

		Number of volunteers giving a particular score									
	Scores	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sample	**Formulations**	**Bitter**				**Bland**			**Sweet**		
A	DL MET: CAR Suc 1			1		1	2	3	4	1	
E	DL CAR: CS Asp 3	1	2	2	1	2	3				1
F	DL MET: CS Asp 2	1	1	2	1	1	3	2			1
Bayer	Commercial chewable acetylsalicylic acid (Aspirin) tablet (orange flavour)			1		4	3	3	1		

The *in vivo* taste masking evaluation were performed on 3 optimized formulations and showed that sucralose and aspartame were able to mask the bitter taste of acetylsalicylic acid in the DL MET:CAR Suc1, DL CAR:CS Asp 3 and DL MET:CS Asp 2, respectively. Overall, the formulations containing DL MET:CAR and MET:CS showed more palatability and acceptance because according to Amelian and Winnicka, MET also possesses appropriate properties to be used for effective taste masking (Amelian & Winnicka, 2017). At the molecular level, the drug-polymer complex with aspartame or sucralose exhibited significant taste-masking, as confirmed in the taste assessment by volunteers. As a result of the *in vivo* taste masking evaluation, further characterizations were performed on the DL MET:CAR Suc1, DL CAR:CS Asp 3 and DL MET:CS Asp 2. The volunteers also judged a commercially available chewable acetylsalicylic acid with orange flavour to compare with the results for the formulated wafers. Though the commercially available chewable acetylsalicylic acid was largely accepted, some volunteers judged it as bitter or bland, and this might be due to taste receptors being different for each person.

3.5 Swelling capacity

The swelling capacity of DL MET:CAR Suc 1, DL CAR:CS Asp 2 and DL MET:CS Asp 3 are shown in Figure 1. The % swelling capacity for (DL MET:CAR 3:1 Suc) was observed to be 283 ± 47 %. The high rate of swelling is related to the total percentage polymer content (by weight), as they were prepared from 2.5% w/v gels while the other taste masked formulations, were prepared from 4.0% w/v gels with the latter being more dense due to the higher total polymer content after freeze-drying. DL MET:CS Asp 2 wafers showed a swelling capacity of 185 ± 33 %. The results showed that MET in the formulations made the wafers more stable, thus they had longer duration of swelling of about 30 to 70 minutes, before disintegrating, compared to the DL CAR: CS Asp 3 formulations. This is because MET acts as a stabilizer for the wafers (Shin Etsu Chemical, 2005). DL CAR:CS Asp 2 wafer was able to maintain its structural integrity for 10 minutes, after which it disintegrated because of excessive absorption of water molecules. Compared to our previously reported study (Farias & Boateng, 2018) of DL formulations without any sweeteners, the taste masked DL wafers showed lower swelling capacity. This could be due to the increased hardness and brittleness of the taste masked DL wafers because of the compact and denser solid matrix that resulted in wafers with smaller pores and therefore, less capacity for water ingress (Kianfar, et al., 2014).

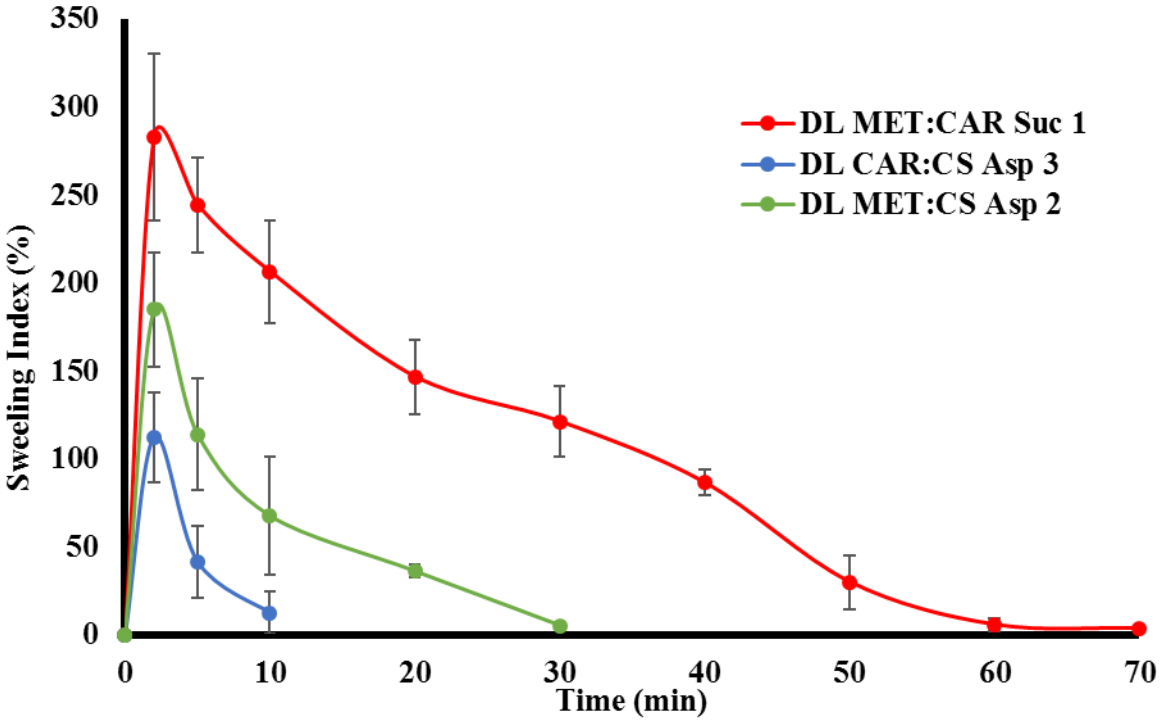


Figure 1. Swelling profiles of taste masked acetylsalicylic acid loaded wafers (DL MET:CAR Suc 1, DL CAR:CS Asp 3 and DL MET:CS Asp 2) in SS. It was determined for three replicates (mean ± SD, n = 3) and calculated using equation 1.

The maximum swelling capacity (%) of the taste masked DL wafers occurred within 2 minutes compared to the DL wafers without sweeteners as previously reported (Farias & Boateng, 2018). The reason for the rapid water ingress in the former is because of the high water solubility of both sweeteners. When compared with results in PBS (Farias & Boateng, 2018), the swelling capacity values were lower in SS which can be attributed to the difference in ionic strength of the media which plays an important role in the swelling profile of porous formulations such as wafers (Peh & Wong, 1999).

3.6 Scanning electron microscopy (SEM)

Figure 2 (a) and (b) shows the surface morphology of the sweeteners' (aspartame and sucralose) crystals. The morphology of acetylsalicylic acid crystals have been previously reported (Farias & Boateng., 2018). It was observed that the aspartame and sucralose crystals were similar as they are mainly composed of maltodextrin and the particles seem to be fused with each other, thus forming larger clusters with no uniform size or shape (Singh, et al., 1993). Figure 2 (c to e) shows the surface morphology of representative taste masked DL wafers (DL MET:CAR Suc 1, DL CAR:CS Asp 3 and DL MET:CS Asp 2).

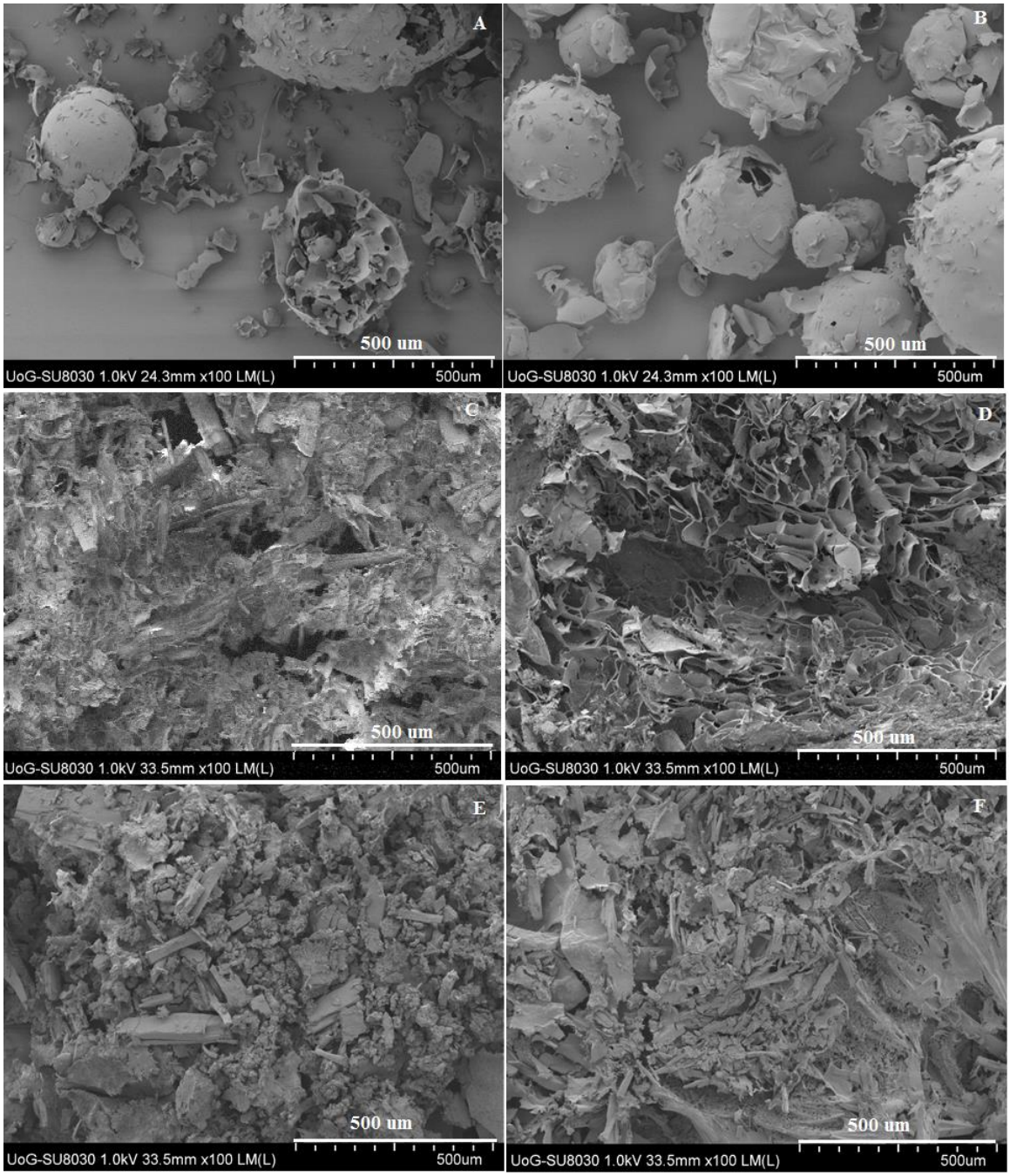


Figure 2. SEM images showing surface morphology of (A) aspartame, (B) sucralose, and internal porous structure of (C) BDL MET:CAR (DL only wafer) (D) DL MET:CAR Suc 1, (E) DL CAR:CS Asp 3 and (F) DL MET:CS Asp 2.

The taste masked DL wafers showed a very compact matrix structure with crystals of excess acetylsalicylic acid and maltodextrin distributed over their surfaces, which is a major reason for the high hardness values described in section 3.1 above. The small pores in these taste masked DL formulations is attributed to the thicker walls formed due to polymer- drug interaction (Farias & Boateng, 2018) and addition of sweetener. The SEM images confirm the swelling capacity results in section 3.5, with the DL MET:CAR Suc 1 able to swell more

due to being more porous, and thus exhibiting faster rate of water ingress and hydration, compared to DL MET:CAR Asp 2 and DL CAR:CS Asp 3.

3.7 X-ray diffraction (XRD)

The transmission diffractograms of acetylsalicylic acid, sucralose, aspartame, and representative taste masked DL formulations (DL MET:CAR Suc 1, DL MET:CS Asp 2 and DL CAR:CS Asp 3) are shown in Figure 3. The results confirm the amorphous nature of the sweeteners (sucralose and aspartame) demonstrated by the broad peak at 2θ of 15° and 25°. Acetylsalicylic acid showed its crystalline nature with the presence of sharp peaks at 2θ of 15°, 20°, 23° and 27°. The crystalline peaks from acetylsalicylic acid can be observed at the same 2θ positions of 15°, 20°, 23° and 27° in the diffractograms of the taste masked DL formulations (DL MET:CAR Suc 1, DL MET:CS Asp 2 and DL CAR:CS Asp 3) suggesting that the addition of sweeteners did not change the crystallinity of the acetylsalicylic acid within the wafers.

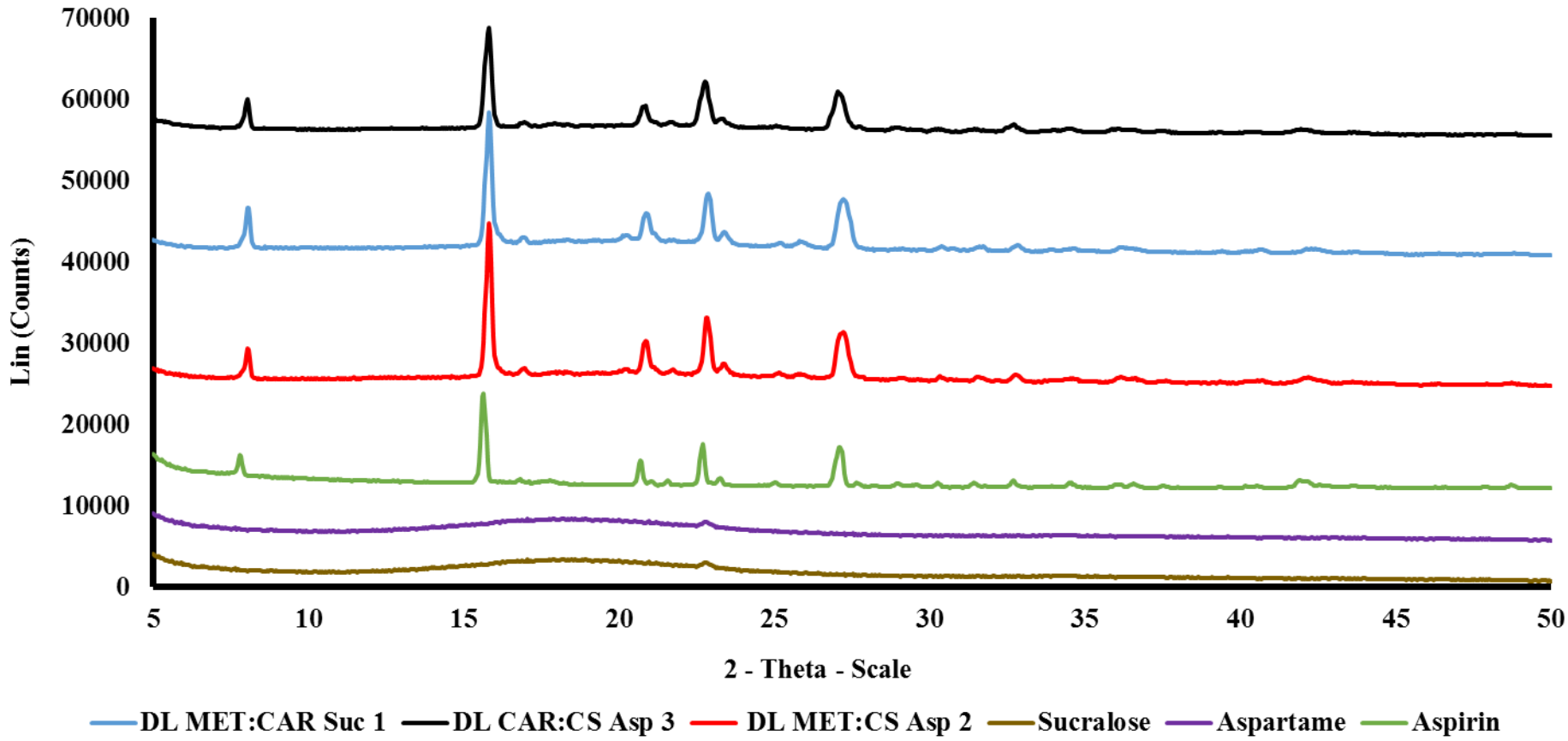


Figure 3. XRD-transmission diffractograms of acetylsalicylic acid, sucralose, aspartame and taste masked DL formulations (DL MET:CAR Suc 1, DL MET:CS Asp 2 and DL CAR:CS Asp 3.

3.8 Attenuated total reflectance – Fourier transform infrared (ATR-FTIR)

Figure 4 (a) shows the ATR-FTIR spectra of pure sucralose, aspartame, acetylsalicylic acid and physical mixtures of acetylsalicylic acid with aspartame and acetylsalicylic acid with sucralose. Sucralose is manufactured by the selective chlorination of sucrose, in which three of sucrose's hydroxyl groups are substituted with chlorine atoms. Dextrose and maltodextrin are used as bulking agents and are the major components of the sweetener. The IR spectrum contains a broad band near 3258 cm^{-1} due to the hydrogen bonded hydroxyl (O-H) groups in the structure. There are C-H symmetric and asymmetric stretching bands between 2800 and 3000 cm^{-1} and a series of bands between 1200 and 650 cm^{-1} which are the result of vibration of the C-O, C-C and C-O-H groups of the sugar. The spectrum of aspartame was similar to that of sucralose as the major components of both sweeteners is maltodextrin.

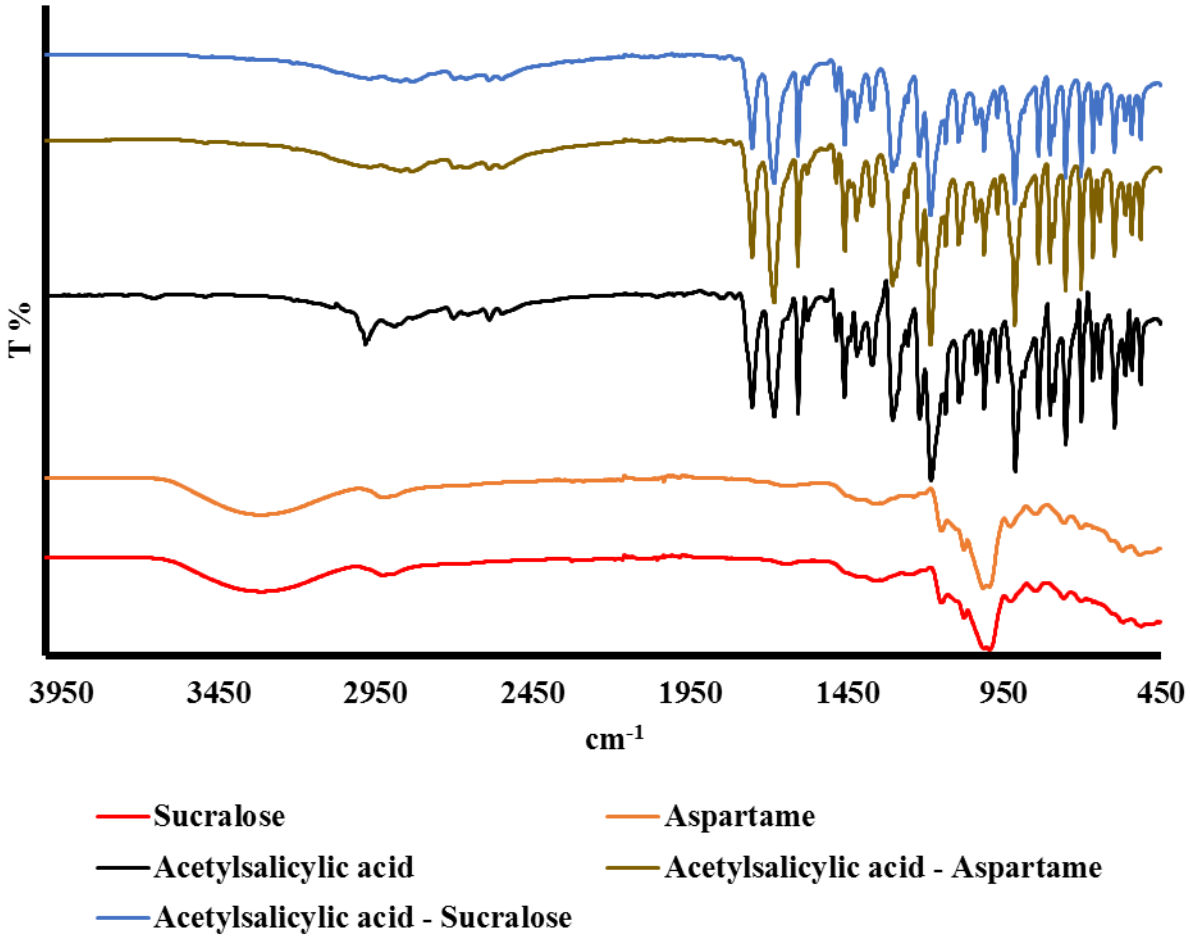


Figure 4a. ATR-FTIR spectra of pure acetylsalicylic acid, sucralose, aspartame, and physical mixtures of acetylsalicylic acid *with the two sweeteners.*

The IR spectra of acetylsalicylic acid which has three functional groups, a benzene ring (aromatic group), a carboxylic acid (COOH) group and an ester (R-C=O-O-R) group is also shown in Figure 4a. The broad and wide peak from 2500 to 3300 cm^{-1} represents the carboxylic acid (COOH) part of the molecule. The aromatic functional group is represented by the sharp peak for the C-H stretch around 1710-1780 cm^{-1}, a medium peak around 1500-1700 cm^{-1} and a carbonyl C=O group stretch around 1710-1780 cm^{-1}. The ester group is represented by a C=O stretch at 1735-1750 cm^{-1}.

Figure 4 (b) shows the ATR-FTIR of taste masked DL formulations (DL MET:CAR Suc 1, DL MET:CS 1:3 Asp 2 and DL CAR:CS Suc 3). It can be observed that there was interaction of acetylsalicylic acid with the polymers as well as with the sweeteners by the 2500 cm^{-1} to 3300 cm^{-1} band representing the COOH group. This interaction is shown by the shifting of the peaks to a high wavenumber and the reduced peak intensity between 1710 – 1780 cm^{-1} (aromatic group) for acetylsalicylic acid. The bands at 1223 cm^{-1} and 843 cm^{-1} were attributed to O-S-O symmetric vibration and the band at 925 cm^{-1} demonstrated the existence of C-O-C of the 3,-anhydro-D-galactose for CAR. The bands around 3389, 1036 cm^{-1} were related to O-H and C-O stretch.

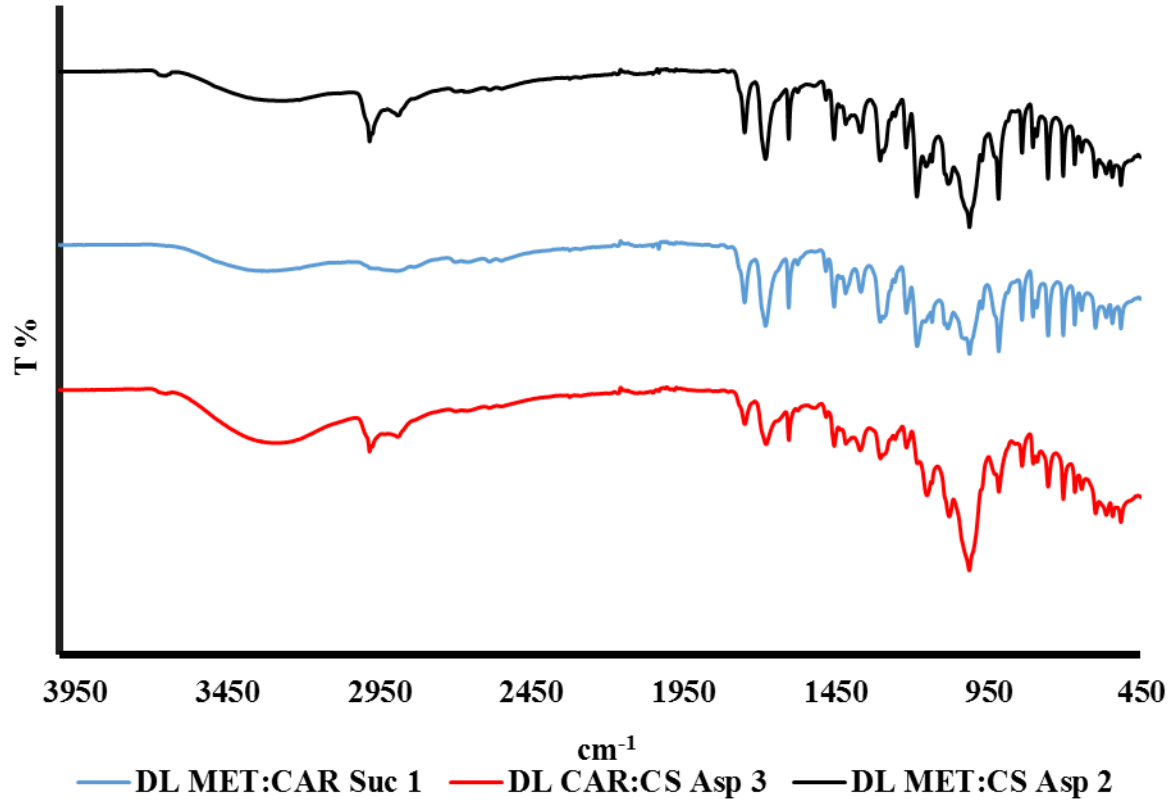


Figure 4b. ATR-FTIR spectra of taste masked DL formulations (DL MET:CAR Suc 1, DL MET:CS Asp 2 and DL CAR:CS Asp 3).

The intense band at 1625 cm^{-1} was related to the deformation of hydrogen bond in water and described as water deformation band (Farias & Boateng, 2018). The interaction is also confirmed by the presence of C-H symmetric and asymmetric stretching bands between 2800 and 3000 cm^{-1} and a series of bands between 1200 and 650 cm^{-1} which is a result of vibration of the C-O, C-C and C-O-H groups of the sugars (dextrose and maltodextrin) present in the sweeteners within the taste masked DL formulations.

3.9 *In vitro* drug release

The drug dissolution study was carried on samples that were optimized from the *in-vivo* taste masking evaluation using SS at pH 6.8 ± 0.1 as observed in Figure 5.

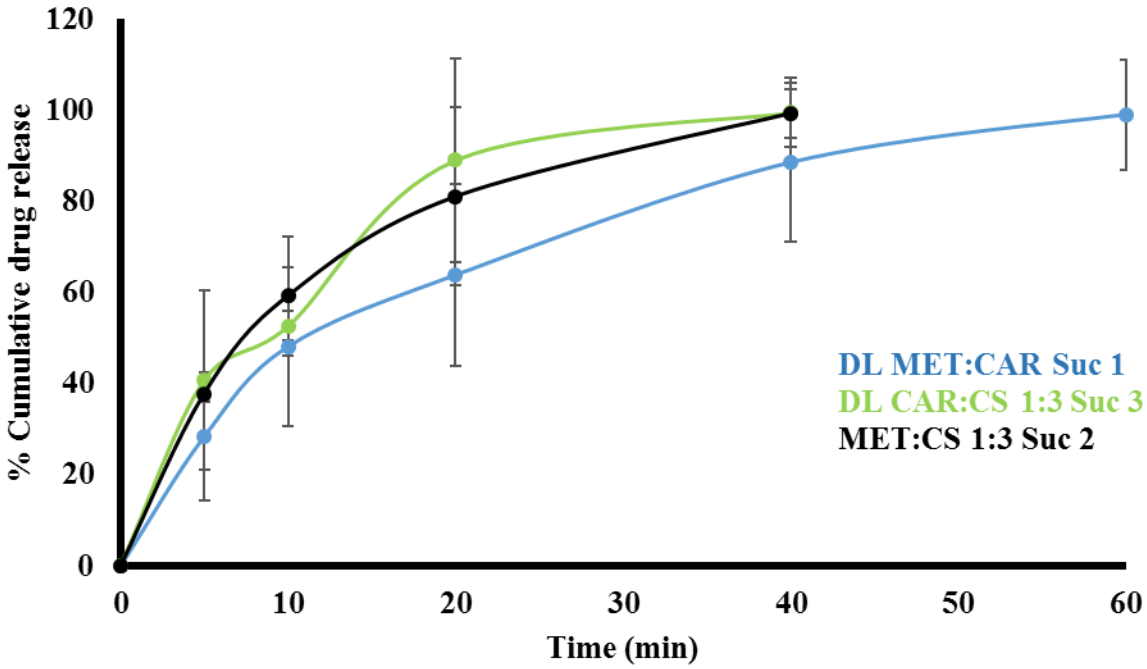


Figure 5. Drug dissolution profiles of selected optimized taste masked DL formulations (DL MET:CAR Suc 1, DL MET:CS Asp 2 and DL CAR:CS Asp 3) in SS at pH 6.8 ± 0.1 (mean ± SD, n = 3).

The mean percent released was 63.9 %, 89.1 % and 81.1 % for DL MET:CAR Suc 1, DL CAR:CS Asp 3 and DL MET:CS Asp 2 respectively, in the first 20 minutes. The DL MET:CAR Suc 1 wafers achieved maximum release of 99.1 % in 60 minutes, while the DL CAR:CS Asp 3 and DL MET:CS Asp 2 wafers achieved maximum release of 99.5 and 99.3 % respectively in 40 minutes. DL MET:CAR Suc wafers were able to release the drug for a longer period. This is due to the MET in the formulations which helps to increase the viscosity and density of the formulations, thus slowing drug diffusion and release. When

comparing the taste masked DL formulations with the BDL formulations with no sweeteners from previous studies (Farias & Boateng, 2018), it can be observed that the % release in SS increased with the addition of sweetener. For example, DL MET:CAR Suc 1 had a percent release of 63.9 % at 20 minutes and the respective BDL formulation had a percent release of 14.5 %. Addition of sweetener in the formulation enhanced the rate of drug release from the polymeric systems and this is because sucralose and aspartame are highly water soluble materials which allowed a faster ingress of dissolution medium into the wafers and subsequent rapid hydration, swelling and drug diffusion from the swollen matrix and its subsequent erosion.

The observed drug release period appears to be a relatively long time for buccal absorption. However, once the wafer becomes fully hydrated, it is expected to form a flowing gel, which will then be mixed with saliva and the patient can unconsciously swallow more readily. This is an important advantage for geriatric patients with dysphagia, who will typically struggle to swallow solid or even semi solid formulations. Since acetyl salicylic acid is stable in the gastric acid, eventual swallowing is not expected to be a limitation.

3.10 Permeation studies

3.10.1 EpiOral™ permeation studies

Epioral™ has been previously used in the permeation studies of lyophilized thiolated chitosan xerogels for buccal delivery of insulin (Boateng, et al., 2014). In their study the permeation parameters of insulin for the optimized drug loaded chitosan xerogels through the Epioral™ was determined. Another study which used the Epioral™ was reported by Giovino and co-workers, who developed an integrated buccal delivery system combining chitosan films impregnated with insulin loaded PEG-b-PLA nanoparticles (Concetta, et al., 2013). Epioral™ was also used by Brian Keyser and colleagues, for the development of 3D human oral tissue model for oral permeation of smokeless moist snuff (Keyser, et al., 2018). The cumulative permeation curves of the optimised taste masked DL wafers using EpiOral™ buccal tissue are shown in Figure 6. The permeation flux (J) of acetylsalicylic acid released from the optimised wafers are shown in Table 4.

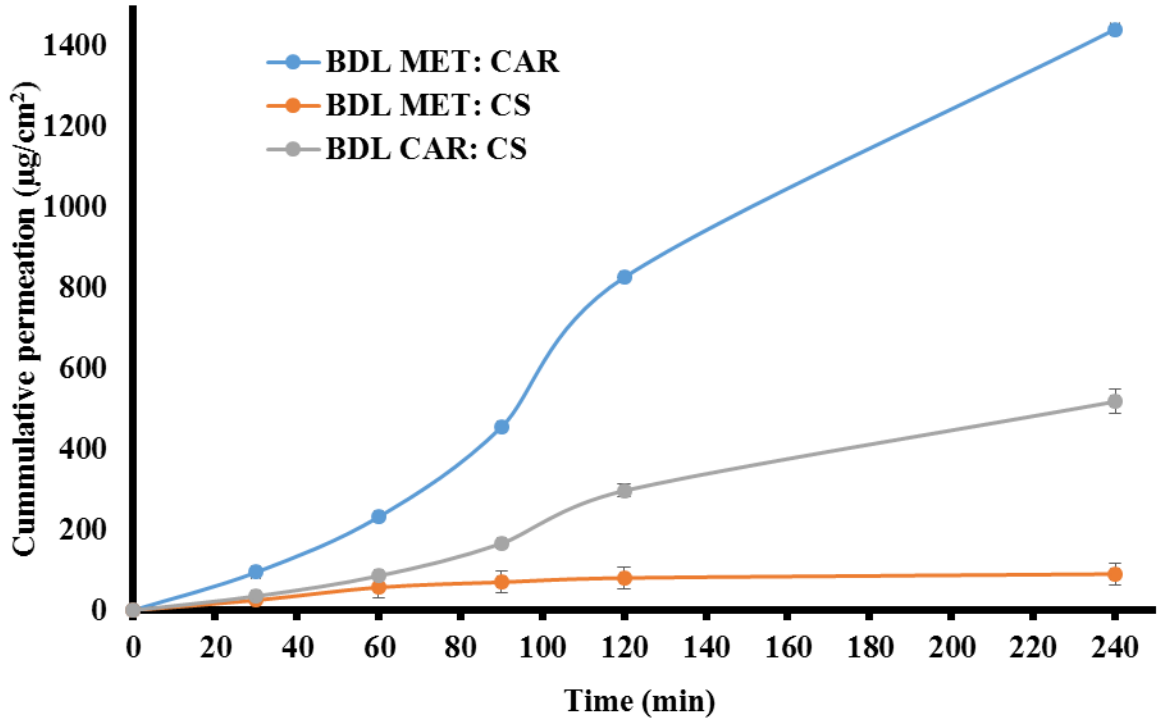


Figure 6. Cumulative permeation curve of optimized DL wafers using EpiOral™ buccal tissue (n = 3, ± SD).

The highest cumulative permeation within 4 hours and permeation flux (J) was observed for BDL MET: CAR wafer with the maximum cumulative permeation of 1440 ± 1.0 µg/cm^2 and permeation flux (J) of 360 ± 0.3 µg/cm^2/h. The BDL MET: CS wafers demonstrated the lowest cumulative permeation and permeation flux within 4 hours, with a maximum cumulative permeation of 90 ± 2.7 µg/cm^2 and permeation flux (J) of 22.4 ± 6.7 µg/cm^2/hr which were significantly different ($p < 0.05$) from the other two formulations. Due to the high cost of EpiOral™ tissue and associated budgetary constraints for the current research, it was not possible to test permeation for the corresponding taste masked formulations, therefore, both sets of optimized formulations (taste masked and non-taste masked wafers) were further tested for permeation using cheaper *ex vivo* porcine model and artificial buccal membrane barriers (Permeapad™) which was freely donated.

Table 4. Permeation flux (J) for optimized DL wafers from porcine tissue, Permeapad™ and EpiOral™ buccal tissue.

	Formulation	***Flux (J) (µg/cm2/h) (mean ± SD, n = 3)***
EpiOral™	BDL MET: CAR	360.0 ± 0.3
	BDL MET: CS	22.4 ± 6.7
	BDL CAR: CS	129.3 ± 7.5
Porcine tissue	DL MET:CAR Suc 1	19.5 ± 1.4
	DL CAR:CS Asp 3	7.9± 0.7
	DL MET:CS Asp 2	5.9 ± 1.9
Permeapad™	DL MET:CAR Suc 1	14.7 ± 1.5
	DL CAR:CS Asp 3	18.0 ± 5.1
	DL MET:CS Asp 2	9.1 ± 0.5

3.10.2 Ex-vivo permeation studies

The cumulative permeation curve of the optimized taste masked DL wafers using porcine buccal tissue are shown in Figure 7 and the permeation flux (J) values are shown in Table 4. The taste masked DL wafers in general showed a significantly ($p < 0.05$) lower cumulative permeation than the corresponding BDL (non-taste masked) formulations. The highest cumulative permeation and permeation flux (J) was shown for DL MET:CAR Suc 1 with the maximum cumulative permeation of 78.0 ± 5.5 µg/cm^2 within 4 hours and permeation flux (J) of 19.5 ± 1.4 µg/cm^2/h while the lowest cumulative permeation and permeation flux (J) was shown for optimized taste masked DL MET:CS Asp 2 with the maximum cumulative permeation of 24 ± 7 µg/cm^2 within 4 hours and permeation flux (J) of 5.9 ± 1.9 µg/cm^2/h.

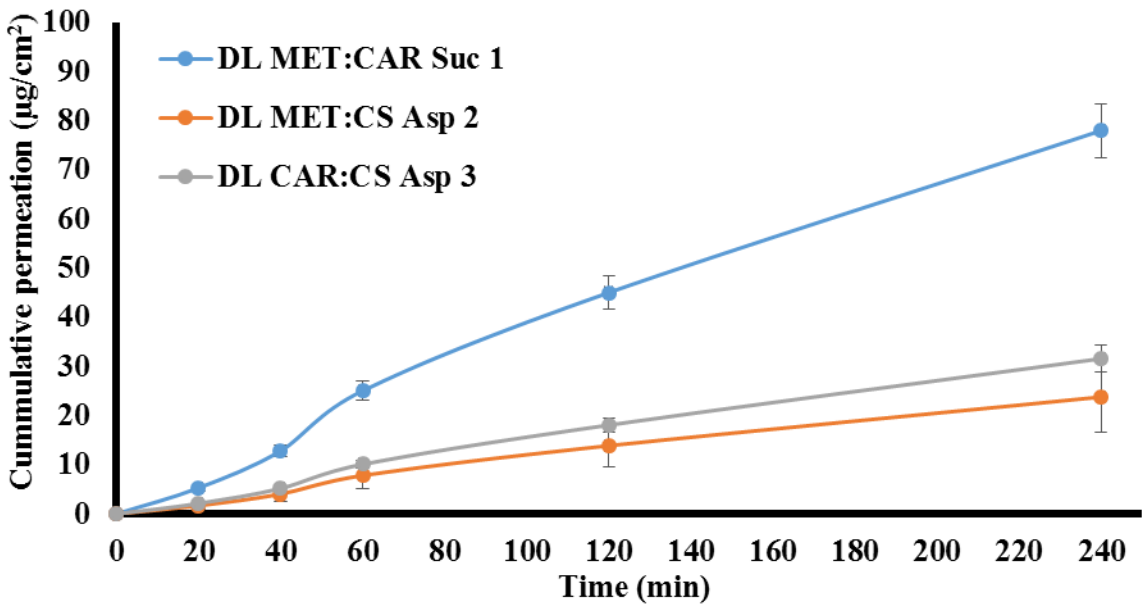
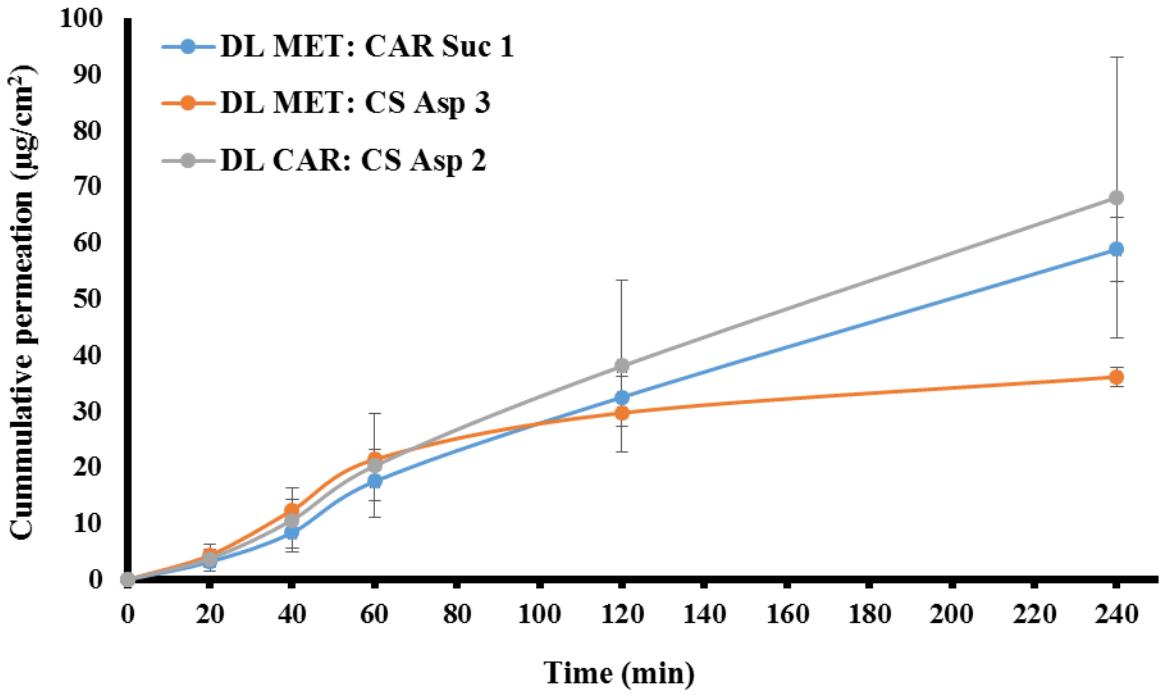


Figure 7. Cumulative permeation curve of optimized taste masked DL wafers using porcine buccal tissue (n = 3, ± SD).

3.10.3 Permeapad™ permeation studies

The cumulative permeation curves of the optimized taste masked DL wafers using Permeapad™ are shown in Figure 8 and the permeation flux (J) of acetylsalicylic acid are shown in Table 4. The highest cumulative permeation within 4 hours and the permeation flux (J) was observed for DL CAR:CS Asp 2 wafers with the maximum cumulative permeation of 59 ± 6 µg/cm^2 and permeation flux (J) 14.7 ± 1.5 µg/cm^2/h for DL MET:CAR Suc 1. Optimized DL MET:CS Asp 2 wafers demonstrated lowest cumulative permeation within 4 hours and permeation flux (J), with a maximum cumulative permeation of 36 ± 2 µg/cm^2 and permeation flux (J) 9.1 ± 0.5 µg/cm^2/h.



620
621

622 *Figure 8. Cumulative permeation curve of optimized taste masked DL wafers using*
623 *Permeapad™ artificial barrier (n = 3, ± SD).*
624

625 Generally, the rates of hydration, swelling, release of acetylsalicylic acid from the
626 formulations and mucoadhesion played a role on the permeation flux via the three different
627 permeation models employed (EpiOral™, porcine buccal tissue and Permeapad™ artificial
628 buccal membrane). EpiOral™ buccal tissue demonstrated a higher flux than porcine buccal
629 tissues which can be attributed to fatty tissues beneath the porcine buccal mucosa tissue. On
630 the other hand, Permeapad™ artificial barrier demonstrated a lower flux than porcine and
631 EpiOral™ buccal tissues. Though Permeapad™ demonstrated lower cumulative permeation
632 than the other buccal permeation models tested, the results indicated that Permeapad™ is well
633 suited as a cheap alternative for fast and reliable preliminary prediction of passive drug
634 permeability (Bibi, et al., 2015). Moreover, the investigated biomimetic barrier has been
635 proven to maintain its functionality over time and in different pH environments (di Cagno, et
636 al., 2015), making it a useful addition to the *in vitro* permeation testing tool kit. The reason
637 for the low permeation and permeation flux (J) in the taste masked DL formulations is
638 confirmed by the SEM results in section 3.6, which showed that the taste masked DL wafers
639 were more compacted with crystals of excess acetylsalicylic acid and maltodextrin distributed
640 over their surfaces, blocking the wafer pores.
641 .

3.11 MTT assay

There are many factors involved in determining the successful and safe application of polymers as drug carriers in humans, with toxicity being an important factor (Khan, et al., 2016). Tissue viability was assessed using 3-[4,5-dimethylthiazol-2-ul]-2,5 diphenyl tetrazolium bromide (MTT) cytotoxicity testing for pure polymers, acetylsalicylic acid, optimized BLK and DL formulations previously reported in (Farias & Boateng, 2018) and the taste masked acetylsalicylic acid loaded formulations. This is a reduction assay where yellow MTT is 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 9 shows the respective cell viability data for the samples described when exposed to Vero cells as measured by MTT assay after 24 hours. Triton-X-100 (positive control) killed 90% of cells compared with untreated cells (negative control) after 24 hours of exposure. Data for 72 hours incubation are not shown as they were similar to that after 24 hours, which is a more ideal exposure time for buccal delivery.

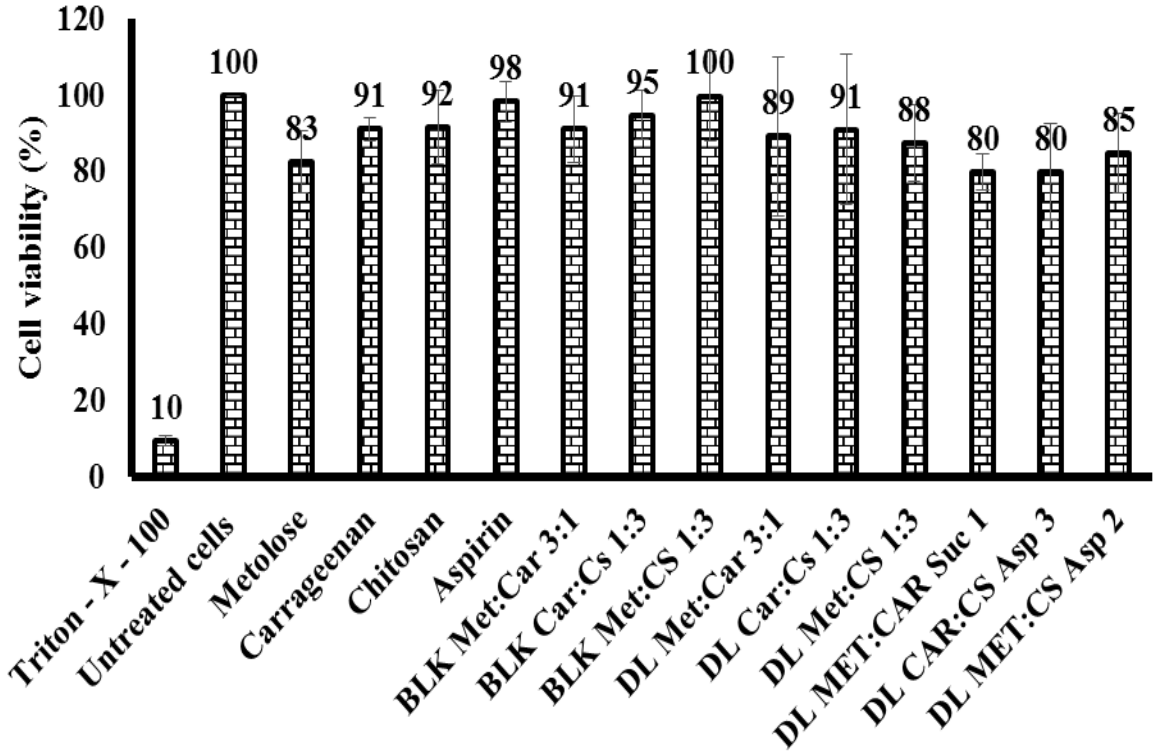


Figure 9. MTT assay results, showing cell viability of pure polymers and pure drug, BDL loaded formulations (non-taste masked), their respective blank (no acetylsalicylic acid *loaded) wafers, taste masked DL wafers, Triton-X-100 and untreated cells (mean ± SD, n = 3) after 24 hours of incubation.*

The results show a clear profile of the cytotoxicity of the pure materials, and the various formulations on adherent mammalian cells with greater than 70% cell viability in all cases. This confirms that the pure polymers, pure acetylsalicylic acid and the drug released from the taste masked DL wafers were non-toxic and can be employed for geriatric drug delivery (Moritz, et al., 2014). This study confirms that acetylsalicylic acid poses no physical threats to endothelial cells when used for potential buccal application in geriatric patients compared with the known toxic Triton-X-100.

In addition to MTT cell viability testing, mucosal irritation caused by formulations meant for local application to the buccal mucosa is important. However, the wafers were designed and formulated using well known and FDA approved mucoadhesive GRAS (generally regarded as safe) polymers of high viscosity with greater flexibility and optimum chain length to avoid mucosal irritation. Among the various mucoadhesive drug delivery systems, buccal wafers and films are better than oral gels due to relatively longer residence time, more flexibility to cover the buccal mucosa and better comfort (Semalty, et al., 2010). Furthermore, the neutral environment of the mouth allows for administration of acidic drugs such as acetylsalicylic acid (Ribeiro Costa, et al., 2019). The salivary pH varies from 5.5 to 7.0 and the wafers produced would therefore not be expected to produce any local irritation to the mucosal surface upon application (Kassem, et al., 2015). However, this will require further investigation in the future in the form of mucosal irritation test, to confirm it definitively.

4. Conclusions

The functional properties of taste masked DL wafers for geriatric delivery, have been characterized. Wafers comprising sucralose and aspartame showed higher hardness compared to their corresponding non-taste masked wafers which was reflected in the SEM, swelling capacity and porosity results. However, adding the sweeteners increased the rate of release compared to the BDL formulations. Both sucralose and aspartame showed similar effect in masking the bitter taste of acetylsalicylic acid while MET:CAR and MET:CS wafers showed more palatability and acceptance because of MET's known taste masking properties. The wafers showed enough drug permeability after release of acetylsalicylic acid through EpiOral^{TM}, porcine buccal tissues and artificial Permeapad^{TM} membrane, which is expected to ensure therapeutic bioavailability and therefore a potentially useful alternative to oral tablets. MTT assay showed that all the wafers were safe for continuous attachment in the cheek region and therefore suitable for geriatric patients. Taste masked DL formulations (DL

MET:CAR Suc 1, DL CAR:CS Asp 3 and DL MET:CS Asp 2) are very promising systems for the delivery of low dose acetylsalicylic acid to geriatric patients with dysphagia.

Conflict of interest

The authors report no conflict of interest

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