DEVELOPMENT OF MEDICATED CHEWING GUM FORMULATIONS FOR THE DELIVERY OF A POORLY SOLUBLE DRUG

PAUL BAHL

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The University of Huddersfield in collaboration with Revolymer®

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I can do all things through Christ who strengthens me...

Philippians IV.XIII

Dedications and Acknowledgements

I would like to dedicate this thesis to my beautiful mother; Kamla Masih Bahl whose continued love, faith, prayers and words of inspiration kept me strong throughout the process and pushed me to succeed. I would like to acknowledge the patience and support of all my family and friends (including my brother Ranjeev, sister Neelam, twin brother Robert and my loyal dog Samson) who have all supported me through my sacrifices.

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Abstract

The concept of chewing gum for medical purposes provides discrete, convenient administration, the potential for buccal absorption and the avoidance of first pass metabolism or gastrointestinal degradation. This work contributes to the limited information available on the release of poorly soluble drugs from medicated chewing gum formulations. Lansoprazole was chosen as a model drug due to its poor solubility and instability (under acidic conditions), thus a chewing gum formulation would be of particular benefit avoiding gastrointestinal degradation.

The solubility and stability of lansoprazole in artificial saliva was found to be dependent on the pH of the solution. An increase in pH caused an increase in solubility with a significant increase between pH 9 and pH 10. At pH 6, concentrations decreased over time confirming the acid instability of lansoprazole. The use of cyclodextrins as solubilisers and stabilisers for lansoprazole were investigated; complexed lansoprazole (with Mβ-CD, 1:1) resulted in a 9 fold increase in solubility compared to free lansoprazole and remained stable at pH 6.

Chewing gum formulations incorporating lansoprazole were prepared and the following excipients were investigated: Revolymer's® hydrophilic polymer Rev7, buffering excipients and complexed lansoprazole (with Mβ-CD, 1:1). Drug diffusion from gum surfaces was found to be limited, highlighting the need for effective mastication to ensure the timely release of the drug. *In vitro* release was evaluated using the EP approved masticator. Various parameters were investigated including: the type of dissolution medium, pH, chew rate and sampling and replacement volumes. Significant differences in release after 30 minutes mastication were found for gums containing Rev7 and potassium carbonate (both of which contributed to increasing the hydrophilic capacity of the gum). These gums were also softer than other formulations due to a plasticising effect on the gum base elastomer resulting in softer, less cohesive gums. Complexation was not found to have an impact on *in vitro* drug release from gums.

The study also assessed the buccal absorption of free lansoprazole and complexed lansoprazole (with Mβ-CD, 1:1) using porcine buccal mucosae. The highest partitioning coefficient was observed for free lansoprazole at pH 6.8 due to a lower ionised fraction in combination with a lower molecular weight. Complexed lansoprazole had the highest drug flux but also had the paradoxical effect of decreasing the permeability coefficient.

Overall the study contributed to increasing the understanding of factors governing the release of a poorly soluble and unstable API, lansoprazole, from a medicated chewing gum formulation. The optimised formulation would contain lansoprazole, 8 % Rev7 and potassium carbonate to provide the maximum release of drug from the gum and also facilitate buccal absorption.

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Glossary of Abbreviations

API	Active pharmaceutical ingredient
BCS	Biopharmaceutical Classification System
BHA	Butylated hydroxyanisole
CD	Cyclodextrin
cLog P	Calculated Log P
COX	Cyclooxygenase
Cpm	Chews *per* minute
DART	Direct Analysis in Real Time Mass Spectrometry
DSC	Differential scanning calorimetry
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medical Agency
EP	European Pharmacopeia
FTIR	Fourier transfer infrared spectroscopy
GIT	Gastrointestinal tract
GORD	Gastro-oesophageal reflux disorder
HPLC	High performance liquid chromatography
IV	Intra venous
K_p	Permeability coefficient
Log P	Partition coefficient
MCGs	Membrane coated granules

MPEG	Mono methoxy polyethylene glycol
MS	Mass spectrometry
Mβ-CD	Methyl beta cyclodextrin
NAB	Nocturnal acid breakthrough
NHT	Nicotine hydrogen tartrate
NMR	Nuclear magnetic resonance
NSAID	Non-steroidal anti-inflammatory drug
ODT	Oral dispersible tablet
PEG	Polyethylene glycol
PG	Propylene glycol
PIB	Poly isobutylene
PIP-g-MA	Poly isoprene-graft-maleic anhydride
PPI	Proton pump inhibitor
PVA	Poly vinylacetate
PVP	Polyvinylpyrrolidone
QC	Quality control
SDS	Sodium dodecyl sulphate
THF	Tetrahyrdofuran
UWL	Unstirred water layer
β-CD	Beta cyclodextrin

Chapter One:

Introduction

1.1 Oral drug delivery route

The oral route is regarded as the most preferred route of drug delivery amongst clinicians and patients due to its convenient method of administration, correlating to high patient compliance and increased patient satisfaction (Shojaei, 1998). One major limitation of the oral administration route is the effect of first pass metabolism and pre-systemic degradation. To ensure therapeutic dosages are maintained after absorption, the dose of affected active can be increased to ensure bioavailability after metabolism. This contributes to a range of problems including possible side effects with the direct contact of high concentrations of the active with the epithelium and also the possible risk of over-dosing.

The opportunity to develop a convenient oral delivery system, which can bypass first pass metabolism (thus avoiding hepatic circulation and associated degradation with acid liable/ susceptible drugs) whilst providing a sustained release delivery system, may be possible using medicated chewing gum as a form of drug delivery.

1.1.1 Drug absorption through the oral mucosa

The purpose of the oral mucosa is to provide a suitable barrier to protect the body from harmful substances; however it can also provide a route for absorption of suitable drugs (see chapter 5). The oral mucosa has been shown to be a reliable delivery route for a range of drugs including glyceryl trinitrate for which a rapid onset of action is required. The mouth and cheeks are lined with an epithelial layer, which provides a permeable membrane for rapid absorption due to its rich vascularity (Figure 1.1). The main absorption of the active occurs through the oral mucosa (buccal and sublingual), which means first pass metabolism can be avoided along with associated enzymatic degradation. This offers opportunity for drugs that are particularly susceptible in the acidic environment in the gastro-intestinal tract (GIT). This increased bioavailability may mean a lower dose can be administered resulting in fewer gastric side effects which may be related to the higher dosages in standard oral tablet formulations (Conway, 2007).

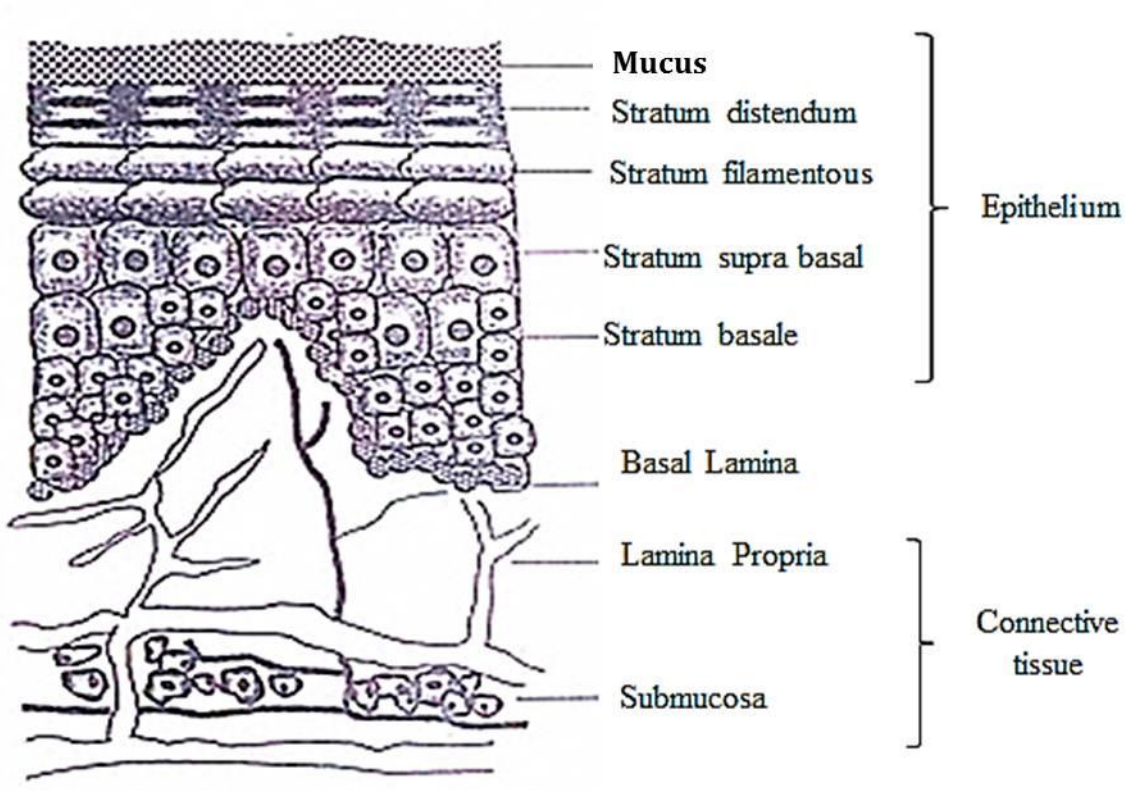


Figure 1.1 Cross section of the oral mucosa

(adapted from Shinkar *et al*., 2012)

1.2 History of chewing gums

Chewing gum as a pass-time has been around for thousands of years with the ancient Mayan Indians chewing chicle sourced from the Sabodilla trees. In 1892, William Wrigley invented his first brand of flavoured confectionary chewing gums "Wrigley's Spearmint", providing the foundations for his thriving business (Rassing, 1996). The concept of chewing gum for medical purposes provides a discrete method for delivery and does not highlight the illness or need for medication for the individual. It can also contribute to delivering medicine on demand during peoples' busy lifestyles (Hyrup *et al.,* 2005). One of the first official oral health gum patents was filed by William F Semple (in 1869), which stated the use of chewing gum for dental hygiene purposes (Khatun and Sutradhar, 2012). The first gum containing a medicated active was patented in 1924, Aspergum® containing acetylsalicylic acid (aspirin) (Biswal and Anantkumar, 2013). One of the most notable recent successes is nicotine replacement gums which have greatly improved the acceptability of medicated gums.

1.3 Chewing gum as a drug delivery system

The right delivery system can significantly impact success by providing product distinctiveness in the market, as evidenced with nicotine gums. An innovative drug delivery system can provide additional benefits to the patient including a discrete and convenient administration as well as the potential for buccal absorption, providing a rapid onset of action. It can also provide new business opportunities for drugs approaching their patent expiry. This has led to interest in the potential of chewing gum formulations containing a range of actives. A review by Chaudhary and Shahiwala lists recent collaborations between Fertin (a market leader in medicated chewing gum development situated in Denmark) and Generex Biotechnology™, focusing on developing a metformin-containing gum for the management of diabetes. Other examples include a functional gum containing *Hoodia gordonii*, a natural ingredient which helps controlled weight loss by releasing a compound (P57), similar to glucose, to stunt the appetite in the hypothalamus (Chaudhary and Shahiwala, 2010).

As a drug delivery system chewing gum has many advantages over other oral administration forms; its main attributes include its convenient manner; being able to chew discretely at any time and any place and the exclusion of the requirement of water. There may also be a particularly high acceptance in the paediatrics market, as some children may be more inclined to chew rather than swallow (e.g. dysphagia). The sensory perception with regards to the flavour, taste and texture/chewability is another important concept. In order to ensure patient compliance, the gum needs to be pleasant to chew and various taste masking concepts may be employed to achieve this, whilst the gum base will be the main contributing factor in the texture of the gum. Replacement of sugar by bulk sweeteners (including polyols e.g. sorbitol and mannitol) in chewing gums has been a successful innovation, improving taste, texture and the healthier image of chewing gum (Khatun and Sutradhar, 2012).

In general gums can be considered as a relatively safe dosage form as the gum can be removed from the mouth as desired to cease treatment. As the gastric mucosa is not exposed to high concentrations of the drug in its solid state, the potential of irritation and intolerance is reduced, compared to the risks involved with some oral formulations (e.g. formulations of NSAIDs). Also if the gum is swallowed, the risks of complications are reduced due to the requirement of masticatory forces needed for drug release. Some risk considerations include

the physical mechanics of increased chewing, including the potential of damaging teeth on hard gums and mandible muscle pain caused by extensive chewing.

Additional functional qualities include the extended shelf life of gums; protection against oxidation can be maintained with the use of a sealed outer coating and the low water content (typically 2-5 % w/w) minimises microbial contamination (Chaudhary and Shahiwala, 2010; Biswal and Anantkumar, 2013).

1.4 Formulation of medicated chewing gums

Gum bases are generally lipophilic, influence the characteristic masticatory texture and feel to the user and typically contribute between 40 – 70 % of the total gum mass. The exact ingredients and formulation of gum bases are usually proprietary information and there is limited systematic technical information in the public domain, but they are normally inert and tasteless (Lee, 2001). Fertin Pharma (Denmark) specialises in the development of gum bases and manufactures Nicotinell® and NiQuitin® on behalf of its partner companies Novartis and GSK (Fertin Pharma, 2003). Revolymer has developed the Rev7™ polymer, gaining approval for human use as an ingredient incorporated into the gum base, up to a maximum of 8 %, conferring additional properties to the gum (2011/882/EU). The specific components of the gum base and the method of preparation are important factors controlling drug release from chewing gums (Morjaria *et al.*, 2004).

The soluble portions of a chewing gum formulation are generally comprised of fillers, sweeteners, flavouring and buffering excipients which contribute to the taste and texture of the gum (hydrophilic capacity can be described as the sum of the water soluble components) (see chapter 1; Table 1.1). Sugar substitutes/sugar alcohols (polyols) such as sorbitol, xylitol, maltitol and mannitol are the preferred sweeteners in sugar-free gums. They are also primarily used as bulking agents, ensuring even mixing resulting in a homogenous gum and can typically contribute between 40 - 60 % of the gum mass. Polyols may agglomerate and recrystallise whilst in the gum and a mixture of polyols can retard/prevent this by increasing dispersibility and reducing the risk of crystal morphology changes upon manufacturing and storage (Smewing, N.D). They vary in sweetness and hygroscopicity and can act as humectants, lowering the relative humidity and slowing the rate of moisture loss. The loss/

absorption of moisture in gum formulations influences the texture and hence the shelf-life of gums (Smewing, N.D).

1.4.1 Conventional/ kettle fusion manufacturing method

The traditional manufacturing method for chewing gums involves the use of a kettle mixer/ Z blade mixer which heats the gum base, resulting in a phase change to molten state allowing for effective mixing (Figure 1.2). Excipients are added in stages and mixed for specific periods of time to ensure a homogenous formulation. The active drug is generally incorporated into the gum base at the start of the process before mixing with other excipients to ensure homogenous distribution throughout the samples (Rassing, 1996). It has been suggested that incorporating the active at the end of the manufacturing mixing process may increase the rate and extent of release, but this may also consequently have a negative effect on homogeneity (Rassing and Jacobesen, 2003). Modifying the form of the active, using blending components (such as aqueous sweeteners which soften the bulk mix and aid in a homogenous blend) and hydrophilic coatings can increase the release rate. Generally flavours are incorporated at the later stages limiting the level of exposure to stress (heat) due to the essential oils having relatively low boiling points and increased are relatively unstable and volatile. Limitations are also associated with using sensitive thermolabile actives as temperatures need to be sufficient to soften the gum base during mixing. The viscous nature of the molten gum base also contributes to difficulties in ensuring a homogenous distribution of active and dose accuracy may be compromised. Once mixed, the use of specialised rollers can be employed to ensure uniformity through consistent form, shape and weight during the later stages of processing (Pagare *et al.*, 2012).

Figure 1.2 The Z blade mixer

Kettles are made in a range of different capacities and this is something that should be considered when scaling up production to ensure adequate mixing.

1.4.2 Direct compression method

This cost effective technique utilises free flowing powders comprising a mixture of polyols, sugars and gum base, which can be directly compressed on a traditional tableting machine, thus reducing manufacturing time and costs (William and Millind, 2012). An example is Pharmagum®, which is available in 3 forms including Pharmagum® M; which has a high gum base content, 50 % more than Pharmagum® S; which consists of gum base and sorbitol and Pharmagum® C containing gum base, mannitol and isomalt. However gums formed using direct compression are generally harder and subject to crumbling during chewing, which may provide an undesirable sensation to the user (William and Millind, 2012).

A 3 layered chewing gum tablet was manufactured by direct compression, containing the active in the internal core, sandwiched between two external protective, anti-adherent layers of maltodextrin, to prevent adhesion to the machine's pistons and also potentially improve taste. The gums were formulated using a range of drugs including ranitidine, caffeine and paracetamol and release was directly correlated with the drugs' physiochemical properties, specifically water solubility and also chewing efficiency and time (Maggi *et al.,* 2005). The 3-layer gum model was investigated further to include formulations containing fenoprofen calcium (100 mg) in the inner core (weighing 1.4 g) and maltodextrin in the two external sandwich layers (0.2 g). Optimised formulations contained freeze-dried complexes of the active with β-CD (1:1) and PVP as a water soluble polymer to enhance release. An *in vivo* study found that relative bioavailability was increased compared to commercial 200 mg capsules (166.06 %) and absorption rates were faster, presumably due to absorption *via* the buccal mucosa and avoidance of hepatic first pass metabolism. Thus, there is the potential to reduce the active administered dose in gum formulations compared to conventional formulations (El-Assassy *et al.,* 2012).

Another method of formulation involves freezing, grinding and tabletting of excipients. The gum base and excipients are maintained in a cooled environment (typically 15 °C or lower) until brittle, then the mixture is ground and mixed to a fine powder. On warming to room temperature, the gum may self-adhere leaving minute air bubbles in the gum matrix.

Alternatively the powdered mix can be blended with additional excipients including binders and lubricants and then compressed (Gavaskar *et al.*, 2011; Khatun and Sutradhar, 2012).

1.4.3 Composition of chewing gums

Within a chewing gum, materials can be grouped as water insoluble portions (gum base) and water soluble components (polyols and sugars) (Table 1.1). The chewing gum base is an important material comprising the majority of the gum's inert and insoluble portion. It is the major masticatory component in the finished product influencing both texture and elasticity (Khatun and Sutradhar, 2012). The composition of the gum, specifically the gum base mass percentage, has shown to be highly influential on the release of the active. A high gum base mass resulted in significantly lower release rates of salicylate compared to a lower gum base percentage. This was due to increasing the hydrophobic portion of the gum altering the lipophilic/hydrophilic capacity of the gum, thus binding lipophilic actives more tightly to the increased lipophilic gum base mass resulting in lower levels of release (Christrup and Rassing, 1988). The hydrophilic capacity of the gum can be described as the sum of all the water soluble components.

Standard gum bases consist of a mixture of elastomers: natural (e.g. chicle gum, jelutong gum and glycerol esters from pine resins) and synthetic resins, plasticisers (natural and synthetic waxes and hydrogenated vegetable oils), emulsifiers, waxes, fats and fillers. These provide the elasticity, softening and cohesion components of the gum. The particle size should be kept below 100 μm to avoid a gritty texture during chewing (Biswal and Anantkumar, 2013).

Table 1.1 Chewing gum components

Water Insoluble Components	**Examples**	**Functions**
Elastomers	synthetic rubbers: butadiene, styrene co polymers, poly ethylene mixtures and poly iso butylene	gum texture, cohesion and elasticity
Plasticisers	rosin esters, lanolin, glycerine, fatty waxes and propylene glycol	textures, binding consistency
Fillers	talc, calcium carbonate and magnesium carbonate	chewability and texture
Water Soluble Components	**Examples**	**Functions**
Softeners and emulsifiers	glycerides	optimise softness of gum
Sweeteners	sorbitol, aspartame and mannitol	sweeteners to taste
Flavourings	essential oils	taste

(adapted from Gavaskar *et al.*, 2011; Khatun and Sutradhar, 2012)

Most medicated gums are designed to release the majority (>75%) of the active at around 20-30 minutes. The release rate needs to be controlled effectively to achieve this. The physiochemical properties of the active (aqueous solubility, pK_a and the potential distribution between chewing gum and saliva) will greatly influence its release (Rowe, 2003).

Hydrophilic actives will generally be released rapidly and to slow release, the gum base content may be increased (increasing the lipophilic/hydrophilic balance of the gum) or encapsulation of the active may also be utilised (Rassing, 1994). If the active is lipophilic, it will be released slowly and incompletely due to adherence to the gum base; it will first partition from the lipophilic gum base into hydrophilic components of the gum before dissolving gradually into saliva.

1.5 Additional health benefits with respect to chewing gum

Various physiological effects have been reported with respect to chewing gum including increasing alertness, easing tension and stimulating salivary flow which increases the pH in the oral cavity resulting in improved dental health *via* a reduction in plaque acidgenicity (Karami-Nogourani *et al.*, 2011). Chewing gum can stimulate an increase in salivary flow

(containing calcium and phosphates), which in turn causes an increase in pH and the buffering capacity of the mouth. This provides a positive effect on enamel demineralisation and remineralisation, with the bicarbonates contained in the stimulated saliva also helping to neutralise plaque acid.

Chewing gums also increases the volume of saliva production; initial studies showed chewing sugar sweetened gum resulted in a total salivary volume of 32.2 mL, compared with chewing sorbitol sweetened gum (29.5 mL) and chewing paraffin 7.4 mL as a control (Frohlich *et al.,* 1992). This can also prove beneficial in the management of dry mouth (xerostomia) caused by hyposalivation. Other inherent effects reported include stress relief, weight management and improved concentration and focus (Chaudhary and Shahiwala, 2010). Working memory, tested by cognitive memory and recognition tests, was seen to improve during chewing of gum, based on a study on 75 healthy volunteers (Wilkinson *et al*., 2002). The study was later further expanded and it was suggested that memory was improved *via* insulin secretion, promoting glucose uptake, in response to chewing in food anticipation and the possibility of activation of neural pathways linked to memory and chewing (Scholey, 2004).

There still may be some social and behavioural concerns over the acceptability of chewing gums as novel drug delivery vehicles. This may involve the ability to chew with elderly patients especially with orthodontic appliances and also the attitude to chewing gum overall. The elderly population may consider chewing gum to be rude and ill-mannered due to the decreased exposure in their generation. A questionnaire by Fertin examined opinions regarding novel medicated chewing gums and resulted in positive feedback from younger generations. End users of the survey expressed special interest in the following properties:

1.6 Examples of medicated chewing gums

A range of experimental formulations incorporating different actives have been studied or marketed including recent trends for functional ingredients including nutrients and vitamins (Table 1.2). A novel anti-microbial decapeptide (KSL) has shown success in pre-formulation studies suggesting it has potential application in a chewing gum formulation to inhibit dental plaque growth with *in vitro/in vivo* releases of 70 - 80 % over 20 minutes chewing (Hee Na *et al.,* 2005). The potential use of chewing gums for anti-plaque and other oral diseases is practical due to the gum preparation having extended residence time in the oral cavity. Other

recent work includes the incorporation of probiotics (*Streptococcus salivarius*) into a gum used to treat throat to ear infections and bad breath (Fayermann, 2010). This suggests that chewing gum formulations may provide an effective delivery route for macro molecules to the oral cavity and buccal membrane.

Table 1.2 Worldwide marketed medicated gums

Active	Trade name & market	Indications
Calcium carbonate	Chooz® , USA	neutralise stomach acid
Vitamin C	Endykay Vit.C®, UK	general health
Caffeine	Stay alert®, USA	alertness
Guarana	Buzz gum®, UK	alertness
Fluoride	Fluorette®, USA	cariostatic
Dimenhydrinate	Travvel®, USA	motion sickness
Xylitol	V6®, UK	dental caries
Chlorhexidine	Vitaflo Chx®, USA	tooth decay
Nicotine	Nicotinell®, UK	Smoking cessation

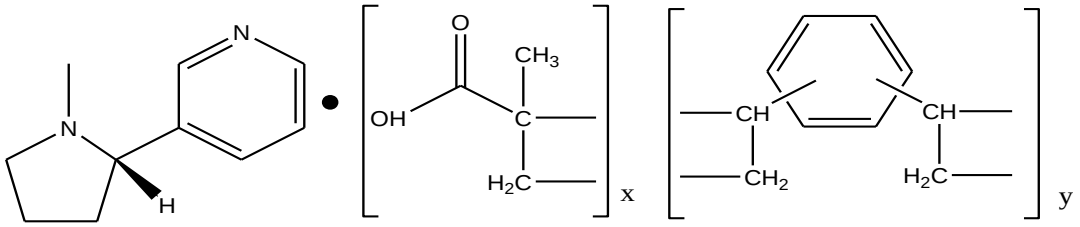
(adapted from Pagare *et al.*, 2012)

1.6.1 Nicotine replacement gums

Nicotine is the main active ingredient that reinforces smoking behaviours due to its physiological effects on dopamine in the brain, therefore when people cease smoking nicotine withdrawal symptoms are observed (Aslani and Rafiei, 2012). The acceptance of medicated gums has been influenced by the success of nicotine gums used for smoking cessation. Nicotine replacement gum was first registered in 1978 and was available without prescription in 1995 (Khatun and Sutradhar, 2012). Gums are available in two dosages: 2 mg aimed at light smokers who want to avoid cravings for cigarettes and 4 mg nicotine gums targeted to give an initial rush of nicotine to satisfy the needs of heavier smokers. Users are advised to chew until the desired effect is achieved, the gum is designed to satisfy the initial craving at the start of the chewing process and then give sustained release for the duration of chewing. Nicotine 2 mg gums have a combination of sodium carbonate and sodium bicarbonate as an internal buffer, whereas 4 mg gums use sodium carbonate. This may be to raise the local pH to facilitate buccal absorption and also to mask the bitter taste. A dosing regimen of between 10-20 gums per day is advised with a gradual decrease over a period of 3-6 months. Popular

brands include Nicorette® (GlaxoSmithKline) and Nicotinell® (Novartis) available in a variety of flavours including: mint, cinnamon, fruit and original; with coatings to aid in masking the bitter taste of nicotine. Specific flavours can affect individuals differently, specifically with salivary flow rate, in response to mechanoreceptors (mechanical) and gustatory (taste) stimuli (Karami-Nogourani *et al.*, 2011). In a patient study evaluating trial formulations of nicotine gums, cherry and eucalyptus trial formulations were preferred with aspartame as the sweetener to modify taste (Aslani and Rafiei, 2012). Shiffman (2009) identified the need for optimisation in effectively controlling release from commercially available gums to ease withdrawal symptoms by providing faster craving relief. The study evaluated the initial phases of chewing test gums *in vivo* by measuring early rises in plasma levels of nicotine. The optimised formulation (NHTG2) had a burst effect in the first ten minutes of chewing, delivering more nicotine compared to other formulations tested, including commercial Nicorette® FreshMint™. It was suggested that the controlled release mechanism was an interaction between gum base, nicotine and the buffering system, potassium carbonate (Shiffman *et al.*, 2009).

Nicotine is freely soluble in water with pK_a values of 3.12 and 8.02. This contributes to a rapid release and thus there is a need for a controlled release preparation to provide sustained release for effective therapy using a gum. To achieve this, nicotine is bound to polacrilex to ensure sustained release necessary to facilitate effective buccal absorption providing relief of nicotine cravings (Figures 1.3 and 1.4). This also increases the distribution of nicotine ensuring even mixing of excipients. The first step in nicotine gum formulation is forming the nicotine cation exchange complex (nicotine polacrilex); a mixture of the ion exchanger (Amberlite IRP 64M) and nicotine. Amberlite IRP 64M contains weak acidic methacrylic acid polymers which bind to nicotine. The loaded nicotine polacrilex is added into the gum mixture as a pre mix (Rassing, 1996).



nicotine ion exchange resin nicotine polacrilex

Figure 1.3 Chemical structure of nicotine polacrilex

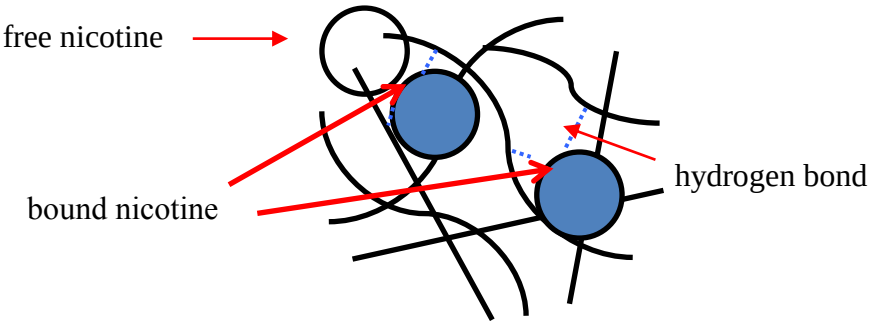


Figure 1.4 Schematic representation of nicotine polacrilex

(Images supplied by Revolymer®, 2011)

1.6.1.1 Taste masking using buffering excipients

The mastication of early nicotine chewing gum formulations resulted in a salivary pH of between 3 – 5. This caused an uncomfortable bitter, burning sensation to the individual. Lichtneckert (1973) reported in his patent that the addition of water soluble buffering agents such as alkali bicarbonates and hydro carbonates resulted in a salivary pH of 7.5 (usually between 8 -10) compared to pH 6.7 (normal saliva). This masked the taste and also improved bioavailability by increasing the availability of free base (non-ionised) nicotine readily available for absorption, ensuring nicotine was absorbed at a steady state (Lichtneckert *et al*., 1973).

1.7 Controlling release from chewing gums

The initial development processes for a medicated gum can be tailored specifically to the active and the required release profile to ensure optimal concentrations are released into saliva. *In vitro* testing is a compendial requirement and can provide insights into discriminatory formulation factors which influence release of the active. An optimal release rate can be tailored to facilitate buccal permeability and subsequent systemic absorption, using modifying systems, e.g. embedding /coating techniques (Pagare *et al.*, 2012). Information in monographs regarding *in vitro* testing is limited and is mainly related to QC applications (Gajendran *et al.*, 2012). Once *in vitro* release is established for a range of conditions to aid in the formulation of optimised gum formulations, it can be used to predict *in vivo* release before the gum is tested by subjects during relevant chew out studies in product developmental stages.

Final developmental stages tend to specific market needs such as increasing compliance through optimising patient acceptability (Karami-Nogourani *et al.,* 2011). The use of emulsifiers can optimise chewability and mouth feel, whereas the use of flavouring oils can improve taste (Pagare *et al*., 2012). CDs have been successfully applied to a range of drugs and foods to mask the taste of bitter actives and to increase patient compliance (Szejtli and Szente, 2005). Any changes to the formulation/manufacturing process are likely to impact the release of the drug and there is little information available in the literature on this.

Nutravail Technologies formulated a bi-phasic nicotine delivery system (NHT gums) to address the initial craving periods of nicotine withdrawal by providing more rapid therapeutic effects to the users in order to improve compliance. The formulation contained a unique gum base and increased levels of buffering excipients to facilitate buccal delivery, resulting in an initial burst release followed by a sustained release period. The study compared commercially available Nicorette® (which contains nicotine polacrilex) with a formulation containing nicotine hydrogen tartrate in a rapid release formulation (NHT gums). The design aimed to increase compliance by balancing sensory effects with increased pharmacokinetic characteristics. The randomised crossover study evaluated the early absorption kinetics (after 10 minutes chewing) of nicotine gum formulations containing different buffering excipients. There was a significant increase in nicotine uptake over the first 10 minutes for gums containing potassium carbonate. Nicotine levels were found to significantly rise above baseline after 4 minutes for gums containing potassium carbonate, after 6 minutes for gums containing sodium carbonate and after 8 minutes for commercial Nicorette®. The study concluded that buffering excipients can be used to effectively control nicotine release rates from medicated gums with potassium carbonate resulting in the most rapid release rate (Shiffman *et al*., 2009). The user sensation was also reported (*via* patient surveys) to be improved compared to previous formulations and was noted to be comparable to confectionary gums with regards to texture and chewability. The authors also recommended normal chewing techniques without the need for the parking strategy.

Coatings have also been used to modify release of actives from gum formulations. A polyvinyl acetate gum base was coated with insoluble acrylic polymers, *In vivo* chew out studies found that the PVA matrix, in combination with coatings, retarded and controlled the release of highly soluble catechins during mastication from gums (Yang *et al*., 2004).

Specific modifying systems can also be used to optimise the drug release. For highly soluble drugs, a sustained release profile may be achieved by a range of modifying systems including:

- Ion exchange resins, e.g. styrene-di vinyl benzene
- Embedding, encapsulation/coatings e.g. spraying with hydrophilic/phobic coatings
- Granulation-mixing with components e.g. polymers
- Solubilisation- adding emulsifying components
- Reducing particle size

(Rassing, 1994; Gavaskar *et al*., 2011)

1.7.1 In vitro release from chewing gums

It has been over 30 years since medicated nicotine replacement gum was registered and its success has been instrumental in the inclusion of relevant specific monographs in pharmacopoeia. In 1991, the European Pharmacopoeia (EP) first defined a medicated gum as: "A solid, single dose preparation consisting of gum that is intended to be chewed but not swallowed, providing a slow steady release of the medicine contained after which it can be discarded" (European Pharmacopeia, 1998). Further relevant monographs were expanded to briefly describe the preparation of chewing gums and more specifically suitable modified dissolution (chewing) testing apparatus in the 3rd edition of the European Pharmacopeia in 2000 (Khatun and Sutradhar, 2012).

The release of active from a medicated gum is similar to an extraction process and due to the complex mechanisms governing release there are minimum experimental *in vitro* settings proposed by regulatory bodies (Gavaskar *et al.,* 2011). *In vitro* drug release testing can provide information on the influence of excipients on drug release and is a powerful screening tool in product development and optimisation in predicting *in vivo* performance (Gajendran *et al.,* 2012). *In vitro* release is influenced by mechanical forces such as the chewing rate, providing the necessary shearing forces to expose new surfaces for contact with

the dissolution medium. In general, drug release is proportional to the aqueous solubility of the drug and the chew rate and inversely proportional to the mass of gum base. Other contributing factors include temperature, wettability and water permeation (Pagare *et al*., 2012). There is normally a residual amount of drug expected in the chewed cud and this can be utilised as a tool for estimation of release by measuring residual content after *in vivo* chew out studies.

The official EP compendial chewing apparatus (apparatus A) was designed by Christrup and Moller. Successful *in vivo in vitro* correlations of the release of water soluble actives from chewing gums have been published using the apparatus (Christrup and Moller, 1986). An alternative chewing gum release apparatus (apparatus B) with further adjustable settings including temperature, twisting angle and jaw distance was later proposed (Kvist *et al*., 2000). Release from a range of formulations using official EP compendial apparatus A and apparatus B has shown correlations with *in vivo* chew out studies. Both apparatus are suitable for predicting drug release from medicated gums but it has been suggested that apparatus B may be more discriminating (due to the greater degree of fine tuning controls) and so is recommended for formulation optimisation (Gajendran *et al.*, 2012). Specific release information parameters are not generally available on the public domain and are used solely for formulation development. Further product quality control performance tests can critically assess manufacturing variations for chewing gums and include content uniformity and uniformity of mass tests (William and Millind, 2012). This is described in further detail in chapters 2 and 4.

1.7.2 Drug release and absorption from chewing gums

The active is released from the gum by chewing (masticatory forces) and this provides renewable surfaces for contact between saliva and gum. Saliva dissolves through the gums hydrophilic components and subsequently the drug partitions out of the gum and dissolves into the saliva entering the oral cavity (Figure 1.5).

The EP approved masticator replicates these conditions *in vitro*. After dissolution of the active into saliva it is available for local treatment in the mouth or it can be absorbed directly through the buccal mucosa or sub-lingual routes for a systemic effect *via* the jugular veins, reducing the lag time for onset. This is described in further detail in chapter 5. The absorption

of aspirin, caffeine and dimenhydrinate from chewing gums has been shown to be faster than from standard tablets (Khatun and Sutradhar, 2012). Any active swallowed will be dissolved in saliva and so will be bioready upon absorption (bypassing the disintegration and dissolution steps normally encountered with conventional tablets). Once in the blood, the active can be transported to the necessary receptor sites (Chaudhary and Shahiwala, 2010).

It is generally thought that there are three main factors which can influence drug release from chewing gums:

- Consumer controlled- e.g. chew time, frequency, intensity and salivary flow
- Production processes –specific composition and manufacturing processes
- Drug related- physiochemical properties (aqueous solubility, pK_a)

(Imfeld, 1999)

In order to facilitate permeability, a concentration gradient must be maintained in the buccal cavity for a period of time to drive absorption *via* passive diffusion mechanisms. Therefore variations in bioavailability from medicated gums can be explained by differences in patients' chewing patterns, time, frequency and intensity, swallowing variations and also saliva production. This can be reduced by patient training and instructional guidelines for proper chewing to maximise release and subsequent absorbance. Based on questionnaires, the average chewing time has been reported as 36 minutes (80 % of the population had a 20 minute chew time) and the average chew rate was 60 chews *per* minute (Barabolak *et al*., 1991). This formed the basis for the recommended guidelines for investigating *in vitro* release from chewing gums with settings of 60 chews *per* minute for a 30 minute mastication period.

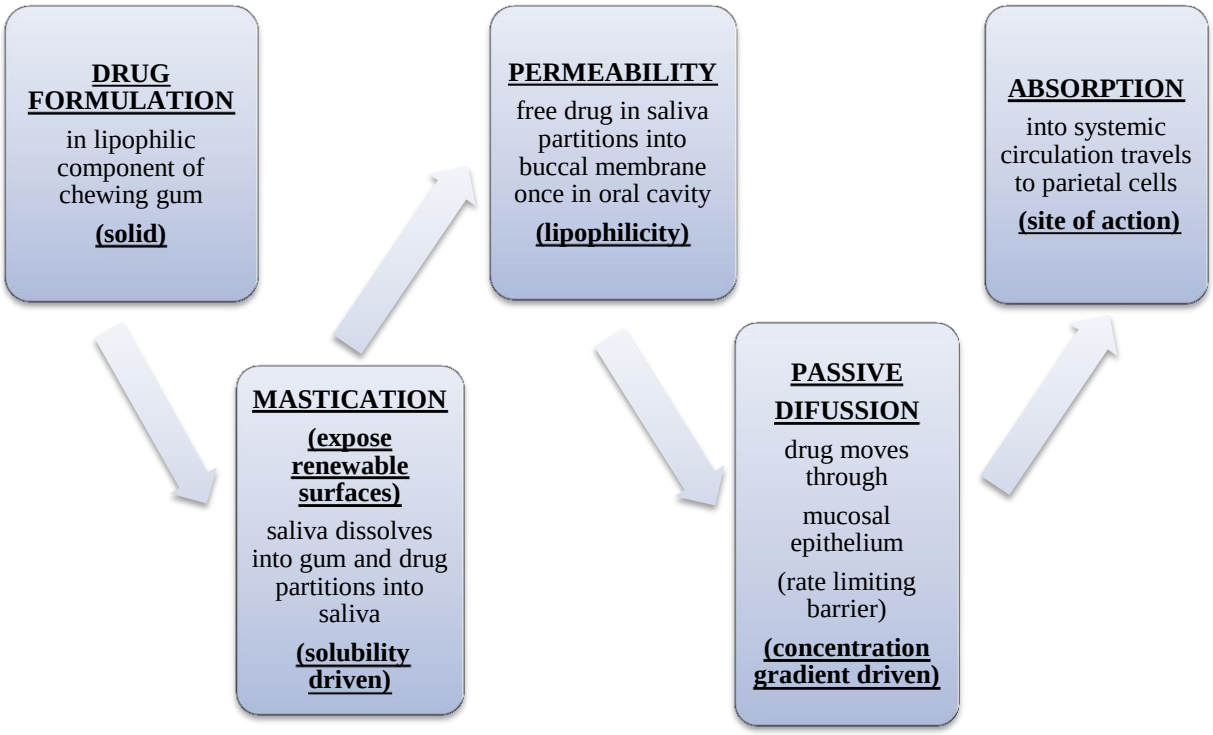
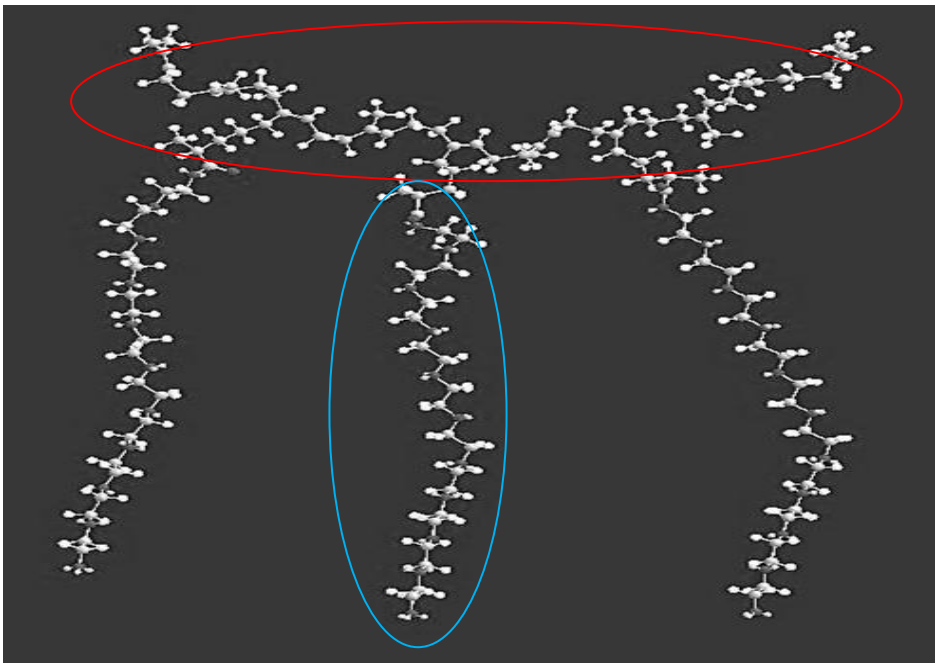


Figure 1.5 Process of *in vivo* drug movement from gum formulation to site of action

1.8 Rev7 polymer in chewing gum formulations

Revolymer® identified problems associated with the disposal of the waste material after chewing gum. Taking into consideration that 13.2 million Kg of gums were sold in the UK in 2010, this can lead to serious problems to keep the environment clean and gum cud-free. It is estimated that it costs about 3p to make a single gum unit, but costs 10p for its removal from unwanted surfaces such as streets. This led to fines for littering of gums to take this into account. It has been estimated that the annual cost for gum removal in the UK is £150m (statistics supplied by Revolymer®, 2010).

Revolymer® has developed a specific amphiphilic polymer Rev7™; when incorporated in to a chewing gum base it can provide functional benefits including increased chewability. The patented formulation of Rev7 has multi-factorial functions including increasing the hydrophilic component of the gum further control the release of actives as well as improving removability of the gum cud after usage (Farber *et al.*, 2009).



RED Hydrophobic backbone (PIP)

BLUE Hydrophilic grafts (MPEG)

Figure 1.6 Incorporation of amphiphilic graft copolymer into the Rev7

(Figure supplied by Revolymer®, 2012)

1.8.1 Structural chemistry of Rev7 polymer

Rev7 is a synthetic polymer and consists of branched polymers of mono methoxypolyethylene glycol (MPEG) grafted onto poly isoprene-graft-maleic anhydride (PIP-g-MA), and unreacted MPEG (< 35 %). The MPEG units are grafted onto the polymer backbone to impart hydrophilcity (Figure 1.6) (Farber *et al*., 2009). This allows the surface chemistry to be modified and water to form a layer around the gum. Any bonds formed to surfaces can be easily removed by street cleaners and passing pedestrians, without the need of specialist equipment. The polymer also provides an improved chewy texture and a feeling of softness to the gum.

1.8.2 Revolymer® gum base composition

The gum base (which incorporates Rev7) contains:

- Elastomers (water insoluble polymers) **poly-isobutylene; a synthetic rubber (**PIB) and polyvinyl acetate; a thermoplastic resin (PVA) - this gives the gum its texture and rubbery nature (Figure 1.7).
- Plasticisers - used to soften the gum base and provide its chewy characteristics e.g castor oil or gylcerol
- Waxes – used to soften the gum and also contributes to its elasticity

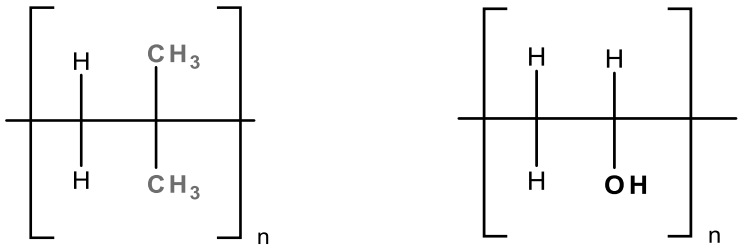


Figure 1.7 Chemical structure of PIB and PVA

1.9 Lansoprazole

It has been estimated that more than 40 % of new drug candidates are lipophilic and have poor solubility. Lansoprazole (Figure 3.1) belongs to class II of the Biopharmaceutical Classification System (BCS) having low solubility and high permeability. It is a gastric acid inhibitor, which prevents the final step in acid production in the stomach. It is a weak base and is commercially marketed as a racemic mixture with both enantiomers inhibiting gastric acid production (Landes *et al.*, 1995). The European Medical Agency (EMA) has identified lansoprazole as a drug in need of reformulating especially for use in paediatrics; a medicated gum formulation may be particularly suitable for this market (European Medical Agency, 2007). Generic manufacturing companies of lansoprazole in the UK include: Actavis UK Ltd® and Zentiva which produce 15 and 30 mg gastro resistant capsules and Zoton FasTab® preparations manufactured by Pfizer Limited.

(http://www.medicines.org.uk/emc/search/lansoprazole)

Lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) belongs to a class of compounds called proton pump inhibitors (PPIs) which inhibit gastric acid secretion regardless of the primary stimulus. PPIs are commonly used in the treatment of acid-related diseases, with the first introduction of omeprazole in 1989. This led the way for other PPIs including lansoprazole (1995), pantoprazole (1997) and rabeprazole (1999) (Shi and Klotz, 2008). PPIs are used in the therapy of gastric and duodenal ulcerative diseases, for the treatment of the heartburn and other symptoms associated with gastro oesophageal reflux disease (GORD), for the treatment of erosive oesophagitis (food pipe injuries) and long term treatment of pathological hyper secretory conditions, such as Zollinger-Ellison syndrome (Horn and Howden, 2005). The majority of gastric acid disorders are caused by an imbalance between pepsin and acid production (aggressive factors) and mucus bicarbonate and prostaglandin production (defensive factors) (Taneja and Gupta, 2002). The ability of anti-secretory drugs to heal duodenal ulcers is directly correlated to their ability to maintain a pH above 3, accumulation in the most acidic place in body (parietal cells) and the acid- catalysed conversion due to specific chemistry of the drug (Tutunji *et al.,* 2006). A pH of above 4 is required for healing of gastric ulcers and erosive oesophagitis (Kinner, 2012).

The key mechanism of action of the PPIs involves direct inhibition of the proton pump (H^+/K^+ adenosine triphosphate) *via* an enzyme present in the gastric parietal cells. These drugs are metabolised in the parietal cells to the active sulfenamide metabolite that directly inactivates the sulfhydryl group of the proton pump and thereby reducing hydrogen ion secretion. Absorption of the PPIs takes place in the proximal small intestine (Horn and Howden, 2005). PPIs will only inhibit actively acid secreting proton pumps and different PPIs bind to different sites on the proton pump which may explain differences in potency; with rabeprazole known to be the most potent and pantoprazole as the least (Kinner, 2012).

All PPIs undergo hepatic metabolism and are extensively protein-bound (> 95 %). All of the currently available delayed-release PPI's have a short elimination half-life ($t_{1/2}$) of between 1 and 2 hours. All formulations have similar pharmacokinetics with the only difference being in bioavailability in the initial days of oral dosing (Horn and Howden, 2005).

Table 1.3 Relative bio availabilities of the PPIs

	Esomeprazole	Lansoprazole	Omeprazole	Pantoprazole
Absolute bioavailability (%)	64-90	>80	40	77
Time to peak plasma level (hours)	1.5	1.7	0.5-3.5	2-4

(Horn and Howden, 2005)

Lansoprazole has an absolute bioavailability of greater than 80%, time to peak plasma level is 1.7 hours, plasma half-life is 1.5 hours and it is 97 % protein bound (Table 1.3) (Horn and Howden, 2005). Lansoprazole is the only PPI suitable for children as young as one year old and is recommended for administration with fruit juices or applesauce. The recommended duration of treatment is short term for between 4 – 8 weeks. The effects are dose related and can last up to 24 hours as the elimination rate does not affect its acid suppression. Current medical information leaflets recommend consumption in the morning before food on an empty stomach to aid in absorption.

Table 1.4 Physiochemical characteristics of lansoprazole

Characteristics	**Drug candidate: Lansoprazole**
Appearance	White to off white, odourless powder
Thermal stability	Around 170 °C
Solubility	Practically insoluble in water Sparingly soluble in ethanol
Absorption	Oral dosing: C_{max} =1.7 hours
Half life	1.5 hours
pK_a	1.33, 4.15 and 8.84

The physiochemical properties of lansoprazole particularly the thermal stability (required during the kettle mixing method) indicate that it would be suitable for formulation into chewing gums (Table 1.4). The characterisation of lansoprazole is described in detail in chapter 3.

1.9.1 Pathophysiology of ulcers

Peptic ulcers can result from high levels of acid or pepsin in the gastric juice which can cause a discontinuity in the thickness of the duodenal or gastric mucosa. The reasons for reflux of acid, pepsin or bile from the stomach into the oesophagus can be multi-factorial and caused by lower oesophageal sphincter relaxations and reduced tone as well as abnormal acid clearance. One common described symptom of GORD (20 %) and peptic ulcers (10 - 15 %) is dyspepsia, defined as a recurrent or persistent discomfort or pain in the upper abdomen. The prevalence of people affected by peptic ulcers at some point is around 10 % of the population in developed countries (Kinner, 2012). The two main types of peptic ulcers are associated with aspirin and (NSAIDs: COX inhibitors can reduce mucosal prostaglandin production) and also those associated with *Helicobacter pylori (H. pylori)*, gram negative spiral bacterium found in the gastric antrum of the stomach. The vast majority (95 %) of duodenal ulcers and 80 - 85 % of gastric ulcers are associated with *H. pylori* (Kinner, 2012). The pathophysiology of ulcers caused by *H. pylori* involves tissue damage caused by activation of the inflammatory cascade which alters gastrin (the hormone involved in stimulating gastric acid) homeostasis; causing hyperacidity.

Lansoprazole demonstrates efficacy and a mechanism of action as an effective anti-microbial activity against *H. pylori.* Lansoprazole was 4-16 times more effective against *H. pylori* compared to omeprazole and pantoprazole and inhibited 90 % of organisms from 58 clinical isolates tested (Gremse, 2001). It was suggested that the secretory response is actively involved in host defence mechanisms (Kinner, 2012). Lansoprazole can be combined as part of a triple therapy for the eradication of *H. pylori*, example regimens include lansoprazole (30 mg) combined with clarithromycin (500 mg) and amoxicillin (1 g) twice daily.

1.9.2 Epidemiology, prevalence and indications of ulcers

Heartburn occurs in 20 % of the population on a weekly basis in developed countries (Kinner, 2012). Lansoprazole is licensed in over 60 countries including the US, UK, Europe, Japan and Canada with worldwide sales of $3.9 billion in 2000. It is considered to provide faster relief and more effective/superior treatment options than H_2- receptor antagonists such as ranitidine. After 2 weeks of treatment with lansoprazole (30 mg/day) a healing rate of 74-78 % was observed for duodenal ulcers compared with a healing rate of 46 - 60 % with

ranitidine (300 mg/day) (Landes *et al*., 1995). Lansoprazole (30 mg/day) has also been found to heal duodenal ulcers at a faster rate than omeprazole (20 mg/day) having an increased healing rate of 74 % compared to 58 % respectively over 2 weeks (Landes *et al*., 1995). Lansoprazole is indicated for long term treatment of healed reflux and ulcer disorders and also for hypersecretory conditions such as Zollinger-Ellison syndrome (caused by gastrinomas) resulting in an increased number of parietal cells in the stomach causing gastric mucosal hypertrophy and increased basal gastric acid secretion (Gremse, 2001).

1.9.3 Mechanism of action of lansoprazole

Following oral administration of the prodrug lansoprazole, due to the drug's (weak base) affinity, it is converted under the acidic conditions of the parietal canaliculus (pH close to 1), (Figure 1.8) (Pearce *et al.,* 1996). It is rearranged by an acid catalysed conversion to a tetracyclic cationic sulfenamide active form and into a di-sulphide inactive form. The sulfenamide form covalently binds *via* di sulphide bonds to critical sulfhydryl groups (critical cysteines 813/822) on the H^+/K^+ -ATPase which causes prolonged inhibition of the proton pump at the secretory surface of gastric parietal cells (Horn and Howden, 2005). The amount of drug accumulated for conversion depends on the proton gradient between the cytoplasm of the parietal cell and the secretory canaliculus. The rate of conversion to active metabolite depends on the acidity of the intracellular space. Therefore the rate and extent of acid suppression depends on the acidity of the parietal cells, the duration of effective concentrations in the canaliculus and stability of the di-sulphide bond formed between the drug and proton pump (Bell *et al.,* 2001). The conversion is irreversible and inhibition is dose related, causing a prolonged effect of gastric inhibition; 85 % inhibition after a 30 mg morning dose and 90 % after 7 days continuous treatment. This inhibits both basal and meal stimulated acid output and volume secretion due to the combined inhibition of histamine, gastrin and acetylcholine (Bell *et al.,* 2001). The reduction in gastric acid secretion results in a negative feedback mechanism causing an increase in serum gastrin levels. The increase in pH of the stomach causes a reduction in stomach pepsin activity along with increases in serum pepsinogen (the precursor to pepsin) levels in response to meal stimulation under basal conditions. This affects both central and peripheral mediated gastric acid secretion due to the signalling pathway convergence of the different stimuli receptors at the proton pump. This contributes to an increase in the mean gastric pH and duration that the gastric pH remains above pH 3-4, which allows for subsequent healing (Taneja and Gupta, 2002).

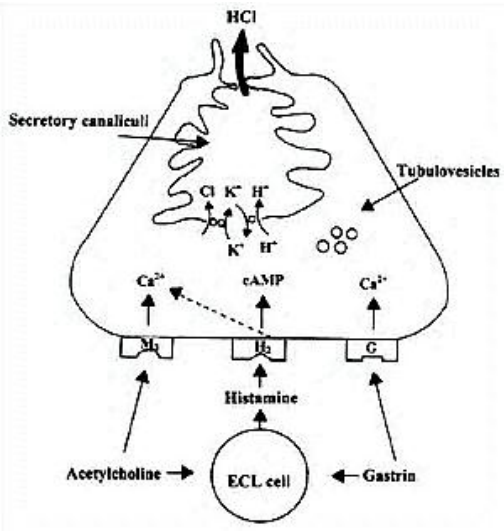


Figure 1.8 Parietal cell

(adapted from Kinner, 2012)

1.9.4 Pharmacokinetics of lansoprazole

To prevent acid degradation of the acid labile drug, lansoprazole is administered orally as enteric coated granules to prevent acid decomposition and increase bioavailability. This can delay absorption with a maximum plasma concentration after 2-3 hours. The pH of the GIT is variable, dependent on times and physiological conditions (fed/fasting states) and this can lead to variations in pharmacokinetic profiles in individuals. Absolute bioavailability is 80-84 % after absorption in the small intestine. Bioavailability is decreased (27 % reduction in bioavailability) when administered within 30 minutes of food compared with fasting states (Gremse, 2001). However bioavailability is inconsistent due to the variation of the genotype of CYP 2C19, acid degradation and limited water solubility (Lu *et al*., 2012). Onset of action is seen within 2-3 hours with an increase in gastric pH following a 15 mg dose. Inhibition is dose dependent with the concentration of active metabolites in the parietal cells contributing to the anti-secretory efficacy. Serum concentrations may be increased twofold following morning dosing regimens compared to evening. Therefore it is suggested to take lansoprazole before breakfast and before meals to take advantage of the meal induced activation of the pump. Its duration of action is 24 hours with no rebound actions following discontinuation (Bell *et al*., 2001).

Lansoprazole is distributed in the tissues of the parietal cells with an apparent oral volume of 0.5 litres per kilogram following 30 mg administration and is 96 % albumin protein bound (Maisch and Smith, 2003).

Lansoprazole is extensively metabolised in the liver by cytochrome P450 (enzymes CYP3A4 and CYP2C19 *via* 5- hydroxylation and sulfoxidation) to two main inactive metabolites; 5 hydroxylansoprazole and lansoprazole sulfone (Figure 1.9). These can be measured in plasma and are non-pharmacologically active. In the acidic environment of the parietal cells the pro drug metabolites are converted to its active sulphonamide derivatives (AG-1812 & AG-2000) which directly inhibit the H^+/K^+ -ATPase the terminal step in the acid secretion pathway (Maisch and Smith, 2003). The CYP2C19 shows genetic polymorphs yielding poor and extensive metabolisers (Niioka *et al.,* 2008). Inter individual variability is greater in men compared to women (Pearce *et al.,* 1996).

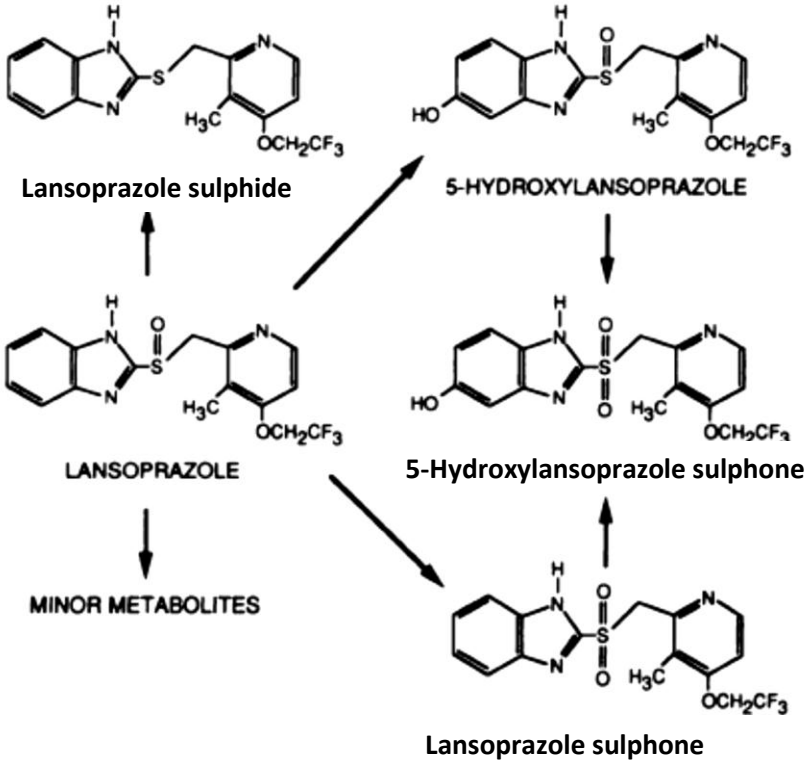


Figure 1.9 Major metabolites of lansoprazole (Pearce *et al.,* 1996)

The half life is 1.5 hours under normal renal functioning, 1.9 - 2.9 hours in elderly patients and 3.2-7.2 hours with hepatic function impairment.

1.9.5 Side effects of lansoprazole

Common GI side effects occur in < 5% including nausea, diarrhoea (caused by infection of *Clostridium difficile*) and stomach or abdominal pains have also been reported. Non GI-side effects include headaches and dermatological effects with a frequency of 1-3 % (Kinner, 2012).

1.9.6 Current formulations of lansoprazole

At 25 °C the degradation half-life of lansoprazole is approximately 0.5 hour at pH 5.0 and approximately 18 hours at pH 7.0 (Ekype and Jacobsen, 1999). The polymeric enteric coating protects the acid labile drug from acid degradation and only dissolves at pH > 6 to allow absorption in the alkaline upper region of the small intestines. Initial formulations of lansoprazole were marketed by Takeda/TAP Pharmaceutical products and were available as 15 mg and 30 mg delayed release capsules; enteric coated granules were filled in a hard gelatin capsule intended to be swallowed whole.

Other modified formulations included delayed release orally disintegrating tablets (ODT); SoluTab® (US) (Abbott Laboratories) and Zoton FasTab® (UK) (Pfizer Ltd) and also as packet powders for suspensions when added to water. These flavoured formulations rapidly dissolve (< 60 seconds) releasing the gastro-resistant micro-engineered granules which are swallowed. The ODT formulations were found to be bioequivalent to capsules (Iwasaki *et al.*, 2004).

Micronised particles have been proposed to increase surface area and the possible dissolution rate of lansoprazole. It was suggested that the drug can be stabilised using a combination of carbonate salts (sodium carbonate and bicarbonate (1:1 molar ratio)) to neutralise the pH of the gastric fluid. Sodium bicarbonate (10 mL of 8.4 % solution) was sufficient to neutralise the gastric acid and to avoid drug degradation, however side effects included flatulence due to the production of gases with excess carbonates (Taneja and Gupta, 2002).

An intravenous (IV) formulation was under review by TAP Pharmaceuticals, with the pharmacokinetic and pharmacodynamic profile being equivalent to oral forms with no significant differences in pH after 5 days of therapy. The IV route raised the pH significantly

higher after 1 hour and maintained a pH above 4 for a longer duration after administration compared to oral dosing (Maisch and Smith, 2003).

Some modified suspensions have been developed utilising sodium bicarbonate to reduce acid degradation. Lansoprazole (3 mg/mL) was stable for 8 hours at 22 °C and 14 days at 4 °C compared with omeprazole (2 mg/mL) which was stable for 14 days at 22 °C and 45 days at 4 °C (as suspensions in 8.4 % sodium bicarbonate solution when stored in amber coloured plastic syringes) (DiGiacinto *et al.*, 2000). This technique was successfully used for omeprazole in an immediate release formulation, Zegerid® developed by Santarus Inc and available for sale in the US. (Small, 2005). The powder formulation for oral suspension combined the PPI (available in 40 mg and 20 mg doses) with the antacid sodium bicarbonate (460 mg; to increase stability). The formulation also contains a suspending agent to avoid precipitation once mixed with water. The suspension is stable at room temperature for up to 2 hours, however it is recommended that each dose is prepared immediately before administration. Pharmacokinetic testing showed the preparation rapidly increased pH, protecting against degradation and eliminating the need for enteric coatings and allowing rapid absorption within 30 minutes (Small, 2005). The preparation was significantly effective at reducing nocturnal gastric acidity when dosed before bedtime compared to pantoprazole. The preparation targeted nocturnal acid breakthrough (NAB) as 80 % of individuals experiencing heartburn have symptoms at night, during the first half of the sleeping period, with 29 % awakened by coughing and discomfort (Katz *et al.*, 2007). The formulation provides a convenient and flexible treatment option with a quick on set of action which is beneficial for patients who suffers the discomfort of GORD at night.

There are limited examples of lansoprazole in buccal delivery systems but omeprazole has been formulated as buccal tablets, prepared with bioadhesive polymers to increase buccal retention; however the stability of the PPI was low in saliva. Croscarmellose sodium (a super disintergrant) enhanced the release of omeprazole from the buccal tablet but decreased the bioadhesive forces and stability of the formulation (Yong *et al.*, 2001). Buccal formulations of omeprazole containing (omeprazole/sodium alginate /HPMC/magnesium oxide) were stable for 4 h in human saliva and possessed increased buccal adhesion compared to other formulations (Choi and Kim, 2000). Both studies on buccal formulations suggested that stabilising lansoprazole in the oral cavity in saliva is challenging, however the time spent in

the mouth with chewing gums will be relatively short compared with buccal tablet formulations. HPMC oral films have been previously prepared containing omeprazole and L arginine (used as a stabiliser, 1:2) for potential buccal delivery for paediatric patients (Khan et al., 2015).

1.10 Challenges for chewing gum formulations containing lansoprazole

Due to lansoprazole's high sensitivity to acidic, chemical degradation and specific incompatibilities with excipients, stabilising the drug within the formulation is challenging. This is described in detail in Chapter 3. Historically, incorporation of buffering excipients as pH adjusters has been utilized in a range of formulations to maintain the micro-environmental pH and improve stability during manufacture and storage (Carstensen, 2000). In the case of alkaline stabilisers, sodium bicarbonate, sodium carbonate, and magnesium and calcium oxides are commonly used in solid formulations and are capable of providing pH > 7 (Carstensen, 2000). Lansoprazole is unstable under strongly basic conditions, but its degradation is minimized under weakly basic conditions. Degradation of lansoprazole has been effectively minimised by using magnesium carbonate to produce a weakly basic pH (Tetsuro *et al*., 1992). The effects of different alkaline stabilisers on lansoprazole multi-coated pellets found dibasic sodium phosphate provided the most stable microenvironment due to its optimal pH and high buffering capacity. Sodium carbonate was found to produce the fastest release rate and the authors suggested mechanisms relating to interactions between the drug causing disordering and resulting in an amorphous state of lansoprazole. Increases in porosity were also suggested with sodium carbonate (increasing drug release); but this also contributed to moisture uptake and subsequent drug degradation in the pellets (He *et al*., 2010). Further work assessed four different alkaline stabilizers (including sodium carbonate, magnesium oxide and magnesium carbonate; added in equivalent weight ratios) in lansoprazole pellet formulations and found that inclusion of sodium carbonate produced the fastest release rate due to increased micropore formation which facilitated diffusion (He *et al*., 2011). The case study by Missaghi utilised multi-particulate dry powder layering and seal-coating technology which provided an isolation barrier against the outer aqueous enteric coating that had previously been observed to degrade the drug. This resulted in improved

stability and subsequent release from the oral solid formulations containing PPIs (Missaghi *et al*., 2010).

The release of the buffering excipients (soluble fractions) will directly influence the local environment (salivary pH) and so this should be considered and carefully controlled to facilitate potential targeted absorption *via* the oral buccal membrane following drug release. The primary absorption mechanism is *via* passive diffusion, the rate and extent of absorption of which is pH dependent. The salivary pH will therefore affect the portion of unionised drug readily available for absorption, due to increased partitioning into the buccal membrane (Pagare *et al*., 2012) (see section 5.3.3.1 and Figure 5.4).

Therefore the manipulation/control of the oral pH can affect drug delivery and this is exemplified by the range of formulations which incorporate buffering excipients, or pH adjusters, to facilitate absorption. Commercial Nicorette® formulations contain different compositions of buffers (sodium carbonate and sodium bicarbonate) between the 2 mg and 4 mg dosages; this is to ensure a similar pH increase to allow optimised conditions for nicotine release and absorption (Morjaria, 2004). As nicotine gums were chewed *in vitro* there was a corresponding increase in pH of the dissolution medium. The greatest pH increases were observed within the first 5 minutes of chewing, due to the increased solubility of the buffering excipient and then gradually plateaued to a final value of around pH 8.4 (a total increase of 1.5 pH units) resulting in nicotine being predominately in its unionised form. The study reported an increase in nicotine release proportional to the increase in buffering excipient content (sodium carbonate); this was related to the increase in micro-pores in the gum matrix which are formed after dissolution of the buffer upon contact with saliva, leaving channels for subsequent drug release. The gums texture was also observed to change, resulting in a softer gum with increasing buffering excipient content (Morjaria, 2004).

The masticatory actions of chewing a gum also affect the pH of the oral cavity. The pH of saliva was observed to increase during *in vivo* chewing of flavoured gums, due to the increased flow rate of saliva which leads to proportional increases in bicarbonates (Karami-Nogourani *et al*., 2011).

Other considerations include the increased solubility and stability of lansoprazole in a basic pH environment. Increases in pH of phosphate buffer were found to facilitate the dissolution of commercial lansoprazole capsules (as granules), with maximum dissolution observed at

pH 8. Dissolution was also dependent on digestion time and the ionic strength of the chosen medium (Ashraf *et al*., 2012).

rapid onset of action, convenience, pleasant sensation on being chewed and the potential of high acceptance in the paediatric market from parents (Mehta *et al*., 2010).

1.11 Positive functional effects of chewing gum on GORD

It has been understood that the production of saliva provides a beneficial effect for sufferers of acidic reflux, as the bicarbonate helps neutralise acid and accelerates acidic clearance time protecting the gastric mucosa against acid induced effects. Chewing gum base for a prolonged period of time significantly increased salivary flow and clearance time in 10 healthy individuals due to increased swallowing of saliva (Schonfeld *et al.*, 1997).

Chewing gum has been shown to be more effective than walking in reducing acid production after breakfast in patients with GORD. The reduction in acid was prolonged for one hour post walking but sustained for three hours post chewing gum (Avidan *et al.*, 2001). Chewing gums containing 600 mg calcium carbonate were demonstrated to be more effective for reduction of heartburn than chewable tablets (1000 mg) (Collings *et al.*, 2002). The beneficial effects on reflux were due to chewing causing increased salivary flow, which increased the buffering capacity and salivary bicarbonate concentration as well as the rate of swallowing saliva.

Some studies have also suggested that chewing gum may have a detrimental effect on reflux due to having a weak stimulating effect on gastric acid secretion. A study examining the effect of chewing gum on volume and pH of gastric content in 46 patients found no significant effect between non chewing gum patients and suggested this was due to the neutralising of the weak acid stimulation by the salivary bicarbonate (Dubin *et al.*, 1994).

1.12 Summary

There is an increased demand for convenient and efficient drug delivery systems to fit into modern active lifestyles (Hyrup *et al*., 2005). The potential to improve various treatments using chewing gum as a novel drug delivery method presents great opportunities. The potential of a medicated chewing gum formulation containing lansoprazole will provide all the functional benefits that a chewing gum can provide including the rapid onset of action

with the potential of buccal delivery as well as increased salivary stimulation to neutralise gastric acidic. It will also provide an alternative convenient treatment strategy to increase compliance.

The possible formulation of a stable novel delivery system for lansoprazole is challenging as lansoprazole belongs to BCS, class II, with solubility and stability being pH-dependent. It degrades in highly acidic /basic environments and is unstable under conditions of high temperature and high humidity (Tetsuro *et al*., 1992). To ensure success the drug must be released from the gum at a rate and extent that facilitates buccal absorption (Conway, 2007). Therefore the main challenges will be in stabilising the drug, increasing solubility in the oral environment and applying techniques to raise the pH of the saliva locally.

1.12.1 Aims and objectives

The main aim of this thesis was to incorporate a poorly soluble, acid-labile active (lansoprazole) into a chewing gum formulation for potential absorption across the buccal mucosa. This will contribute to the limited knowledge around the release of a poorly soluble active from a medicated chewing gum formulation.

The study utilised the EP approved chewing apparatus to quantify *in vitro* release of lansoprazole from chewing gums leading to the production of a range of further optimised trial formulations. Various experimental chew machine settings were explored including choice of dissolution medium, sample replacement volumes, pH and chew rate to mimic different chew related physiological states *in vivo.*

The study also assessed formulation considerations including the use of Rev7 technology, the effect of buffer excipients and the use of cyclodextrin complexation to enhance release of a poorly soluble active from medicated gums.

The study contributed to the understanding of factors governing the release rate and extent of a poorly stable, poorly soluble drug from a novel medicated chewing gum formulation. The study also assessed the potential for buccal absorption of lansoprazole once released from the medicated gum in the oral cavity.

1.12.1.1 Drug-related objectives

- Investigate the characterisation of lansoprazole particularly the solubility and stability in artificial saliva.
- Investigate the use of cyclodextrins as a solubiliser and stabiliser for lansoprazole.
- Utilise the *in vitro* Franz cell apparatus to provide an estimation of permeability of lansoprazole using artificial saliva through porcine buccal mucosa.

1.12.1.2 Gum formulation objectives

- Load required dose of stable active pharmaceutical ingredient (API) into a range of trial gum formulations - including investigating the effect of gum base mass percentage and also different excipients; Rev7 polymer, release modifiers (cyclodextrin as solubilisers) and buffering excipients.
- Evaluate *in vitro* release of API from the gum using the EP approved masticator utilising a variety of test settings.

Chapter Two:

General methods

Experimental methods

All routinely used methods for this thesis are described in this chapter, including HPLC parameters. Any specific variations are detailed in the relevant chapters.

2.1 HPLC analysis of lansoprazole and nicotine

HPLC was the primary tool used for quantitative and qualitative analysis of drugs.

Equipment

The Shimadzu HPLC system was set up as follows:

Pump: LC-20AT SN: L201146

Autosampler: SIL-20A SN: L201646

Oven: CTO-10AS SN: 02120446

UV Detector: SPD-20AV SN: L20144473015

2.1.1 HPLC parameters for the analysis of nicotine

The method was based on a validated HPLC assay method used previously and was developed further in-house (Morjaria, 2004).

Materials

Nicotine hydrogen tartrate, ammonium phosphate monobasic, ammonium hydroxide and acetonitrile were all supplied by Sigma (Dorset, UK) and were of pharmaceutical, analytical or HPLC grade as appropriate. Double distilled water was generated in house using a Fison Fi Stream still (Birmingham, UK).

Chromatographic conditions

Column: Waters Xterra RP-18 4.6 x150 mm, 3.5 µm SN: W21631B022

Mobile phase: 70 % ammonium phosphate buffer: 30 % acetonitrile (pH 8.5)

Injection Volume: 20 µL

Wavelength: 260 nm

Flow rate: 1 mL/ minute

Run time: 6.0 minutes

Methods

Preparation of mobile phase

Monobasic ammonium phosphate (6.7 g) was dissolved in approximately 950 mL double distilled water. The pH of this solution was then adjusted to pH 8.5 using ammonium hydroxide solution (3.0 % w/v). The volume was then made to 1 L using distilled water.

Preparation of nicotine standards

Working standards of nicotine were prepared by serial dilution of a 100 µg/mL nicotine stock solution to produce serial dilutions of 80, 60, 40, 20 and 10 µg/mL. All standards were diluted using mobile phase. The stock solution was prepared by weighing 28.5 mg of nicotine hydrogen tartrate made up to 100 mL to produce a stock solution of 285 µg/mL of nicotine hydrogen tartrate equivalent to 100 µg/mL of nicotine.

The calibration range was chosen to incorporate the maximum theoretical concentration of nicotine in chewing chamber from both strengths of nicotine gums (4 mg and 2 mg) once diluted 1:1 with mobile phase (Figure 2.1).

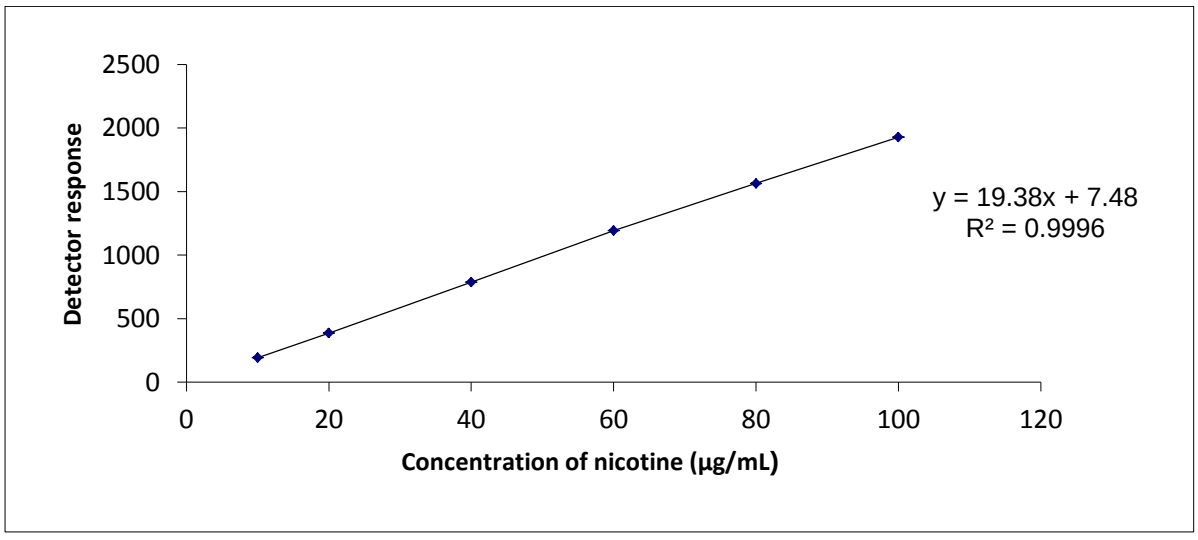


Figure 2.1 Nicotine calibration curve (n = 5; mean ± s.d)

2.1.2 HPLC parameters for the analysis of lansoprazole

The method was based on validated methods developed by (Ekype and Jacobsen, 1999; DiGiacinto *et al.,* 2000; United States Pharmacopeia, 2007; Song *et al.,* 2008; Idrees and Majdoleen, 2010; Hong *et al.,* 2012).

Materials

Sodium phosphate and acetonitrile were supplied by Fisher Scientific (Loughborough, UK) and were of pharmaceutical, analytical or HPLC grade as appropriate. Ultra-pure water was generated in house (Huddersfield, UK). Lansoprazole was supplied by Discovery Fine Chemicals (Dorset, UK) and was used as received.

Chromatographic conditions

Column: Waters Xterra RP-18 4.6 x150mm, 3.5µm SN: 02231262161

Mobile phase: 45 % v/v acetonitrile: 55 % distilled water, (pH adjusted to pH 7.5 using 0.1 M sodium phosphate)

Wavelength: 285 nm

Injection Volume: 5 µL

Flow rate: 1.0 mL/ minute

Temperature: 35 °C

Run time: 10.0 minutes

Preparation of lansoprazole standards

Working standards of lansoprazole were prepared by serial dilution of a 200 µg/mL stock solution to produce 150, 100, 50 and 20 µg/mL lansoprazole solutions in mobile phase. The range was chosen to represent the maximum theoretical concentration of lansoprazole in chewing chamber from masticated gums: (15 mg) in 40 mL of dissolution medium assuming 100 % release and dilution 1:1 with mobile phase (Figure 2.2).

Preparation of 200 µg/mL lansoprazole stock solution

0.02 g of lansoprazole standard was weighed and made up to 100 mL using mobile phase (Mettler Toledo-analytical electronic balance). All glass volumetric flasks were wrapped in foil to restrict light exposure and were stored at 4°C. Standards were remade every 30 days to prevent errors due to any drug degradation.

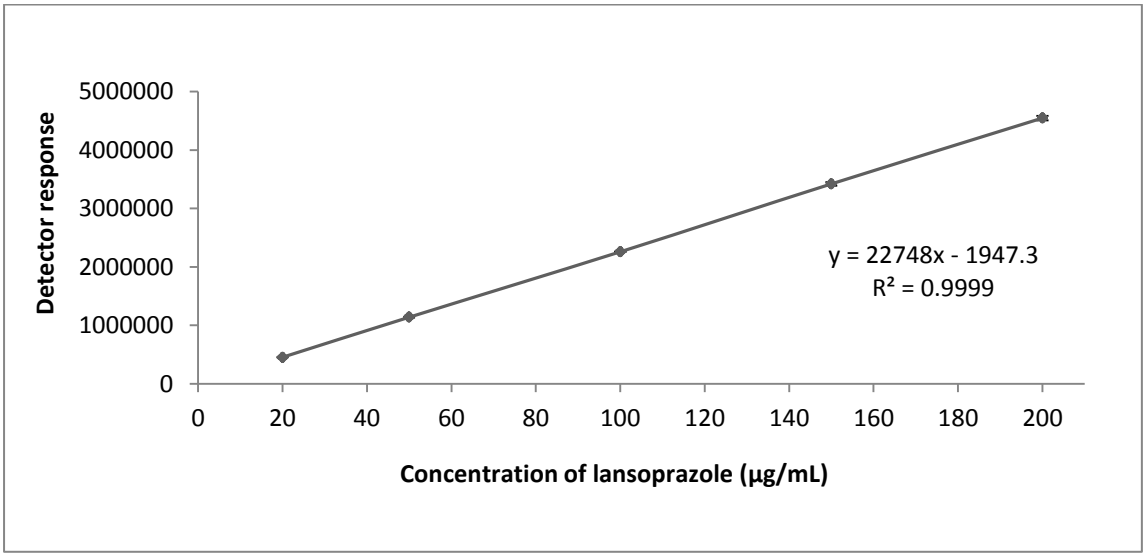


Figure 2.2 Lansoprazole calibration curve (n = 5; mean ± s.d)

2.1.3 HPLC validation

All HPLC validation tests and acceptance limits within this section were performed in accordance with guidelines in the International Conference of Harmonization, (ICH,1996) and as *per* general validation instrumentation guidelines given by Shimadzu Customer Support (Shimadzu Ltd, N.D). The HPLC system and method of lansoprazole determination were validated by evaluating linearity, precision, accuracy, limit of detection and limit of quantification. Accuracy can be defined as the proximity of measurements to the true value. Precision can be described as being able to get the same result for a particular sample every time, when analysis of that sample is repeated.

System suitability testing is an integral part of all analytical procedures. The utilised method was checked for system suitability by determining the capacity factor (K′), Tailing factor (T) and number of theoretical plates (N).

The capacity K′ is a measure of sample retention and sample separation (Eq. 2.1). It accounts for the location of the peak of interest with respect to the void volume and the solvent front. For an ideal separation the capacity factor should be above 1.5 (Shimadzu Ltd, N.D).

$$K' = \frac{t_A - t_0}{t_0}$$

Equation 2.1 Capacity factor (K′)

Where t_0 - initial retention time (solvent front), t_A - retention time of the analyte

The tailing factor confirms the ideal calibration range (Eq. 2.2). Tailing tends to be more pronounced when the mobile phase is overloaded with a high analyte concentration. The calibration range used in the current method is 200 µg/mL to 20 µg/mL. The concentration range and injection volume were chosen based on the tailing factor. The recommended tailing factor for a good separation should not be more than 2 (Shimadzu Ltd, N.D).

$$T = \frac{W}{2f}$$

Equation 2.2 Tailing factor (T)

Where W- width of the peak, *f*- flow rate (mL/min)

The theoretical plate number (N) is a measure of column efficiency (Eq. 2.3). N remains constant for each plate on a chromatogram with a fixed set of defined conditions. Factors which may influence N include the particle size of column, molecular weight of analyte, flow rate of mobile phase, position of peak and column temperature. The greater the number of theoretical plates, the higher the efficiency of separation with good peak resolution and sharp symmetric peaks. The generally accepted criteria are > 2000 recommended for a good separation (Shimadzu Ltd, N.D).

$$N = 5.54\left(\frac{t_A}{w_{0.5}}\right)^2$$

Equation 2.3 Number of theoretical plate (N)

Where t_A- retention time of the analyte, $W_{0.5}$ – half the peak width

Linearity is the detectable range that obeys the Beer-Lambert Law, the linear relationship between absorbance and concentration of an absorbing species (Eq. 2.4).

$$A = \varepsilon bc$$

Equation 2.4 Linearity

Where A is absorbance, Ɛ is the molar absorptivity, b is the path length and c is the concentration of the sample.

The linearity of the method was tested to demonstrate a proportional relationship of response *versus* analyte concentration over the working range. The calibration curves were constructed with working concentration of 200 µg/mL to 20 µg/mL and linearity was evaluated between calibrations ran on the same day as well as over multiple days, all the experiments were carried out in triplicate.

Interday validation was evaluated to determine variation that may arise from day to day working. A known concentration of lansoprazole solution was made and analysed on the first day and then was re analyzed on the following five consecutive days. The % R.S.D was accepted if it was < 1.0 % (Shimadzu Ltd, N.D).

To determine the precision of the system, 6 multiple injections were made from the same vial for one concentration of lansoprazole and were analysed on the same day (intraday), under the same conditions. Acceptable % R.S.D was taken as < 1.0% (Shimadzu Ltd, N.D).

To determine accuracy, a known concentration of lansoprazole was added to hexane and mobile phase (50 mL: 50 mL) and % recovery was measured. This was repeated five times to ensure accuracy. This also provided validation of the extraction method as well as testing accuracy of the HPLC method. Acceptable % RSD was taken as < 1.0% (Shimadzu Ltd, N.D).

Selectivity and specificity are measures of the interference from substances which may be expected to be present, including other excipients, impurities and degradation products. As lansoprazole is acid labile it is important that the concentration of lansoprazole can be determined in the presence of its acid degradation products (Idrees and Majdoleen, 2010). This was demonstrated by identifying lansoprazole in presence of its acid induced

degradation products and also with using impurities A and B; which were purchased from the British Pharmacopeia (see Figure 3.5).

Limit of detection (LOD) is the lowest concentration of an analyte which can be detected under the experimental conditions. Limit of quantification (LOQ) is the lowest level of analyte that can be accurately measured under the experimental conditions. The LOQ value was accepted if the % R.S.D was < 5% (Shimadzu Ltd, N.D).

The limit of quantification (LOQ) and limit of detection (LOD) were calculated for lansoprazole. Three calibration runs were performed in triplicate and the calibration curve was constructed. The standard deviation of the y intercepts (σ) and the slope of the regression lines (S) was calculated from the calibration curves. The following equations (Eq. 2.5) were used to calculate LOQ and LOQ:

$$\mathrm{LOD} = \frac{3.3\sigma}{\mathrm{S}}$$

$$\mathrm{LOQ} = \frac{10\sigma}{\mathrm{S}}$$

Equation 2.5 LOD and LOQ

(Shimadzu Ltd, N.D and ICH, 1996)

2.1.3.1 Validation of HPLC method for determination of nicotine

Table 2.1 HPLC method validation for nicotine

Test	Values	Result: R.S.Ds	Acceptance criteria
Linearity (n=5)	0.999 ± 0.0003	0.03 %	All R.S.Ds of less than 1.00%
Injection precision (n=5)	1928 ± 11.10	0.58 %	
Repeatability (n=6)	387 ± 2.97	0.77 %	

All of the R.S.Ds were less than 1.0 % therefore the HPLC method was accepted as accurate and precise (Table 2.1). LOD was 0.273 µg/mL and LOQ was 0.826 µg/mL which were similar to those reported previously (Morjaria, 2004).

2.1.3.2 HPLC system suitability and method validation for determination of lansoprazole

Table 2.2 System suitability for determination of lansoprazole

Test	Result	Acceptance criteria
Capacity factor (K′)	2.89	> 1.5
Tailing factor (T)	0.35	< 2
Number of theoretical plates (N)	2846	> 2000

All of the results were accepted and showed that the system is suitable for detection of lansoprazole (Table 2.2).

Table 2.3 HPLC method validation for lansoprazole

Test	Values	Result: R.S.Ds	Acceptance criteria
Linearity (n=5)	0.999 ± 0.004	0.026 %	All R.S.Ds of less than 1.00%
Injection precision (n=5)	451833 ± 3155.2	0.70 %	
Repeatability (n=6)	419162 ± 1625.25	0.39 %	

All of the R.S.Ds were less than 1.0%, the method was accepted as accurate and precise (Table 2.3). The accuracy was 0.97 %. LOD values were 0.267 µg/mL and LOQ was 0.809 µg/mL.

Both the nicotine and lansoprazole HPLC analysis methods were accurate and reproducible, as evidenced by the calibrations which show good linearity and reproducibility between the working standard ranges.

2.2 *In vitro* release testing of chewing gums

The specialised dissolution apparatus is designed to determine the active dosage form released from chewing gum by providing the masticatory mechanical shearing forces. The apparatus can simulate the necessary chewing forces for release; normal chewing forces during mastication of food substances can range from 70 to 150 Newtons with maximum biting forces of 500-700 Newtons (Crispian, 2002).

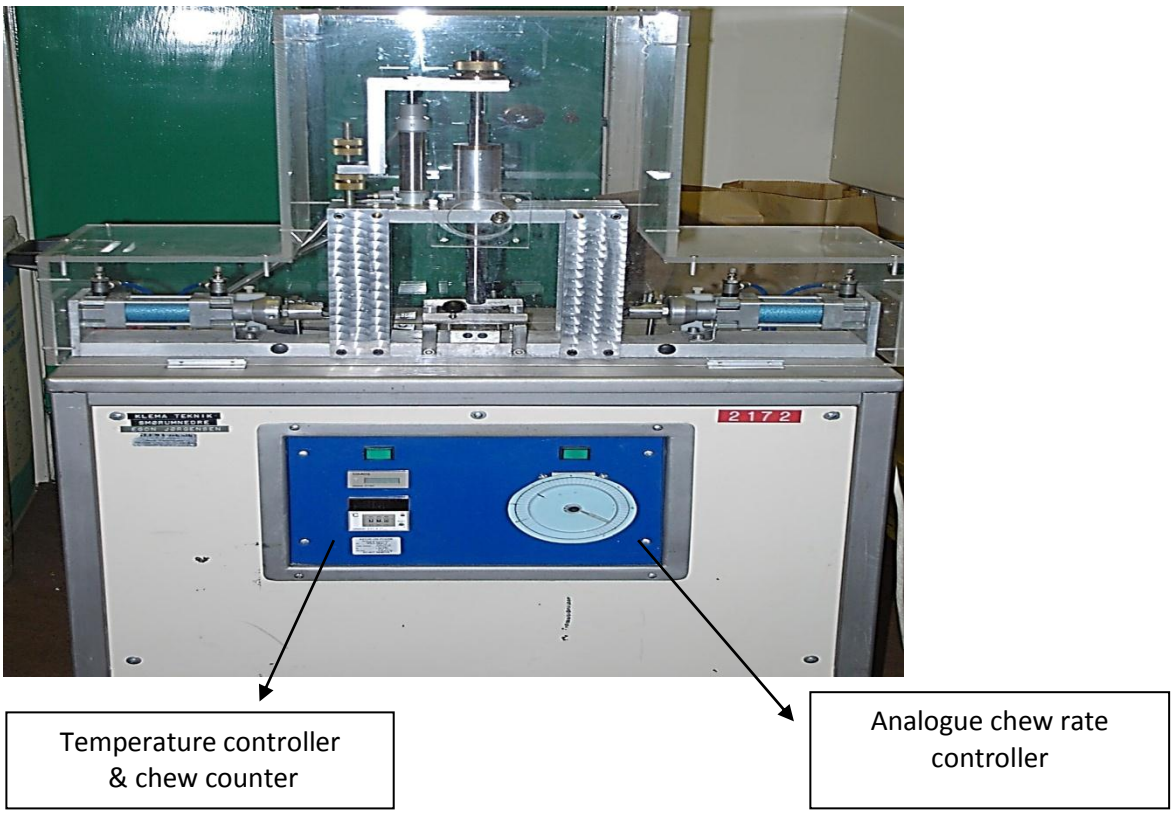


Figure 2.3 The European Pharmacopeia approved masticator (control panels)

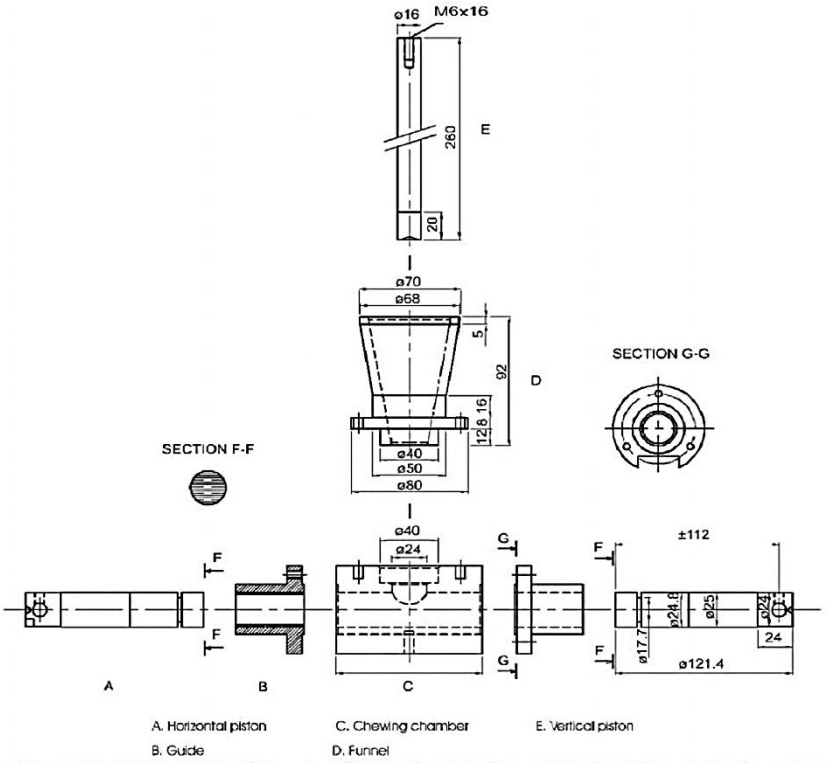


Figure 2.4 Cross-section of European Pharmacopeia approved masticator

(William and Millind, 2012)

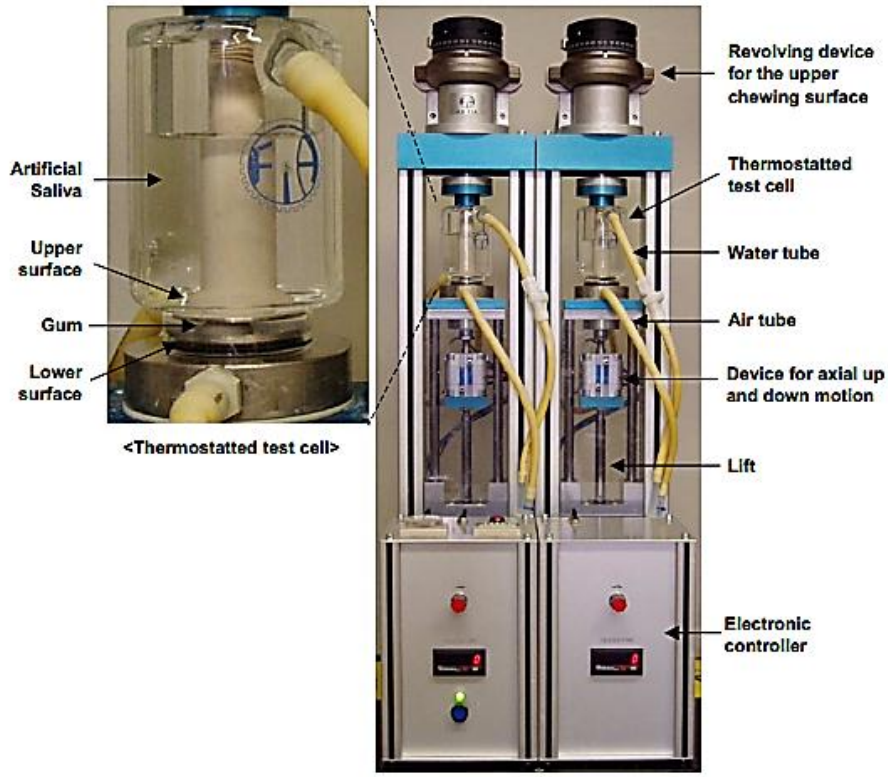


Figure 2.5 Apparatus b, Double module (Wennergren masticator)

(Hee Na *et al.*, 2005)

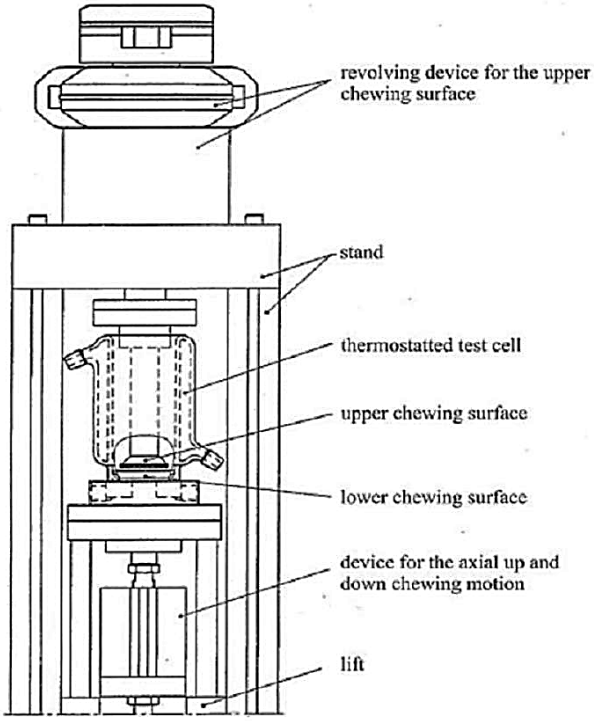


Figure 2.6 Apparatus b, Single module designed by Wennergren

(William and Millind, 2012)

The compendial machine (masticator M36 or apparatus a) designed by Christrup and Moller, has been approved and was first briefly described by the EP in chapter 2.9.25 in 2002 for the purpose of product control of medicated gums and has been further refined in subsequent editions (European Pharmacopoeia, 2011) (Figures 2.3 and 2.4). There is an alternative design of *in vitro* testing apparatus (apparatus b) designed by Wennergren that is also well investigated and published in the European Pharmacopoeia as well as the United States Pharmacopeia (USP) (United States Pharmacopeia, 2011; Gajendran *et al*., 2012) (Figures 2.5 and 2.6). The chewing intensity is influenced by chewing frequency, distance between the chewing surfaces and the torsion angle (Kvist *et al*., 1999). The force will increase with decreasing distance between the pistons however apparatus a has fixed parameters (distance between jaws was 0.5 mm) whereas apparatus b has definable parameters including the torsion angle and distance between jaws. This allows for the capacity to fine tune the release profiles for gum formulations.

2.2.1 Preparation of artificial saliva

The method used was based on the recommendations from the EP and further adapted from previous reports optimized for *in vitro* - *in vivo* correlations (IVIVC) (Morjaria, 2004). Artificial saliva was prepared fresh daily and was based on the method developed by Parker (Table 2.4) (Parker *et al*., 1999).

Table 2.4 Artificial saliva composition

Components	Quantity	
	(mM per litre)	**g/L**
KH_2PO_4	2.5	0.34
Na_2PHO_4	2.4	0.43
$KHCO_3$	15	1.50
NaCl	10	0.58
$MgCl_2$	1.5	0.14
$CaCl_2$	1.5	0.22
Citric Acid	0.15	0.03
pH adjusted to 6.7 with NaOH or HCl		

(Adapted from Parker *et al.*, 1999)

2.2.2 Preparation of phosphate buffer

Phosphate buffer solution was prepared based on an in-house method at Revolymer. Potassium phosphate (0.2 M) was prepared by weighing 13.609 g of potassium di hydrogen phosphate and made to 500 mL using distilled water. 0.2 M sodium hydroxide was prepared by weighing 1.6 g of sodium hydroxide and made to 200 mL using distilled water.

Potassium phosphate solution (250 mL, 0.2 M) was added to 28.5 mL of 0.2 M sodium hydroxide solution and made up to 1 L using distilled water. The pH of the buffer was checked and adjusted to pH 6.0 as necessary.

2.2.3 In vitro release testing

The EP recommends the following guidelines for testing release from gums: 20 mL of an unspecified buffer (pH close to 6), in a chewing chamber of 40 mL and with a chew rate of 60 strokes per minute (European Pharmacopoeia, 2011). This was modified as *per* previous studies and in house Revolymer based methods (Morjaria, 2004) and the recommended volume for reproducible release studies was between 30 – 40 mL. This was based on the size of the chamber and ensuring a consistent contact between gum and medium. Studies showed that there was no significant difference between 20, 40 and 80 mL dissolution volumes on the release of nicotine from medicated gums (Morjaria, 2004).

Dissolution medium was added (40 mL) and the temperature of the chewing chamber was set to 37 °C ± 0.5 °C with a chew rate of 60 chews per minute (standard settings). Once the medium had reached the desired temperature the gum was placed in the chewing reservoir after which the vertical piston (tongue) was re-assembled.

Samples were taken at 0, 5, 10, 15, 20, 25 and 30 minutes and replaced with equal volumes of fresh dissolution medium using a needle and syringe (2 mL). The samples were diluted 1:1 with mobile phase before being filtered through a 0.45 µm filter and the drug levels quantified using the relevant HPLC method. To correct the progressive dilution by sampling and replacement an equation was used to calculate the cumulative amount of drug accurately. The chewed cud was analysed for residual content after mastication to give a total drug load.

(Sample replacement volumes were increased to 20 mL during method development to take into the account the poor solubility of lansoprazole and to maintain sink conditions).

The progressive sample dilution was accounted for due to the sampling and replacing of fresh dissolution medium to the chewing chamber (Eq. 2.6).

$$M_t[n] = \frac{V_r.C[n] + V_s.\sum_{m=1}^{n-1} C[m]}{1000}$$

Equation 2.6 Sampling replacement

Where,

M_t [n] is the current, cumulative mass released from the gum at time t,

C[n] is the current concentration in the dissolution medium

Σ {C[m]} is the summed total of the previous measured concentrations {m=1- (n-1)}

V_r is the volume of dissolution medium and

V_s is the volume of sample removed for analysis

2.2.4 Chewing apparatus validation

The masticator chewing rate was calibrated by hand to ensure it was accurate and reproducible, as the chew rate can influence the release of active from medicated gums (Kvist *et al*., 1999). An electronic chew counter was installed and the chew rate was determined at set increments and tested for intra-day and inter-day variation. The acceptance criteria was less than 2.0 % R.S.Ds to be considered accurate (Shimadzu Ltd, N.D). Random chew rate checks were also preformed over the duration of the work.

In order to investigate the intra-day variation, the chewing machine was switched on and the temperature of the chewing chamber was set (37 °C ± 0.5 °C) and allowed to equilibrate. The chew rate was selected, the chewing pistons switched on and the number of chews every minute was noted for 30 minutes. After each run the chewing machine was switched off and was left for 15 minutes. The whole process was then repeated in triplicate for each chew rate

setting; 40, 60 and 80 chews per minute (cpm). For inter-day variation, the number of chews every minute for 10 minutes was noted for each interval setting twice a day for three days.

2.2.4.1Results and discussion

Table 2.5 Chew rate intra day validation over 30 minute periods

Chew rate (cpm)	Total chew count over 30 minutes	Mean	S.D	R.S.D
40	1220	1243.33	20.82	1.67
	1250			
	1260			
60	1830	1856.67	25.17	1.36
	1880			
	1860			
80	2442	2445.33	4.16	0.17
	2444			
	2450			

Table 2.6 Chew rate inter-day validation over 10 minute periods

	Total chews over 10 minutes			Mean	S.D	R.S.D
Chew rate (cpm)	Day 1	Day 2	Day 3			
40	407	422	413	416.5	5.54	1.33
	418	420	419			
60	610	615	622	621	7.16	1.15
	624	626	629			
80	814	818	819	816.33	1.97	0.24
	815	817	815			

The chew rate was considered to be acceptable in all settings tested; R.S.Ds < 1.67 % (Table 2.5 and 2.6). The accuracy increased as the chew rate increased as indicated with decreasing R.S.Ds.

2.2.4.2 *In vitro* release from nicotine chewing gums

The masticator was investigated to ensure that it was providing the necessary masticatory forces for *in vitro* drug release from a chewing gum formulation. To evaluate *in vitro* release, a commercially available nicotine chewing gum (Nicorette® (2 mg) BN: LL613A) was used. The standard masticator set up was used and artificial saliva was used as the dissolution medium.

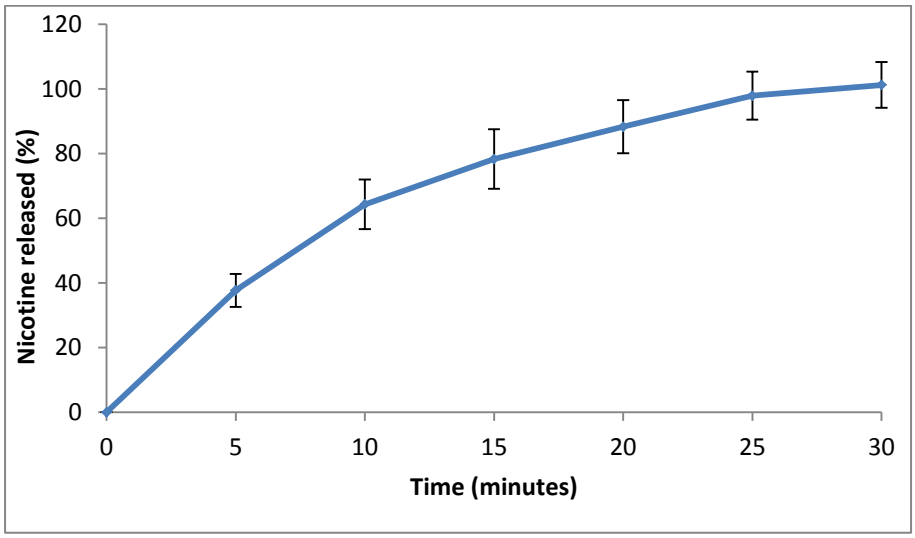


Figure 2.7 Release of nicotine from commercial Nicorette® gums (2 mg) (n = 9; mean ± s.d)

There was a steady increase in the release of nicotine over 30 minutes (Figure 2.7) (Appendix A). Most medicated gums are designed to release the majority of the active (> 75 %) after 20-30 minutes of chewing (Rowe, 2003). This demonstrates that the masticator was capable of providing the necessary masticatory forces to predict the extent of active release from chewing gums.

2.3 Quantification of nicotine in commercial gums

Nicotine content was determined following solvent extract of the active from the gums. The method was based on previous reports (Morjaria, 2004).

Method

Ten gums were selected at random and were weighed (approximately 10 g) and placed in 1000 mL volumetric flask. Tetrahyrdofuran (THF, 200 mL) was added and this was shaken

on a Varimax horizontal mixer at 135 shakes / minute for 90 minutes. HCl (0.1M, 300 mL) was added and the flask was allowed to stand before shaking for a further 5 minutes. This suspension was then centrifuged at 3000 rpm for 10 minutes. A sample (1.25 mL) of the supernatant was diluted to 50 mL using distilled water. This was then analysed using HPLC parameters as described earlier. The method was repeated for composites of 5 gums and also single gum pieces to test for accuracy.

Results

The mean total drug loading in commercial gum samples (Nicorette® 2 mg) was found to be 2.51 ± 0.10 mg of nicotine per gum, which was over estimated in comparison to the label claims.

2.4 Quantification of lansoprazole in formulated gums

Lansoprazole content of gums was determined using a modified extraction method avoiding acidic conditions. The residual drug content was also determined following *in vitro* release testing to account for mass balance (using the same method).

50 mL of hexane was added to 50 mL of mobile phase (acetonitrile: water) (45:55) and a formulated gum was added to the solvent mix. The suspension was stirred (75 rpm) for 30 minutes and then allowed to settle and divide into 2 separate phases on the bench for 30 minutes. An aliquot was taken from the lower phase (containing the drug in mobile phase) and was filtered before being analysed by HPLC.

In order to ensure efficiency of extraction, 50 mL of hexane was added to 40 mL of mobile phase. This solution was spiked with 10 mL of 200 µg/mL lansoprazole standard solution. The solution was stirred for 30 minutes and then allowed to settle and divide into 2 separate phases on the bench for 30 minutes. An aliquot was taken from the lower phase (containing the drug in mobile phase and filtered before being analysed using HPLC. This was repeated five times to determine reproducibility.

Table 2.7 Lansoprazole extraction spiking and recovery (n=5)

Spiking experiment	Recovery (%)
1	95.99
2	95.62
3	93.65
4	95.34
5	94.72
Mean ± s.d	**95.07 ± 0.92**

The extraction method resulted in a mean recovery of 95.07 ± 0.92 % (n =5; mean ± s.d) (Table 2.7).

2.5 Conclusion

The HPLC methods were proven to be accurate and reproducible for the detection and quantification of lansoprazole and nicotine for the analysis of drug release from gums. The masticator was proven to be reproducible and was able to provide the necessary forces needed for *in vitro* drug release from gums. Gum extraction methods were proven to ensure the efficiency of extraction and can provide total drug loading and residual contents in gums after mastication.

Chapter Three:

Drug solubility and stability

3.1 Introduction

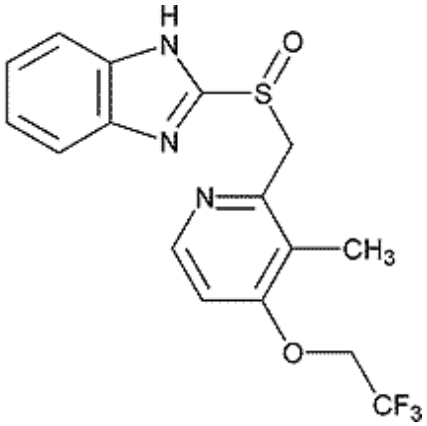


Figure 3.1 The structure of lansoprazole

Lansoprazole (Figure 3.1) belongs to class II of the Biopharmaceutics Classification System, characterised by low solubility and high permeability due to its lipophilic nature. It exists in two polymorphic forms designated as form A and form B. Form B is unstable and is completely converted to the stable form A under physical stress (milling) or even after some time at ambient temperature (Kotar *et al*., 1996). Lansoprazole has a Z shape crystal structure with the benzimidazole moiety and the pyridine ring being co-planar (Swamy and Ravikumar, 2007).

The decreased stability of lansoprazole leads to an increased rate of transformation to the active forms lansoprazole sulfone and 5- hydroxy lansoprazole (Song *et al*., 2009). This is described in detail in section 1.7.3 and Figure 1.9. Lansoprazole had the fastest accumulation rate in the parietal cells and the highest rate of conversion amongst the other PPIs (Tutunji et al., 2006). Furthermore, lansoprazole degrades in pH's below 7 and highly basic environments (pH's above 10) (Kristl and Vrecer, 2000). It is unstable under conditions of high temperature above 180 °C. Lansoprazole is also photo-sensitive and is recommended to be protected from light and to be stored at 4 °C. Various studies have observed a significant decrease in concentration and also discoloration of the material noted on storage under such conditions (Tetsuro *et al*., 1992). Dissolution studies evaluating particle size found insignificant differences in dissolution rate when reducing particle size and this was due to the particles having a tendency to agglomerate (Kristl and Vrecer, 2000). The drug is stable

under high relative humidity's (75 % and 96 %) and does not bind water (only 0.02 % at 96 %) or degrade under these conditions (Kristl and Vrecer, 2000).

The need for further studies into reformulation of off-patent drugs (including lansoprazole) has been identified by the European Medicines Agency (EMA) since 2007; with particular emphasis for the paediatric market (European Medical Agency, 2007). Formulation of a stable delivery system for lansoprazole can be challenging due to the highly unstable drug with the main challenge in designing a pre-oral lansoprazole formulation being stability in presence of acids whist in solution and in the dosage form (Kristl and Vrecer, 2000).

3.1.1 Lansoprazole stability

Drug degradation can occur by the following pathways:

- Hydrolysis
- Oxidation
- Photolysis
- Trace metal catalysis

Hydrolysis and oxidation are the most common mechanisms of drug degradation for most drugs (Carstensen, 2000). Regulatory bodies, including the ICH, have set guidelines for stress testing for reporting, identifying and quantifying impurities and degradants to ensure patient safety and efficacy (ICH, 1996).

Lansoprazole is the least stable from the class of benzimidazole proton pump inhibitors (PPIs), with omeprazole second and pantoprazole being the most stable (Ekype and Jacobsen, 1999). At pH 7.5, both pantoprazole and omeprazole were stable, but lansoprazole still underwent limited degradation. This is due to the absence of substituents on the benzimidazole ring (compared with omeprazole and pantoprazole which have electron withdrawing groups attached). This increases the basicity of benzimidazole nitrogen and rates of degradation were directly dependent on the basicity of the benzimidazole nitrogen (Tutunji *et al.*, 2006). The introduction of the tri-fluoroethoxy group and the sulfinyl group increase the acidic properties of the drug (Kristl, 2009).

Current lansoprazole formulations (15 and 30 mg) include enteric coated granules (which dissolve only in alkaline pH) filled in a gelatin capsule to protect the drug from degradation in the acid in the stomach (Ito *et al*., 2005 ; El-Sherif *et al*., 2006). Current formulations are described in detail in section 1.7.6. There is therefore a need for a pharmaceutical delivery system which protects the active substance both during storage as well as avoiding degradation during passage through the stomach. Oral bioavailability of lansoprazole was improved by formulating solid dispersion pellets with the solubiliser PVP (polyvinylpyrrolidone) (1:1.75) using solvent evaporation in a fluid bed coater (Zhang *et al*., 2008). The study reported an increase in dissolution rate (80 % within 5 minutes) compared to drug alone (5 % after 60 minutes). Another study also used solid dispersions of a novel polymer Soluplus® to improve the dissolution rate of lansoprazole (Mendiratta *et al*., 2011).

3.1.2 pH-dependant solubility of lansoprazole

Lansoprazole is known to have pH-dependant solubility in aqueous solutions (Hong *et al*., 2012) and there is a significant increase at pHs greater than 9 (Tetsuro *et al*., 1992). Kristl confirmed the pH- dependent stability of lansoprazole, displaying degradation first order rate constants and determining pK_a values of (acidic) pK_{a1} =8.84 (dissociation from the protonated nitrogen atom N-1 on the benzimidazole ring) and (basic) pK_{a2} =4.15 (protonation from the pyridine moiety) & pK_{a3} =1.33 (protonated nitrogen atom N-3 on the benzimidazole ring) (Figure 5.4) (Kristl, 2009). Significant increases in solubility were found above pH 9, when lansoprazole was in its dissociated form. Limited solubility was displayed at low pHs and at neutral pH solubility was constant; where lansoprazole is in an unionised form and had low wettability/contact angles (Kristl and Vrecer, 2000). Lansoprazole was more stable at higher pH, as the pH increases the rate of degradation was found to decrease (Ekype and Jacobsen, 1999).

3.2 Characterisation of lansoprazole

The pH-dependence on solubility and stability of lansoprazole (particularly important when designing a buccal delivery system) was characterised and compared with literature.

3.2.1 Methods

3.2.1.1 Determination of lansoprazole pH-solubility profile

The method was based on methods by Higuchi and Connors, (1965), Kristl and Vrecer, (2000) and Mendiratta *et al*., (2011).To determine lansoprazole solubility at a range of pHs, artificial saliva was prepared as described in Chapter 2. The pH was adjusted using 0.1 M sodium hydroxide solution to the following pHs: 6, 7, 8, 9 and 10. Artificial saliva (10 mL) was fully saturated with an excess with lansoprazole (n=3). The vials were stirred at 250 rpm in a water bath at a controlled temperature of 25 °C and samples were taken at 8.5 hours and 15.5 hours. All vials were protected from light. The sample suspensions were filtered (the first 1 mL was discarded) before being analysed by HPLC. The sample times were chosen to limit the possible effects of degradation at the lower pHs and to compare the possible effects of the increased pH on stability over time. It was presumed that any sampled drug would be replaced by more available free drug (Higuchi and Connors, 1965).

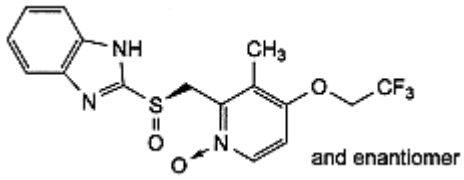
3.2.1.2 Acid induced degradation studies

The degradation of lansoprazole was induced to assess to ensure that the detection of lansoprazole was possible in presence of its degradation products (stability indicating method) as recommended by the literature (El-Sherif *et al*., 2006; United States Pharmacopeia, 2007; Srinivas *et al*., 2010). The instability under acidic conditions is due to proton attack on the sulfoxide group, the most fragile part of the lansoprazole molecule (Lu *et al*., 2012). The degradation of lansoprazole was initiated using hydrochloric acid and application of heat. This product was then combined with aliquots of lansoprazole working standard (see section 2.1.2) to ensure the potential identification of lansoprazole in presence of its acid-induced degradation products. Chromatogram resolution was assessed ensuring there was no interference from any additional peaks with the peak of interest (lansoprazole).

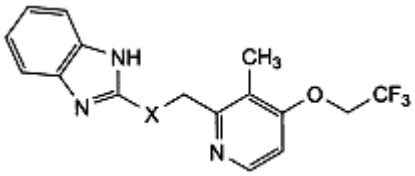
Lansoprazole (20 mg) was added to 25 mL of 0.1 M HCl, the solution was stirred and heated at 40°C for 6 hours to ensure complete degradation. The solution was then neutralised using 0.1M sodium hydroxide and made to 100 mL using mobile phase (final concentration 200 µg/mL). Aliquots of the degraded lansoprazole were added to lansoprazole working standard (100 µg/mL) to give the following percentages: 90, 80, 60, 50, 40, 20 and 1 % v/v

lansoprazole. The samples were analysed by HPLC to quantify lansoprazole in presence of its degradation products.

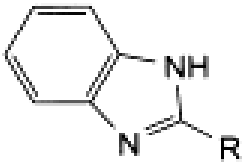
Impurities A & B (Figure 3.2) were identified using British Pharmacopeia (BP) reference standards (purchased directly from the British Pharmacopoeia; Catalogue: 873 Batch: 3188).



Impurity A (oxidation on N) (N oxide)



Impurity B (X = SO_2) (Sulphone) Impurity C (X = S)



Impurity D (R = OH) Impurity E (R = SH)

Figure 3.2 Lansoprazole impurities

(British Pharmacopeia, 2010)

3.2.2 Results

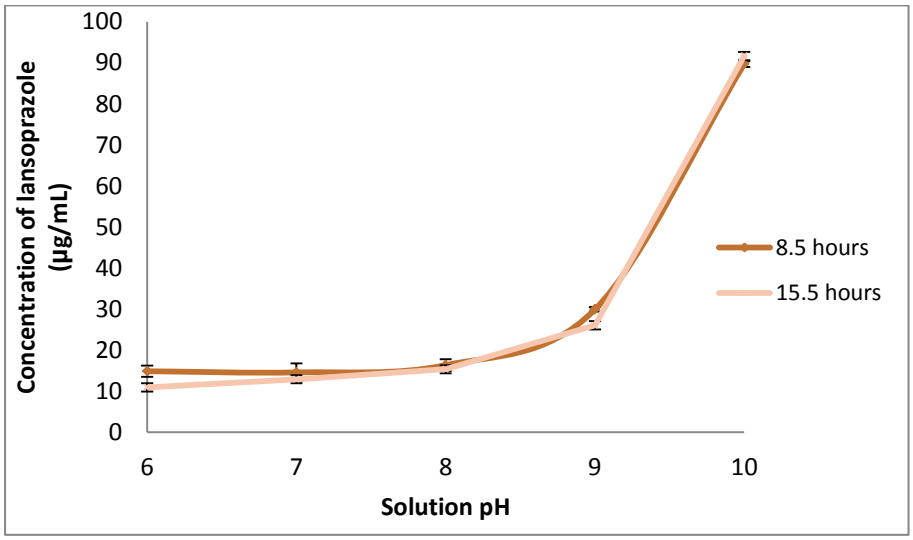


Figure 3.3 pH solubility profile of lansoprazole in artificial saliva (n=3; mean ± s.d)

The solubility of lansoprazole is dependent on the pH of the solution, an increase in pH causes an increase in lansoprazole solubility (Figure 3.3) with a significant increase between pH 9 and 10 as seen previously (Kristl and Vrecer, 2000). At the lower pH, the concentration was decreased (by 26 % at pH 6) after 15.5 hours confirming the instability of lansoprazole at acidic pH (See Appendix A). This suggests that pH encountered in the mouth (pH 6.8) will lead to challenging conditions with regards to solubility and stability; however this will be limited due to the transient chewing time of the formulation whilst being chewed in the mouth.

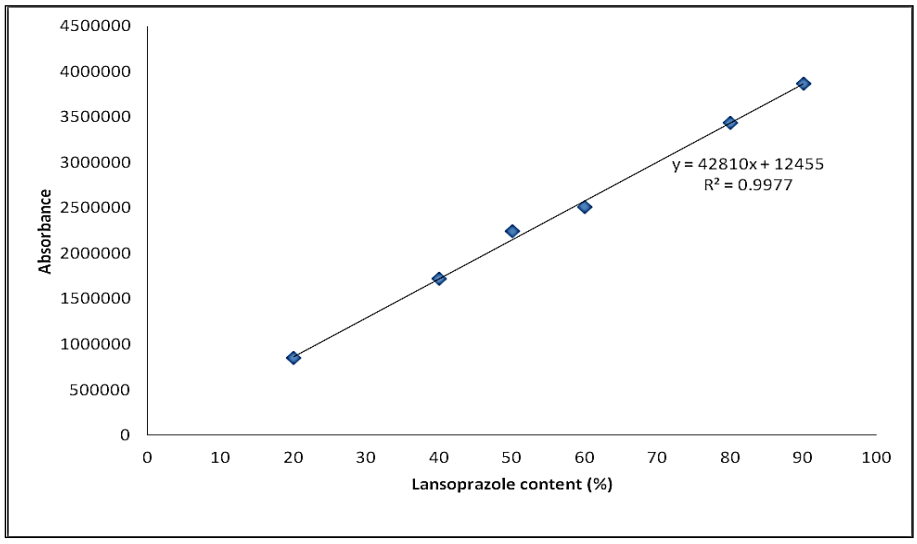


Figure 3.4 Recovered lansoprazole in the presence of its acid induced degradation products

Only 0.23 % of lansoprazole remained following degradation in acid for 6 hours. The linear relationship in Figure 3.4 shows that lansoprazole can be separated from its degradation products. This demonstrates that the detection of lansoprazole is possible in presence of any of its degradation products using the HPLC method. This is also evident when looking at each chromatogram as each peak is fully resolved and there is no interference from any additional degradation peaks to the peak of interest.

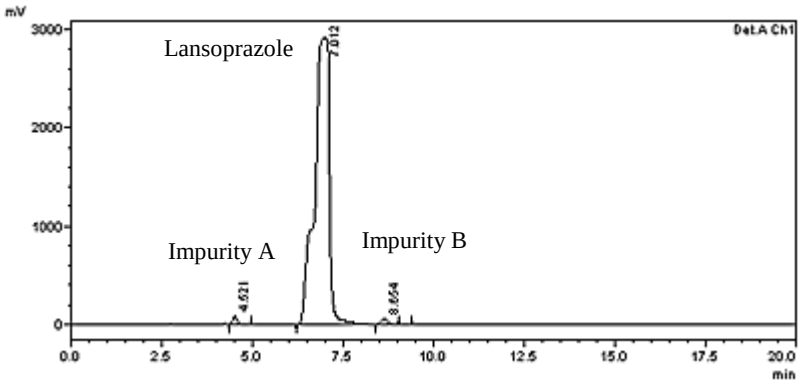


Figure 3.5 HPLC chromatogram with lansoprazole, impurity A and impurity B

The chromatogram (Figure 3.5) matched the reference chromatogram and Relative Retention Times (RRT) recommended by the BP monograph, Impurity A RRT around 0.5 and Impurity B RRT around 1.2.

Lansoprazole Retention time (RT) = 7.01 minutes

Impurity A

RT=4.52 minutes, (Actual RRT) = 0.64

Impurity B

RT=8.65 minutes, (Actual RRT) = 1.23

(British Pharmacopeia, 2010)

Impurities A (N oxide) and B (sulfone) were successfully identified using the HPLC method. These are consistent with other published data on lansoprazole impurities (Selenka *et al*., 2007).

3.3 Compatibility studies with gum base components

Early formulations of lansoprazole gums (57 and 41 % gum base; H gums and L gums), containing 6 % Rev7 polymer and 15 mg of lansoprazole per gram of gum) developed a mottled violet/brown discolouration upon visual examination of the gums after 2 weeks (Figure 3.6). The formulation process is described in detail in Table 4.1. It was observed that an increased proportion of the 57 % gum base formulation (H gums) was discoloured compared with those containing 41 % gum base (L gums). The formation of a coloured product indicated possible instability (degradation products) as well as being an undesirable factor as gums lacked aesthetic appeal and also consumers may associate the effect with poor

quality and a lack of content uniformity. This red complex colour has been detected previously in lansoprazole formulations (Tetsuro *et al*., 1992; DellaGreca *et al*., 2006; He *et al*., 2010).

Gums were stored under a variety of conditions to investigate the impact of environmental factors on discolouration. Formulations were wrapped in foil to protect from light, stored at 4°C and also under controlled humidity's. All of the conditions still resulted in appearance of coloured product and suggested a solid-solid interaction between an excipient in the gum and the drug. The coloured product developed even in a simple mixture of gum base, Rev7 and drug (lansoprazole loaded gum base), thus suggesting incompatibility between these components (Figure 3.7).

A. Discoloured H gum	B. Discoloured loaded gum base	C. Non discoloured L gum

Figure 3.6 The formation of a coloured product in lansoprazole gums A. Discoloured H gum, B. Discoloured lansoprazole loaded gum base and C. Non discoloured L gum

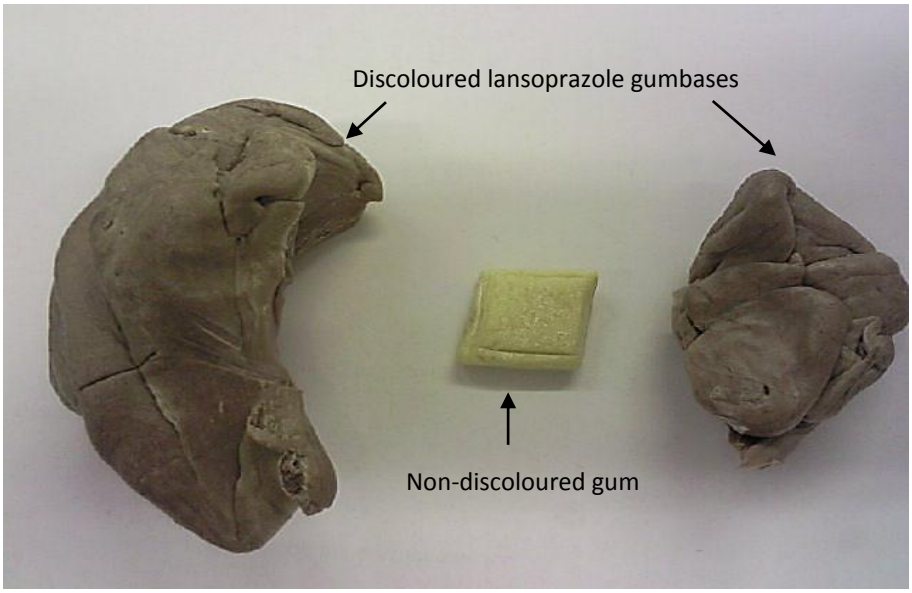


Figure 3.7 The formation of a coloured product in lansoprazole loaded gum base compared with a non-discoloured gum

3.3.1 Videometer analysis of gums

VideometerLab (Analytik, UK) is a multi-spectral imaging system which can be utilised to measure surface colour and chemical composition of solid samples. The Videometer is capable of scanning specimens using a range of different wavelengths from near infrared to soft ultraviolet. The technique was used on gums and combined with false colour imaging to emphasis the formation of a coloured product and any possible chemical changes.

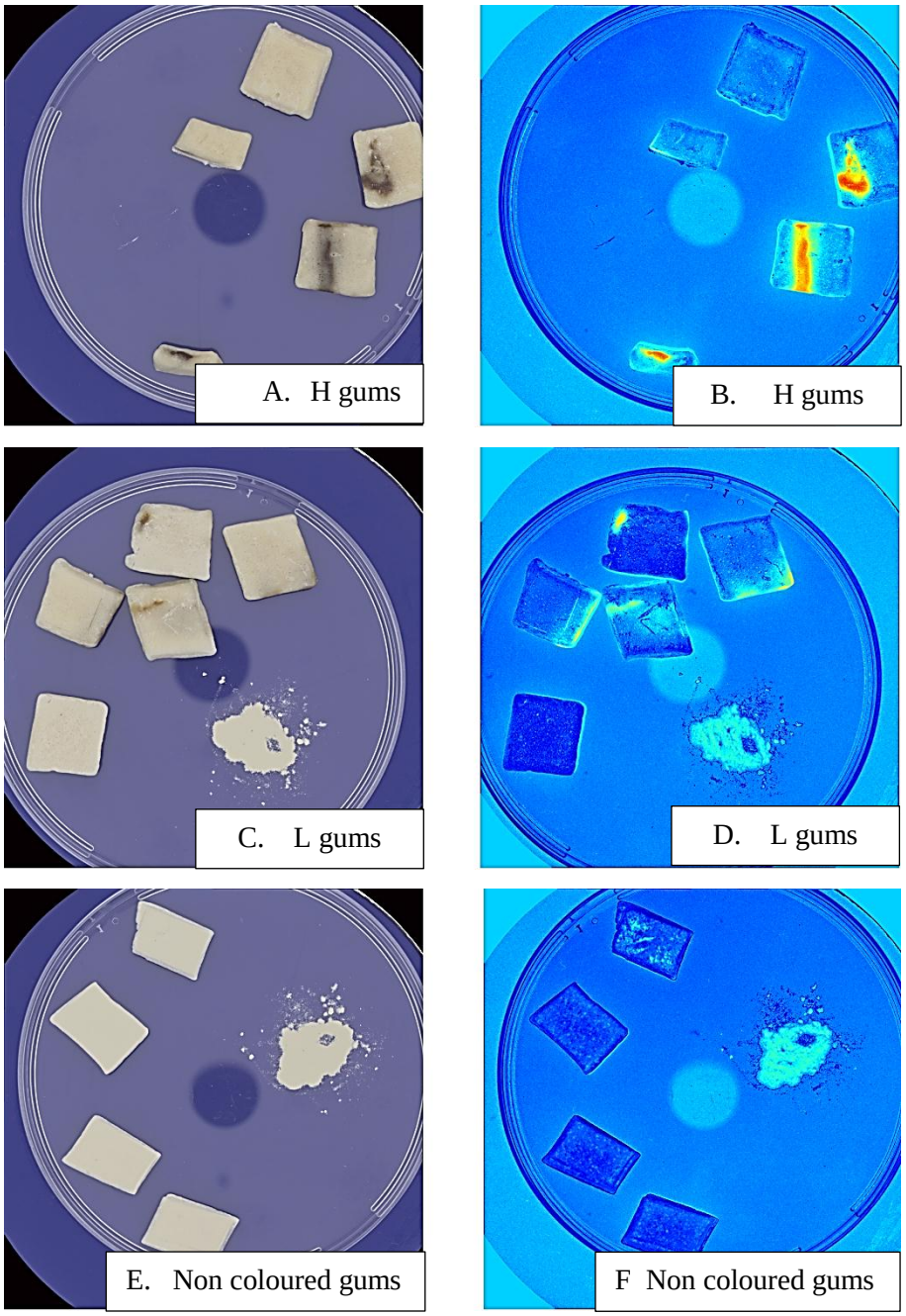


Figure 3.8 Discoloration in gums using Videometer (A and B. H gums, C and D. L gums and E and F. Non coloured gums)

The formation of coloured product was more extensive in formulations with increased gum base content (H gums) (Figure 3.8 A-F).

3.3.2 Compatibility studies with commercial gum bases and lansoprazole

To further investigate the interaction between the gum base (RVG1T000799), Rev7 and lansoprazole, formulations were prepared using commercial gum bases.

3.3.3 Bench top gum mixing method

The gum base was warmed in a steel tray using an electric hot plate at 80°C until molten. The drug was added gradually and mixed using palette knives (approx. 5 minutes) to obtain a homogenous sample containing 15 mg lansoprazole per gram gum base.

The following commercial gum bases were mixed with lansoprazole using bench top mixing:

- DILL containing BHA (anti-oxidant)
- MAGNA (no anti-oxidant)
- EURODENT containing Tocopherol (anti-oxidant)

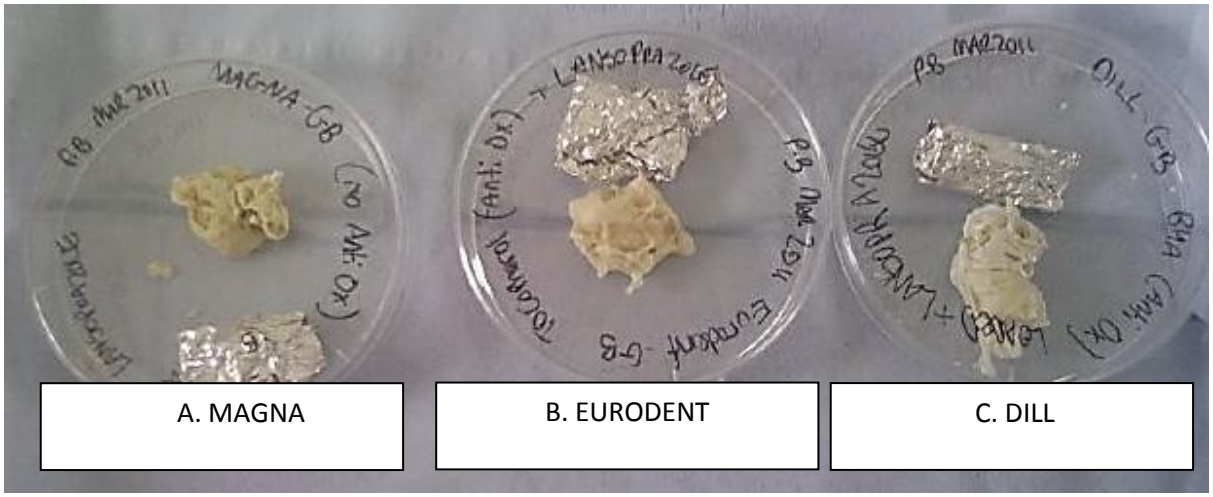


Figure 3.9 Commercial gum bases mixed with lansoprazole (A. Magna, B. Eurodent and C. Dill)

No visual discolouration was observed in any of the formulations with commercial gum bases suggesting a specific incompatibility between Rev7 components and lansoprazole (Figure 3.9 A-C).

3.3.4 Compatibility studies between Rev7 and lansoprazole

Rev7 was heated until molten using the bench top mixing method and mixed with lansoprazole to contain 15 mg of lansoprazole per gram.

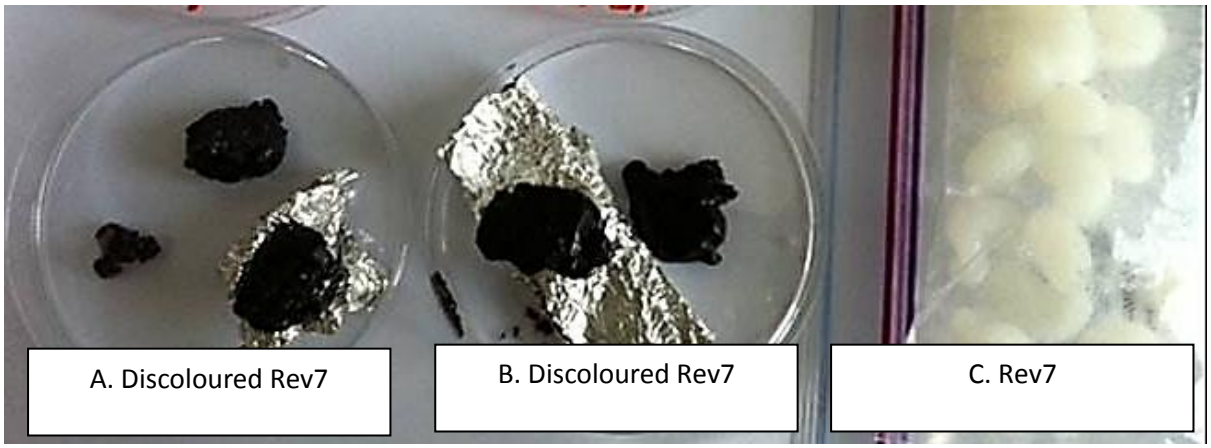


Figure 3.10 The formation of a coloured product with Rev7 and lansoprazole after 2 weeks (A. Discoloured Rev7, B. Discoloured Rev7and C. Rev7)

The formation of a heavily coloured product was visible after minutes displaying a violet tinge and this increased after 24 hours with a deeper violet colour developing over time (Figure 3.10 A-C).

Magnesium oxide (a glidant and pH adjuster) and EDTA (a chelating agent) were also mixed with Rev7 (additive: drug 1:1 and 3:1 w/w ratios) to try to minimise the formation of the coloured product (by chelating any free metal ions), however discolouration was still observed in all formulations.

3.3.5 Compatibility studies between Rev7 components and lansoprazole

Individual Rev7 components supplied by Revolymer® (MPEG, LIR 403 & MERIT co polymer) were mixed using the bench top mixing method with lansoprazole to contain 15 mg of lansoprazole per gram.

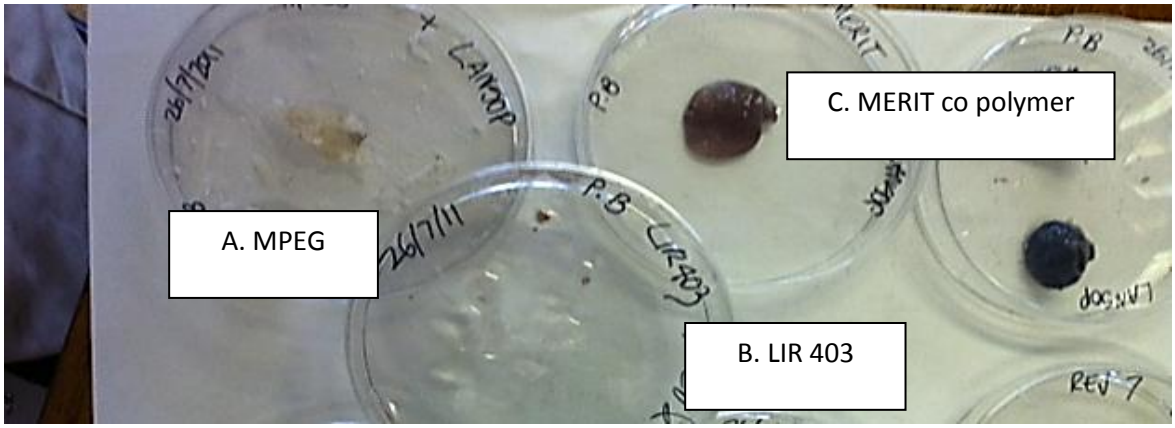


Figure 3.11 The formation of a coloured product with individual Rev7 components and lansoprazole after 1 month (A. MPEG, B. LIR403 and C.MERIT co polymer)

There was an immediate onset of the formation of a coloured product upon mixing with the Merit co polymer, however a small level of discolouration was observed with MPEG after 2 weeks and LIR 403 after 3 months (Figure 3.11 A-C).

3.3.6 The impact of discolouration on drug loading and content uniformity

To investigate if the formation of the coloured product caused a decrease in drug content and resulted in any quantifiable degradation products, drug was extracted from formulated gums and lansoprazole content quantified using HPLC. Samples were chosen as either visibly discoloured or non-discoloured gums for both formulations (57 %, H and 41 %, L gums compositions). Lansoprazole was extracted as described in Chapter 2.

Table 3.1 Drug content in discoloured and non-discoloured gums (H and L) (n= 4; mean ± s.d)

Sample description	Lansoprazole content (mg per g)
Discoloured H gums	15.36 ± 0.18
Discoloured L gums	15.13 ± 0.19
Non-discoloured H gums	15.09 ± 0.16
Non-discoloured L gums	14.57 ± 0.08

The overall mean content of all gums (discoloured and non-discoloured) was 15.04 ± 0.33 mg of lansoprazole per gram of gum (n=8; mean ± s.d) (Table 3.1). The content uniformity ranged from 96.75 - 103.26 % for all gums tested. There was no major loss of active in any formulation and degradation products were less than 5 % for all formulations.

3.3.7 Discussion of compatibility of lansoprazole with gum components

Results suggested that there was a compatibility issue with the Rev7 polymer; specifically the Merit co polymer, which resulted in the formation of a coloured product. Information on specific components was limited due to company confidentiality. Further formulations containing 8 % Rev7 polymer did not display the violet discolouration and so it was speculated that this may have been due to batch to batch variations.

Further attempts to extract the coloured component using solvent extraction and to further characterise the discoloured material using the HPLC-UV stability indicating method were unsuccessful. Possible incompatibilities resulting in formation of a coloured product have also been reported and assigned to an incompatibility with PEG (Tetsuro *et al*., 1992). However, there are no known current HPLC methods to characterise this coloured fraction (United States Pharmacopeia, 2007). This has been previously attempted but also failed due to the complexity and changeable nature of the material; it was suggested that the material consisted of a mixture of very labile degradation products (DellaGreca *et al*., 2006). A new lansoprazole impurity was identified as (des-(trifluoroethoxy) using mass spectrometry (MS), nuclear magnetic resonance (NMR) and fourier transform infrared spectroscopy (FTIR), but the study did not describe an associated colour production (Srinivas *et al*., 2010). MS linear ion trap technology has also been used to identify degradation products but again the formation of a coloured product was not reported (Selenka *et al*., 2007).

3.4 The potential of cyclodextrins as solubilisers, stabilisers and penetration enhancers

Cyclodextrin (CDs) represent an economical and practical method to improve undesirable physiochemical properties of problematic drugs (Bilensoy, 2011). CDs have seen a growing interest reflected in the increased popularity, particularly in formulation patents and publications over the last 10 years with a significant contribution using cyclodextrins as solubilisers and stabiliser (Messner *et al*., 2011). CDs have been used in chewing gums to improve drug release by increasing hydrophilicity and also for taste masking (Szejtli and Putter, 1993; Rassing, 1996 ; Chaudhary and Shahiwala, 2010).

3.4.1 CD structure

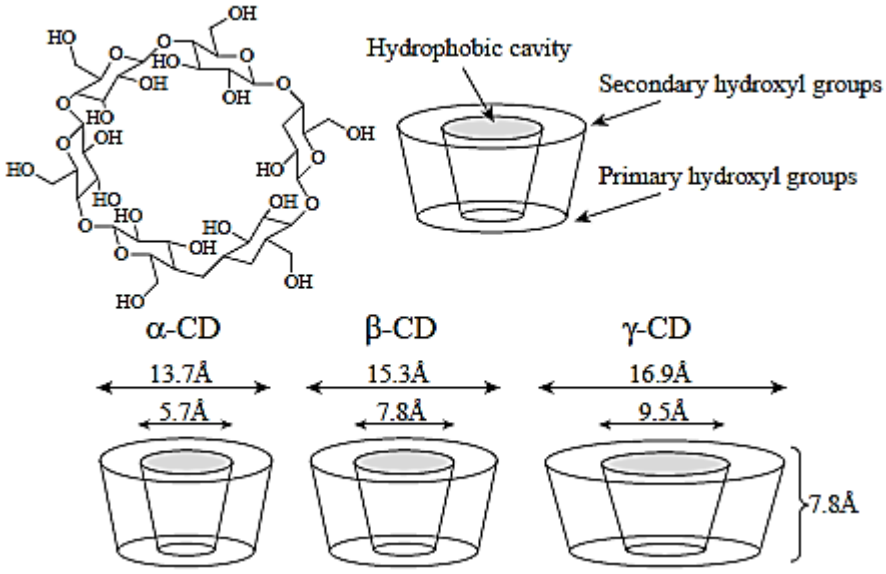


Figure 3.12 Structures of different CDs

(Bilensoy, 2011)

CDs are cyclic oligosaccharides which can contain six (α- CD), seven (β- CD), eight (γ- CD) (natural CDs) or more (α-1,4)-linked α-D-glucopyranose units (Figure 3.12 and Table 3.2). Molecular weights can range from 1000 to over 2000 Da. CDs occupy the shape of a truncated cone with hydroxyl functions orientated to the exterior of the cone. The central axial cavity has a skeletal lining of carbon and oxygen from the glucose residue. This gives the defining qualities of a lipophilic internal cavity and the ability to form an outer

hydrophilic exterior (hydration shell) due to hydrogen bonding between water and the hydroxyl groups on the CD. The cavity is occupied with water (inclusion water) resulting in an energetically unfavourable state, both in solution and crystalline states. β-CD contains approximately 14 % water as crystalline water (bound between CD molecules) and inclusion water (Szejtli and Szente, 2005). Further chemical modification of the hydrogen bond-forming hydroxyl groups in CDs causes a decrease in intermolecular forces in the crystal lattice, which results in large increases in the aqueous solubility of CDs, transforming the crystalline CD into an amorphous mixture (Loftsson and Brewster, 1996). One example of CD derivative is randomly methylated β- CD (Mβ-CD), with an average of 1.8 methyl (-CH_3) substitution per glucose repeat units, having a MW of 1312 Da and enhanced solubility of > 500 mg/mL compared to methylated forms (Loftsson *et al*., 2002). The differences in CDs structure may result in differences in inclusion complex formations (Lu *et al*., 2012).

Table 3.2 Properties of different CDs

Cyclodextrin	α	β	γ
Glucopyranose units	6	7	8
Molecular weight (Da)	972	1135	1297
Internal Central cavity diameter (Á)	4.9	6.2	7.9
Water solubility (at 25°C, g/100 mL)	14.5	1.85	23.2

(Anjana *et al*., 2013)

There are currently a range of formulations containing CDs available in the European market (Table 3.3). The majority of marketed drug products employ the CD as a complexing agent, increasing aqueous solubility, stability and bioavailability of the parent drug with the multi-functional drug carrier (Vyas *et al*., 2008). CDs can be particularly useful when bioavailability is limited due to limited dissolution compared to permeability, such as in cases with BCS class II drugs with low solubility and high permeability (Douroumis *et al*., 2013). Other uses include reducing irritation and eliminating undesirable smells and tastes *via* complexation and lack of interaction with sensory taste receptors (Szejtli and Szente, 2005).

The initial discovery by Villers who isolated a bacterial digest from starch in1891, naming it cellulosine, was later expanded by Franz Schardinger who isolated two dextrins, α and β from potato starch. Major biotechnological advancements, particularly in genetic engineering in the 1970's, lead to improvements in the production of highly pure, affordable, pharmaceutical grade CDs (Brewster and Loftsson, 2007). CDs are considered as having low toxicity and β-CD was approved as a food additive by the FDA as generally regarded as safe (GRAS) in

2001. Notably formulations include a taste masking complex in nicotine chewing gums, Nicogum® with β-CD (1:3, nicotine: β-CD). When chewing the gum, the dissolved sodium bi carbonate (buffering excipient) reacts with nicotine polyacrylate salt, releasing the water soluble nicotine base, which complexes with β-CD instantaneously in saliva to mask the bitter taste. The process is dynamic and complexation was predicted to be 99% (Szejtli and Szente, 2005).

Table 3.3 Example β-CD formulations marketed in Europe

Drug	Trade name	Formulation	Company
Cetirizine	Cetrizin™	Chewable tablet	Losan Pharma
Diphenhydramine	Stada travel™	Chewable tablet	Stada
Nicotine	Nicorette®	Sublingual tablet	Pfizer
Omeprazole	Omebata™	Tablet	Betafarm

(Brewster and Loftsson, 2007; Anjana *et al*., 2013)

3.4.2 CD complexation methods

Various methods to prepare complexes exist including co-precipitation, spray drying, freeze drying, co crystallisation, kneading and simple grinding (Szejtli and Szente, 2005; Carrier *et al*., 2007). Complexation may occur in the solid state due to the crystalline water in the CD however this is a slower process and depends on the mobility of the guest molecule (Szejtli and Szente, 2005). The method of complexation can affect complexation efficiency directly as well as the degree of amorphous content of the complexation. Using unionised drugs, additives such as ethanol, soluble co-polymers to act as a third component and also sonication to provide a super saturated solution, can increase complexation efficiency and stability (Loftsson and Brewster, 1996). A review of 28 randomly selected studies utilising CDs concluded that a combination of complicated factors may influence delivery enhancements of drug CD complexes (Carrier *et al*., 2007). The study by Arias (2000), involving γ-CD with omeprazole (2:1) found co-precipitation the most favourable method based on the increase in dissolution rate compared with freeze drying and spray drying (Arias *et al*., 2000).

There exists a dynamic equilibrium with the free drug and bound CD complex through self-association. The guest molecule is mainly believed to be instantaneously released/ disassociated through dilution of the complex. However other release mechanisms include replacement by another molecule to fit the cavity or being transferred to an area of increased affinity which may occur when coming into close proximity to a lipophilic biological membrane such as the oral mucosa and other forms of partitioning of the drug (Brewster and Loftsson, 2007).

In most cases, a 1:1 guest to host complex is formed (Brewster and Loftsson, 2007; Messner *et al*., 2011). The complex formation is independent of the chemical properties of the guest molecule and it is suggested that hydrogen bonding and Van de Waals forces are important in complex formation. Other system specific driving forces include the exclusion of high energy water bound in the CD cavity, release of conformational strain electrostatic charges, dipole-dipole and hydrogen bonding (Carrier *et al*., 2007). Complex formation is associated with a large negative enthalpy change. The specific component that is included in the CD will influence the changes in physiochemical properties by the molecular shielding from the cavity. This can be analysed by molecular modelling, single crystal X ray analysis, calorimetric titrations and by NMR techniques (Loftsson *et al*., 2004; Jambhekar and Thomas, 2013). Molecular modelling has suggested that the benzimidazole ring was included in a complex formed between omeprazole and hydroxypropyl- β- CD. However the study showed that this complex did not protect against degradation from light, heat and humidity, suggesting that this may have been due to the specific orientation of the structure in the CD inclusion complex (Ramos *et al*., 2011).

The following guidelines have been suggested for successful complexes based on favourable characteristics for the guest molecule:

- Molecular weight between 100 – 400
- Solubility less than 10 mg/mL
- More than 5 atoms forming the skeleton of the molecule ensuring optimal geometry
- Melting point below 250 °C
- Drug Log P > 2.5
- Low dose of drug < 100 mg (Carrier *et al*., 2007)

All factors involve increasing geometric compatibility and the affinity of the guest to the CD cavity and hence increasing the driving force for complexation (Vyas *et al*., 2008; Jambhekar and Thomas, 2013). This suggests that a possible complex of lansoprazole and CD may be possible. The effects of CDs in chewing gum formulations have previously been investigated (Chaudhary and Shahiwala, 2010). Inclusion complexes of miconazole with hydroxypropyl-β-CD resulted in supersaturated solubility and increased stability. This correlated with an increased release (25 %) compared to drug alone (0.7 %); when tested during *in vitro* mastication testing (Jacobsen *et al*., 1999).

3.4.3 CD metabolism and toxicity

CDs are resistant to enzymes which hydrolyse starch (α and β amylase). γ-CD is metabolised by saliva, whereas α-CD and β-CD remain intact and are metabolised by cyclomaltodextrinase, secreted from colonic bacteria (Carrier *et al*., 2007). CDs exhibit good overall oral safety profiles with Japan being the first country to approve the use of CD in the 1980s (Shabir and Mohammed, 2010). Detailed toxicity studies in rats and dogs shows that CDs can be administered safely *via* the oral route, with no significant changes after prolonged use (Jambhekar and Thomas, 2013). Both α-CD and β-CD have monographs in the United States and European Pharmacopeia and are also included in the Handbook of Pharmaceutical Excipients (Bilensoy, 2011). β-CD and its derivatives are more commonly used and are considered to have lower toxicity than γ- CD (Shabir and Mohammed, 2010).

3.4.4 CD solubility enhancement

The CD can host a guest drug molecule and has the ability to form non-covalent inclusion complexes (Loftsson *et al*., 2002). No covalent bonds are formed or broken during complexation and the complex is able to readily disassemble when diluted in aqueous solutions. The complex of a poorly soluble drug has increased hydrophilicity compared to the drug alone; a change in physical state from crystalline to amorphous has been reported when using Mβ-CD and this will contribute to an increase in dissolution rate (Carrier *et al*., 2007). The interior cavity can provide a microenvironment for suitably sized drugs whilst the outer surface provides water soluble wetting properties (increased contact angles in solution) and molecular shielding during complexation (Figure 3.13). Bioavailability can also be increased due to the increase in solubility with complexed drug formulations compared to cases where

bioavailability is limited due to low solubility of the drug alone. The AUC of flurbiprofen increased by 1.7 fold on complexation with CDs (Carrier *et al*., 2007).

Recent data also suggests that agglomeration and aggregation of CDs and complexes may also influence the ability to solubilise poorly water soluble drugs (He *et al*., 2008; Messner *et al*., 2011). Non-inclusion complexes may exist with the hydroxyl groups on the outer surface of the CD, forming hydrogen bonds forming drug aggregates. The study by Messner found 10 out of 11 guest compounds formed aggregation compounds and suggested that the size of agglomeration increased with CD concentration (Messner *et al*., 2011). The aggregates readily disassociated on dilution and so were metastable due to weak solute-solute interactions.

3.4.5 CD enhanced drug stability and safety

Molecular shielding can protect the drug from reactive species and has the ability to protect against incompatibilities with non complexed excipients (Jambhekar and Thomas, 2013) (Figure 3.13). The cavity provides insulation of labile drugs against corrosive environments such as oxidation, heat and light, reducing degradation processes. The stabilizing effect is dependent on the inhibition of interaction with the specific functional group of the drug protected by inclusion in the CD cavity as well as the strength of the hydrophobic interactions (Shabir and Mohammed, 2010). One study with unionised aspirin and β-CD (1:1) used NMR to model the complexed product and suggested the benzene ring was located inside the cavity (Loftsson and Brewster, 1996). As the most susceptible component in lansoprazole is the sulfoxide group (under acidic conditions) this will need to be included in the cavity to ensure maximum protection.

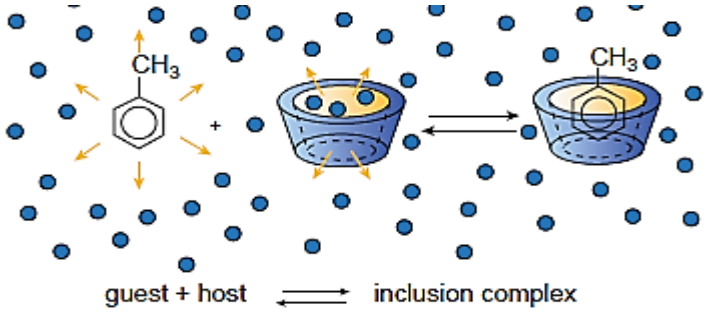


Figure 3.13 Molecular shielding during inclusion complexation

(Bilensoy, 2011)

Increased safety profiles of complexes compared to drug alone can be achieved by reducing toxicity due to increased potency and efficacy caused by the increase in solubility. The included functional group of the drug is protected from direct contact with biological membranes and can therefore also reduce toxicity (Rajewski and Stella, 1996). Degradation of omeprazole was found to decrease following complexation with β -CD compared to drug alone, but this may have been formulation specific. It was suggested that this was due to the specific orientation and also the high liability of omeprazole which may have interacted with free hydroxyl groups on the CD (El-Badry *et al*., 2009).

Bitter tastes and irritation can limit acceptance of dosage forms but this can be controlled if the specific component can be included in a complex formation. β -CD has a slightly sweet taste and is used in food/flavouring complexes. The ability to mask bitter drugs in saliva whilst in the oral cavity has been proven previously using β-CD and cetirizine (Szejtli and Szente, 2005). β-CD is also known to be particularly beneficial in increasing shelf life by displaying increased microbiological purity and stability/protection from polymerisation and sublimation (Lindner, 2006).

3.4.6 CD enhanced absorption across biological membranes

There are four possible mechanisms reported by which CDs can enhance absorption:

- Modifying the rate of drug release by acting as a potent drug carrier, increasing the availability and contact time of the drug at the surface of the barrier by increased wettability/ solubility of hydrophobic drugs.
- Protecting against degradation of particularly unstable drugs.
- Lowering the barrier function directly by membrane fluidisation by removing/solubilising membrane lipid components thereby modifying and facilitating absorption. β-CD has been shown to selectively remove cholesterol from rat intestines and hence modify absorption (Carrier *et al*., 2007).
- Competitive CD complexation with other components to release the drug resulting in increased site specific delivery.

Mβ-CD was found to display superior properties with regards to solubility, stability and permeability of omeprazole over β-CD complexes for a buccal delivery system (Figueiras *et*

al., 2007a). Solubility in artificial saliva increased 1.7-(β-CD) and 3.4-(Mβ-CD) fold following complexation. Structure analysis using NMR and ROESY showed that the benzimidazole moiety was included within the CD. The pyridine ring was found to cause a repulsion effect due to its size, whereas the benzimidazole had a more favourable affinity, but this was dependent on the orientation of the methoxy group on the CD. Increased stability of Mβ-CD complexes was reported to be due to the fact that the drug was included deeper within the cavity through the wider region of the CD (Figueiras *et al*., 2007b). Porcine buccal permeability studies with omeprazole found increases of 1.1 fold with complexed β -CD and a 1.7 fold increase with Mβ-CD. The increased permeability using the Mβ-CD complex was due to a solubilising effect on the membrane by the CD (Figueiras *et al*., 2009). Permeability increased with the addition of a third component, the alkalising agent, L-arginine, which caused a significant increase in solubility and stability of omeprazole. The work suggested potential for novel buccal formulations of omeprazole complexed with Mβ-CD in the presence of L-arginine (Figueiras *et al*., 2010).

3.5 Complexation of lansoprazole

β-CD and Mβ-CD were studied for their potential to form inclusion complexes with lansoprazole. They were chosen due to their popularity amongst current formulations, increased solubilising effects, cavity size, reduced toxicity and cost.

3.5.1 Complexation method

β- CD (Lot: 30H3400) and Mβ-CD (Lot: A0273729) were supplied from Sigma (Dorset, UK) and were of pharmaceutical grade.

The method of complexation was based on methods by Figueiras *et al*., (2007) and Ramos *et al*., (2011). The CD was dissolved in a basic hydro alcoholic aqueous solution (2:1 v/v distilled water: ethanol) at pH 10 ± 0.5 adjusted using 0.1M sodium hydroxide; to increase the solubility of lansoprazole (Figure 3.3). The required amount of lansoprazole was added and stirred for 48 hours in a tinted glass bottle at ambient temperature. The increased pH of the solution was necessary to increase the solubility and stability of lansoprazole. It was accepted that a decrease in drug affinity for the CD cavity would occur compared to the non-ionised form of drug but the increase in solubility and stability was deemed more desirable.

The non-ionised form of lansoprazole would be more lipophilic and so would have a higher propensity to displace water in the CD cavity, but would have an overall limited total solubility in the aqueous solution. The resultant clear solution was frozen using liquid nitrogen and then immediately freeze dried at - 40 °C and 0.129 mbar (Edward Modulyo, UK) (approx. 48 hours). Freeze drying was chosen due to its avoidance of heat as a technique to sublime water and to dry the solution leaving a lyophilised amorphous product. Previous studies with acidic drugs have resulted in high yields of complexed drug (Jambhekar and Thomas, 2013).

Ratio of CD: lansoprazole

In most cases a 1:1 guest to host complex is formed (Szejtli and Szente, 2005; Brewster and Loftsson, 2007; Messner *et al*., 2011; Jambhekar and Thomas, 2013). The work conducted by Figuieras also formed complexes of omeprazole and CDs in 1:1 molar ratios (Figueiras *et al*., 2010). Both 1:1 and 3:1 molar ratios (CD: lansoprazole) were prepared. The 3:1 ratio may improve complex efficiency during complexation in aqueous solution of CDs, molar ratios greater than 1 resulted in greater than 90 % complexed drug yields (Szejtli and Szente, 2005).

3.6 Characterisation of lansoprazole and complexed lansoprazole

Analytical techniques such as phase solubility, X ray diffraction (XRD), FTIR and differential scanning calorimetry (DSC) can be used to characterise changes to indicate successful complex formation when compared to drug alone and physical mixtures. Solubility experiments are the most commonly used methods to study complexation (Anjana *et al*., 2013). Physical mixtures (controls) were prepared by gently mixing components in a mortar with a pestle for a few minutes. It was accepted that physical mixing with grinding (addition of energy) can result in complex formation and this was accounted for (Carrier *et al*., 2007).

Experiments were performed to evaluate successful complex formation of lansoprazole with the CDs:

- Determination of solubility
- Differential scanning calorimetry

- X Ray Diffraction
- Fourier Transform Infrared Red
- Scanning Electron Microscope (SEM)
- Loading efficiencies of complexes

3.6.1 Methods

3.6.1.1 Determination of solubility of lansoprazole and complexed lansoprazole

An increase in solubility was used as an indication for successful complex formation as used previously (Zheng *et al*., 2005; Brewster and Loftsson, 2007). In brief, the different CDs complex ratios were added in excess to 10 mL of artificial saliva while stirring at 250 rpm in a water bath at a controlled temperature of 25 ℃. Samples were taken at 8.5 hours and 15.5 hours to correspond with previous solubility data (section 3.2.1.1). All CD solubility experiments were conducted in triplicate and analysed by HPLC. Two pH conditions were assessed, pH 6 and pH 8, to reflect the physiological pH in the oral cavity before chewing (pH 6) and after chewing gum (pH 8).

3.6.1.2 Loading efficiency of CD complexes

Accurately weighed samples of the 1:1 Mβ-CD complex (10 mg) were used to determine drug loading in the complex. Samples were dissolved in 50 mL mobile phase (see section 2.1.2) and drug content determined using HPLC. Samples were analysed in triplicate.

3.6.1.3 DSC

The thermal characteristics of pure drug and CDs, 1:1 physical mixtures and inclusion complexes were determined by DSC (Mettler Toledo DSC 822e). Ten milligrams of sample was weighed in aluminium pans and sealed. The instrument was calibrated using indium. The samples were heated from 50°C to 300°C with a heating rate of 10 °C per minute, nitrogen was purged at a flow of 30 mL /min. Samples were analysed in triplicate.

3.6.1.4 XRD

Powder X Ray Diffractometry (Brucker AXS D2 phaser) was performed on pure drug and CDs and inclusion complexes (1:1) to evaluate any changes in crystalline/ amorphous structure. The voltage was set at 20k, with current at 5mA. Samples were scanned over 5.0°-50° 2θ range with step sizes of 0.025 and step counting times of 2 seconds, at a temperature of 25 °C.

3.6.1.5 FTIR

FTIR (Nicolet 380) was used to characterise shifts in bands of absorbance for pure drug and CDs, 1:1 physical mixtures and inclusion complexes and were carried out using the FTIR-ATR (Attenuated Total Reflectance) attachment. Attenuated total reflectance (ATR) was used with the diamond attachment. Scans were performed over 400 - 4000 cm^{-1} with a 4 cm^{-1} resolution.

3.6.1.6 SEM

The morphology of pure drug and CDs and inclusion complexes (1:1) were observed using SEM (JOEL JSM 6060LV, Joel UK Limited, Herts, UK). The sample was placed on carbon tape and plated/coated with gold and palladium (Au and Pd) to allow conducting of electrons.

3.6.2 Results

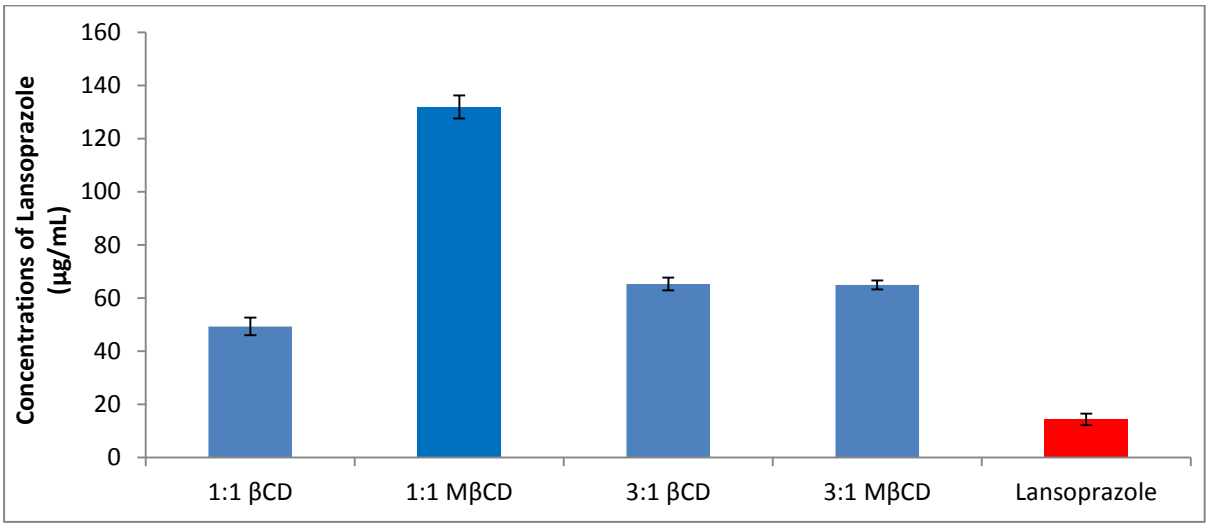


Figure 3.14 Solubility of complexed lansoprazole in artificial saliva pH 6.1 after 8.5 hours (n=3; mean ± s.d)

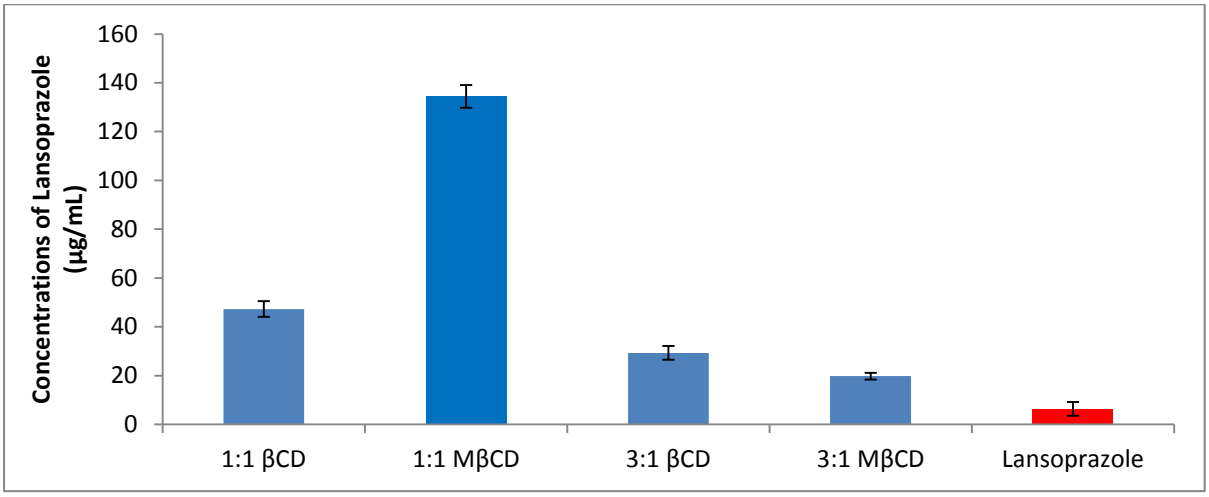


Figure 3.15 Solubility of complexed lansoprazole in artificial saliva pH 6.1 after 15.5 hours (n=3; mean ± s.d)

Solubility was increased with all ratios of CDs compared to pure lansoprazole due to the increased wettability of CDs; the 1:1 Mβ-CD complex was the most soluble (134 µg/mL) and this was maintained up to 15 hours (134 µg/mL) (Figures 3.14 and 3.15). Solubility of the pure drug decreased over the 15 hour duration as seen previously due to degradation at the lower pH (Figure 3.4). Complexes formed at a 1:1 ratio maintained a consistent solubility over the time course whereas the solubility of the 3:1 complex decreased over time, this may

have been due to disassociation of the drug from the CD. Increasing CD concentration has been linked with an increase in degradation (El-Badry *et al*., 2009).

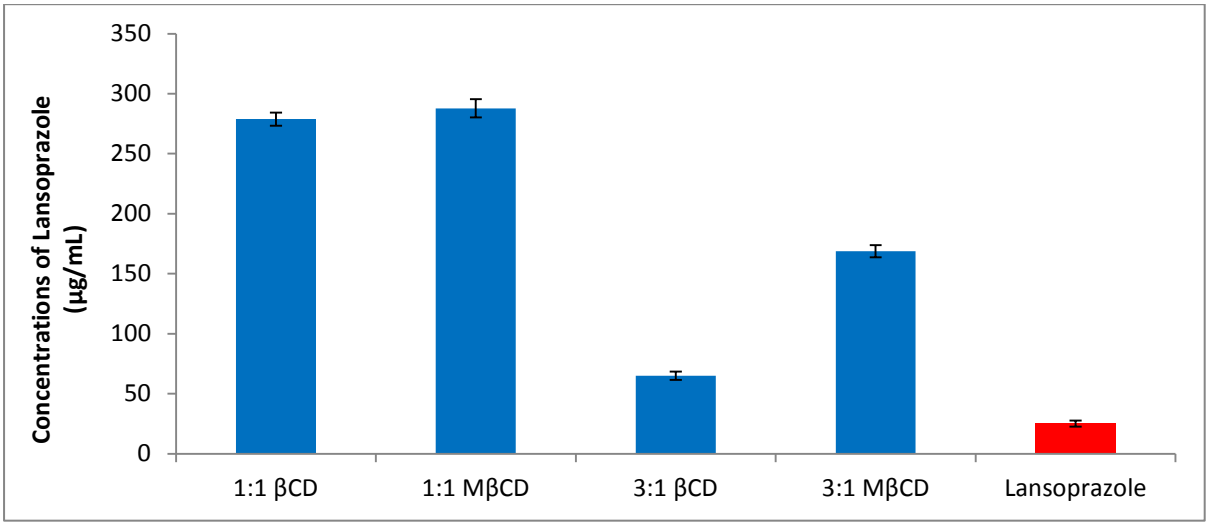


Figure 3.16 Solubility of complexed lansoprazole in artificial saliva pH 8.2 after 8.5 hours (n=3; mean ± s.d)

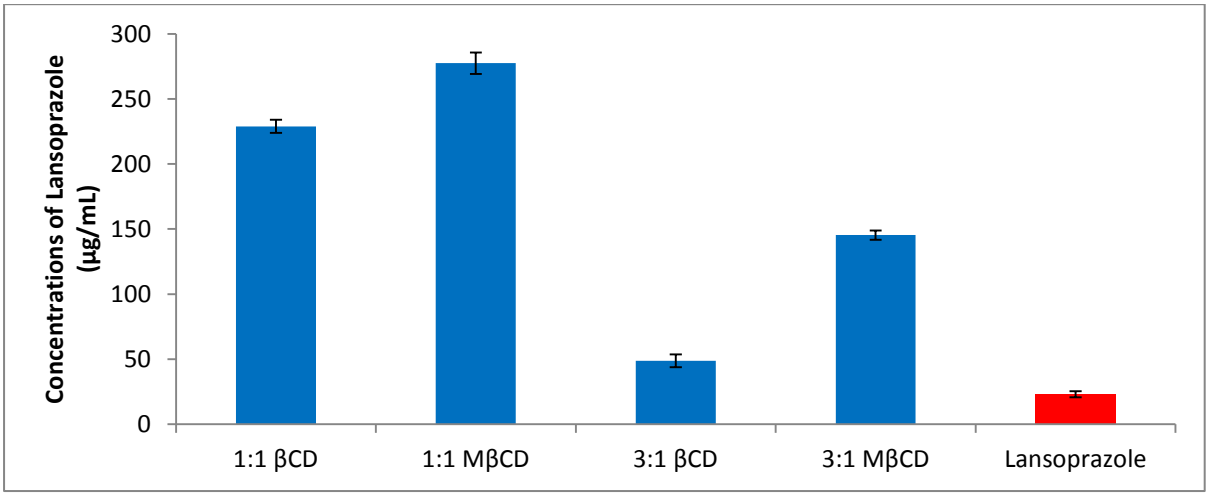


Figure 3.17 Solubility of complexed lansoprazole in artificial saliva pH 8.2 after 15.5 hours (n=3; mean ± s.d)

As expected, an increased pH resulted in increased solubility (Figures 3.16 and 3.17). All complexed forms had higher solubility than the drug. The highest mean solubility was observed with the 1:1 Mβ-CD (289 µg/mL). The 1:1 complexes maintained a constant solubility over the time of the study indicating increased stability.

The Mβ-CD (1:1) gave an 9 fold increase in solubility (134 µg/mL) compared to lansoprazole alone at pH 6.1 and maintained solubility up to 15 hours at pH 6.1 suggesting increased stability due to possible molecular shielding. The increase in solubility is due to the

hydrophilic component in CD. The total amount of drug in solution was equal to free drug and complexed drug. The increase in wettability is due to increased surface contact/interaction with drug and artificial saliva. The Mβ-CD complexed solution resulted in increased concentrations compared to β-CD complexed solution due to the increased solubilising effect of the modified Mβ-CD (Shabir and Mohammed, 2010). Freeze drying resulted in an amorphous complexed material, and the reduced crystallinity would have also contributed to increased solubility; this was previously observed in omeprazole inclusion complexes also (Figueiras *et al*., 2007b).

Total lansoprazole drug recovery from prepared complexes was 91.26 ± 12.34 % (n=3; mean ± s.d). The total amount of drug in solution was equal to free drug and complexed drug existing in a dynamic equilibrium.

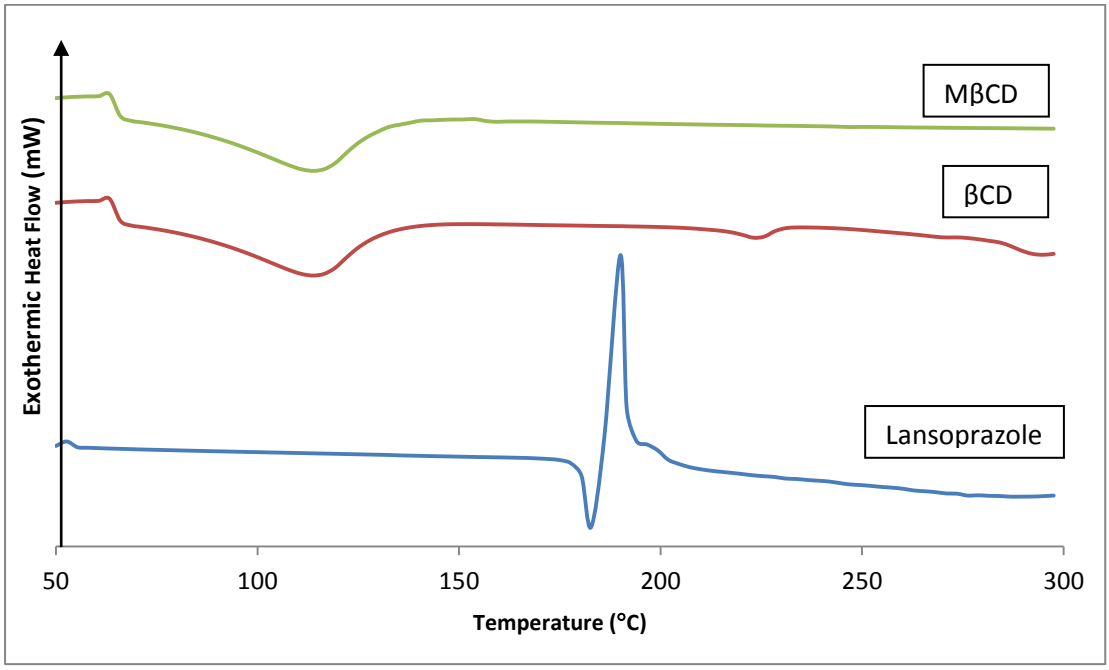


Figure 3.18 DSC thermograms of pure lansoprazole and cyclodextrins

A sharp fusion endothermic peak at 180 ± 1.2°C was observed which corresponds to the melting point of the lansoprazole (178-182°C) (Figure 3.18). This is followed by an exothermic peak at 185 ± 1.3 °C (n=3; mean ± s.d) due to thermal decomposition, with a small shoulder at 195 ± 1.9 °C corresponding to previous studies (Zhang *et al*., 2008; Mendiratta *et al*., 2011; Lu *et al*., 2012). The CDs display broad endothermic peaks at onset temperature of 67 ± 2.3°C and a maximum at 113 ± 2.2 °C. Further smaller endothermic peaks were seen at 215 ± 0.9 °C and 286 ± 1.1 °C for β-CD. These effects are normally

associated with crystal water losses (dehydration) in the cyclodextrin (Szejtli and Szente, 2005) and thermal decomposition at 250 °C by oxidation (Jambhekar and Thomas, 2013).

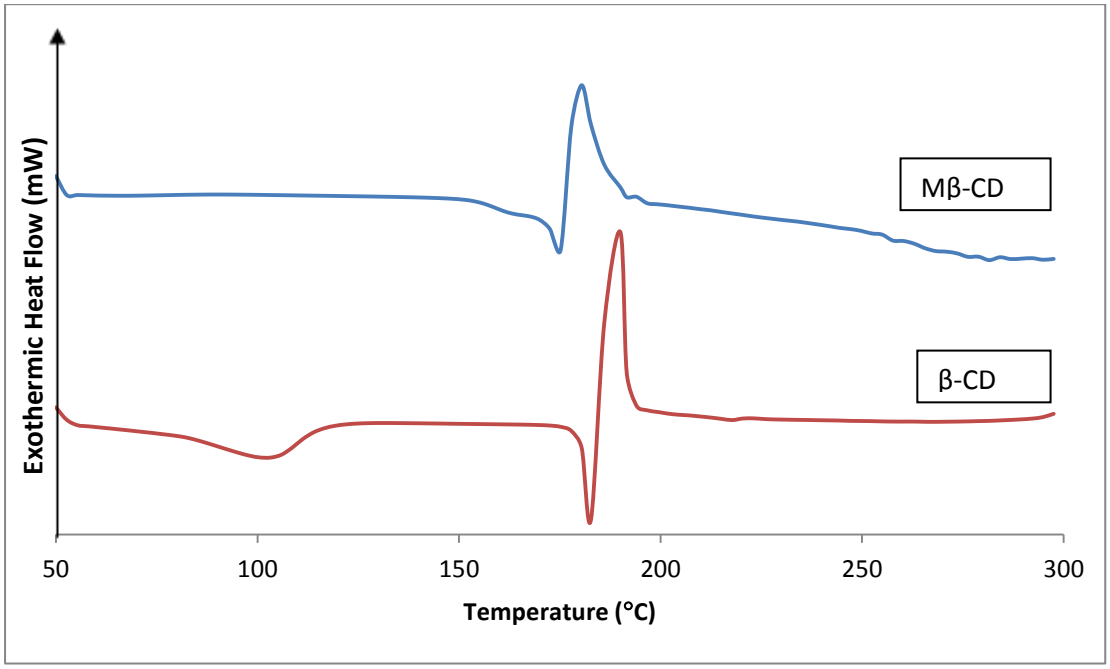


Figure 3.19 DSC thermograms of physical mixtures of lansoprazole and cyclodextrin (1:1)

The occurrence of the lansoprazole peak (178-182 °C) in the physical mixtures suggests that the drug was not included in a complex within the CDs (Figure 3.19). A broader peak for Mβ-CD suggests a possible solid state interaction or loss of drug crystallinity.

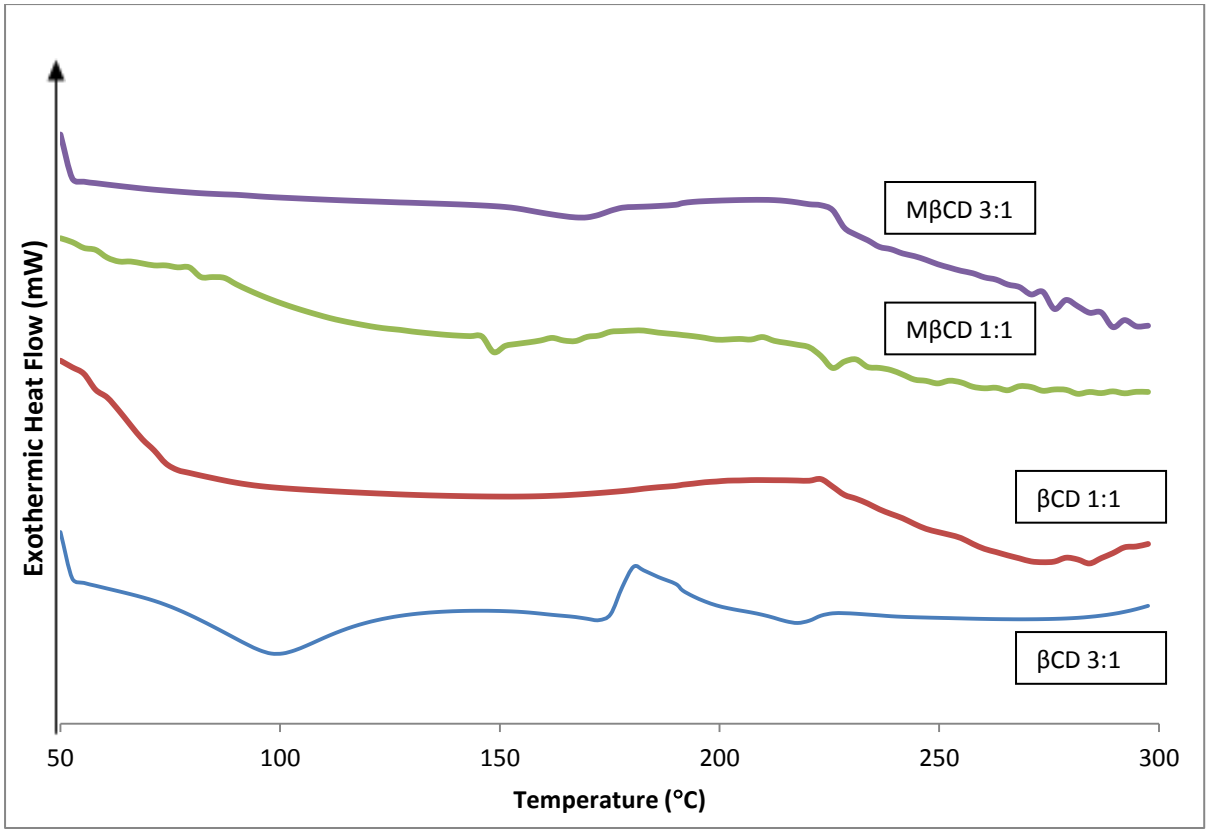


Figure 3.20 DSC thermograms of lansoprazole and CD complexes (1:1 and 3:1)

The eradication of the signature lansoprazole peak at (178-182 °C) indicates formation of a complex due to the absence of crystal structure suggesting inclusion of the drug (observed for both Mβ-CD ratios and the β-CD (1:1) (Figure 3.20). This technique is commonly used to provide solid state characterisations during complexation by the absence of key peaks, which can signify transition of the guest into the CDs (Jambhekar and Thomas, 2013; Anjana *et al*., 2013). The occurrence of the lansoprazole peak in the 3:1 β-CD suggests that not all the drug was included in the complex.

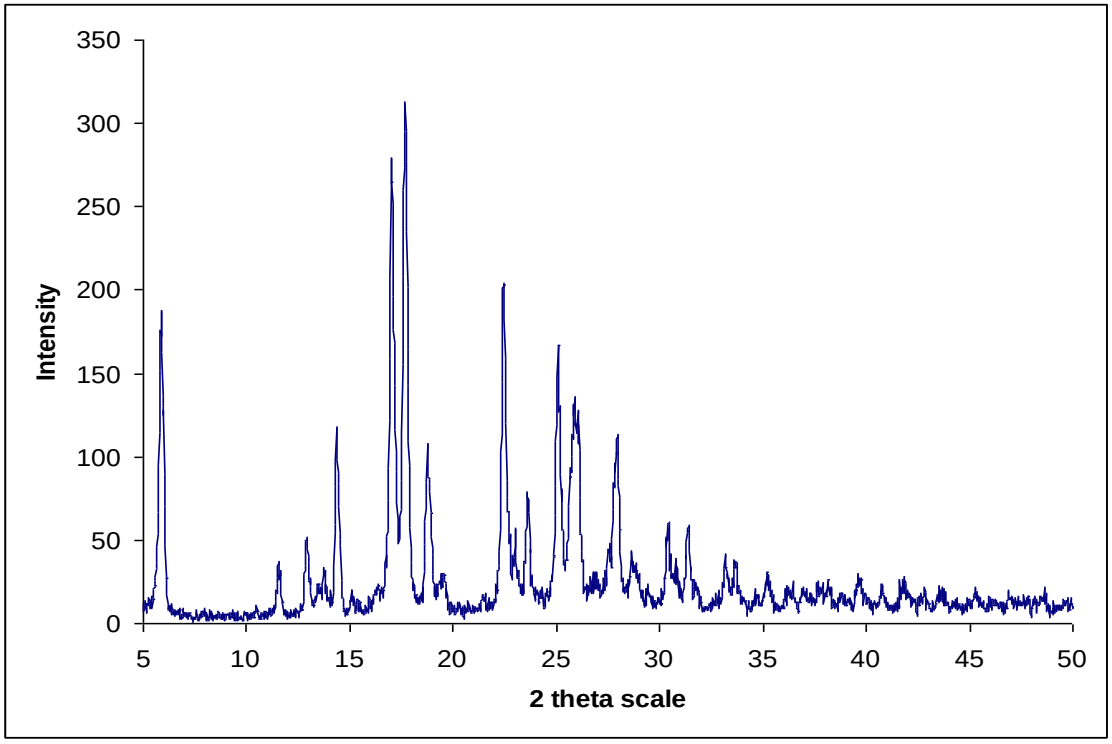


Figure 3.21 XRD spectrum of lansoprazole

Sharp characteristics peaks were observed at a diffraction angle 2θ 5.7,14.9, 17.4, 17.9, 18.9, 22.3, 24.9 and 27.9° and show the pure drug is in a crystalline state (Figure 3.21).

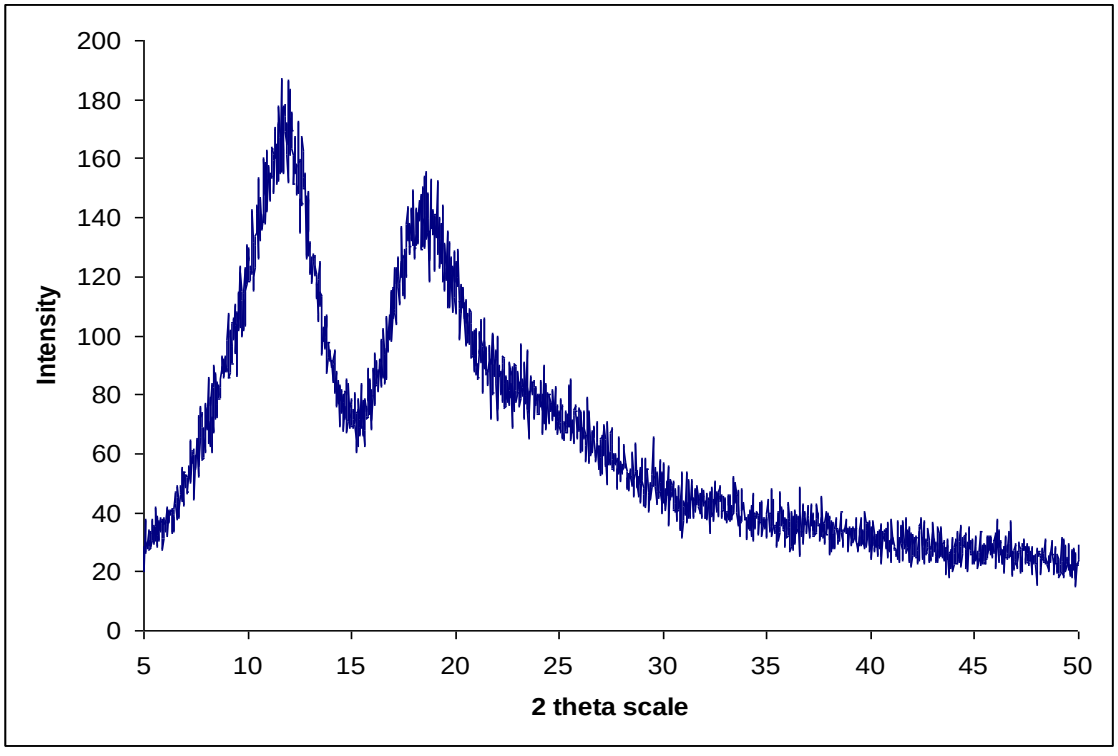


Figure 3.22 XRD spectrum of Mβ-CD

The diffractogram did not show any distinct diffraction peaks indicating the hollow amorphous structure of the Mβ-CD (Figure 3.22) and agreed with previously reported data (Figueiras *et al*., 2007a).

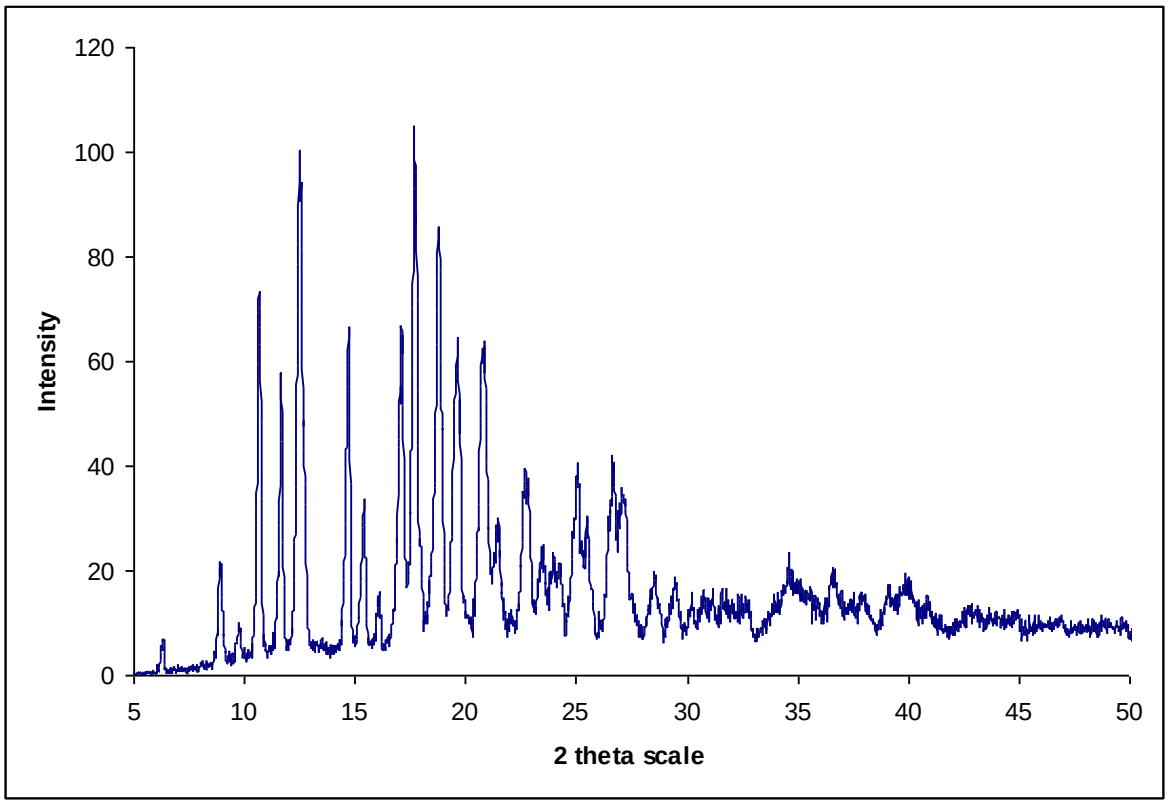


Figure 3.23 XRD spectrum of β-CD

Figure 3.23 indicates the crystalline structure of the β-CD with a range of peaks intensities over 10 - 30°.

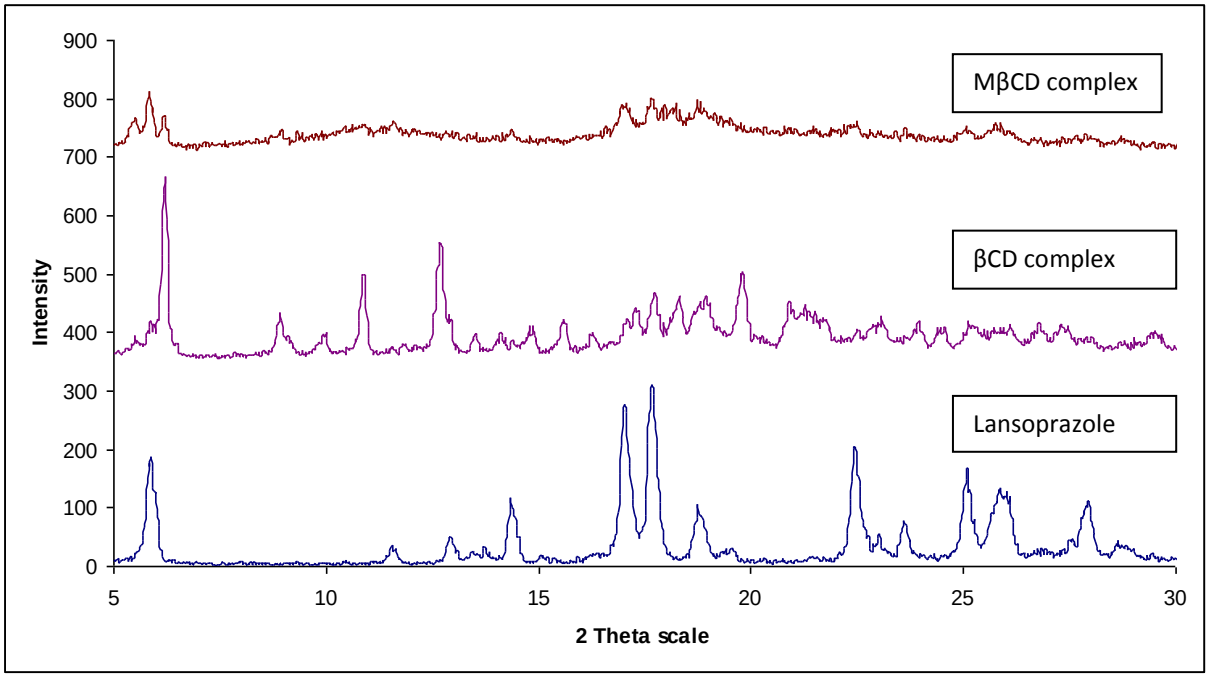


Figure 3.24 XRD spectra of complexed lansoprazole and CDs (1:1)

The intensity of the finger print regions of lansoprazole at 17.4, 17.9, 18.9° were greatly reduced in the Mβ-CD complex (Figure 3.24). The Mβ-CD complex showed that the drug no longer existed in its crystalline state and had an amorphous structure as the result of inclusion.

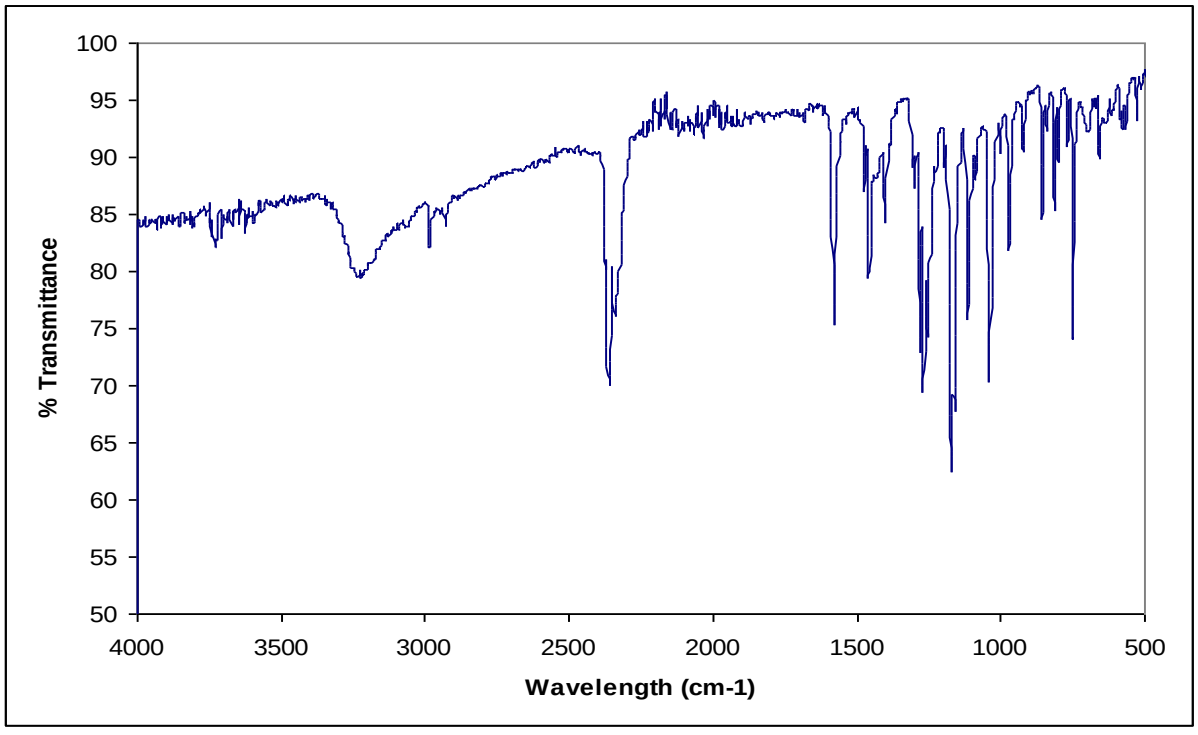


Figure 3.25 FTIR spectrum of lansoprazole

Characteristic peaks were identified for lansoprazole and agreed with previous data (Figure 3.25) (Zhang *et al*., 2008; Mendiratta *et al*., 2011; Lu *et al*., 2012).

Wavelength (cm^{-1})	**Chemical groups**
749.8	aromatic stretching of C-H
1038.3	sulfinyl (S=O)
1117.4	ether band (-O-)
1272.1	stretching vibrations of C-N (in the benzimidazole)
1579.2	stretching vibrations of C=N (in the benzimidazole)
2373.7	stretching of CH_2
3226.4	stretching vibrations of NH_4 (amine)

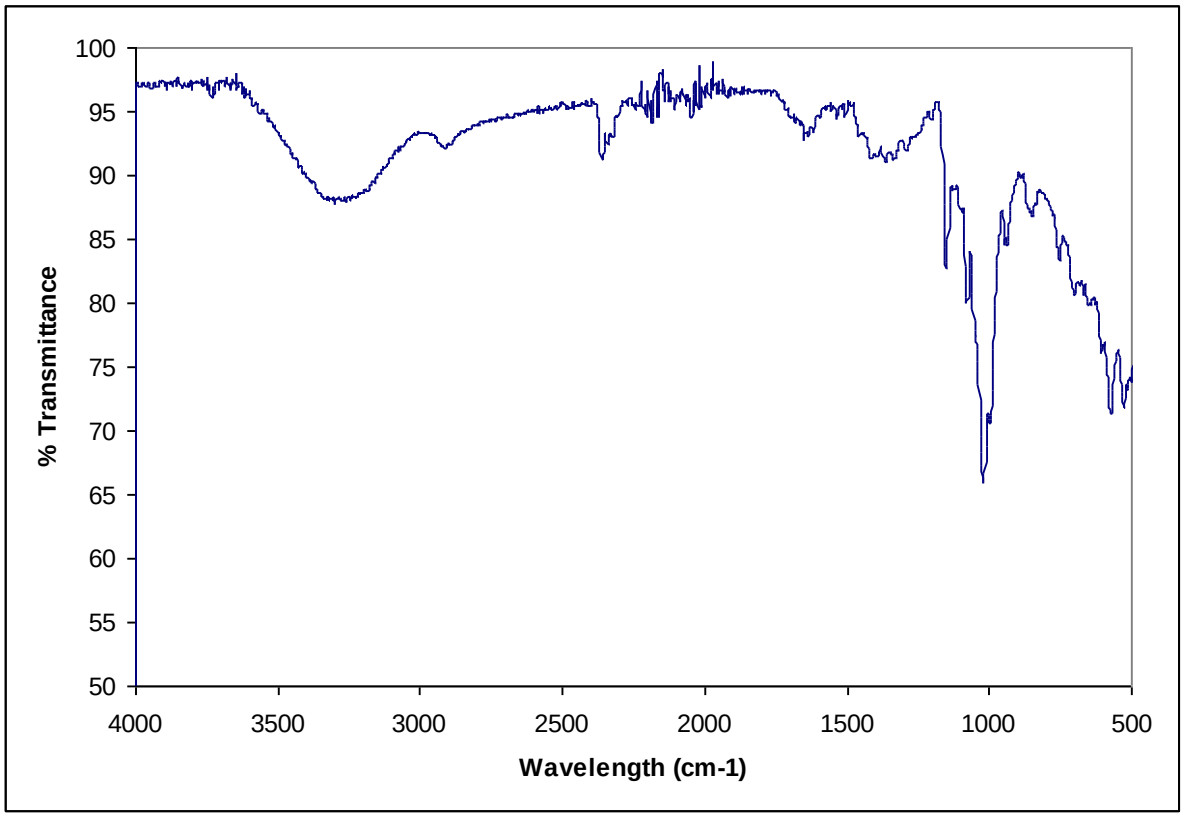


Figure 3.26 FTIR spectrum of β-CD

The following characteristic peaks were identified for β-CD (Figure 3.26).

Wavelength (cm^{-1})	**Chemical groups**
1020.6	bending of C-O-C
3298.5	stretching of O-H

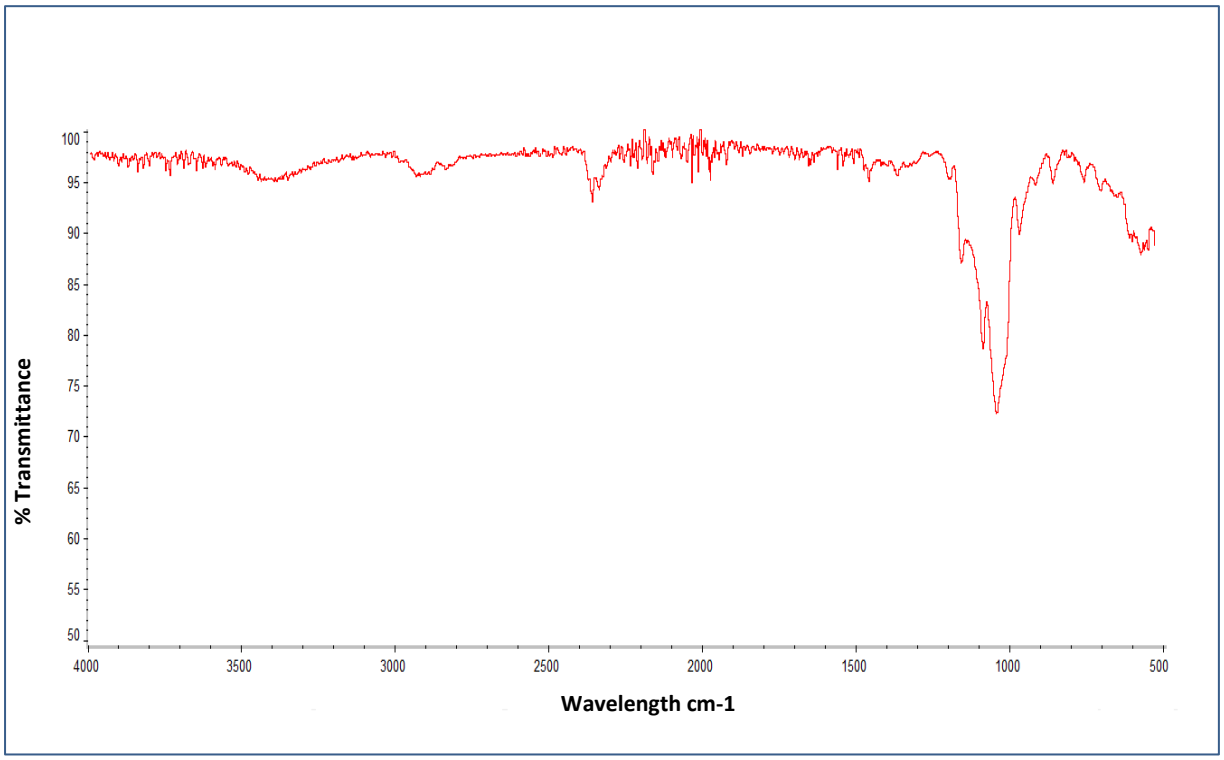


Figure 3.27 FTIR spectrum of Mβ-CD

The FTIR analysis was preformed (June 2015) and the following characteristic peaks were identified for Mβ-CD (Figure 3.27).

Wavelength (cm^{-1})	Chemical groups
1083.8	bending of C-O-C
2645.6	stretching of C-H

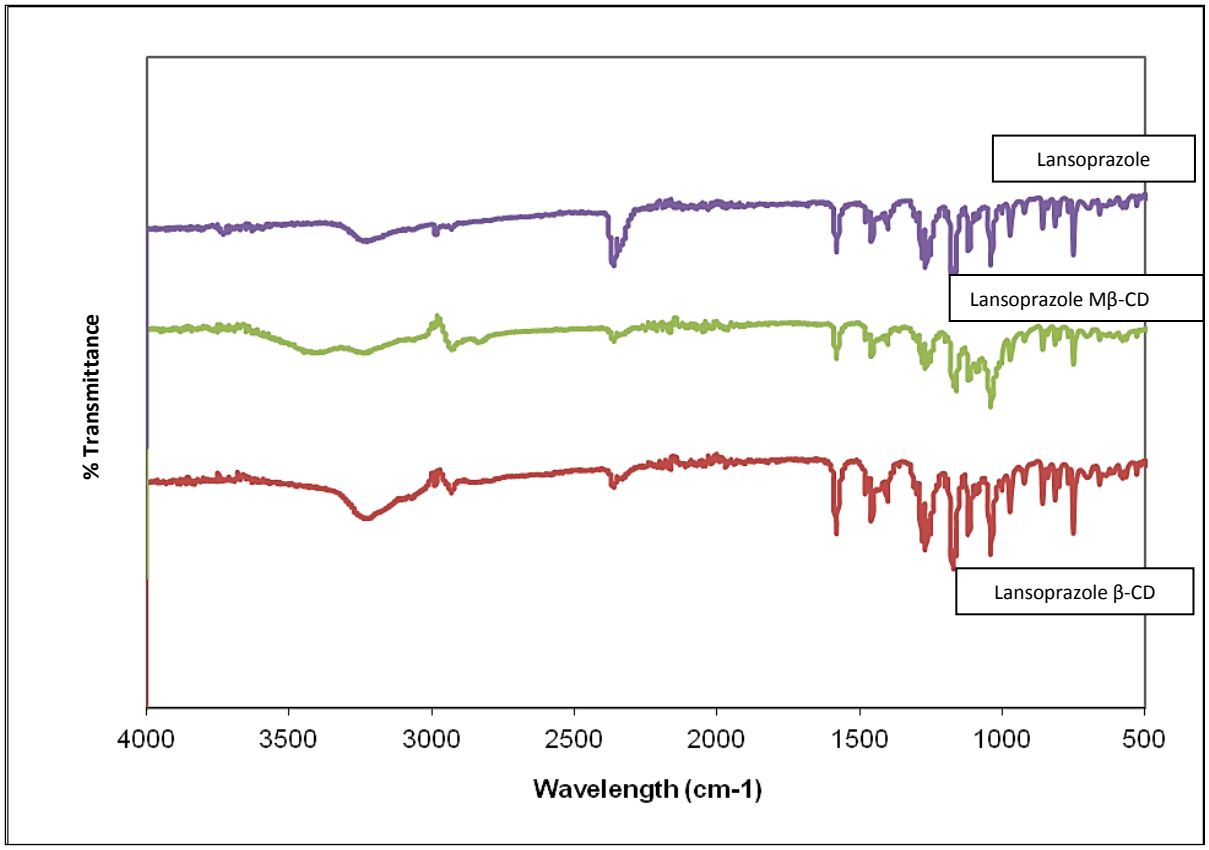


Figure 3.28 FTIR spectra of physical mixtures of Mβ-CD and β-CD (1:1)

The characteristic finger print regions of lansoprazole in both CD physical mixtures are visible (Figure 3.28). However Mβ-CD physical mixtures showed reduced intensity of the 1117 ether band (-O-) 1163 and 1253 suggesting a degree of formation of complex. It was noted that the broad and intense bands of the CD (within the same wavelength range) may have masked the characteristic bands of the drug.

Wavelength (cm^{-1})	**Chemical groups**
749.8	aromatic stretching of C-H
1038.3	sulfinyl (S=O)
1117.4	ether band (-O-)
1282.3	stretching vibrations of C-N (in the benzimidazole)
1579.6	stretching vibrations of C=N (in the benzimidazole)
3225.8	stretching vibrations of NH_4 (amine)

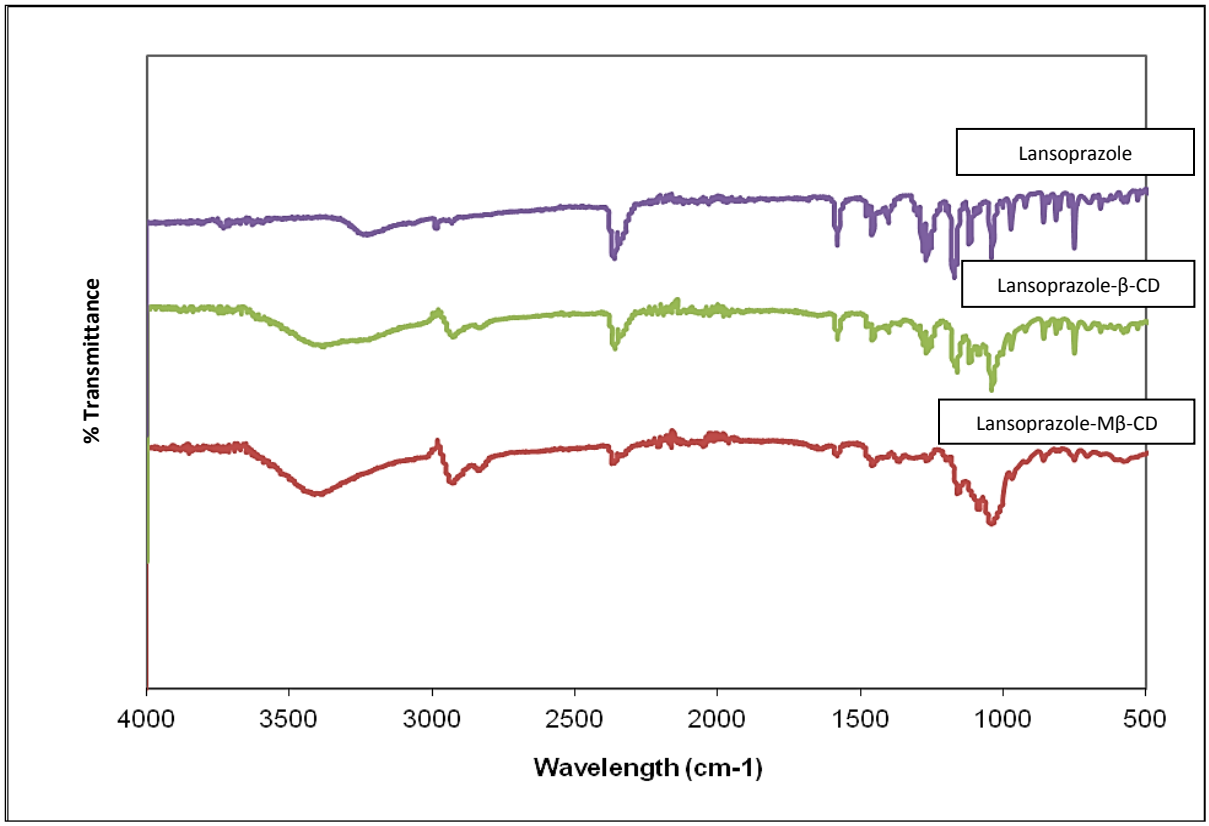


Figure 3.29 FTIR spectra of complexes of lansoprazole with β-CD and Mβ-CD (1:1)

The decreased intensity and shifting of bands can indicate which groups are directly involved in formation of hydrogen bonds with the CD cavity (Anjana *et al*., 2013). The Mβ-CD complex showed decreased band intensities at 1579 (C=N benzimidazole ring), 1272 (C-N pyridine ring), 1117 (ether band) and 749 cm^{-1} (aromatic C-H) suggesting inclusion complex formation (Figure 3.29).

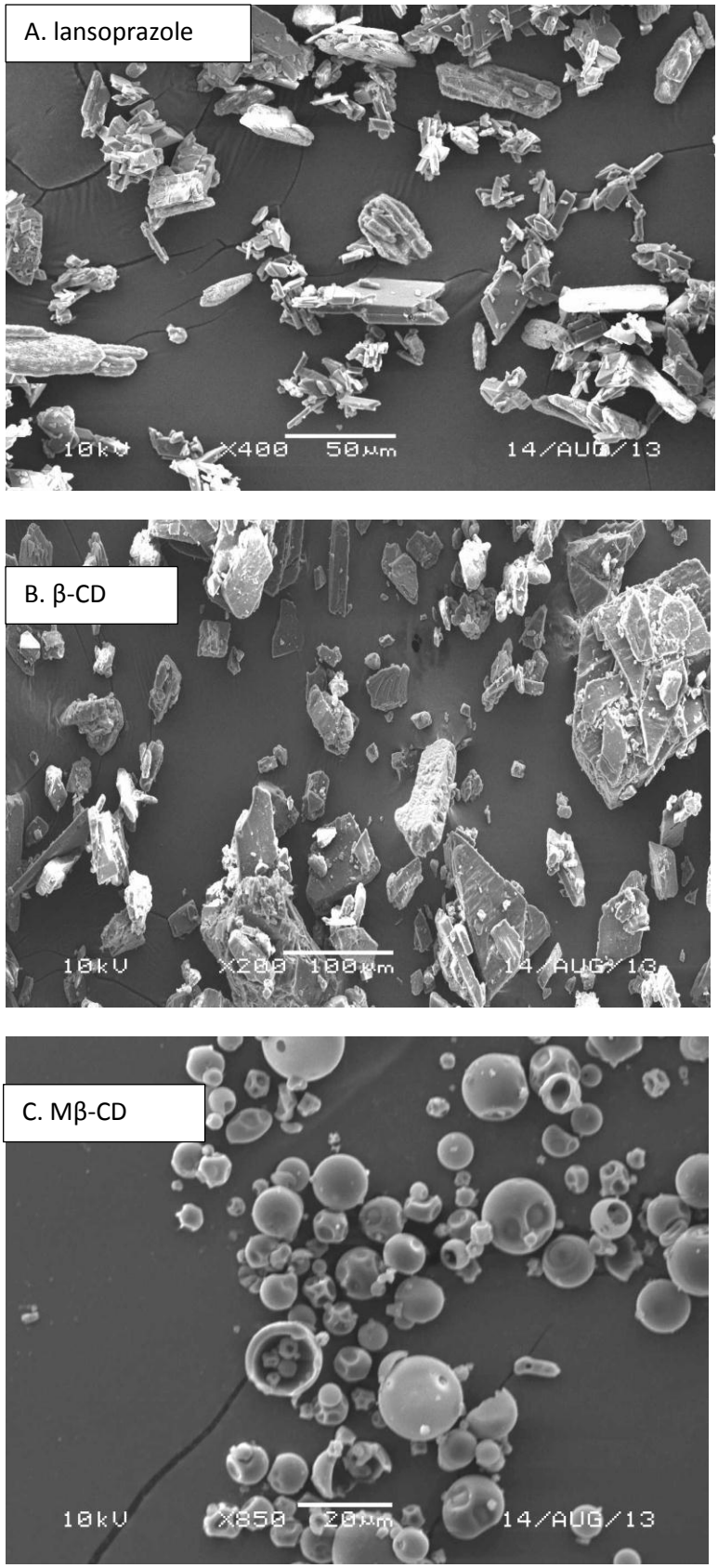
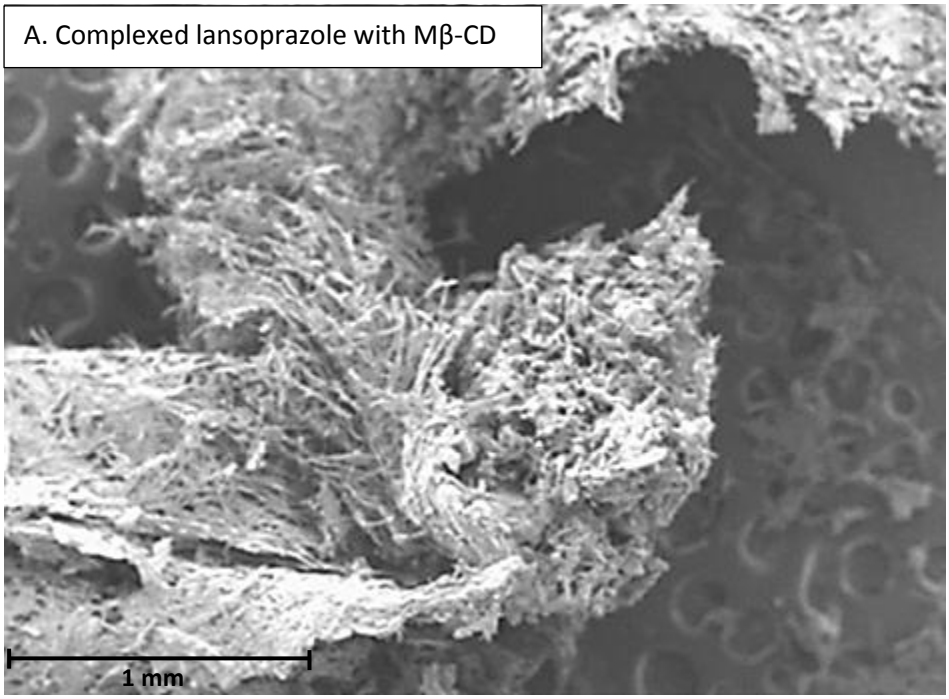


Figure 3.30 SEM images of A. lansoprazole,B. β-CD and C. Mβ-CD

Lansoprazole is characterized by regular shaped crystals ranging from 25-70 µm in size. β-CD presented irregular shaped crystal structures ranging from 10 - 200 µm in size. Mβ-CD presented spherical particles ranging from 5-20 µm in size (Figures 3.30 A, B and C).



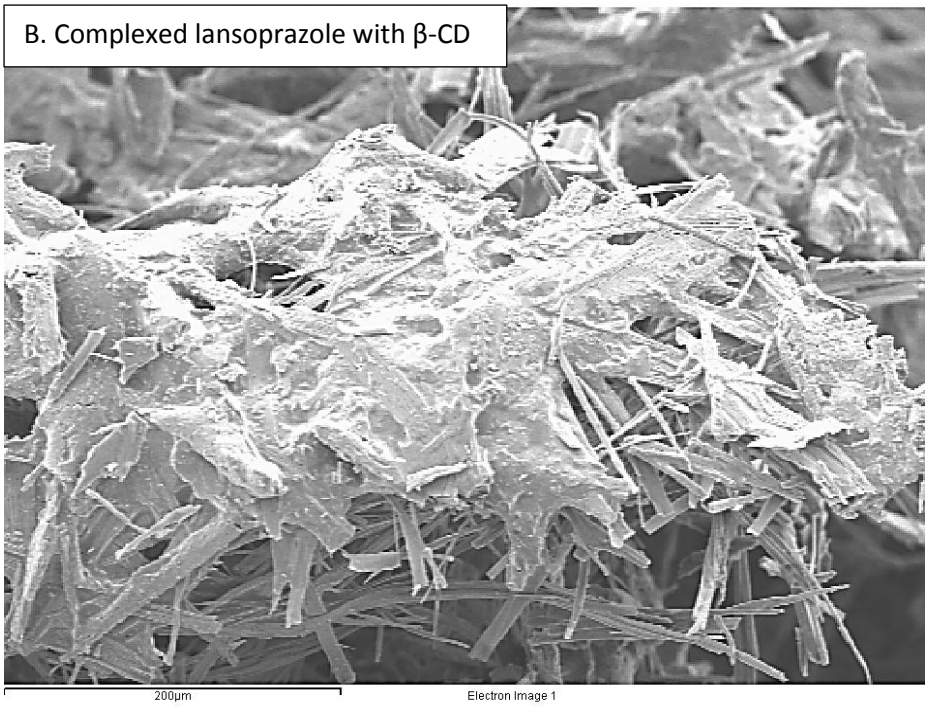


Figure 3.31 SEM images of complexed lansoprazole with A. Mβ-CD and B. β-CD

Freeze drying resulted in changes in morphology in the crystalline drug to an amorphous complex. Complexed lansoprazole with Mβ-CD appeared to be less crystalline (compared to pure drug) with a visible fluffy appearance. Complexed lansoprazole with β-CD presented needle shaped crystals. Single components were not distinguishable amongst the aggregation, however this may have been due to the difference in magnification (Figures 3.31 A and B).

3.6.3 Discussion of lansoprazole CD inclusion complexes

The increase in solubility was due to the increased wettability of CD and also the reduction in drug crystallinity after freeze drying (Figure 3.24). The Mβ-CD (1:1) had a higher solubility than the other samples tested. This agreed with the data from Figueiras who found Mβ-CD displayed superior properties with regards to drug solubility and stability (and permeability) of omeprazole over β-CD complexes for a buccal delivery system (Figueiras *et al*., 2007a). The increased solubility may potentially result in increased release from chewing gum formulations due to reduced affinity of the drug for the lipophilic gum base. The solid state characterisations of Mβ-CD complexes confirmed that interactions were taking place between the guest (lansoprazole) and the host (CD); suggesting the formation of an amorphous inclusion complex through molecular encapsulation of the drug into the hydrophobic cavity of the CD through hydrogen bonding. Further work would involve further characterisation of the complexation to form a greater understanding of the complex formed between Mβ-CD and lansoprazole, including factors affecting the disassociation of the complexed form. NMR spectroscopy has been widely used to characterise complexes using β-CD and along with molecular mechanics/dynamics modelling and can be used to provide further evidence of complexation orientation (Zheng *et al*., 2005).

Lansoprazole has previously been complexed with β–CD and hydroxypropyl β-CD using a fluid bed coating method with solubility and stability improved for both complexes. Computational modelling suggested that the benzimidazole group was included in both CDs (Figure 3.32). However the hydroxypropyl- β-CD was more photostable compared to β-CD. It was suggested that this was due to the hydroxypropyl- β-CD forming a deeper inclusion complex providing molecular shielding to the sulfinyl moiety of lansoprazole (Lu *et al*., 2012).

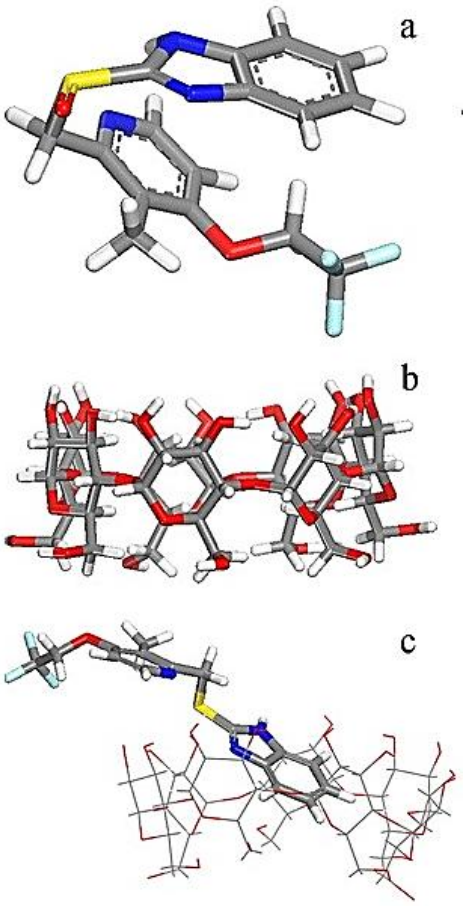


Figure 3.32 Schematic models of A. lansoprazole, B. β-CD and C. complexed lansoprazole: β-CD (1:1)

(Lu *et al*., 2012)

3.7 Formulation stability considerations in chewing gums

There are three main factors that impact the stability of chewing gum formulations and consideration was given to each when manufacturing the gums.

- Formulation method

The manufacturing method employed for chewing gums used a heated Z blade mixer at 50 °C mixing at 40 rpm, to avoid extremes temperatures which could potentially accelerate decomposition of lansoprazole.

- Environmental

Lansoprazole is sensitive to pH, light and moisture. Once made into a chewing gum formulation exposure to external factors can be further controlled using packaging and specific recommended storage conditions. All formulated gums were manufactured without coating or packaging and were stored in plastic containers throughout use.

- Drug and excipient specific

Lansoprazole is particularly unstable compared to other PPIs. The specific ratio of drug to excipients was 15 mg per 1 gram gum piece. The formation of a coloured product indicated possible instability; however drug content was not compromised in the presence of the coloured product once extracted. The use of alkaline stabilising compounds has been investigated previously and can be incorporated into gum formulations to help maintain the localised pH at a desirable range of the microenvironment (Tetsuro *et al*., 1992; He *et al*., 2010; Lu *et al*., 2012). Buffering excipients were incorporated in gum formulations and upon release will increase the pH of saliva, which will increase the stability and solubility of lansoprazole.

Moisture absorption was found to be a detrimental factor affecting stability of lansoprazole coated pellets (He *et al*., 2010). Generally chewing gums have a low water content, around 2-5 % w/w (Rassing, 1996) hence the internal microenvironment is limited to solid-solid interactions due to limited moisture within the gum.

3.7.1 Stability testing of gums

Formulated gums were stored under ambient storage conditions in sealed transparent plastic containers prior to analysis. Gums were chosen at random and the drug extracted as described in Chapter 2. Both discoloured and non-discoloured gums (H and L) were included in the study and were chosen at random (n= 8).

Table 3.4 Drug content of gums (H and L) following storage under ambient conditions for 1 year (n= 8; mean ± s.d)

Test Gum	Lansoprazole content (mg per g)
H gums	15.30 ± 0.41
L gums	14.66 ± 0.14
Overall mean	14.98 ± 0.44

3.7.1.1 Discussion

All gums tested showed acceptable content uniformity after 1 year with content ranging from 96.87- 104.62 % (Table 3.4) and complied with EP guidelines recommending that solid dosage forms have a satisfactory content range of 100 ± 5 % (European Pharmacopoeia, 2002; El-Assassy *et al*., 2012). There was no obvious difference between visually discoloured and non discoloured gums (as seen previously) with regards to drug content. The results showed that lansoprazole was stable in the medicated chewing gum formulations and there was no major loss of active and levels of degradation products were less than 5 % for all formulations. The overall content uniformity from all gum formulations is described in section 4.4.1.

3.8 Conclusion

The solubility and stability of lansoprazole in artificial saliva was found to be dependent on the pH of the solution, an increase in pH caused an increase in lansoprazole solubility with a significant increase between pH 9 and 10. At the lower pH, the concentration was decreased (by 26 % at pH 6) after 15.5 hours, confirming the acid instability of lansoprazole. The detection of lansoprazole was possible in presence of its degradation products and impurity A (N oxide) and impurity B (sulfone) were identified using the HPLC method.

Gums (H and L) were tested for stability and showed acceptable content uniformity after 1 year under ambient conditions with drug content ranging from 96.87- 104.62 %. However the

gums displayed a formation of a coloured product after formulation which was later found to be a compatibility issue with lansoprazole and the Rev7 polymer; specifically the Merit co polymer. Further formulations containing 8 % Rev7 polymer did not display the violet discolouration.

Ratios of CD (β-CD and Mβ-CD) with lansoprazole were complexed using freeze drying resulting in an amorphous material. The solubility and solid state characteristics were investigated. The Mβ-CD (1:1) resulted in a 9 fold increase in solubility compared to lansoprazole alone at pH 6.1 and maintained solubility over 15 hours at pH 6.1 suggesting increased stability due to possible molecular shielding. The solid state characterisations of Mβ-CD complexes (1:1) confirmed that interactions were taking place between the guest (lansoprazole) and the host (CD); suggesting the formation of an inclusion complex through molecular encapsulation of the drug into the hydrophobic cavity of the CD through hydrogen bonding.

It was decided to incorporate (Mβ-CD: lansoprazole, 1:1) into chewing gum formulations and investigate its effect on *in vitro* release. This is described in detail in Chapter 4. The effects on permeability of (Mβ-CD: lansoprazole, 1:1) compared to pure lansoprazole were also investigated. This is described in detail in Chapter 5.

Chapter Four
Formulation development
&
in vitro release

4.1 Drug release from medicated chewing gums

The process of active being released from a chewing gum formulation can be described as a patient controlled release, with the drug being released dependent on the patients' needs or chews. Dissolution of a drug from a gum is dependent on contact of the dissolution medium with the exposed area of the gum (which is renewed each time that the gum is chewed and so is constantly changing). Release will consist of processes involving partioning (dependent on the drugs affinity to the gum base) and diffusion (based on the resistance (texture) of the gum base and molecule weight of the drug) of the active from the gum base matrix into saliva (Figure 1.4). The partioning step will influence the amount, whereas diffusion will define how fast the active is released (Lee, 2001). The medium will penetrate/ingress into the gum and dissolve the gums' hydrophilic portions which will then diffuse out into the exterior bulk medium. The hydrophilicity of the chewing gum and its porosity will be controlling factors for release, influencing penetration of dissolution medium as well as the leaching out of the drug through channels or pores. Release will consist of a mixture of leaching and diffusion controlled mechanisms (both into and out of the gum beginning with the hydrophilic domains and then the lipophilic components) and will also be dependent on the mechanical forces/action of chewing. Confectionary gums have four phases of chewing consisting of the initial chew phase, intermediate chewing phase, the main extraction phase where most of the bulk sweeteners and flavourings are released and the final chewing phase, leaving the gum base and a low level of excipients once most of the sweeteners have been extracted (National Confectioners Association, 2010).

General factors which will influence release from a medicated gum include physical forces, including: chew rate and temperature (causing textural changes; resulting in a softer gum) and also physiochemical interactions between the active and excipients (including the drug's affinity to the gum base/ lipophilic component).

Factors affecting the release of the drug from a gum can be categorised into 3 general groups:

- **Patient chew related factors**

The physiological inter-individual variation can include the chew count, pH and volume of saliva which differs greatly between individuals. The normal chewing range is between 40-80 chews *per* minute. An average of 60 chews *per* minute is recommended by relevant EP

guidelines when conducting *in vitro* mastication testing (European Pharmacopoeia, 2011). As the gum is chewed, its surface area is renewed which allows the drug to be released and so an increased chew rate will result in an increased exposed surface area and thus a greater extent of release would be expected.

The pH of saliva can range from pH 5.8 - 7.4 (Shojaei, 1998). This can be further controlled and manipulated with the use of buffering excipients which will also be released alongside the drug, influencing the solubility of the active and facilitating absorption. The flow rate and volume of saliva will vary between individuals (see section 5.2.1) including stimulus effects, disease state, time of day, age and sex amongst other factors. Chemical stimuli (such as acids) and mechanical (chewing) can increase salivary flow rates, however there is large inter-individual variation with parotid saliva flow. The average volume of saliva in the oral cavity ranges from 5- 10 mL and flow rates can range from 0.06 - 0.10 mL *per* minute whilst at rest compared to 0.43 - 0.66 mL *per* minute whilst chewing (Rudney *et al*., 1995; Yang *et al*., 2004). Daily salivary flow is between 0.5 – 2 litres for healthy subjects (Shojaei, 1998). Saliva flow rate in response to chewing gums is shown to peak in the first minute and reduces as the gum base softens over time (Guinard *et al*., 1997).

- **Formulation specific gum properties**

Formulation considerations, specifically gum base properties, will have an influence on the release of active from chewing gums. The lipophilic/ hydrophilic capacity can be adjusted by altering the percentage gum base mass and composition (Hyrup *et al*., 2005). This will cause varying adherence of the drug to the corresponding section of the gum. Rates of hydration are also important factors to consider as they will measure the rate of absorption of saliva into the gum matrix and will directly influence release (National Confectioners Association, 2010).

- **Physicochemical properties of the active**

The specific physiochemical properties of the drug will influence its release. These include the aqueous solubility of drug in saliva (Imfeld, 1999; Ochoa *et al*., 2008). One study suggested that poorly soluble drugs can be entirely released but may require an extended period of time and so release will be at a slower rate and possibly incomplete during the 30 minute mastication period, and this may have to be extended to ensure complete release (Maggi *et al*., 2005). The drug's pK_a will also have an influence on the release as well as the

absorption of the drug, with unionised species being absorbed more effectively (Figure 5.4). The drug stability in the oral environment will also need to be considered. This is described in chapter 3.

Lipophilic drugs will have increased distribution and affinity in the lipophilic components of the gum and will subsequently be released slowly and incompletely (Jeckelmann and Haefliger, 2010). The active's relative affinity for the two phases, soluble (sugars) and insoluble (gum base), will influence release characteristics, with hydrophobic compounds having increased binding interactions with the gum base resulting in slower rates and extent of release whilst masticating. Mechanisms of release of cinnamaldehyde from chewing gum have been related to the specific gum base to water partition coefficient (cLog P). This compares the distribution and binding affinity of the flavouring agent between the gum base and the soluble phases (aqueous phase). The study involved a modified shake flask method; the gum base was ground to reduce particle size and suspended in saturated water (containing the flavouring agent). Results found that compounds with a low cLog P (higher distribution in the aqueous phases) were found to release at an increased rate and extent when formulated into medicated gums compared with compounds with a high cLog P (higher distribution in the insoluble portion). The study concluded that gum base affinity was a major factor impacting release kinetics from medicated gum formulations. The authors suggested a two stage model for release of the flavouring agent (based on *in vivo* chew out studies), with an initial rapid phase during the dissolution from the soluble sugar alcohol phases (sorbitol) followed by a diffusion controlled mechanism with sustained release from the insoluble gum base (Potineni and Peterson, 2008). Similar findings were reported for menthol release from chewing gums where the rate limiting factor was found to be the diffusion of the active from the insoluble gum base portion (Yoshii *et al*., 2007).

The transfer of active from lipophilic gum base to saliva will be dependent on the partition co-efficient. As the octanol/water partition of lansoprazole is high (Log P = 2.7), the drug will favour the gum base and so dissolution into saliva may be limited.

4.1.1 Measuring drug release from chewing gums

4.1.1.1 *In vivo* release

In vivo studies to monitor release from chewing gums normally involve chew out studies. These involve a panel of tasters and chewers to chew gums following specific protocols controlling duration, chew rate and intensity; however this can be highly variable between subjects (Kvist *et al*., 1999). Generally a 30 minute chewing duration has been recommended for use in clinical trials based on a survey involving 4000 volunteers who had a mean chewing time of 36 minutes (Barabolak *et al*., 1991). During the mastication process, the active is released into the saliva and absorbed through the oral mucosa or swallowed and absorbed in the gastrointestinal tract. Estimated drug release can be calculated by analysing the residual drug content and further data can be obtained *via* blood samples to provide pharmacokinetic evaluations. Training of volunteers with specific chew rates *per* minute and chew durations has been recommended in order to standardize such tests (Yang *et al*., 2004).

Disadvantages of *in vivo* chew out studies include the lack of standardisation and control of chew-related physiological factors including physical chewing stress, clench times, frequency and physiological differences in the flow and composition of saliva between subjects. This has led to large inter-individual variations. Also the ethical issues and costs involved in obtaining human volunteers have to be considered (Morjaria *et al*., 2004).

An example of relevant pharmacokinetic data achieved from an *in vivo* chew out study involving 84 healthy subjects in a double blind, randomized parallel study comparing caffeine administration in a chewing gum formulation with a capsule formulation. The results showed that the rate of absorption was significantly faster with gums than capsules and both had comparable extent of absorption. The study also suggested dual absorption routes for the active with the primary route being absorption through the buccal mucosae and also some absorption after being swallowed in the GI tract (Kamimori *et al*., 2002).

A novel evaluation of *in vivo* chew out studies involved a compressed three layer tablet with a gum core (3TabGum®) which incorporated model drugs such as ascorbic acid. It trialled a novel method involving yellow dyes acting as a tracer for drug release and found good correlations with release of drugs and dyes *in vivo*. It concluded that the use of dyes could

provide an indicator of the necessary chewing time needed to complete drug delivery from evaluation of the colour loss of the residual gum. It also identified how individual chewing performance can influence drug release and displayed a high degree of variability amongst the volunteers (Ochoa *et al*., 2008). Another novel method to study *in vivo* release was used to explore the release kinetics of actives from chewing gum into saliva using Direct Analysis in Real Time Mass Spectrometry (DART). Subjects were asked to provide saliva samples after consumption of a flavoured chewing gum. Release profiles achieved a range of differing plateau stages (where the rate of release was constant) and this was attributed to the varying amounts of saliva secreted between subjects (Jeckelmann and Haefliger, 2010).

Variability between individuals was found in a translational chew study trialling the use of a chewing gum assessment method to evaluate masticatory ability, efficiency and performance between individuals. The study focused on 20 volunteers perceived to have normal masticatory efficiency, who chewed samples of 2 coloured confectionary chewing gums for different chew cycles. The mixing fraction of the 2 multi-coloured gums was analysed using digital image processing and was shown to provide reliable quantifiable data to assess chewing efficiency. The study recommended this method as an alternative to the food chew and sieve test (separating particles based on size to assess efficiency) for evaluating chewing efficiency in clinics (Schimmel *et al*., 2007).

4.1.1.2 *In vitro* release

Drug dissolution is an important tool in academic development, drug development and quality control. The value of *in vitro* release testing is well established for a range of dosage forms, allowing correlations between *in vitro* and *in vivo* conditions. Different techniques and apparatus are used on a case by case basis specific to the dosage form or product type. Each method must demonstrate accuracy, reproducibility and precision to allow for relevant interpretations of *in vivo* performance (Siewart *et al.*, 2003). The drug's release from the formulation is an essential first step in the absorption and bioavailability of the drug. However for release testing of gums, chewing/masticatory forces are essential for release by providing the renewable surfaces necessary for release and so the use of standard dissolution apparatus would be limited. The European Pharmacopeia describes a stainless steel 3 piston apparatus to provide the shearing forces and activity necessary to continuously expose the

interior of the gum to the surrounding dissolution medium to promote drug release (Figure 4.1) (European Pharmacopoeia, 2011).

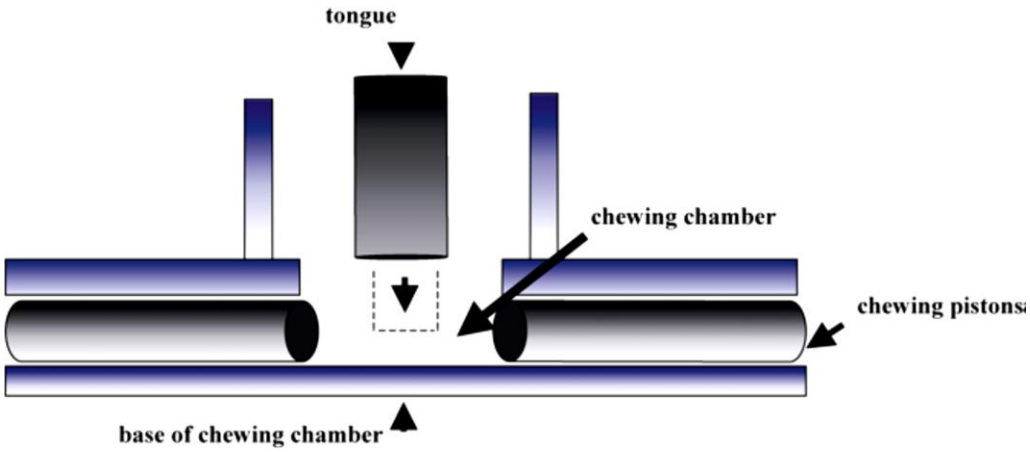


Figure 4.1 Schematic diagram of the EP masticator

(Morjaria *et al.*, 2004)

In 2008, the European Pharmacopeia published a monograph to describe suitable apparatus to apply mechanical kneading to study *in vitro* release of drugs from chewing gums (European Pharmacopoeia, 2011). A number of other non-compendial devices have been reported that also mimic the chewing action necessary for release from gums with various improvements and versatility. One such development, designed by Kvist and Wennergren (apparatus B), can contain up to 6 chambers. The chewing machine is constructed with glass vessels capable of containing up to 70 mL of dissolution medium. This allows visual inspection of the process during chewing. The chewing mechanism is dependent on 2 vertical pistons which can "chew" at up 120 strokes *per* minute. The pistons have removable, sand-blasted jaws at the surface which can be replaced to ensure the gum is uniformly kneaded and does not stick to the pistons. The test cell can be removed to aid in cleaning compared with the EP masticator which has a fixed chamber and so is time consuming to clean (Kvist *et al.*, 2000). Further details are included in Chapter 2 (Figures 2.5 and 2.6). One study advises release testing on both apparatus (EP approved and apparatus B-Kvist and Wennergren) to ensure sound interpretation of the *in vitro* release data to have meaningful predictions for *in vivo* studies (Gajendran *et al.,* 2008). Another system variation uses Teflon plungers and claims to have a good correlation with *in vivo* chew out studies (Rider *et al.*, 1992). Comparable release correlations have been made with *in vitro* and *in vivo* release testing of chewing gums containing KSL (an antimicrobial agent). The active was released steadily with 70 – 80 % released after 20 minutes in both conditions (Hee Na *et al.*, 2005).

The EP masticator has been adapted from the original designs by Christrup and Moller (Christrup and Moller, 1986) (Figures 2.2 and 2.3). It consists of a temperature-controlled chewing chamber; housing the location where the gum is chewed by two electronically-controlled horizontal pistons ("jaws") powered by compressed air. The two pistons transmit twisting and pressing forces to the gum, while a third vertical piston, operates alternately to the two horizontal pistons ensuring that the gum stays in the appropriate position ("tongue") (Figure 4.2). The distances between the jaws are adjustable; a distance of 1.0 mm was set between the horizontal pistons allowing a uniform force to be applied between experimental runs. Each piston has indentations at the chewing surface to prevent the adhesion of the gum to its surface. The temperature of the chamber was maintained at 37 ± 0.5°C and the variable chew rate can be set up to a maximum of 83 chews *per* minute. The European Pharmacopoeia guideline recommends using 20 mL of an unspecified buffer (at approximately pH 6) with a chewing chamber volume of 40 mL and a chew rate of 60 chews *per* minute (European Pharmacopoeia, 2011). However 40 mL of dissolution medium was used due to the size of the chewing chamber as established by previous studies (Morjaria, 2004). There are limited guidelines on monitoring the release from the gum (i.e. directly from the dissolution medium or from the gum's residual content) and the studies in this thesis used a combination of the two where appropriate. Previous work performed on the chewing machine showed that *in vitro* release profiles were similar to *in vivo* chew out studies and that artificial saliva pH 6.7 showed good correlation to real saliva obtained from human subjects (Morjaria, 2004) in testing for *in vitro* release from prepared nicotine gums. This is described in detail in Chapter 2.

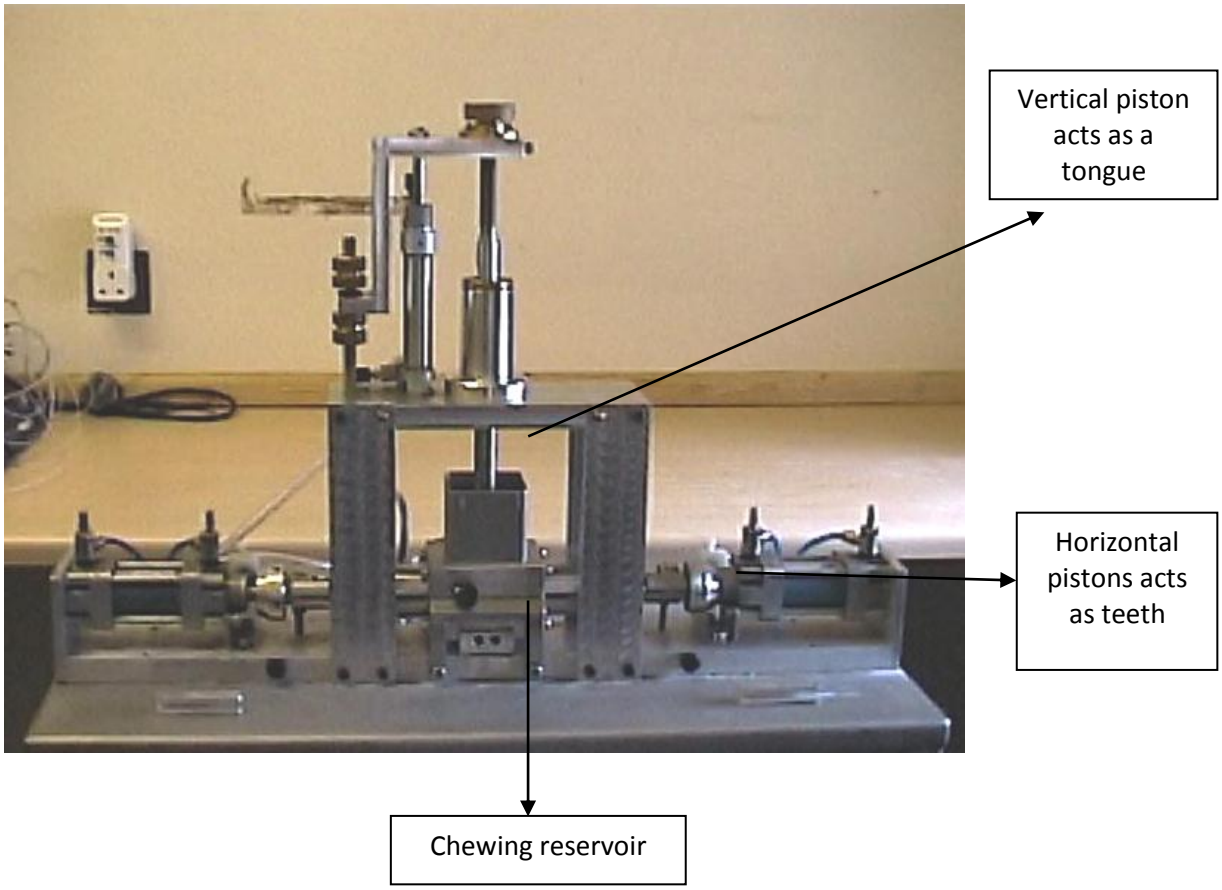


Figure 4.2 The European Pharmacopeia approved masticator

The specialised dissolution apparatus is designed to test release of the active from chewing gum by providing the necessary masticatory forces needed for release.

4.2 Methods

A range of chewing gum formulations was assessed utilising *in vitro* release testing to discriminate between formulation variables and to evaluate release governing factors for a poorly soluble drug from a chewing gum formulation.

4.2.1 Formulation of lansoprazole chewing gum

All lansoprazole gums were prepared at the Revolymer site (Mostyn, UK) using the conventional kettle mixing method (at 50 °C). Initial lansoprazole chewing gum formulations were prepared to assess the effects of varying the lipophilic/hydrophilic capacity on release. This was achieved by varying the gum base mass percentage (57 % w/w (H) and 41% w/w (L) compositions).

Lansoprazole was loaded at a dose equivalent to the conventional 15 mg capsule formulation. A low level of flavouring (3 % peppermint oil) was used to minimise the risk of any interactions with lansoprazole. All formulated gums were rolled to a uniform thickness before being cut to size. The gums were all sectioned using the same method (resulting in similar size, shape and weight) and so had a similar surface area to ensure a fair test and reproducibility. Talc was sparingly added to the working surfaces during cutting to minimise adhesion. This resulted in uniform gum pieces weighing approximately 1 gram.

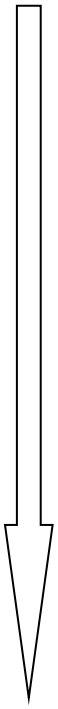
To load the gum base with active, 25 g of lansoprazole (BN: 71878, Discovery Chemicals) was added to 650 g of pre-warmed gum base (RVG1T000799) containing 6 % Rev7 in the Z blade mixer. This was mixed for 30 minutes at a speed of 20 rpm at a temperature of 50°C.

412 g of the loaded gum base was removed, leaving a quantity of 263 g remaining in the mixer. The excipients were added sequentially to make the 41 % w/w lansoprazole gum and were mixed at a speed of 40 rpm at a temperature of 50 °C (Table 4.1).

The theoretical drug load for each gum weighing approximately 1 gram was 15 mg of lansoprazole *per* gum piece.

Table 4.1 Preparation of lansoprazole chewing gum formulations (L gum batches)

Stages	Ingredient	Percent (%)	Quantity (g)	Duration of mixing (Time in minutes)
1	Loaded gum base	41	263.3	3
	Sorbitol	27	177.5	
2	Maltitol	1	6.5	
	Aspartame	1	6.5	2
3	Mannitol	1.5	9.8	
	Xylitol	1.5	9.8	5
	Maltitol	1	6.5	
4	Sorbitol	3.9	25.4	7
5	Mannitol	2.5	16.3	
	Xylitol	2.5	16.3	
	Maltitol	1	6.5	3
6	Flavour	1	6.5	
	Sorbitol	3.9	25.4	
	Flavour	0	0	
	Menthol	1	6.5	2
7	Sorbitol	3.9	25.4	
	Na_2Co_3	3	19.5	5
	Flavour	3	19.5	4
		Total 100%	**Total 646.8 g**	**Total time 31 min**



The excipients were added in a staged approach (stages 1-7) as shown in Table 4.1 and mixed for set periods based on Revolymer in-house protocols. Each stage had a calculated minimum mass of excipients to ensure the required bulk density resulting in efficient mixing.

To manufacture H gums, 263 g of loaded gum base was added with an additional 104 g of pre-warmed loaded gum base (total quantity of 367 g) into the Z blade mixture at the beginning. The amount of sorbitol was decreased accordingly.

4.2.2 Impact of gum base content on lansoprazole gum formulations

The aim of this study was to evaluate the effects of gum base composition by manufacturing two different gum base mass formulations (H and L gums) (varying the lipophilic/hydrophilic capacity). This would allow initial release parameters to be evaluated and would allow a starting basis for further optimised formulations. The effect of pH of dissolution medium (phosphate buffer) was also assessed.

Two preliminary batches of lansoprazole-loaded gums (containing 15 mg of lansoprazole *per* gum), with 6 % Rev7 polymer incorporated into the gum base, were prepared to provide initial data on the release of lansoprazole from chewing gums. Each gum, weighing approximately 1 gram, contained 15 mg of lansoprazole to allow dose equivalence to the enteric capsule formulations available.

Release was tested using the standard masticator set up as described in chapter 2. Standard conditions were utilised as a starting point, i.e. phosphate buffer, 60 chews / min, 37 °C and 2 mL sampling with replacement volume. All gums were tested in triplicate.

To assess method development and chew-related factors, the pH of the dissolution medium was adjusted to pH 6.0 and then pH 8.0 by addition of 0.1M HCl or NaOH.

4.2.3 Formulation variables (L gum batches)

Further experimental gum formulations were produced (all containing 41% w/w gum base) with variables (Table 4.2).

- The influence of complexed drug with MβCD (1:1) (drug alone *versus* complexed drug)
- The influence of Rev7 polymer (8 % Rev7 *versus* 0 % Rev7)
- The influence of buffering excipients (potassium carbonate *versus* sodium carbonate)

Table 4.2 Formulation variables (L gums batches)

Code	Drug form	Rev7 (%)	Buffering excipients
L,8,Na	lansoprazole	8	Na_2CO_3
L,8,K	lansoprazole	8	K_2CO_3
C,8,Na	complexed	8	Na_2CO_3
C,8,K	complexed	8	K_2CO_3
L,0,Na	lansoprazole	0	Na_2CO_3
L,0,K	lansoprazole	0	K_2CO_3
C,0,Na	complexed	0	Na_2CO_3
C,0,K	complexed	0	K_2CO_3

4.2.3.1 Factors affecting *in vitro* release from L gum batches

A series of experiments was conducted to determine the formulation and method variables affecting release. This included a range of chew related factors such as choice of dissolution medium, pH of dissolution medium, sample replacement volume and chew rate.

Standard conditions were utilised as a starting point to allow a direct comparison to previously tested gums. In order to study the impact of dissolution medium on release it was decided to compare phosphate buffer pH 6.0 and artificial saliva at pH 6.7 utilising the standard masticator set up, 60 chews / min, 37 °C and 2 mL sampling and replacement volume. Content uniformity was determined using solvent extraction. This is described in chapter 2.This method was also used to determine residual content post-mastication.

To ensure sink conditions prevailed throughout the entirety of further experiments, sample volumes were subsequently increased from 2 mL to 20 mL. It was noted that this dilution factor would also affect the buffering capacity and pH of the dissolution medium during chewing.

To further investigate method developmental factors and promote increased release the effects of pH was evaluated; the pH of artificial saliva was increased to pH 8.0 (the sampling and replacement volumes were kept at 20 mL).

To investigate the effects of chew rate, the chew rate was increased to the maximum chew rate of 83 chews *per* minute (maintaining the pH of artificial saliva at pH 8.0 and the sampling and replacement volumes at 20 mL).

ANOVA statistical analysis was performed on maximum release after 30 minutes from all gum formulations, Post Hoc LSD testing was used and significance was the at the 95 % confidence level. Independent t-tests were performed between experimental conditions to assess significant differences (using SPSS, version 20, Chicago).

4.2.4 Surface diffusion from lansoprazole chewing gums

A study on diffusion of drug from the surface of gums was performed to allow the evaluation of mastication/chewing of the gum and the importance of renewal of surface areas essential for effective drug release from chewing gums.

Gums were weighed and placed in a beaker containing 40 mL of artificial saliva stirred at 300 rpm at ambient temperatures. 1.5 mL was removed and replaced with fresh dissolution medium at 0, 5, 10, 20, 30, 60 minutes and 24 hours. All gum formulations were tested in duplicate with the mean being reported.

For rapidly disintegrating gums (L,8,K and C,8,K), a modified (smaller volume vessels) USP dissolution bath apparatus with 250 mL of saliva, at a stirring rate of 150 rpm and a temperature of 37 °C was employed. This would limit potential contact/collisions of the stirrer to the gums.

4.2.5 Surface morphology of chewing gums

The surface morphology (texture and roughness) of un chewed formulated gums (L,8,K) was examined using scanning electron microscopy (SEM) to detect any micropores/ channels for ingress and drug release upon contact with saliva.

Samples were mounted on aluminium stubs using double sided carbon tape and coated under vacuum (using a sputter coater) with gold and palladium (Au and Pd) through the use of an Emscope SC 500 (Emscope Engineering Limited, Hertfordshire UK). The study was carried out under magnification with a JOEL JSM 6060LV (Joel UK Limited, Herts, UK).

4.2.6 Texture evaluation

In addition to product manufacturing control tests including content and mass uniformity other sensory tests are needed due to the extended contact time in the oral cavity. The taste of the active can impact sensory taste buds and can often be unpleasant therefore additional sensory parameters are measured continuously until satisfactory results are available. These can include product feel, evaluation of flavours/sweeteners and texture analysis. This can be conducted throughout the developmental phases in a variety of ways including evaluation taste panels and also texture analysers (Gajendran *et al*., 2008).

4.2.6.1 Texture profile analysis

Texture profile analysis (TPA) is an objective method of sensory analysis with defined textural parameters (Szczesniak, 1963). It was later adapted by Bourne to compress standard sized food samples (Bourne, 1978).

The textural characteristics of food can be described mechanically by the following parameters:

- Hardness
- Chewiness
- Cohesiveness
- Adhesiveness

(Szczesniak, 1966)

The TPA performs recognition of multiple parameters and then produces a texture profile which can be useful in on testing between samples. *In vivo* correlations are difficult to measure because of the range of forces involving shearing, compression, tearing, cutting, piercing, grinding and as well as the lubricating component with saliva all at body temperature. The test has been shown to correlate well with sensory parameters and involves compressing a piece food in a reciprocating motion (simulating actions of the jaw) and produces a force over time curve. Chewiness can be measured in terms of the energy required to masticate the solid food.

The TA.XT plus texture analyser (Stable Micro Systems, UK) was utilised for TPA at ambient temperatures. The following in built method was utilised to compare the hardness of chewing gum pellets, the method was similar to one used previously to test medicated nicotine gums (Morjaria *et al*., 2004). Unchewed gums were chosen at random from each optimised formulation and were tested in triplicate with the initial penetration in the centre of each gum. Texture analysis was performed on gums to assess differences in hardness of first penetration (g), chewiness (g/sec) and area under curve (positive energy; cohesion and negative; adhesion).

A 2 mm cylinder probe (P/2) was utilised with a 25 kg load cell and a heavy duty platform (HDP/90) with a blank plate. The gum was placed centrally below the testing probe, the probe approached the sample at a speed of 10.0 mm/s and once a 5g force was exhibited the system measured the resistance in compression force as the probe penetrated through the gum. The probe measured up to a penetration distance of 3 mm after the trigger point.

Data Analysis was obtained by in built macros, which analysed the profile curve once obtained to quantify the 4 parameters of interest; hardness, chewiness, cohesion and adhesion. The hardness parameter was calculated by the mean penetration energy from the area under the curve between 2 mm – 3 mm, force was plotted against time to give the initial bite (hardness of first penetration). On withdrawal, a negative peak is shown measuring adhesion of the gum. Chewiness was calculated by the TPA based on the following macros calculation:

Force 2 x (Area 4:6 / Area 1:3) x (Time diff 4:5 / Time diff 1:2)

(Stable Micro Systems, UK)

4.3 Results and Discussion

4.3.1 Content uniformity of formulated lansoprazole gums

Content uniformity for all gums tested was found to be in the range of 14.51 -15.49 mg/g (96.75 - 103.26 %). This complied with EP guidelines recommending that solid dosage forms have a satisfactory content range of 100 ± 5 % (European Pharmacopoeia, 2002; El-Assassy *et al*., 2012). The manufacturing process provided a homogenous material with each gum piece containing the required dose (equivalent doses of lansoprazole 15 mg) and content uniformity was achieved throughout all the formulations.

4.3.2 Impact of gum base (57 % w/w and 41 % w/w gum base mass) on *in vitro* release

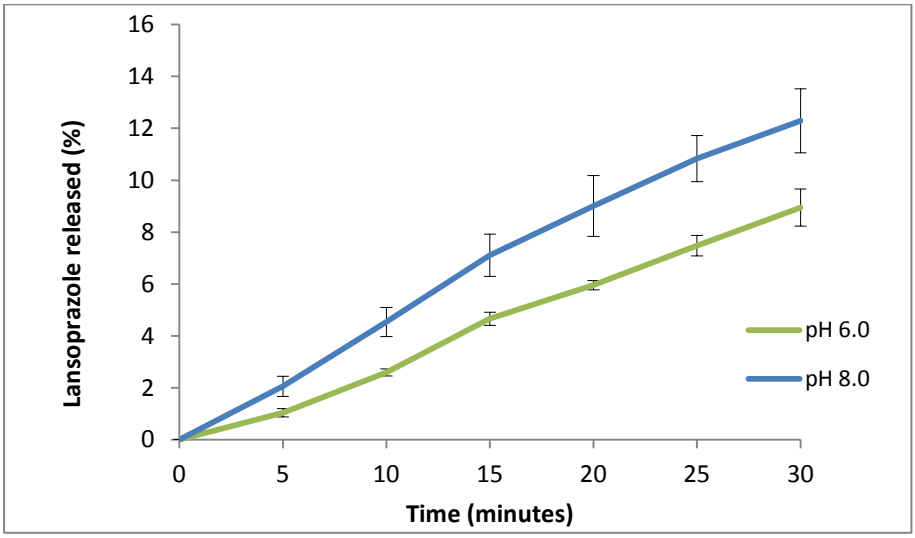


Figure 4.3 The effect of pH on release from gums (H gums)

Increasing pH increased the rate of release for H gums (containing 57 % w/w gum base). The maximum release was at pH 8 with 12.29 ± 1.23 % after 30 minutes (Figure 4.3).

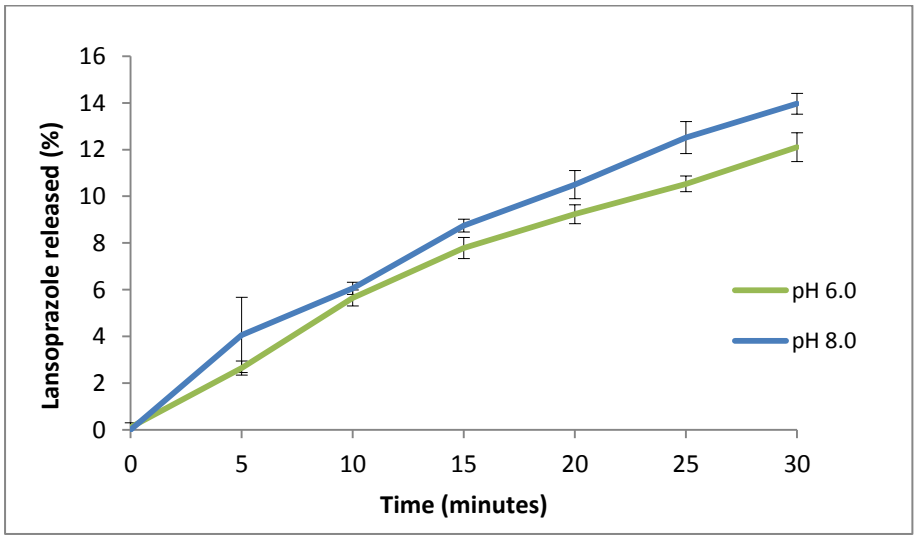


Figure 4.4 The effect of pH on release from gums (L gums)

An increase in pH of dissolution medium caused an increase in release as observed previously, however the difference was less pronounced than that found for formulations with a higher gum base content. The maximum release was at pH 8 with 13.97 ± 0.44 % after 30 minutes (Figure 4.4).

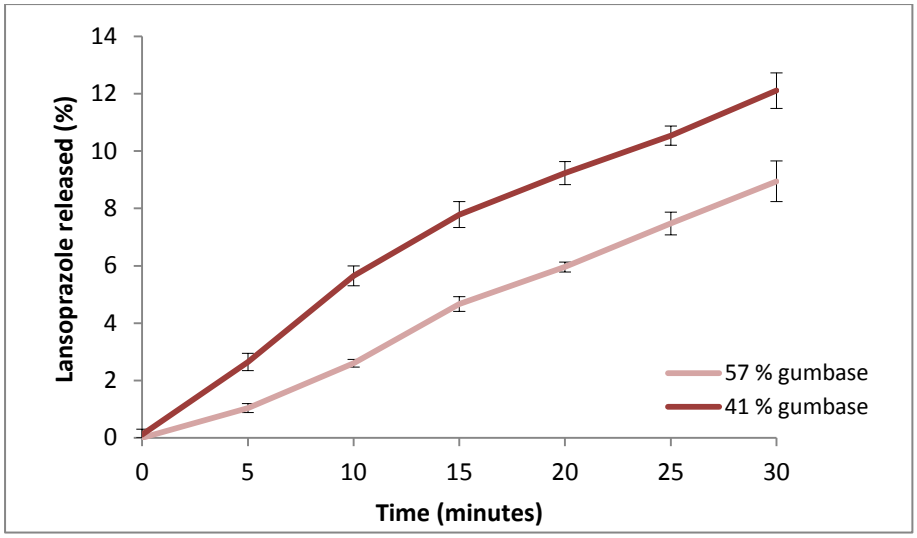


Figure 4.5 The effect of gum base content on release at pH 6.0

At pH 6.0, the L gum (41 %) released more lansoprazole, reaching a maximum of 12.11 ± 0.62 % after 30 minutes (Figure 4.5).

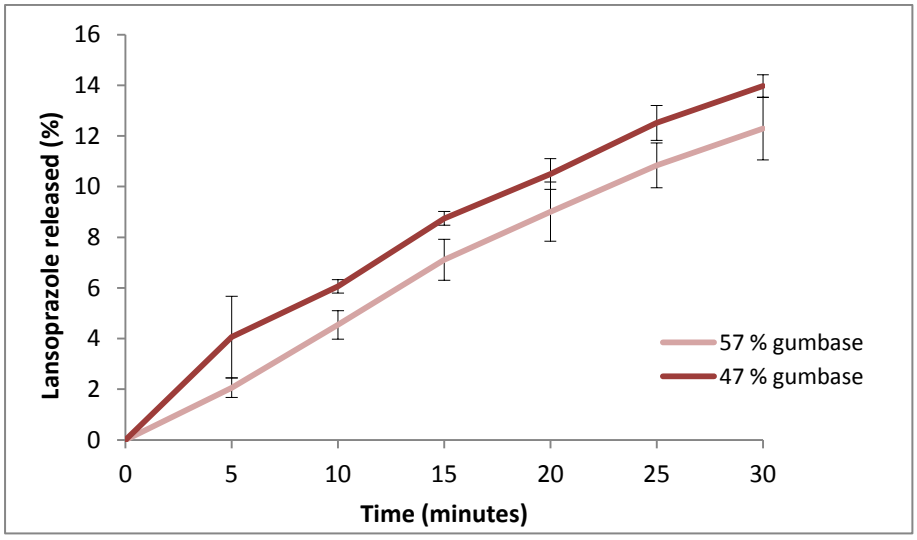


Figure 4.6 The effect of gum base content on release at pH 8.0

As pH of the dissolution medium was increased, the impact of amount of gum base in the formulation on release was reduced (Figure 4.6).

4.3.2.1 Discussion of the impact of gum base content on release

The drug was released slowly from both formulations and was stable over the 30 minute release period, as indicated by less than 5 % degradation products on HPLC chromatograms

(See Appendix A). The maximum drug released was 14 % after 30 minutes from L gums at pH 8.0.

The amount of gum base contained in the formulation was found to significantly impact release ($P<0.05$). The percentage of drug released after 30 minutes was higher for those gums with a reduced lipophilic component (L gums with 41 % w/w gum base). This is due to the lipophilic nature of the drug and consequent affinity to the gum base, hence release was incomplete and gradual; this was further retarded with gums with higher gum base content. This is in agreement with other studies which suggested that increasing the lipophilic capacity of the gum will retain more lipophilic drug after mastication (Maggi *et al*., 2005). This is due to increased binding between the gum base and the lipophilic active (El-Assassy *et al*., 2012). Rassing also observed a low release of less than 5 % after 30 minutes with a poorly water soluble drug (Rassing, 1996). Lipophilic actives will generally be released slowly and to increase the release, the gum base content may be decreased (decreasing the lipophilic/hydrophilic balance of the gum). It is possible to manufacture a gum with a lower gum base composition to increase release, however, in practice, a gum containing less than 20 % gum base will have inadequate chewing properties and may crumble upon chewing (Maggi *et al*., 2005). Increased drug solubility at higher pH may have contributed to the faster release (Figure 3.3) as described in chapter 3.

4.3.3 Diffusion of lansoprazole from surface of chewing gums (L gums)

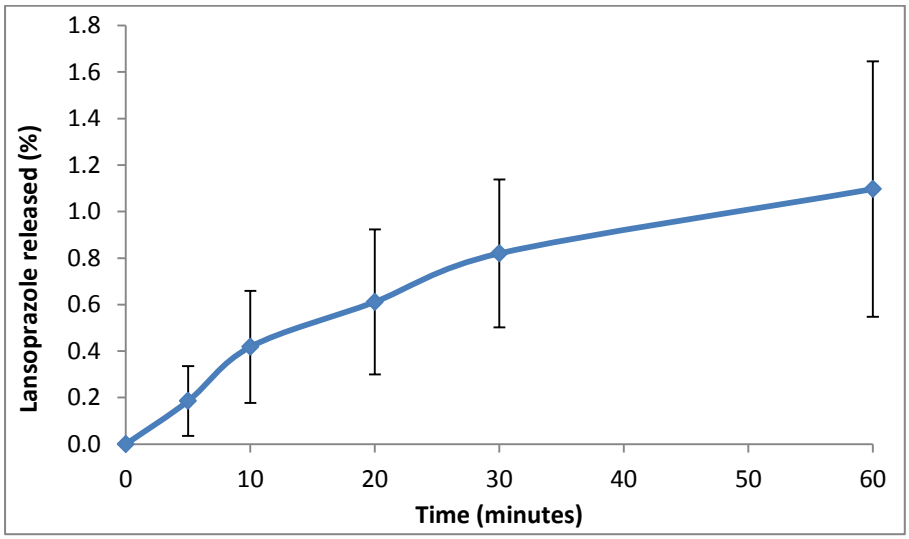


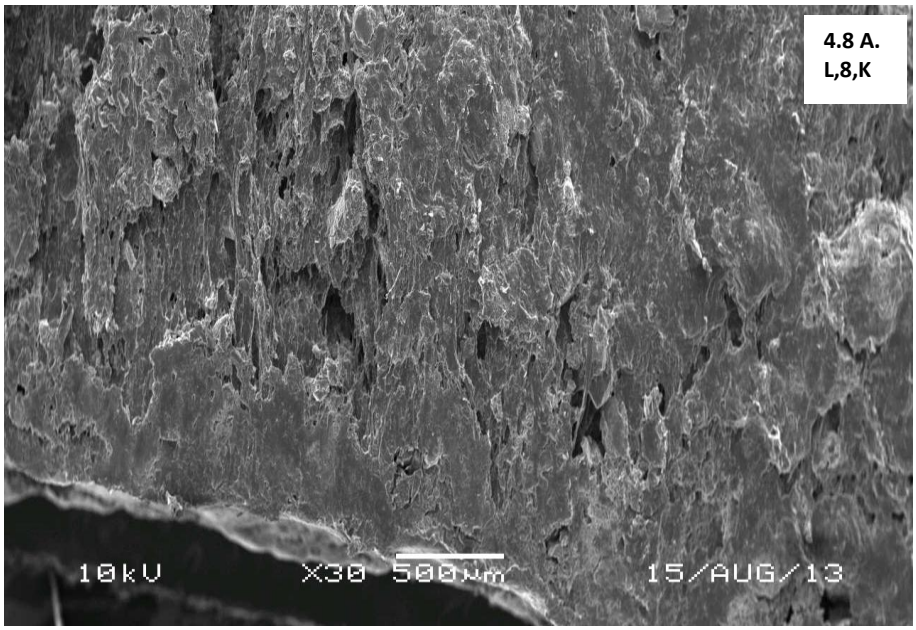
Figure 4.7 Mean drug diffusion from gum surfaces (without mastication)

Release was limited without mastication (Figure 4.7). The release of drug from gums was 1.10 ± 0.55 % after 60 minutes and 10.46 ± 1.64 % after 24 hours. The increased variability at 60 minutes was due to gums L,8,K and C,8,K (both containing potassium carbonate), with values of 1.88 and 2.07 % respectively. The release of drug from all other gums was lower (0.80 ± 0.14 %). It was also noted that gums containing Rev7 released more drug after 24 hours; (11.80 ± 0.83 % compared to gums without Rev7; 9.13 ± 0.92 %). This may be due to the hydrophilicity of Rev7 (see section 1.6).

The main components of chewing gum are a mixture of waxes, emulsifiers and elastomers which constitute the gum base. The gum base is an insoluble solid and so diffusion of drugs through the total gum thickness should be low. Diffusion primarily occurs from the surface of the gum in contact with the dissolution medium. The increased release at 24 hours may have been due to prolonged penetration of dissolution medium through micropores in the gum matrix; allowing an increased level of contact between internal surfaces of the gum causing drug to leach out from the surface.

It can be concluded that drug diffusion from the surface of the gum is limited and mastication is necessary to facilitate adequate release from gums, providing renewable surfaces and increasing contact with dissolution medium with fresh internal surfaces of the gum. This also suggests that gums may be less prone to accidental overdosing if swallowed whole providing that the gum is not metabolised or degraded by in the GI tract.

4.3.4 Surface morphology of chewing gums



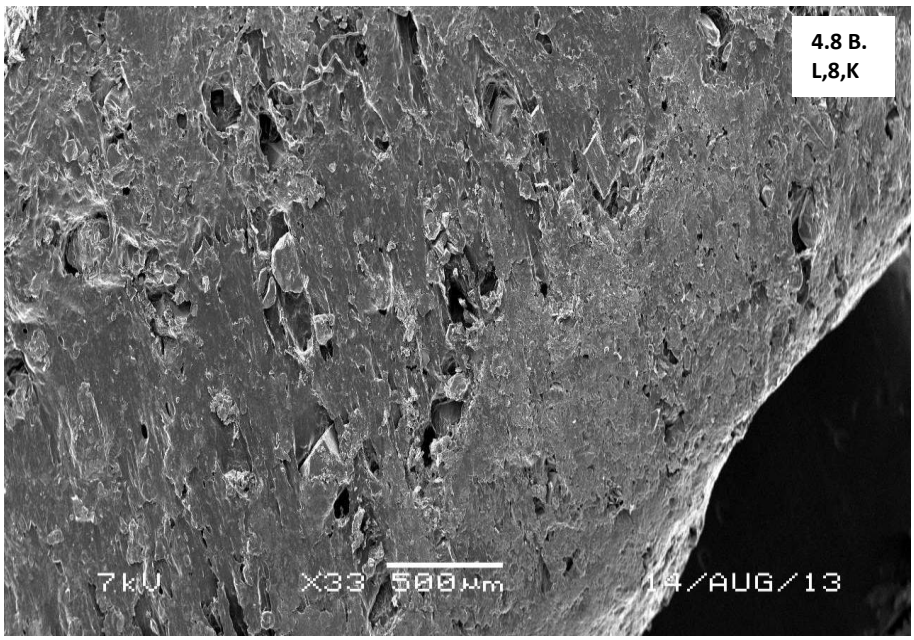


Figure 4.8 A & B. SEM images of L,8,K gums showing the surface micro pore structure

The SEM images (Figures 4.8 A and B) identified the range of possible pores/cavities on the surface of the unchewed gum that may provide channels for saliva to penetrate/ingress and diffuse into the gum thus facilitating release.

4.3.5 *In vitro* release from lansoprazole chewing gum (L gums batches)

4.3.5.1 *In vitro* release from lansoprazole chewing gum (2 mL replacement)

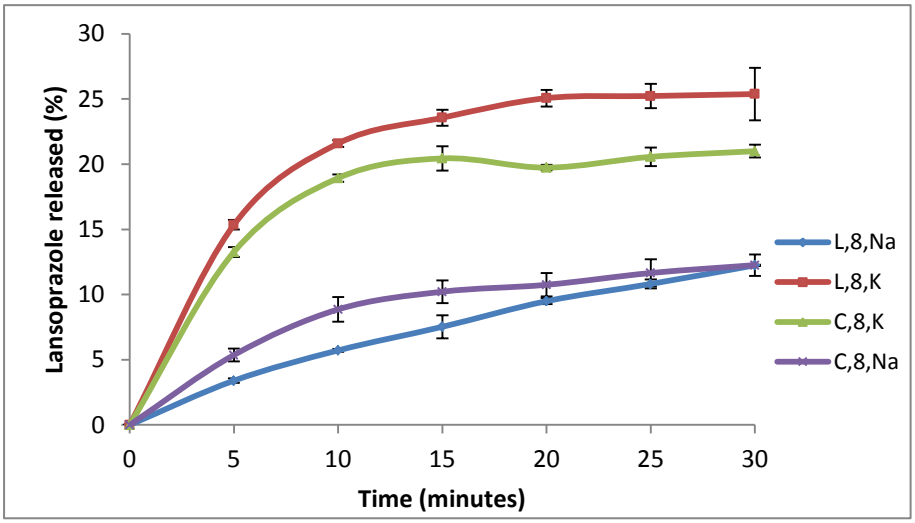


Figure 4.9 Drug release from gums containing 8 % Rev7 in phosphate buffer pH 6.0 (n=3; mean ± s.d)

L,8,K and C,8,K (containing potassium carbonate) displayed a burst release with the majority of the drug release occurring within the first 5-10 minutes followed by a plateau phase with limited drug release (Figure 4.9). Gums containing sodium carbonate released less drug after 30 minutes of mastication. The maximum amount released was observed with L,8,K, 25.4 %, after 30 minutes. There was a significant difference in the amount of drug released release between L,8,K and C,8,K and all other gums ($P < 0.01$).

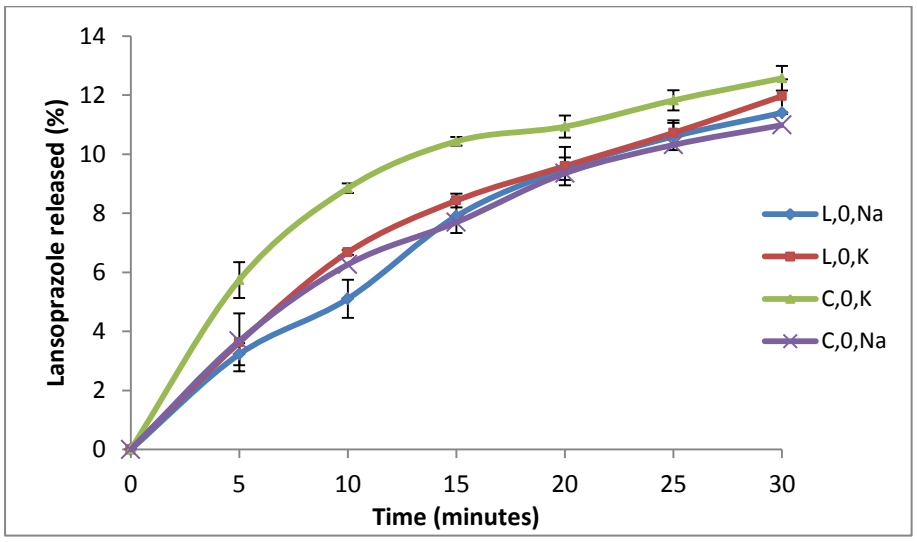


Figure 4.10 Drug release from gums without Rev7 in phosphate buffer pH 6.0 (n=3; mean ± s.d)

C,0,K had the highest release, 12.5 % after 30 minutes of mastication (Figure 4.10). Overall drug release was markedly less than for L,8,K and C,8,K but was similar to L,8,Na and C,8,Na (containing 8 % Rev7). This showed that Rev7 increased the extent of drug release from gums containing potassium carbonate but not from those containing sodium carbonate, thus suggesting a specific interaction between the components.

Drug release from all gums increased steadily over the 30 minute mastication period using phosphate buffer pH 6.0. L,8,K and C,8,K (containing Rev7 and potassium carbonate) released more drug than all other gums tested (Figure 4.11). In the presence of Rev7, the drug form, i.e. complexed drug *versus* drug alone did not impact *in vitro* drug release, however the choice of buffering excipients, influenced release with potassium carbonate increasing release compared to sodium carbonate.

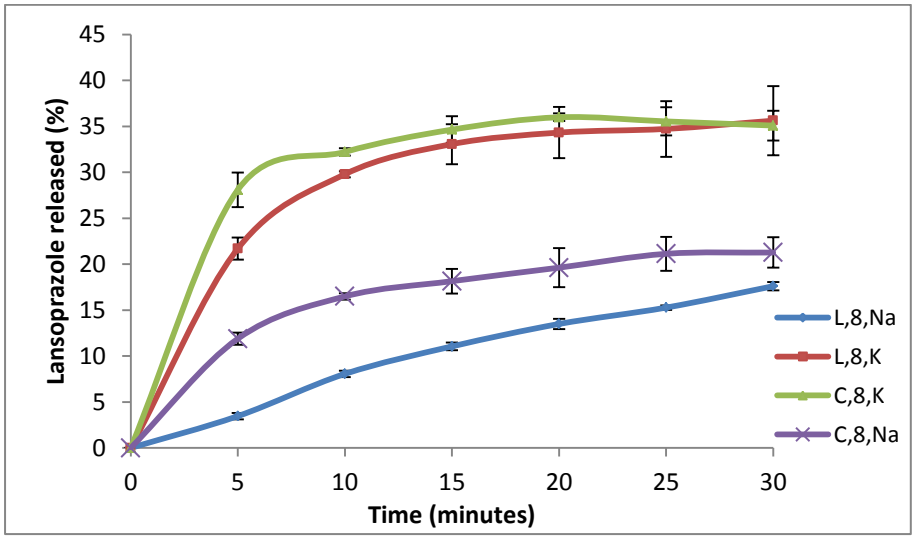


Figure 4.11 Drug release from gums containing 8 % Rev7 in artificial saliva pH 6.7 (n=3; mean ± s.d)

Maximal drug release occurred within the first 5 minutes for L,8,K and C,8,K (containing potassium carbonate) followed by a plateau phase during the remaining 25 minutes due to compromised sink conditions due to drug saturation (Figure 4.11). L,8,Na and C,8,Na increased steadily, consistent with release observed in phosphate buffer pH 6.0.

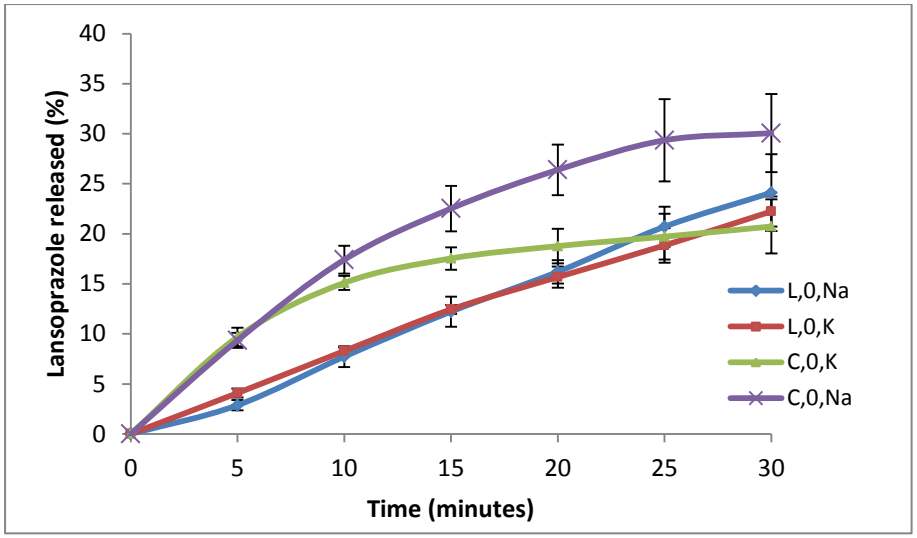


Figure 4.12 Drug release from gums without Rev7 in artificial saliva pH 6.7 (n=3; mean ± s.d)

C,0,Na released more drug, almost 30 % after 30 minutes, however release from C,0,K did not increase after 10 minutes due to compromised sink conditions and limitations in solubility (Figure 4.12). The buffering excipients (sodium carbonate and potassium carbonate) did not result in different release profiles (L,0,Na and L,0,K). An increase in drug release was observed from all gums when tested in artificial saliva pH 6.7 compared to phosphate buffer at pH 6.0 with mean (%) releases of 25.71 ± 6.80 and 14.99 ± 5.36 respectively. As the pKa of lansoprazole is 8.84, therefore increases in solubility are more pronounced as the ionisation increases exponentially above the pK_a (Table 1.4 and Figure 3.4).

L,8,K and C,8,K (containing Rev7 and potassium carbonate) had more extensive drug release compared to all other gums tested which was consistent with the study using phosphate buffer at pH 6.0 (Figure 4.9 and 4.10). Significant differences in release were found between gums, L,8,K and C,8,K were significantly different from all other gums ($P < 0.01$) but not from each other.

The impact of complexation was evident for gums without Rev7, gums containing 8 % Rev7 highlighted differences in buffering excipients, i.e. potassium carbonate and sodium carbonate. L,8,Na resulted in the lowest release from all other formulations with a maximal release of 17.6 % after 30 minutes mastication.

4.3.5.1.1 The effect of dissolution medium on drug release

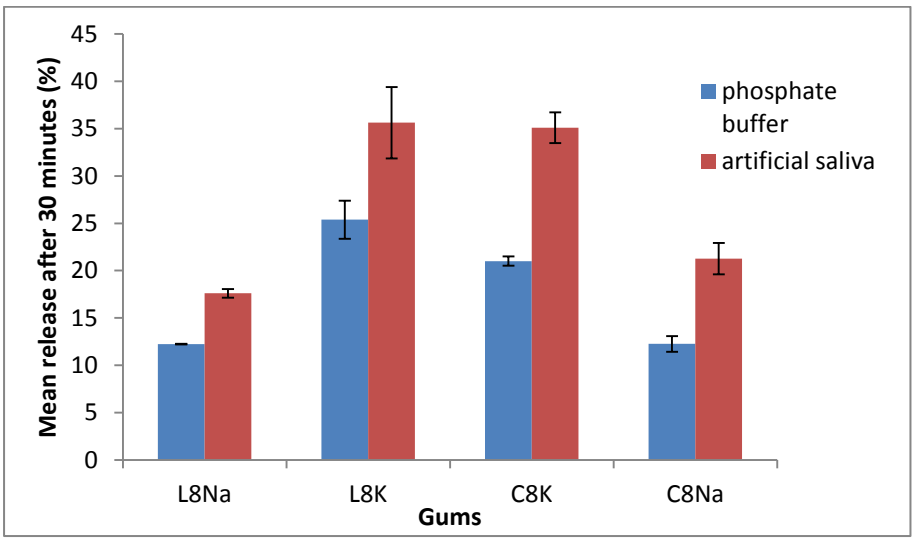


Figure 4.13 The effect of dissolution medium on release from gums containing 8 % Rev7 with chew rate of 60 chew/min and 2 mL replacement volume (n=3; mean ± s.d)

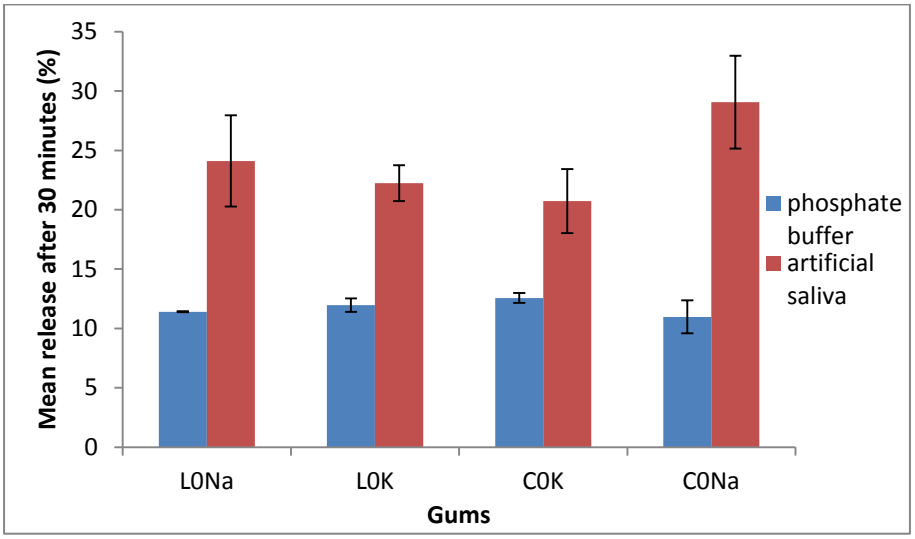


Figure 4.14 The effect of dissolution medium on release from gums without Rev7 with chew rate of 60 chew/min and 2 mL replacement volume (n=3; mean ± s.d)

Drug release increased in artificial saliva pH 6.7 compared to phosphate buffer at pH 6.0 with all gum formulations (Figures 4.13 and 4.14). All gums (apart from L,8,K and C,8,K) released 10 - 15 % in phosphate buffer at pH 6.0. Artificial saliva was a more discriminating medium. Significant differences (using t tests) were found between the two dissolution mediums ($P < 0.01$).

4.3.5.2 *In vitro* release from lansoprazole chewing gum (20 mL replacement)

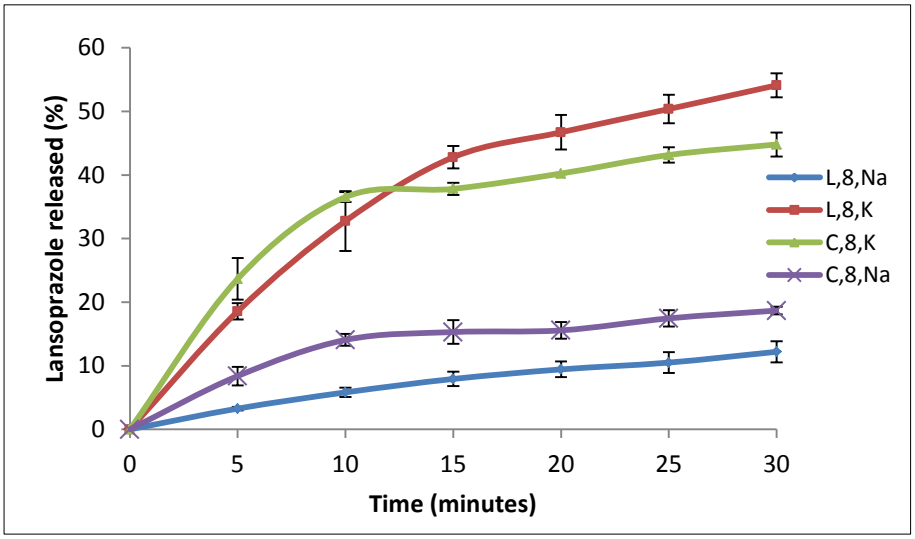


Figure 4.15 Drug release from gums containing 8 % Rev7 in artificial saliva pH 6.7 (20 mL replacement volume) (n=3; mean ± s.d)

Drug release was 54.1 and 44.8 % for L,8,K and C,8,K respectively at 30 minutes (Figure 4.15). An increase in the extent of release was observed in comparison to the 2 mL replacement volume method for both gums. Drug release from L,8,K and C,8,K showed less of a plateau phase as seen previously and more drug was released after the initial burst period. L,8,Na and C,8,Na showed limited release, as seen previously, with increased replacement volume having no effect on release. Gums (with 8% Rev7) containing potassium carbonate had a similar profile (independent of drug type (L,8,K and C,8,K) compared to gums containing sodium carbonate (L,8, Na and C,8,Na) and so can be paired together in groups.

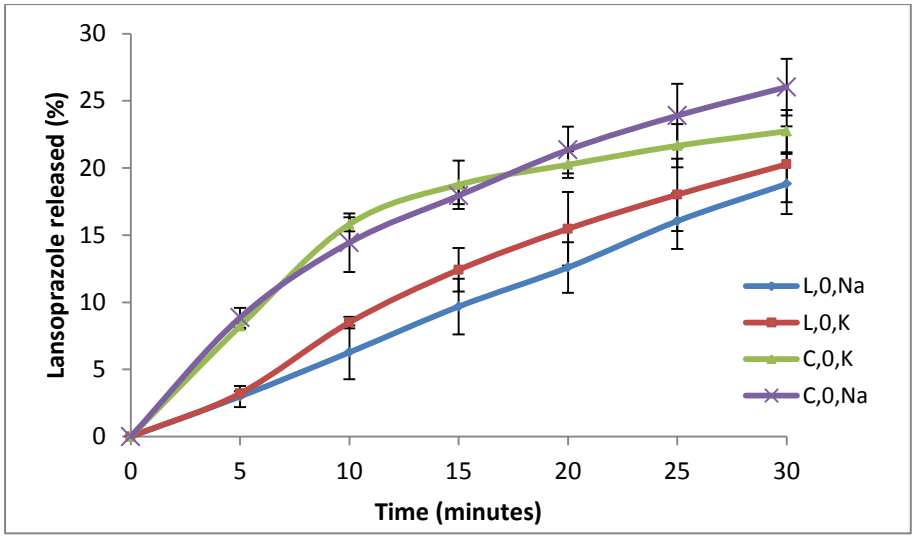


Figure 4.16 Drug release from gums without Rev7 in artificial saliva pH 6.7 (20 mL replacement volume) (n=3; mean ± s.d)

Release from all gums increased steadily over the 30 minute period and drug release was consistent with release observed previously with the 2 mL replacement volume (Figure 4.16). Gums (without Rev7) containing lansoprazole also had similar profiles (independent of buffering excipients (L,0,Na and L,0,K) compared to gums containing the complex form (C,0,K and C,0,Na) and so can be paired together in groups. This suggests both complexed and uncomplexed drug had similar profiles irrespective of buffering excipients in the absence of Rev7 polymer. C,0,Na released the most drug, 26.03 % after 30 minutes of mastication.

Significant differences in release were found between gums ($P < 0.01$). L,8,Na, L,8,K and C,8,K were significantly different to all other gums ($P < 0.01$). L,8,Na resulted in the lowest release from all other formulations with a maximal release of 12.2 % after 30 minutes mastication consistent with the 2 mL replacement volume.

4.3.5.2.1 The effect of sample replacement on drug release

The maximum concentration in the 40 mL chamber (assuming 100 % release) from the 15 mg gum would be 375 µg/mL lansoprazole, the maximum solubility during phase solubility studies was 131.94 µg/mL. 131.94 µg/mL / 375 µg/mL X 100 % = 35.18 % which correlated with the maximum releases after 30 minutes from (L,8,K 35.62 ± 3.77 and C,8,K 35.08 ± 1.61 %; mean ± s.d). This showed that sink conditions were compromised and that the method must be adapted for the release of poorly soluble actives. Increasing the sample and

replacement volume to 20 mL ensured drug solubility could not be a limiting factor by maintaining sink conditions.

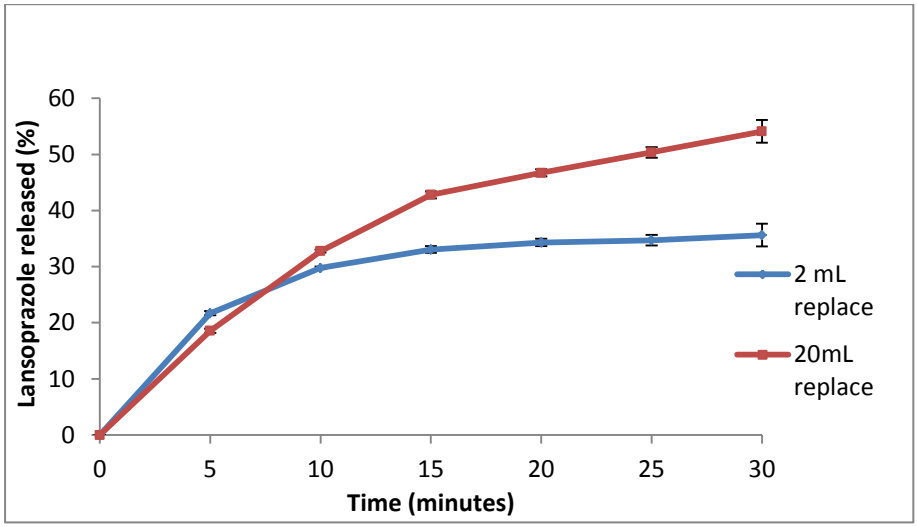


Figure 4.17 The effect of sampling and replacement volumes (2 mL and 20 mL) on release from L,8,K

Increasing the sample and replacement volume resulted in the most significant increases in the extent of drug release from L,8,K. The increase in release for L,8,K is shown in Figure 4.17.

4.3.5.3 *In vitro* release from lansoprazole chewing gum (pH 8.0 and 20 mL replacement)

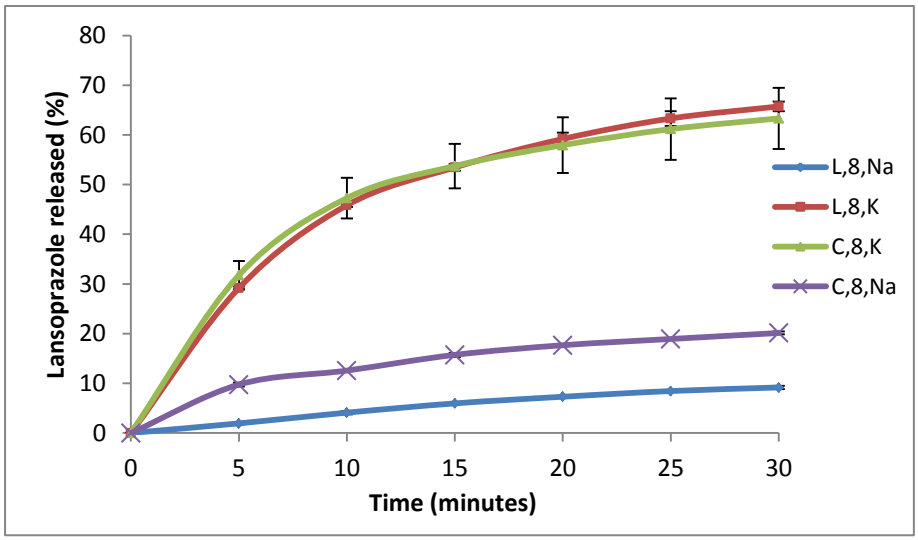


Figure 4.18 Drug release from gums containing 8 % Rev7 in artificial saliva pH 8.0 (20 mL replacement volume) (n=3; mean ± s.d)

The extent of drug release increased with L,8,K and C,8,K to 65.24 and 63.33 % after 30 minutes mastication. The initial burst release (at 5 minutes) increased to 29.17 and 31.75 % respectively in at higher pH (artificial saliva pH 8 and 20 mL replacement volumes) (Figure 4.18). Significant differences in maximum release were found between L,8,K and C,8,K and all other gums, but not from each other ($P < 0.01$). L,8,Na showed a decrease in release with limited release of 9.16 % and C,8,Na also showed limited release of 20.12 %. Both release profiles again showed a slow steady release and were consistent to results seen previously. The distinction between the two groups (L,8,K /C,8,K and L,8,Na/C,8,Na) was more pronounced.

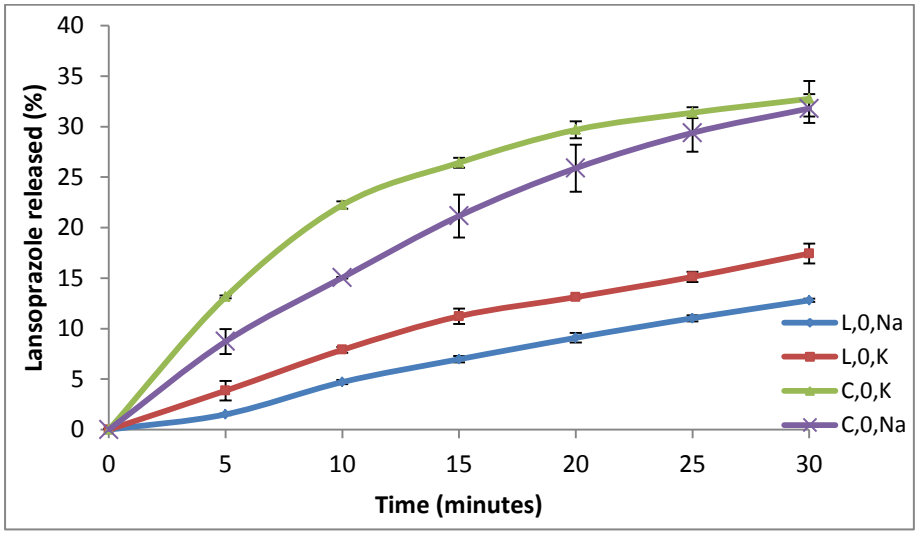


Figure 4.19 Drug release from gums without Rev7 in artificial saliva pH 8.0 (20 mL replacement volume) (n=3; mean ± s.d)

Complexation increased drug release, extending it to 32.8% for C,0,K (Figure 4.19). The paired relationship between the two groups (L,0,Na and L,0,K) and (C,0,K and C,0,Na) was also more pronounced consistent with (Figure 4.19). Significant differences in maximum release were found between these sets of gums ($P < 0.01$). L,8,Na released the lowest amount of drug and was significantly different from all other gums, other than L,0,Na ($P < 0.01$). This suggested that sodium carbonate may retarded release of the drug, independently from Rev7.

4.3.5.3.1 The effect of pH of artificial saliva on drug release

The pH increase is caused by the release of buffering excipients from the formulation and can impact drug release (Morjaria, 2004). The control of salivary pH should be considered to facilitate passive diffusion of unionised drugs for the potential absorption via the buccal membrane (see section 5.3.3.1).

Table 4.3 pH change during 30 minutes *in vitro* mastication

Gum	Phosphate buffer pH 6.0 (after 30 minutes mastication)		Artificial saliva pH 6.7 (after 30 minutes mastication)	
	% released	**pH change**	**% released**	**pH change**
L,8,Na	12.24 ± 0.04	0.43	17.6 ± 0.45	1.96
L,8,K	25.38 ± 2.02	0.39	35.62 ± 3.77	1.77
C,8,Na	12.26 ± 0.82	0.47	21.27 ± 1.66	1.89
C,8,K	21.00 ± 0.49	0.37	35.08 ± 1.61	1.75
L,0,Na	11.40 ± 0.40	0.46	24.11 ± 3.85	2.12
L,0,K	11.96 ± 0.56	0.37	22.24 ± 1.50	1.96
C,0,Na	10.98 ± 1.38	0.47	29.06 ± 2.50	2.15
C,0,K	12.57 ± 0.43	0.38	20.73 ± 2.70	1.74

** Only 2 mL sample replacement experiments were reported due to the increased replacement volume (20 mL) having a diluting effect on pH measurements over the duration

The mean pH change after 30 minutes mastication with formulated gums was 0.42 ± 0.04 pH units for phosphate buffer pH 6.0 and 1.92 ± 0.16 pH units for artificial saliva at pH 6.7 (Table 4.3). This showed that the phosphate buffer retained its buffering capacity (ability to maintain a pH range regardless of stimulation of ions) at pH 6.0. The buffering strength of artificial saliva was 38 mM which was relatively low compared to the strength of phosphate buffer which was 200 mM. The solubility of lansoprazole is pH dependent and is increased above pH 8 (Figure 3.4) the increase in pH after 30 minutes mastication of approximately 2 pH units would account for an increase in solubility of lansoprazole and this may have contributed towards the increased drug release from formulations.

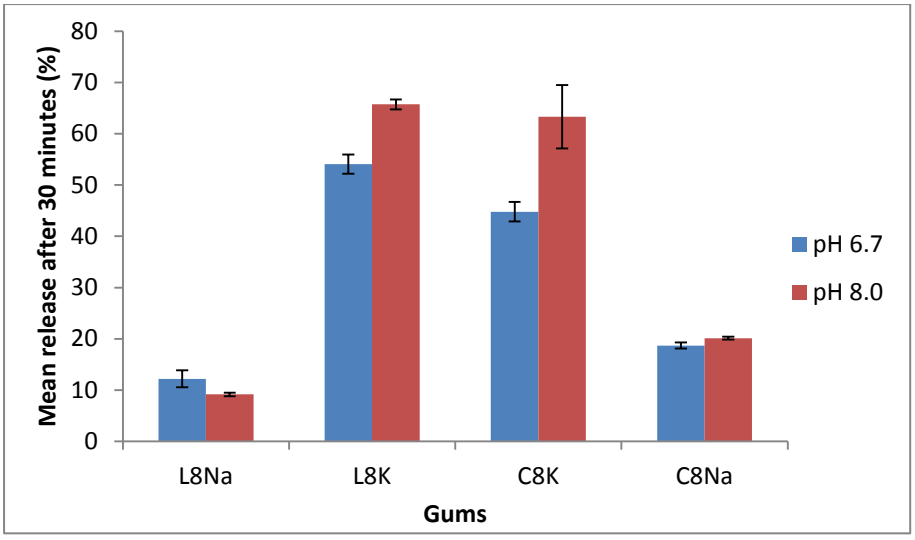


Figure 4.20 The effect of pH on release from gums containing 8 % Rev7 with chew rate of 60 chew/min, artificial saliva and 20 mL replacement volume (n=3; mean ± s.d)

Increases in pH of artificial saliva resulted in increased drug release from L,8,K and C,8,K, (containing potassium carbonate) whereas minor differences were observed for L,8,Na and C,8,Na. (Figure 4.20) In the presence of Rev7 and potassium carbonate an increase in pH of artificial saliva resulted in an increase in the extent of drug release.

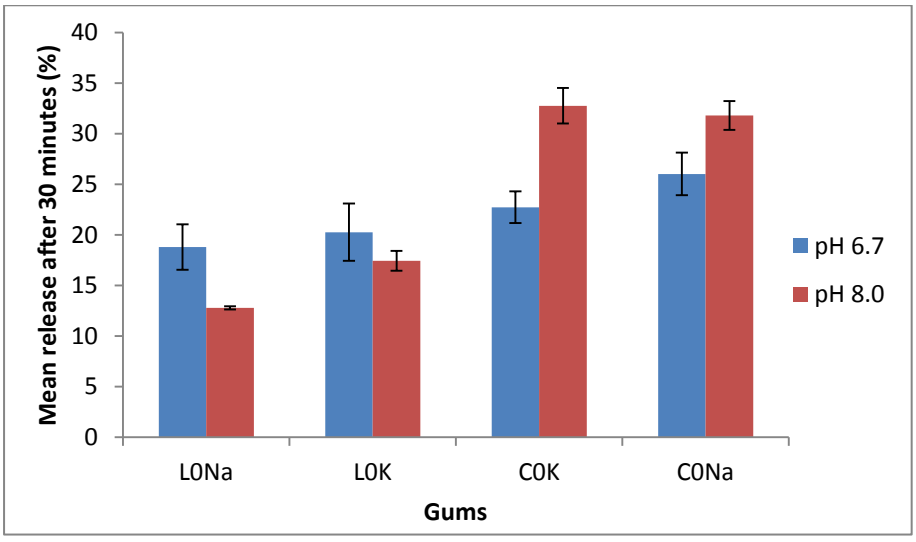


Figure 4.21 The effect of pH on release from gums without Rev7 with chew rate of 60 chew/min, artificial saliva and 20 mL replacement volume (n=3; mean ± s.d)

In the absence of Rev7, gums which contained complexed drug (C,0,K and C,0,Na) resulted in increased drug release and were more influenced by the increase in pH than those with uncomplexed/free drug (Figure 4.21). Gums containing drug alone (L,8,Na, L,0,Na and

L,0,K) had increased release at the lower pH of 6.7 compared to pH 8.0. This may suggest a retarding interaction/mechanism within the gum formulations.

In the absence of Rev7, gums containing complexed drug were influenced more by the increasing of the pH of artificial saliva irrespective of the buffer excipient. In the presence of Rev7, gums containing potassium carbonate released more drug at the higher pHs compared to gums containing sodium carbonate.

The mean % release over all formulations increased in artificial saliva pH 6.7 and pH 8.0 (27.20 ± 14.07 and 31.58 ± 21.14 respectively after 30 min) when compared with phosphate buffer pH 6.0. Increases in pH of phosphate buffer facilitated the dissolution of commercial lansoprazole capsules (as granules) with maximum dissolution observed at pH 8 (Ashraf *et al*., 2012). No significant differences were found when comparing all the gum formulations (using t tests) between pH 6.7 and pH 8.0 of artificial saliva ($P > 0.05$).

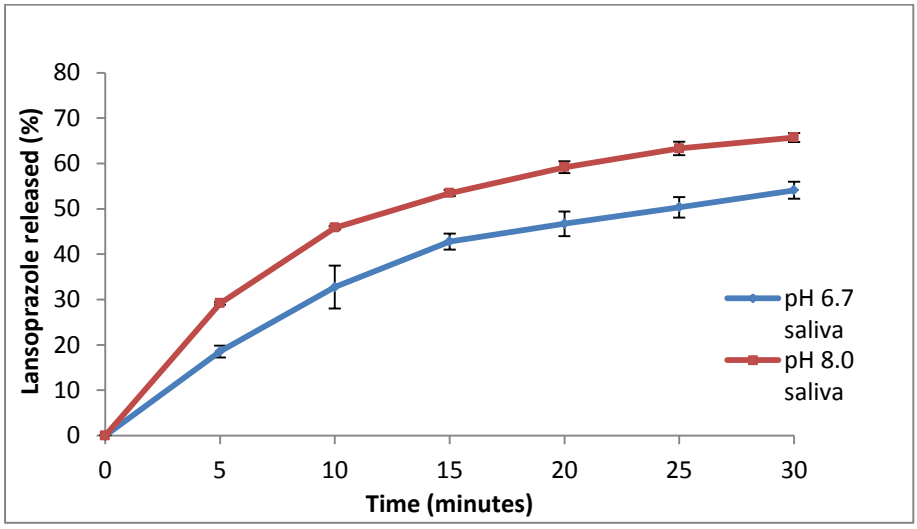


Figure 4.22 The effect of pH of artificial saliva on release from L,8,K

Both release curves looked similar in shape; however the increased pH (pH 8.0) resulted in an increased burst effect in the initial stages (Figure 4.22).

4.3.5.4 *In vitro* release from lansoprazole chewing gum (83 chews per min)

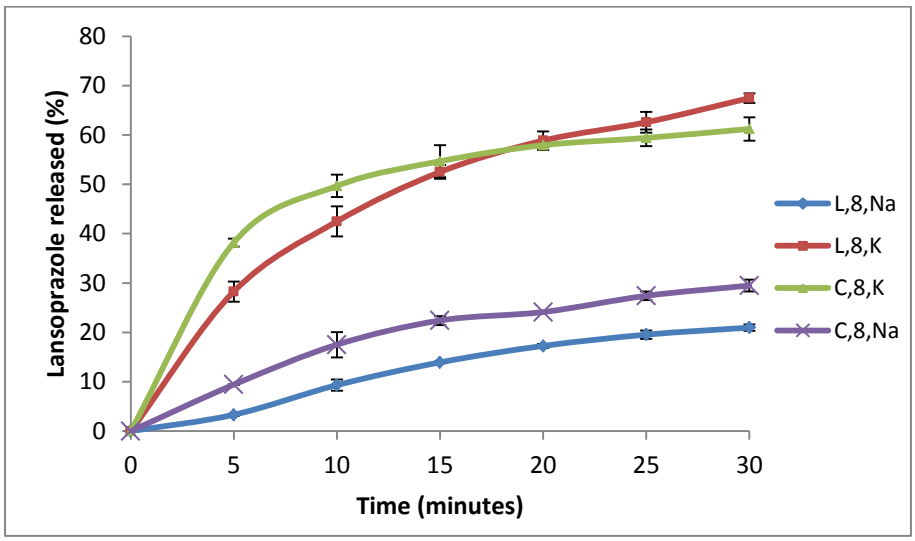


Figure 4.23 Drug release from gums containing 8 % Rev7 with chew rate of 83 chews/min in artificial saliva pH 8.0 (20 mL replacement volume) (n=3; mean ± s.d)

Increasing the chew rate from 60 to 83 chews *per* minute increased the release from L,8,Na and C,8,Na. The extent of drug release from L,8,Na doubled (20.97 % after 30 minutes) compared to the previous 60 chew/min settings (Figure 4.23). Increases in chew rate resulted in minimal increases of the extent of drug release from L,8,K and C,8,K possibly due to interactions with the lipophilic base.

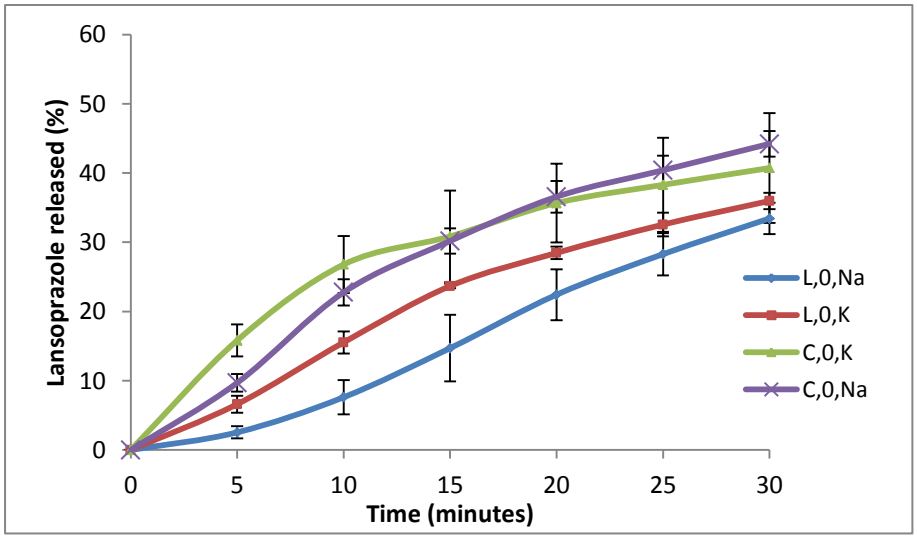


Figure 4.24 Drug release from gums without Rev7 with chew rate of 83 chews/min in artificial saliva pH 8.0 (20 mL replacement volume) (n=3; mean ± s.d)

Minor differences in release were observed between the paired groups gums L,0,Na and L,0,K (containing lansoprazole) and gums C,0,K and C,0,Na (containing complexed drug) (Figure 4.24). L,0,Na and L,0,K had increased drug release (doubled) when compared to the previous setting of 60 chews *per* minute.

L,8,Na was again significantly different to all other gums and released less drug after 30 minutes compared to all other formulations ($P < 0.05$). L,8,K and C,8,K were significantly different to all other gums ($P < 0.01$) but not from each other and consistently released more drug after 30 minutes. Significant differences in release (without Rev7) were found between C,0,Na and L,0,K and L,0,Na ($P < 0.05$).

ANOVA statistical analysis on maximum release after 30 minutes found the following significant differences between formulation variables: significant differences between 8% Rev7 and 0 % Rev7 ($P < 0.01$) and significant differences between buffering excipients; sodium carbonate and potassium carbonate ($P < 0.01$).

4.3.5.4.1 The effect of chew rate on drug release

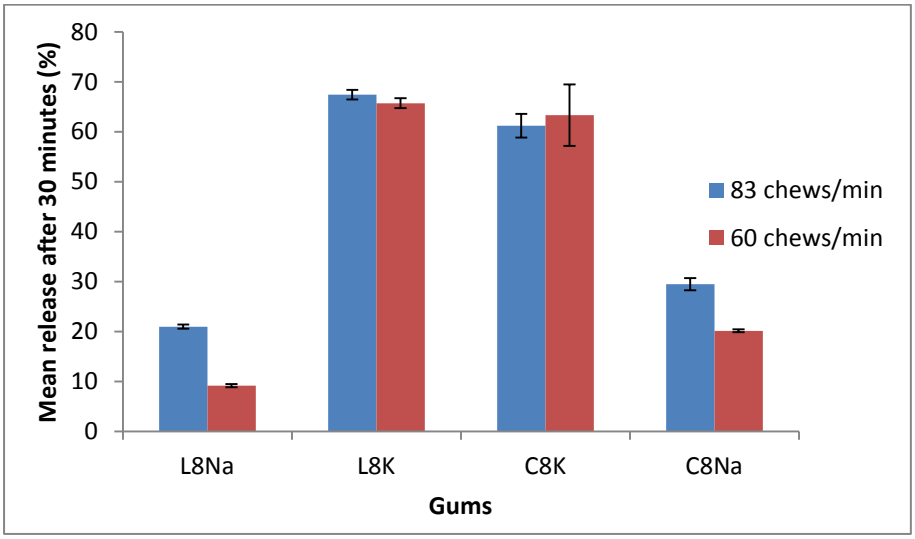


Figure 4.25 The effect of chew rate on release from gums containing 8 % Rev7 with artificial saliva pH 8.0 and 20 mL replacement volume (n=3; mean ± s.d)

Increasing the chew rate had little effect on extending release from L,8,K and C,8,K (Figure 4.25); this may have been due to the maximum release of drug being reached as it is expected that gums will always retain a percentage of active after mastication due to adherence to the

lipophilic gum base. L,8,Na and C,8,Na were affected by chew rate with increased release at the faster chew rate of 83 chews *per* minute.

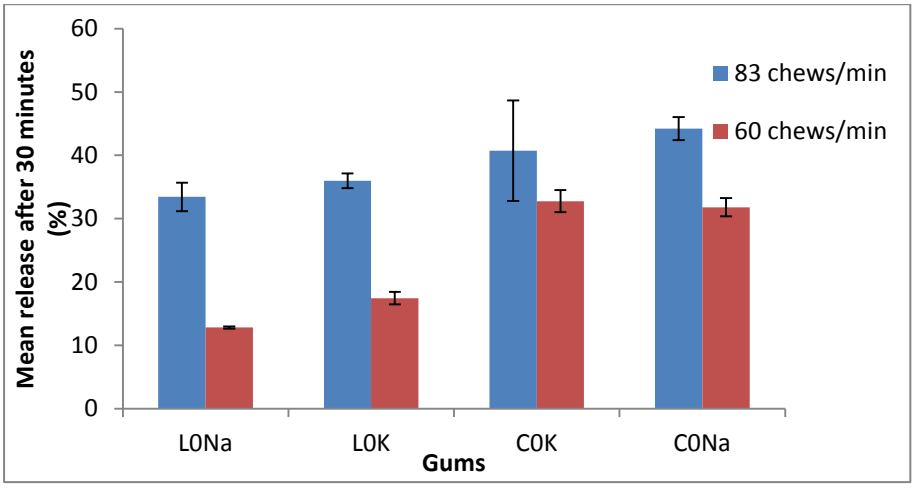


Figure 4.26 The effect of chew rate on release from gums without Rev7 with artificial saliva pH 8.0 and 20 mL replacement volume (n=3; mean ± s.d)

In the absence of Rev7, release increased with increasing chew rate (Figure 4.26). This is due to the increased mechanical forces and renewable areas caused by chewing. However L,8,K and C,8,K had similar release at both chew rates and may have reached the maximum releases.

Mean maximum releases for release in artificial saliva pH 8.0 at 60 chews *per* minute compared to 83 chew *per* minute were 31.58 ± 21.14 % and 41.68 ± 15.37 % respectively. No significant differences (using t tests) were found between 60 chews *per* minute and 83 chews *per* minute in artificial saliva pH 8.0 ($P > 0.05$).

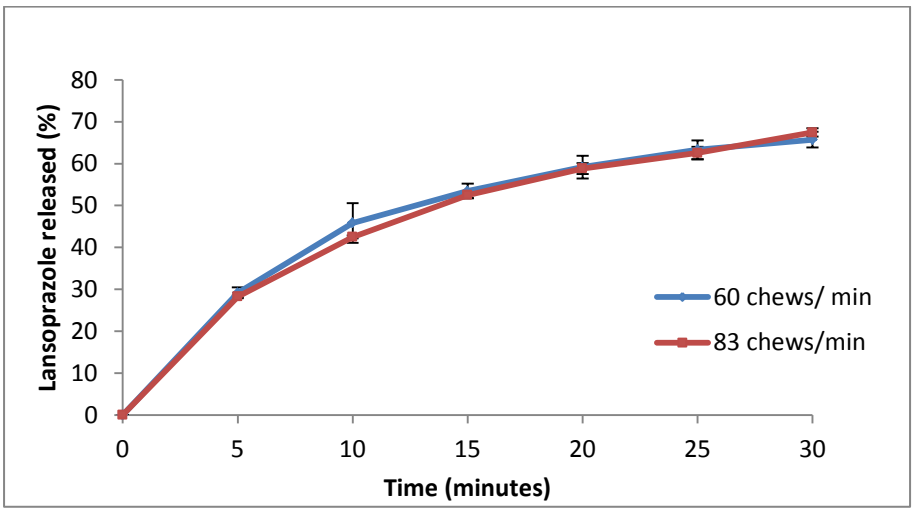


Figure 4.27 The effects of chew rate on release from L,8,K

Increasing the chew rate did not have any significant effect on the release from L,8,K (conducted using artificial saliva pH 8.0 and 20 mL sample and replacement volumes) (Figure 4.27).

4.3.5.5 Overall results summary

Ten different gum formulations were prepared to provide *in vitro* release data necessary for development of medicated gums containing lansoprazole. Preliminary formulations showed release was retarded with increasing gum base due to increasing lipophilicity of the gum. Eight further gums were formulated to evaluate the influence of the form of the active (drug alone or complexed drug with MβCD 1:1), internal buffering excipients and the inclusion of Rev7 polymer. Drug diffusion from gum surfaces was found to be limited, highlighting the need for effective mastication to ensure timely release of the drug. *In vitro* release was then evaluated with the use of the EP approved masticator.

The individual will contribute towards a range of factors whilst chewing the gum *in vivo* (including differing volumes of saliva and chew rates) therefore a range of parameters were investigated such as type of dissolution medium, sampling and replacement volumes, the pH of the medium and lastly chew rate (See Appendix A). The dissolution medium should ideally represent physiological conditions at the site of administration (Siewart *et al*., 2003), therefore dissolution testing was conducted in phosphate buffer pH 6.0 and artificial saliva (pH 6.7 and pH 8.0) satisfying EP guidelines of an unspecified buffer at around pH 6 (European Pharmacopoeia, 2011).

L,8,K and C,8,K (containing Rev7 and potassium carbonate) consistently released more drug than the other gum formulations, reaching a maximum of 67.5 % and 63.3 % for L,8,K and C,8,K respectively. They also partially disintegrated after 60 minutes when in surface diffusion studies whilst all other formulations remained intact. There was also some diffusion of drug from these gums without chewing. These differences may have been due to specific physical interactions between potassium carbonate within the gum base leading to formation of micropores in the gum matrix, allowing increased penetration/ingress of dissolution medium into the gum.

The following settings: 60 chews *per* minute, artificial saliva pH 8.0 with 20 mL sample and replacement volumes-(ensuring *sink* conditions), allowed discrimination of formulation differences (Figures 4.17 and 4.18). Gums containing potassium carbonate (L8K and C8K) had a biphasic release. This may be due to initial leaching (from the hydrophilic domains) followed by sustained release, as a consequence of the diffusion controlled release from within the lipophilic gum base. Previous studies also found an increased burst release with nicotine gums containing potassium carbonate compared to sodium carbonate in the initial chewing stages (Shiffman *et al*., 2009).

L,8,Na and C,8,Na (containing sodium carbonate) showed a limited release after 30 minutes of 9.16 ± 0.33 % and 20.12 ± 0.28 % respectively; both profiles showed a gradual steady linear increase over time compared with the burst release of L,8,K and C,8,K. C,8,Na contained the drug in complexed form and the increase in solubility/hydrophilic capacity of the gum may have influenced the initial increased burst release rate after 5 minutes compared with L,8,Na (drug alone). L,8,Na consistently released less drug after 30 minutes throughout all the conditions tested which suggested a mechanism to retard release from the gum.

Formulations incorporating 8 % Rev7 were influenced by the type of buffering excipients, with potassium carbonate increasing drug release compared to sodium carbonate. Formulations without Rev7 were influenced by the form of the drug, whether complexed or uncomplexed and increased release was observed with the complexed drug form. The complexity in understanding release mechanisms and release kinetics in gums is still not fully understood and a range of authors suggest an complex interactions between the gum base, drug and internal buffering systems (Cherukuri *et al*., 2002; Pinney *et al*., 2005; Chau *et al*., 2008; Shiffman *et al*., 2009).

The study by Lu evaluated ternary systems of lansoprazole and βCD (1:1) with the solubility enhancing effects of two polymers (PVP and PEG 6000). Increasing polymer concentrations (2-10%) increased release due to specific interactions (intermolecular hydrogen bonds) formed between polymer and drug (Lu *et al*., 2012).

The increased extent of release of the poorly soluble drug may have resulted from increasing the hydrophilic capacity of the gum (Rev7) and a greater extent and rate of micropore formation (potassium carbonate). The combined effect could increase release due to lowering

the adherence of the poorly soluble drug to the lipophilic components of the gum and also increasing contact between the gum and dissolution medium.

4.4.6 The effect of formulation components on lansoprazole release

4.4.6.1 The effect of Rev7 polymer

Rev7 is known to improve the hydrophilic capacity of the chewing gum base (Farber *et al.*, 2009), this may increase release and form a softer gum as softer gums will require less force to penetrate and renew surface areas compared to harder gums.

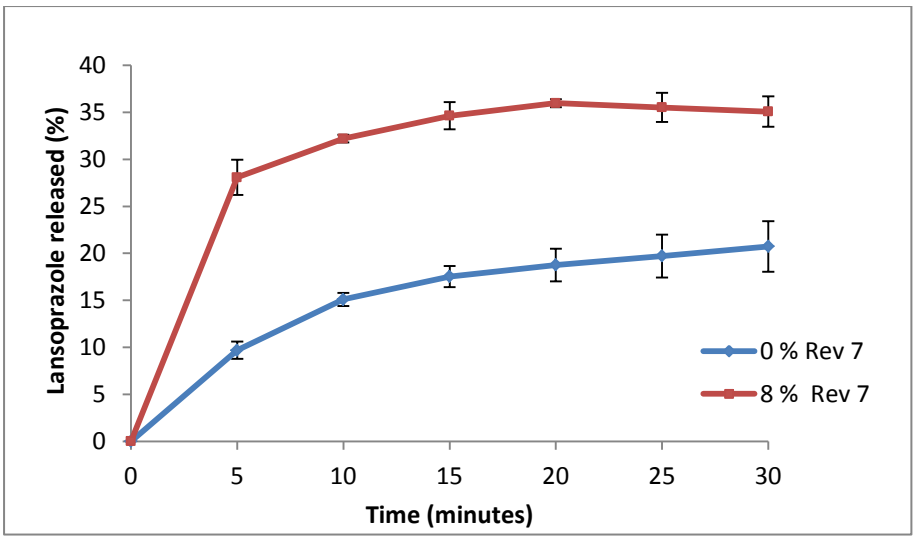


Figure 4.28 The effect of Rev7 polymer on release from gums containing complexed drug and potassium carbonate (C,8,K and C,0,K) in artificial saliva pH 6.7 (2 mL replacement volumes)

The increase in burst release in the initial stages (5 minutes) of chewing demonstrates the effect of Rev7 with increasing release from the hydrophilic components via leaching (Figure 4.28). The release of active from the hydrophilic components of the gum is typically displayed in the initial stages of chewing. The subsequent release profile is super imposable after 5 minutes suggesting that subsequent release of complexed drug is via diffusion controlled mechanisms from the lipophilic regions of the gum. As sink conditions were compromised this would have limited release also causing a plateau.

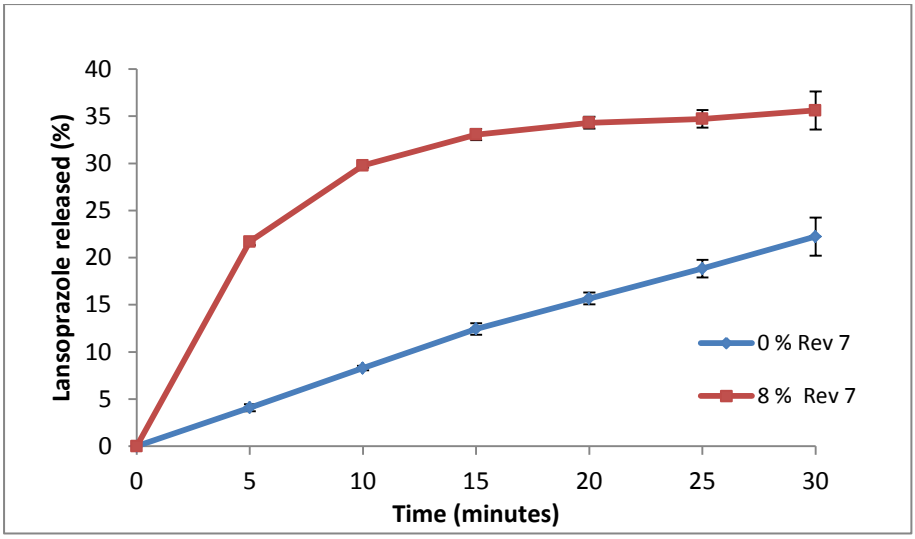


Figure 4.29 The effect of Rev7 polymer on release from gums containing lansoprazole and potassium carbonate (L,8,K and L,0,K) in artificial saliva pH 6.7 (2 mL sampling and replacement)

The inclusion Rev7 increases release (Figure 4.29) and suggests that the hydrophilic capacity of Rev7 dominates whether drug is complexed or not. Release of lansoprazole in gums without Rev7 was slow and steady. This is in contrast to the burst release displayed with the complexed drug form (Figure 4.30), suggesting that complexation contributes to the burst release in the initial stages of mastication.

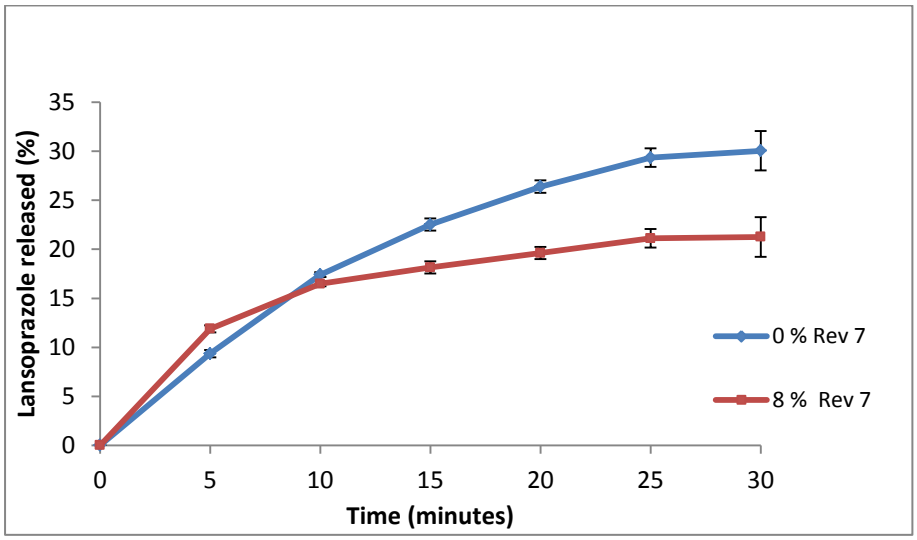


Figure 4.30 The effect of Rev7 polymer on release from gums containing complexed drug and sodium carbonate (C,8,Na and C,0,Na) in artificial saliva pH 6.7 (2 mL sampling and replacement)

Rev7 was observed to retard the release of active in the presence of sodium carbonate as buffering excipient (Figure 4.30). The release profiles of complexed drug were similar in the

initial stages and differences were more pronounced after 10 minutes (predominately the diffusion controlled area) suggesting that there was an increase in adherence and binding of complexed drug to the lipophilic gum base containing Rev7 polymer and sodium carbonate.

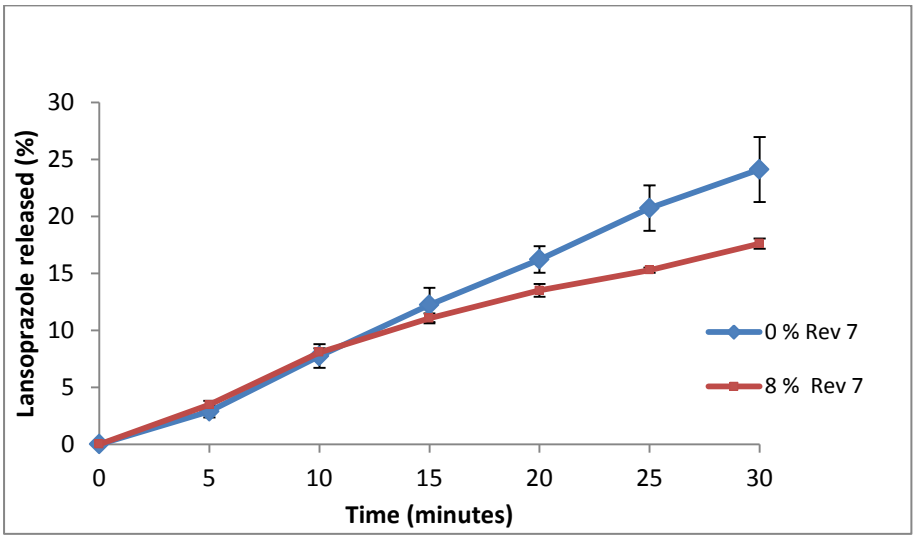


Figure 4.31 The effect of Rev7 polymer on release from gums containing lansoprazole and sodium carbonate (L,8,Na and L,0,Na) in artificial saliva pH 6.7 (2 mL sampling and replacement)

Rev7 was consistent in retarding the release of active in the presence of sodium carbonate (Figure 4.31). Both profiles displayed a steady release similar in the initial stages with differences occurring at the later stages of the mastication process.

The effect of Rev7 was highly influenced by the type of buffering excipient; sodium carbonate release was retarded whilst in the presence of potassium carbonate release was faster.

4.4.6.2 The effect of buffering excipients

The role of the buffering excipient is to increase local pH of the saliva in the oral cavity, facilitating diffusion, as well increasing solubility and enhancing stability. Upon mastication, the water-soluble buffer dissolves leaving channels and micropores in the gum. This facilitates ingress of the dissolution medium and enhances release. L,8,K and C,8,K (containing potassium carbonate and Rev7) consistently released most drug after 30 minutes.

Table 4.4 Physiochemical properties of sodium carbonate and potassium carbonate

	Na_2CO_3	K_2CO_3
RMM	106	138
Density (g/cm^3)	0.97	0.86
Molar concentrations (3 g were added)	0.028	0.0217
pKa	6.37 & 10.25 (carbonic acid)	
Solubility at 20°C in water (g/L)	215	1120

Equivalent weight ratios were added of each buffering excipient to all formulations. A fivefold increase in solubility is found when comparing potassium carbonate with sodium carbonate (Table 4.4). This can facilitate increased channel production and similar results were found for lansoprazole pellet formulations (Wei *et al*,. 2010). There are also small differences in density and molar concentrations. Generally, as soluble excipients are added to the gum, the texture of the gum will also change becoming increasingly soft.

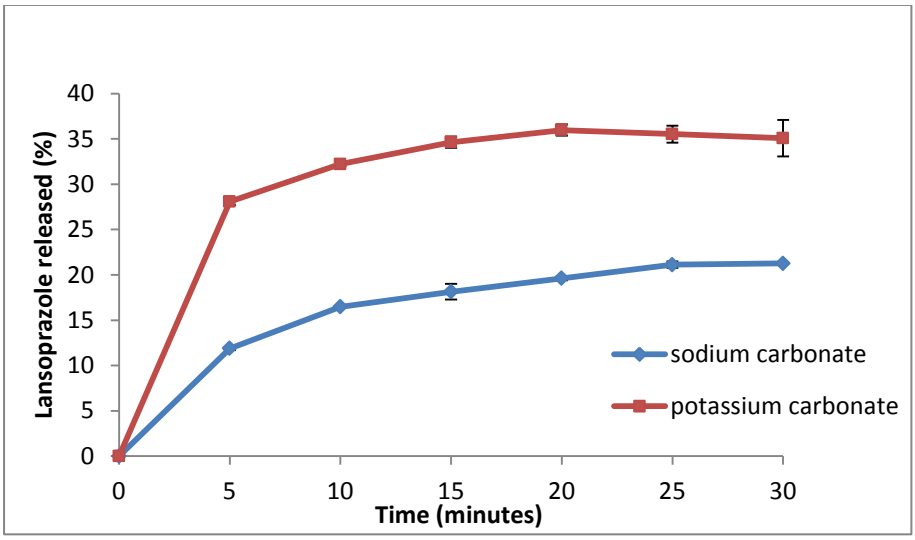


Figure 4.32 The effect of buffering excipients on release from gums containing complex and Rev7 (C,8,K and C,8,Na) in artificial saliva pH 6.7 (2 mL replacement volume)

The increased burst release effect of potassium carbonate can be observed over the first 5 minutes of mastication (initial stages) compared with sodium carbonate (Figure 4.32). A similar burst release was previously observed by Shiffman with nicotine gum formulations containing potassium carbonate (Shiffman *et al*., 2009).

4.4.6.3 The effect of drug form

Complexation with MβCD (1:1) resulted in a 9 fold increase in solubility; the enhancement in solubility can be explained by the reduction in crystallinity caused by the inclusion of the complex and by the freeze drying method resulting in the increased wettability of the drug.

Formulations without Rev7 highlighted differences in the drug with the complexed form increasing the extent of release (Figure 4.18). C,0,K and C,0,Na (containing complexed drug) had a higher burst release effect than the equivalent formulations, C,0,K released 32.76 ± 1.76 % after 30 minutes (containing complexed drug with potassium carbonate). L,0,Na (containing drug alone and sodium carbonate) had a slow and steady release dominated by a diffusion mechanism.

4.4.7 Texture analysis of lansoprazole chewing gum formulations

During mastication the gum's texture changes from a hard solid (necessary for stability and storage) to a softer gum over the first initial minutes of chewing (1-3 minutes). This is caused by the ingress of saliva (hydration) and the release of soluble components such as sweeteners and flavourings. The gum then reaches a final firmer stage once the soluble components are all extracted and leaving the lipophilic gum base (Lee, 2001). The gum base provides the functional textural properties of the overall gum (see section 1.2.1 and Table 1.1). The heterogeneous morphology of the gum base (due to some components not being miscible with each other) contributes to the unique characteristics of each different component providing further tailored functionality in the gum. This can be controlled via manufacturing processes including the mixing intensity-time and temperature and the adding sequence of components. The elastomers contained in the gum base provide the structure and elasticity due to long polymer chain entanglements, while plasticisers in the gum base regulate cohesion and softness due to separating the polymer chains in the elastomer (Lee, 2001).

Texture analysis can provide information regarding the feel of the gum and excipients can be added to modify the mouth feel including vegetable oil products such as glycerine to soften the gum and maintain flexibility. The gum must maintain integrity and remain intact throughout the chewing cycle to maintain the required sustained release to facilitate buccal absorption (Morjaria *et al*., 2004). The inclusion of Rev7 polymer will modify this texture (softening) and the subsequent release parameters due to increasing the hydrophilic

component (via MPEG) (Farber *et al.*, 2009). In the experiments, no sugar coating was used and this may restrict comparisons with marketed products.

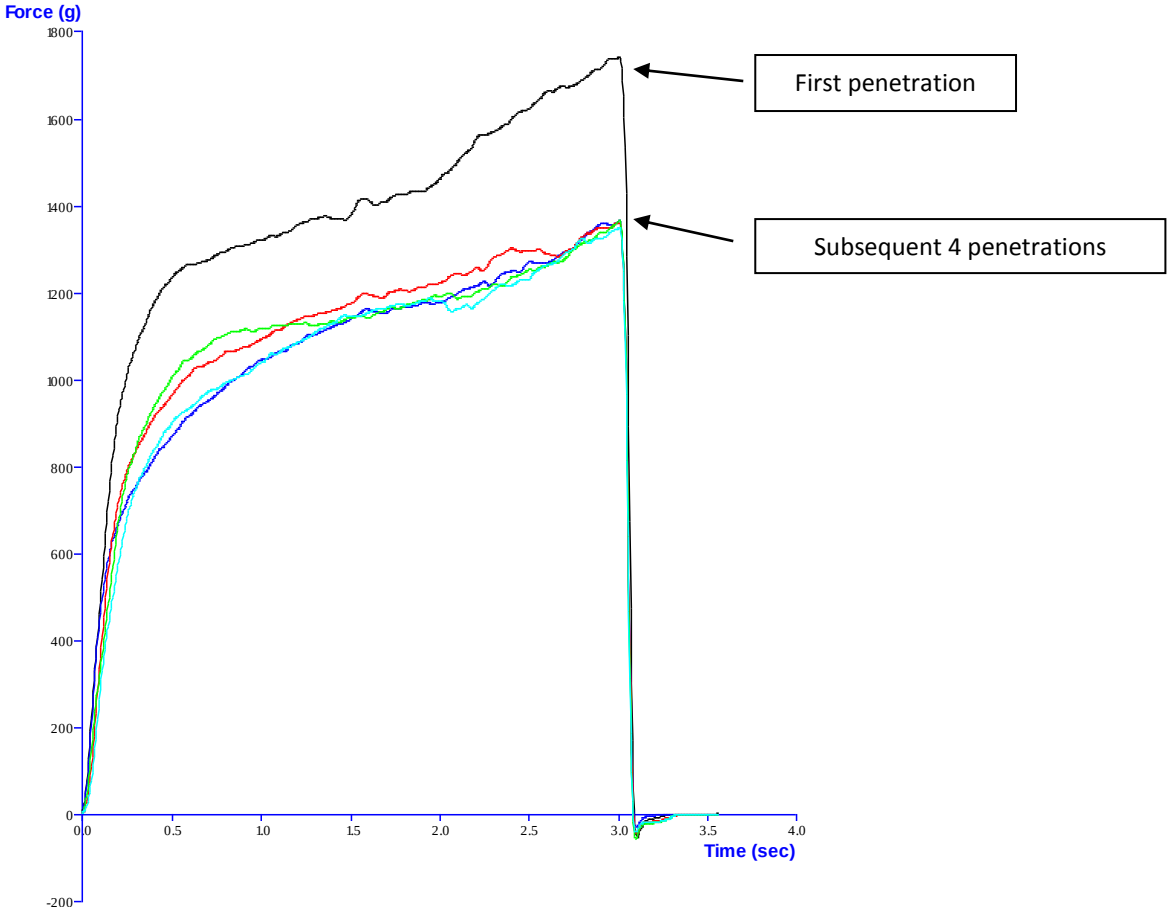


Figure 4.33 Texture analysis profile for L,8,Na

The initial measurement was made in the centre of each gum (black line) with 4 subsequent penetrations on each corner of the gum, however only the initial penetration in the centre was reported due to the compromised surface integrity after deformation from the first point. This is evident in the following 4 points which are markedly decreased (Figure 4.33 and Figure 4.34).

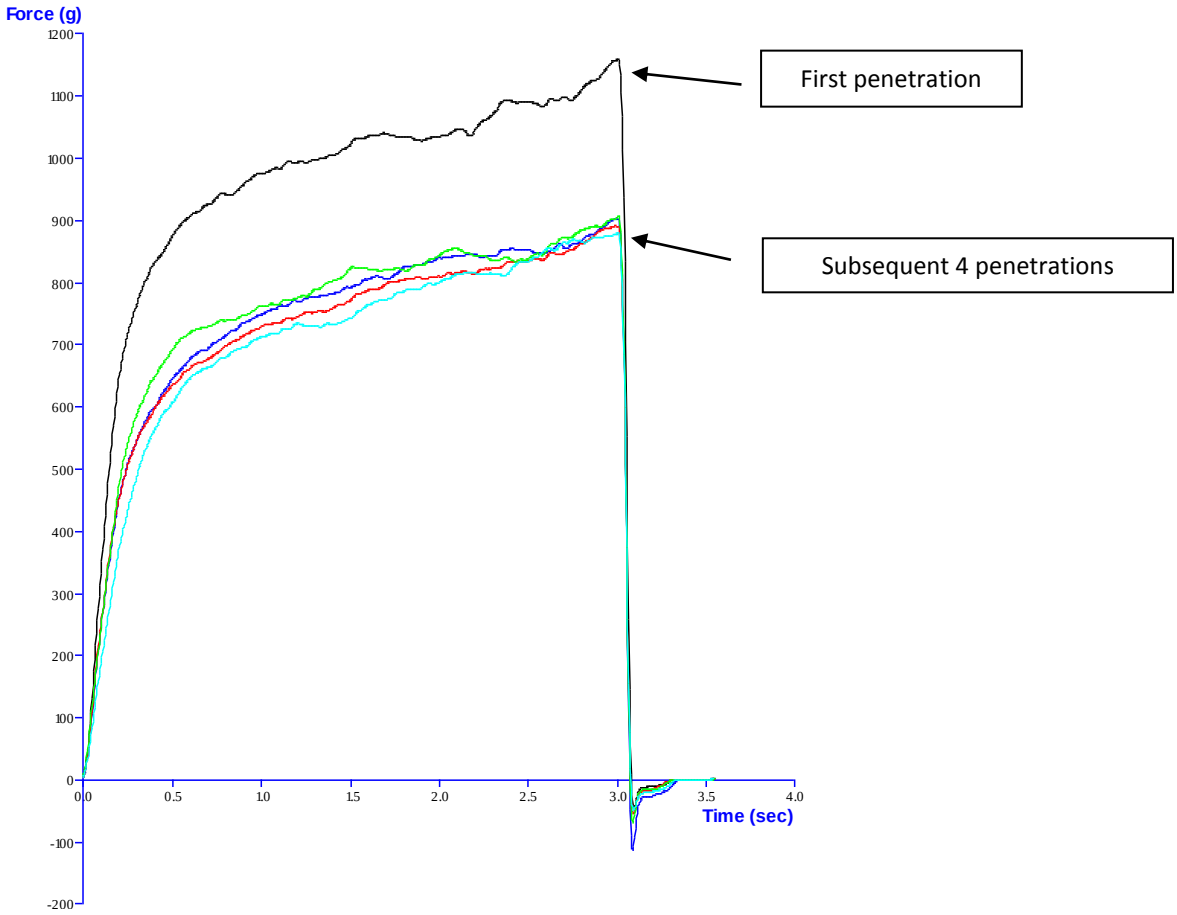


Figure 4.34 Texture analysis profile for C,8,K

Hardness was found to decrease with gums containing Rev7 and potassium carbonate (L,8,K and C,8,K) (Figure 4.33 and Figure 4.34). Hardness can be directly related to release and a softer gum will lead to more surface contact with the dissolution medium leading to increased diffusivity and a greater extent of release.

Table 4.5 Texture analysis of lansoprazole L gums

Test Gum	Hardness of first penetration (force in g)	Chewiness (g/second)	Positive cohesion (g/second)	Negative adhesion (g/second)
L,8,Na				
Mean	1697.41	5345.93	4100.46	-4.09
s.d	41.13	414.88	86.61	1.99
L,8,K				
Mean	1226.70	4234.02	3150.13	-2.81
s.d	53.47	300.08	75.19	1.09
C,8,K				
Mean	1291.99	4592.13	3233.49	-4.24
s.d	187.02	1550.15	606.16	2.65
C,8,Na				
Mean	1912.31	5857.69	4935.80	-4.11
s.d	73.01	625.81	146.48	0.73
L,0,Na				
Mean	2062.38	6825.59	5184.77	-4.21
s.d	187.87	1032.21	318.66	1.83
L,0,K				
Mean	2161.21	6722.48	5634.48	-5.27
s.d	102.87	948.85	357.26	2.51
C,0,K				
Mean	2098.29	6143.67	5500.82	-4.90
s.d	117.52	1361.54	93.71	2.23
C,0,Na				
Mean	2007.76	5371.58	5139.97	-4.24
s.d	137.14	2075.49	266.10	0.57

Inclusion of Rev7 polymer decreased hardness, without Rev7, hardness is consistent irrespective of any other formulation differences (Table 4.5). However release was increased with formulations containing the complexed form of lansoprazole suggesting that the increase in solubility may have influenced the increased extent of release. Complexation influenced release in the absence of Rev7 polymer.

For gums containing 8 % Rev7, there is a marked decrease in hardness for L,8,K and C,8,K (irrespective of drug form) both formulations contained potassium carbonate. There was also no significant change in the extent of drug release from L,8,K and C,8,K which showed that the form of drug did not affect the extent of release in the presence of Rev7 polymer.

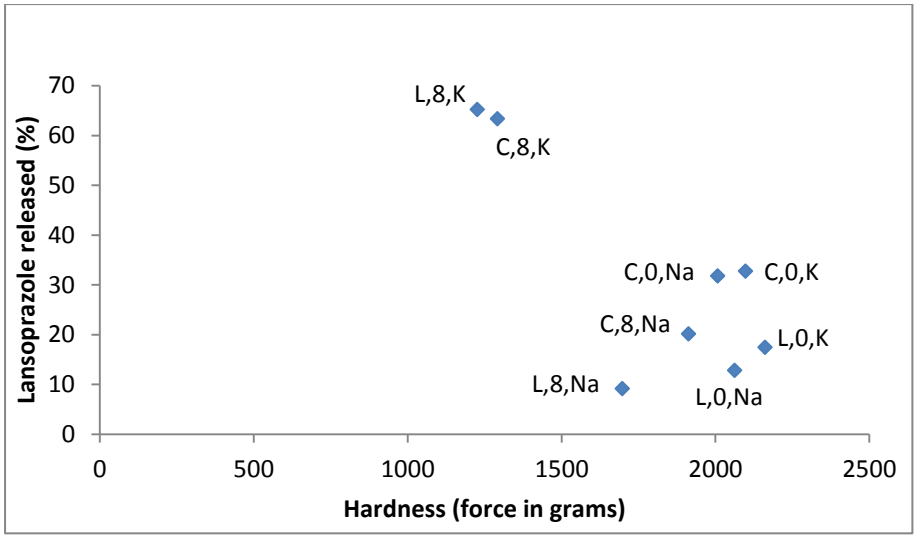


Figure 4.35 Correlations between gum hardness and release in artificial saliva pH 8.0 (20 mL replacement)

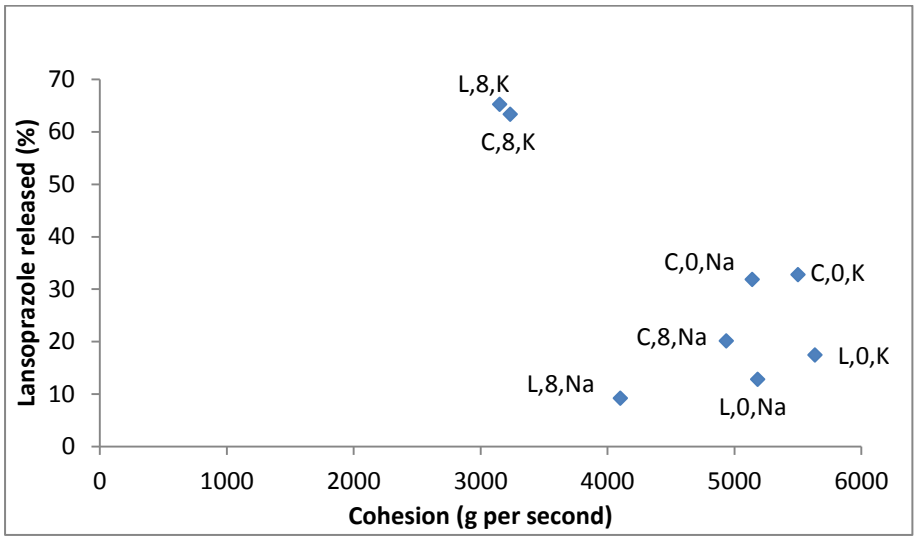


Figure 4.36 Correlations between gum cohesion and release in artificial saliva pH 8.0 (20 mL replacement)

The less cohesive and softer gums (L,8,K and C,8,K) consistently resulted in the highest release of drug during *in vitro* mastication (Figures 4.35 and 4.36). ANOVA statistical analysis found significant differences in hardness and cohesion between 8 % and 0 % Rev7 polymer ($P < 0.01$) (see Appendix B). Cohesion can be described as an attraction process that occurs between similar molecules as a result of chemical bonds formed between components in the gum. These are interactions that bind the gum mass together (Fraunhofer, 2012). Cohesive gums will therefore release the drug more slowly. Significant differences in hardness were found between L,8,K and C,8,K which were significantly different from all other gums ($P < 0.01$) but not from each other. This suggested that potassium carbonate had a

plasticising effect on the gum base (elastomer) to modify the texture (physical interaction), resulting in softer gums, which can be directly related to release via increased renewable surfaces during mastication. Potineni (2008) suggested a specific plasticising effect is needed on the polymeric phase (PVA) of the gum base to increase the release of flavourings and sugar alcohols from chewing gums (Potineni and Peterson, 2008). The texture of nicotine gums was observed to change producing a softer gum with increasing buffering excipient content (sodium carbonate), this was also shown to be proportional to nicotine release. This was related to the increase of micro pores in the gum matrix, formed after dissolving of the buffer upon contact of saliva, leaving channels for subsequent drug release (Morjaria, 2004).

4.5 General discussion

Studying the *in vitro* release profiles of a range of gum formulations allowed discriminatory evaluations to be made to optimise the developmental phases of a chewing gum containing a poorly soluble drug, lansoprazole.

Various method developments and chew related factors were assessed to represent the range of inter-individual variability seen within *in vivo* studies. This resulted in a range of discriminating factors and illustrated the importance of *in vitro* assessment using a range of experimental conditions. The extent of release was initially limited using 40 mL of phosphate buffer at pH 6.0 (standard conditions- 60 chews / min, 37 °C and 2 mL sampling and replacement volumes). Artificial saliva (pH 6.7) increased release for all formulations and was a more discriminating medium, significant differences were found between the two dissolution mediums ($P < 0.01$). One of the limitations of the masticator was the ability to remove saturated dissolution medium to replicate *in vivo* conditions resulting from swallowing or absorption of the active and maintaining sink conditions (particularly important when assessing poorly soluble drug actives). This was overcome by increasing sampling and replacement volumes which maintained sink conditions and ensured that solubility would not limit release. This would also have affected the local pH. However it was accepted that the buffering excipient would be released along with the drug and so the buffering capacity would be maintained. This resulted in the increased extent of release from L,8,K to a maximum of 54.1 % after 30 minutes. It was then decided to increase the pH of the artificial saliva from pH 6.7 to pH 8.0, as this was previously shown to increase drug release.

This further increased drug release from L,8,K to 65.24% after 30 minutes. The last parameter evaluated was increasing the chew rate from 60 to 83 chews *per* minute. This had a limited effect on increasing drug release on L,8,K with a maximum release of 67.46 % after 30 minutes.

There were clear differences in release following inclusion of Rev7 and potassium carbonate when compared to all formulations and release parameters tested ($P < 0.05$) (see Appendix B). Rev7 is known to increase the hydrophilic capacity of the chewing gum base (Farber *et al.*, 2009) and this is why it increased release due to increasing the contact of dissolution medium to the gum.

Surface diffusion studies from formulated gums (containing 41 % w/w gum base) highlighted the limited release of active from surfaces and the need for mastication. However L,8,K and C,8,K (both containing potassium carbonate) partially disintegrated after 60 minutes whilst all other formulations remained intact under the same conditions. This may have been due to the potassium carbonate near surface domains reacting to the dissolution medium. These differences may have been due to potassium carbonate leading to an increased extent of micropores formation in the gum matrix and this was further explored using SEM. The increased release mechanism would involve increased water permeation with penetration/ingress of dissolution medium into the gum matrix; resulting in a greater extent of drug release *via* leaching. This would be in agreement with Wei who suggested mechanisms relating to differences in porosity with regards to explaining differences in release rates using different alkaline stabilisers in lansoprazole pellet formulations (Wei *et al*,. 2010). The study by He also suggests mechanisms relating to increases in porosity and interactions between the drug causing disordering and resulting in an amorphous state of lansoprazole in pellet formulations (He *et al*., 2010). This was further assessed with four different alkaline stabilizers and concluded that the inclusion of sodium carbonate produced the fastest release rate due to increased micropore formation which facilitated diffusion in lansoprazole pellet formulations (He *et al*., 2011).

Gum texture will also affect release. Softer gums will require less force to penetrate and renew surface areas compared to harder gums which will have a reduced renewal of surface area at the same force. Texture analysis showed that L,8,K and C,8,K were significantly softer than all other gums tested and had less cohesion. Potassium carbonate in the presence of Rev7 polymer had a plasticising effect (physical interactions) within the gum base. There

was no significant difference in the extent of drug release from L,8,K and C,8,K which showed that the form of drug did not affect the extent of release. Also as potassium carbonate has a 15 fold increase in solubility (compared to sodium carbonate), the number and formation of channels will be of a greater extent and at a faster rate upon contact with saliva, which will directly influence release of active from the gum. In addition to micropore formation the increased hydrophilicity and wettability of potassium carbonate will dissolve the gums' hydrophilic portions at a greater rate which will then diffuse out into the exterior bulk medium in a rapid burst release. This is in agreement with the study by Shiffman which found that buffering excipients can be used to effectively control nicotine release rates from medicated gums with potassium carbonate resulting in the most rapid release rate in the initial phases of mastication (Shiffman *et al*., 2009).

Differences regarding chew related parameters were observed when comparing formulations containing Rev7 and without Rev7. Formulations containing Rev7 could be split into two groups (pairs) which were influenced by the type of buffering excipients, with potassium carbonate having an increased release compared to sodium carbonate. This may have been a result of the increased solubility of potassium carbonate compared to sodium carbonate resulting in an increased rate of release of the buffer along with the active. The concept of the influence of buffering excipients has been discussed previously but many studies show mixed opinions on the effect caused (Rassing, 1996; Cherukuri *et al*., 2002; Shiffman *et al*., 2009). Formulations without Rev7 were greatly influenced by the form of drug, with complexation increasing release. The effects of buffering excipients were less apparent compared to the significant differences observed with formulations containing Rev7. C,0,K and C,0,Na had an increased extent of release when 20 mL sampling and replacement volumes were used ensuring no limits in solubility and sink conditions. L,0,Na and L,0,K (containing drug alone) doubled in the extent of release when tested at the increased chew rate compared to C,0,K and C,0,Na.

L,8,K and C,8,K consistently released more drug over the duration of the studies. Both gums had similar releases showing that the form of the drug (complexed and drug alone) had less influence than the buffering excipient when used in the presence of Rev7. C,0,Na had the 3rd greatest extent of release, which was interesting as it had a greater extent of release compared to gums containing Rev7 and sodium carbonate. This showed that complexation did have an

effect on increasing release in the absence of Rev7. This could be due to the increased solubility that was observed with complexed lansoprazole.

The faster chew rate increased release from all gums, (except C,8,K) suggesting that the maximum extent of drug release during mastication may have been reached. The choice of dissolution medium is an important factor to consider as all gums tested with phosphate buffer at pH 6.0 (other than L,8,K and C,8,K) had similar release after 30 minutes. Differences in formulations were only highlighted when using artificial saliva at pH 6.7.

Minimum variations were seen amongst each gum formulation tested in triplicate with standard deviations of 1.76 % over all parameters tested, showing a high degree of standardisation and reproducibility. There were only 5 datasets with standard deviations greater than 3.0 % over all the tests. Chewing gum sticking to the surfaces of the piston can introduce some variation, affecting the uniform forces exerted on the gums during chewing and subsequent release. Another interesting observation was found with L,8,Na; this formulation consistently had the lowest release of all the formulations tested at each parameter. This may have been due to specific interactions with the drug and other excipients and this would need to be explored further. It was decided that the application of release kinetics was unsuitable due to the constantly changing shape of the gum cud during chewing.

4.5.1 Conclusion

Various chew related factors were assessed on the EP approved masticator representing a range of inter individual variability seen within *in vivo* studies. This resulted in a range of discriminating factors and illustrated the importance of assessing *in vitro* release using a range of experimental conditions. This allowed an increased understanding of release dynamics and influential contributing factors which can be used in product development before *in vivo* testing. The complexity of release mechanisms are still not fully understood and will require more work to increase understanding from chewing gum formulations.

This work contributes to the limited information available on release of poorly soluble drugs from medicated chewing gum formulations and focussed on identifying factors governing the release of a poorly soluble active (lansoprazole) from gum formulations. Various chewing gum formulations were considered and the effect on the extent of *in vitro* release was

observed. Significant differences in release after 30 minutes *in vitro* mastication were found in gums containing Rev7 and potassium carbonate (both of which contributed to increasing the hydrophilic capacity/domains of the gum). Significant differences were also observed in hardness in gums containing Rev7 and potassium carbonate due to physical interactions causing a plasticising effect on the gum base resulting in softer, less cohesive gums which was correlated with increased release rates from these gum formulations. Therefore in the case of formulation optimisation, I would recommend 8 % Rev7 and potassium carbonate to achieve optimum release.

A combination of mechanism relating to gums containing Rev7 and potassium carbonate (L,8,K and C,8,K) were proposed including textural changes due to physical interactions in the gum base, increasing the hydrophilic capacity of the gum and the increased solubility of the buffering excipient (leading to an increase in the rate and extent of micro pore formation in the gum matrix after dissolving of the buffer upon contact of saliva) all of which contributed to increasing the release of the poorly soluble drug lansoprazole from chewing gum formulations.

Chapter Five:

Diffusion through buccal mucosa

5.1 Drug delivery *via* oral mucosae

Oral drug delivery is the preferred route for drug delivery by both patients and clinicians if appropriate due to increased compliance, ease of administration and low costs. The key parameters of the biopharmaceutics classification system (BCS) controlling oral absorption are solubility/dissolution and permeability. Within the oral cavity, systemic delivery (Figure 5.1) can be classified into two main categories based on site of delivery, sublingual and buccal.

The sublingual route involves absorption into the systemic circulation through the lining of the floor of the mouth. Although the membrane is relatively more permeable than the buccal mucosa, it is washed constantly by saliva (Table 5.1), limiting the dosage formulations to short delivery periods with highly permeable drugs, e.g. sublingual sprays.

Buccal delivery describes administration through the mucosal lining of the lateral walls of the cheeks. It is readily accessible and has fast cellular recovery. The rich blood supply ensures rapid absorption and bypasses the first pass effect. It is also suitable for sustained release formulations and is well supplied with lymphatic drainage and vascular blood supply. In general, oral mucosal permeability is in the following order sublingual > buccal > palatal based on relative thickness of the tissue and degree of keratinisation (Shojaei, 1998 and Sohi *et al*., 2010).

In this project, the main anticipated route of drug absorption is buccal delivery through the oral mucosae once the drug is released from the medicated chewing gum into saliva.

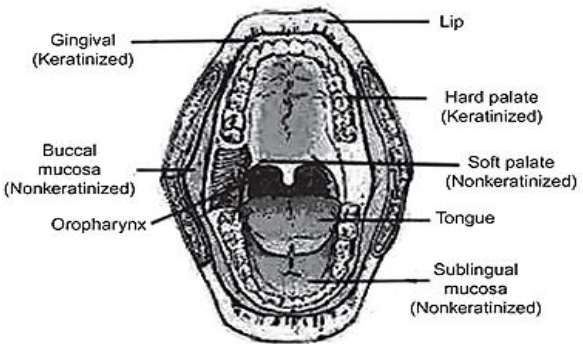


Figure 5.1 The oral cavity

(Sohi *et al*., 2010)

5.1.1 Buccal absorption and chewing gum formulations

Buccal absorption allows advantageous effects seen with medicated chewing gum formulations including faster onset of action compared to the normal administration of a tablet by the oral route. As the buccal mucosa has to be resistant to tissue damage by the frequent exposure to food materials, there is a relatively high rate of cell turnover when compared to skin, thus allowing for faster recovery (Shojaei, 1998).

A precondition for oral absorption is that the drug is in solution prior to absorption therefore low aqueous solubility can limit bioavailability. The release of active during the action of chewing provides the sustained release to facilitate delivery through the buccal membrane. Once the active is in the oral environment it will have good accessibility to the oral mucosa which has a total area of 200 cm^2 and to the epithelium of the buccal mucosa which has an area of 50 cm^2 (Kokate *et al*., 2009 and Sohi *et al*., 2010).

Nicotine replacement gum is indicated for regular use to reduce overall withdrawal symptoms and cravings for nicotine. The 'parking strategy' recommended by the manufacturers of Nicorette® (GlaxoSmithKline Consumer Healthcare) involves chewing the gum until the desired release of nicotine and flavouring is achieved and then a time of (parking) pressing the gum to the side of the cheek to aid absorption until the flavour declines. This is followed by continual cycles of chewing and parking until the craving relief effects are achieved. This strategy facilitates improved permeability by placing the gum in close proximity to the tissue at times when the active is readily available at a high local concentration to ensure maximum absorption.

Factors controlling the rate and extent of drug permeability are highly complex. They can be affected by physiological factors (salivary stimulus- flow rate), physiochemical factors (salivary composition- pH) and dosage forms (buccal adhesion formulations). They can also be influenced by the properties of the barrier as well as specific drug-related properties. The main limitations in buccal delivery are the uncontrolled salivary flow and subsequent swallowing, 'wash out effect', and also the relatively small surface area of the oral mucosae (Patel *et al*., 2012).

5.2 The buccal mucosa

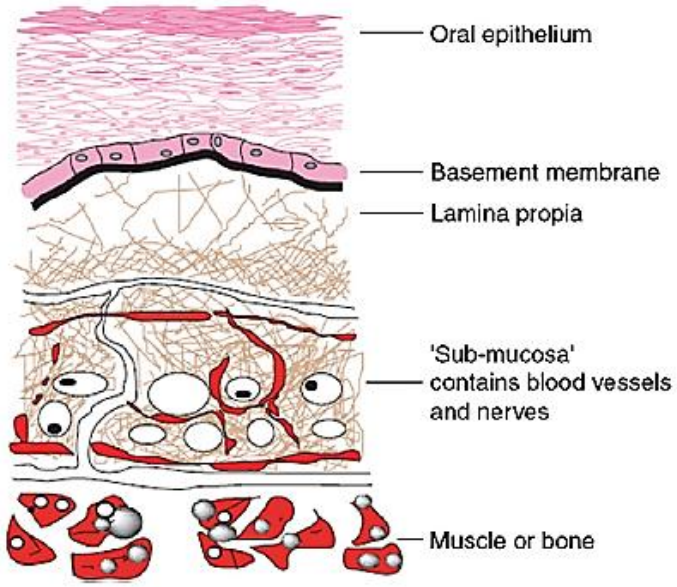


Figure 5.2 Cross section of the buccal mucosa

(Patel *et al*., 2011)

The buccal mucosa (Figure 5.2) is composed of 3 main sections:

- Oral epithelium

This acts as the penetration barrier and is located in the upper third of the membrane (Patel *et al*., 2011). It is a relatively thick (250 – 280 µm), non-keratinised, stratified and squamous epithelium. It has three defining layers: basal layer, an intermediate layer and a superficial layer (Kulkarni *et al*., 2010). It consists of polar lipids such as cholesterol sulphate and glucosyl ceramides (Shojaei, 1998).

- Lamina propia

It provides mechanical support and consists of underlying connective tissue (similar to the dermis of skin) and is separated from the epithelium by a basement membrane (Sohi *et al*., 2010).

- Sub-mucosa

The sub-mucosa is attached to underlying muscle and contains minor salivary glands. This section also contains the extensive capillary network (Kokate *et al*., 2009).

5.2.1 The oral environment

The physiological environment in the oral cavity is based on salivary composition, volume and pH (Table 5.1). Saliva is secreted by the major salivary glands (parotid and sub-maxillary provide the watery secretions, whereas sublingual produce viscous secretions) and minor salivary glands. It is the main protective fluid/lubricator reducing abrasion by food and chemicals and allows for remineralisation of tooth enamel. Saliva comprises 99 % water and 1 % organic and inorganic materials such as potassium bicarbonate and calcium carbonate (Patel *et al*., 2011). Increased salivary flow will facilitate drug dissolution but this can lead to a decrease in permeability due to involuntary swallowing 'wash out' causing drug loss (Sohi *et al*., 2010).

Epithelial cells in the oral environment are surrounded by mucus, which may be free or attached to the cell surface (Figure 5.3). Mucus can act as an additional physical barrier with a thickness of 40-300 μm (Patel *et al*., 2011). Mucus is an intercellular ground substance consisting of proteins and carbohydrates; the key glycoprotein is mucin (1 - 5%), although most of mucus is water (90 - 95 %). This matrix acts as a lubricant as well as playing a role in cell-cell adhesion.

Table 5.1 Physiological conditions of the human oral cavity

Parameters	Physiological range
pH of saliva	5.5 to 7.0
Volume of saliva (continuous available volume)	696 ±312 μL
Daily total secretion	0.5 – 2 L
Viscosity of saliva	1.09 ±0.11 (m Pa.s)
Protein in saliva	0.70 ± 0.30 (mg/mL)
Amylase in saliva	325 ± 199 (IU/mL)

(adapted from Patel *et al*., 2012)

5.3 Transport pathways across the oral mucosa

The two main passive transport permeation pathways across the oral mucosae are the paracellular and transcellular routes (Figure 5.3). Permeants will use the route with least hindrance dependent on their physiochemical properties and drugs can permeate by the lipoidal or aqueous pathway. The intercellular space is filled with 50 % polar lipids and the aqueous pathway involves transport through the intercellular spaces and cytoplasm. The lipoidal pathway involves transcellular transport through intercellular lipids by partitioning through the cell membrane with intracellular spaces posing as the major barrier. Generally routes can usually contain a combination of the two due to the stratified structure of the membrane (Kokate *et al*., 2009).

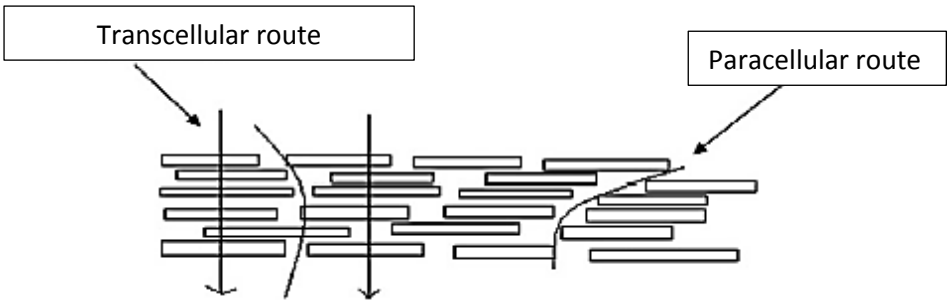


Figure 5.3 Possible routes of drug transport

(Patel *et al*., 2011)

5.3.1 General drug diffusion across a membrane

Drug movement occurs mainly via passive diffusion and this can be described as the random movement of molecules from a high concentration to a low concentration (down a concentration gradient) until an equilibrium is reached. Typically a lag time is initially observed (non-steady state) followed by a linear phase representing a balance between exit and entry of the drug (steady state) through the membrane. As the drug will be dissolved in saliva upon release from the gum, it will be readily available for absorption. The drug needs to be released at a rate and extent to facilitate buccal absorption and minimise any 'wash out' effects. The release of active from chewing gums is described in detail in chapter 4.

The initial stages of drug absorption involve partitioning and can be estimated by the partition coefficient (K or Log P value).

The partition coefficient is a measure of the drug's intrinsic lipophilicity and is taken as the ratio of the drug partitioning in the lipophilic phase (oil phase) from the aqueous phase. The standardised shake flask method (K_{ow}) uses octanol/water as the two separate phases and is performed at a neutral pH. It is inversely proportional to molecular weight and aqueous solubility.

Drug movement can be described via the use of equations:

The simplified model of movement through a membrane following passive diffusion can be described by Fick's first law of diffusion (Eq. 5.1):

$$J = \frac{\Delta m}{At}$$

Equation 5.1 Drug flux

Where,

Δm = change in mass

A = area

t = time

This can be defined as the change in mass of an entity moving through a given cross-sectional area during a given period of time.

The steady state flux is achieved when the movement (entry/exit) of the drug is equal and this can be manipulated as follows (Eq. 5.2):

$$J_{SS} = \frac{D \times K}{h} C_D$$

Equation 5.2 Steady state flux (J_{ss})

Where,

J_{ss} = steady state flux

C_D = concentration at donor site

D = diffusion co efficient

K = partition coefficient

h = path length (thickness)

The equation shows that drug flux is proportional to the concentration gradient. Diffusion will occur in the direction of the lowest concentration down a concentration gradient. Therefore the flux will correspond to the slope of the steady state diffusion curve and will be a positive value as the movement will occur down a concentration gradient. The drug flux is described as a rate and the units are in (µg.cm^2/minute).

The steady state Flux (Eq.5.2) can be further manipulated to provide the permeability coefficient (K_p) (Eq.5.3). The units are in cm/minute, this eliminates the need to measure difficult parameters such as the diffusion coefficient:

$$k_p = \frac{J_{ss}}{C_d}$$

Equation 5.3 Permeability coefficient (K_p)

Where,

J_{ss} = steady state flux

C_d = initial concentration from donor compartment

However various limitations have been identified including the exclusion of ionised drugs as the equation only considers the partition coefficient (Log P) and molecular weight. Due to the increased polar lipid composition in the buccal mucosa, incorporation of Log D has been shown to be a better correlation, compared to Log P, as it considers lipophilicity and ionisation (Kokate *et al*., 2009). The distribution/diffusion co efficient (Log D) is another measure of intrinsic lipophilicity, it also considers the extent of ionisation of the drug and is considered to be a better descriptor of partioning and lipophilicity at a given pH (Kokate *et al*., 2008).

$$J_T = (1-\varepsilon)\frac{D_T \times K_T \times C_D}{h_T}$$

Equation 5.4 Transcellular drug flux

Where,

ε = fraction of surface area

D_T = diffusion coefficient in the intracellular spaces

K_T = partition coefficient between the lipophilic regions and hydrophilic regions

C_D = drug concentration at donor site

h_T = path length (Sohi *et al*., 2010)

Equation 5.4 shows that drug flux is directly proportional to the partition coefficient and diffusion coefficient and inversely proportional to the path length/thickness. It also recognises that drug movement is through a series of lipophilic and hydrophilic regions using partitioning and diffusion to transverse through the stratified cell membrane and intercellular spaces and cytoplasm.

5.3.2 Factors controlling drug diffusion: barrier properties

The physiochemical properties of the barrier as well as its thickness will influence permeability. It is generally accepted for most drugs that movement is by passive diffusion and does not include active processes.

Specialised carrier mediated transport mechanisms in the oral mucosae have been reported for nutrients and some drugs (Patel *et al*., 2011). This includes the stereo specific transport of D-glucose which has a saturation limit, not normally characteristic of passive diffusion (Sohi *et al*., 2010). The review outlined by Sohi also describes the active uptake of anti-bacterial agent (minocycline) after 8 to 40 fold higher intra cellular levels were found compared to extracellular levels (Sohi *et al*., 2010). Other carrier mediated processes in the human buccal cavity include sodium ion dependent active transport mechanisms for the transport of L–ascorbic acid (Sadoogh-Abasian and Evered, 1979).

The rate limiting permeability barrier in skin is located in the intra cellular regions of the stratum corneum, whereas the rate limiting mucosal barrier in oral mucosae is known to exist in the oral epithelium (Figure 5.2) (Kulkarni *et al*., 2010). Skin is regarded as less permeable than oral mucosae and sublingual regions (floor of the mouth) are significantly more permeable than all other regions (Kulkarni *et al*., 2010). The buccal mucosa is 4 – 4000 times

more permeable than skin (Shojaei, 1998 and Kokate *et al.*, 2009). The wide range reflects the regional differences in permeability throughout the oral cavity.

5.3.2.1 Keratinised and non-keratinised regions in the oral cavity

The superficial epithelial cells in the oral cavity can be divided into two types (keratinised and non-keratinised) dependent on the function of the area (Figure 5.1). Keratinised regions (such as the masticatory mucosa, gingival and hard palate) contribute to harder surfaces in areas which undergo masticatory stress and this contributes to higher resilience to the abrasion. This layer can act as a major penetration barrier to some drugs (Sohi *et al*., 2010). It is categorised with a thick lamina propia and the mucosa is tightly bound to the underlying periosteum. Non keratinised regions such as the buccal mucosa, sit on a relatively thin and elastic lamina propia and sub mucosa (Patel *et al*., 2011).

Isotopic labelling was used to assess morphological variations in thickness and permeability of keratinised and non-keratinised regions of porcine oral mucosae. The results showed that the buccal mucosae had a significantly thicker total epithelium (772 ± 20 μm) compared to skin and other oral regions (gingival, sublingual). The specific rate limiting permeability barrier was contained in the upper third portion of the epithelium (282 ± 17 μm). The main difference in permeability between the skin and oral mucosae was considered to be due to hydration levels, with buccal regions being bathed in fluids (saliva) compared to skin (Squier and Hall, 1985). Similar regions containing the rate limiting permeability barrier have been reported in the upper portion (250 – 280 μm) of the epithelium of the buccal mucosa (Kokate *et al*., 2009 and Kulkarni *et al*., 2010).

5.3.2.2 Regional permeability differences in the oral cavity

Permeability differences within oral mucosae can be attributed to the intercellular materials, derived from membrane coating granules (MCGs) between tissues (Patel *et al*., 2011). MCGs are located in intermediate cells and are formed when cells differentiate (Sohi *et al*., 2010). During formation they fuse and discharge their contents into intercellular spaces. These MCGs extrude glycolipids and lipids that control the cohesion of the epithelium cells (Kokate *et al*., 2009). This discharge forms a barrier permeability barrier in both keratinised and non-keratinised tissues. The penetration of tracer molecules was shown to coincide with high levels of MCGs (volume and density) adjacent to the plasma membranes of the epithelium

cells (Shojaei, 1998). In the oral cavity, MCGs are thought to play a more significant role in controlling permeability compared to keratinisation (Patel *et al*., 2011).

MCGs are more abundant in skin epidermis compared to oral epithelium. It has also been observed that different types of MCGs exist with those in keratinised epithelium comprising a series of parallel lamellae, similar to MCGS in skin epidermis. Non-keratinised oral regions contain MCGs with a different morphology, consisting of an amorphous core enclosed by a membrane correlating with differences in patterns of glycolipids such as cholesterol esters (Squier and Hall, 1985 and Shojaei, 1998).

5.3.3 Factors controlling drug diffusion: drug related properties

Drug absorption involves a combination of events and factors, the drug must first partition into the lipid bilayer (cell membrane) and then traverse into the interior of the cells including cytoplasm and intercellular spaces (hydrophilic barrier). The final hurdle is an enzymatic barrier prior to reaching the mucosa. The buccal regions have less enzymatic activity (proteolytic) and enzymes present (including dehydrogenases, esterases, aminopeptidases and carboxypeptidases) compared to the GI tract, however more research is required to fully characterise all the different levels and types of enzymes present in the human buccal mucosae (Patel *et al*., 2011).

The drugs physiochemical properties will influence its absorption pathway as follows:

- Size has an inverse relationship with permeability and increasing molecular size inhibits absorption. Generally molecular weights of less than 500 Daltons are preferred.
- Ionisation/charge, permeation is favoured by non-ionised drugs through the transcellular route due to the increase in lipid solubility and this follows the pH-partitioning theory characteristic of passive diffusion. The degree of ionisation depends on the drug's pK_a and the pH of the environment.
- Lipophilicity, the drugs partition coefficient, dictates the route taken, with a highly lipophilic drug traversing through the transcellular pathway. However cytoplasm and intercellular spaces will pose a major barrier for highly lipophilic drugs; so a partition coefficient of less than 5 is recommended (Kokate *et al*., 2009).

5.3.3.1 Diffusion related properties of lansoprazole

Upon release and partitioning from the chewing gum formulation, during chewing, the active must dissolve in saliva, before partitioning into the membrane. The next stage will involve a series of traversing, partitioning and diffusing through the membrane components before absorption into systemic circulation (see chapter 1, Figure 1.4).

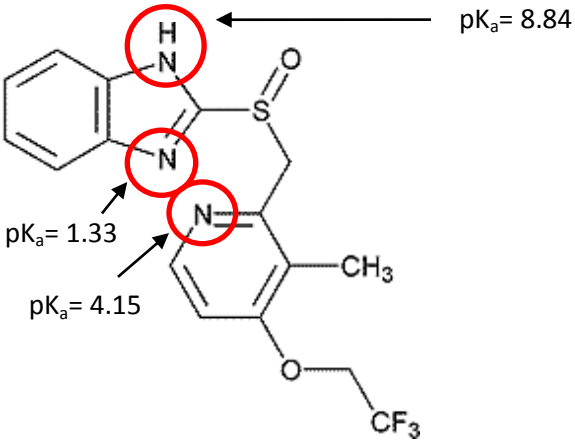


Figure 5.4 Sites of protonation for lansoprazole

The factors that will contribute towards permeability for lansoprazole are lipophilicity and solubility. The drug solubility and stability is pH dependent and degradation occurs at acidic pHs (see section 3.2.2). Lansoprazole has 3 pK_as; 1.33 and 4.15 both of which will be un-ionised at physiological pHs in the oral cavity (Table 1.4 and section 3.1.2). The pK_a of 8.84 (acidic-protonated nitrogen atom N-1 on the benzimidazole ring) will be subject to protonation at physiological pHs (Figure 5.4). The pH partitioning theory by Henderson-Hasselbach (H-H) predicts the extent of ionisation and charge as a function of pH and fraction of dissociation and can be described by Eq. 5.5:

For weak acids;

$$pH = pKa + \log([A-]/[HA])$$

For weak bases;

$$pH = pKa + \log([B]/[BH+])$$

Equation 5.5 pH partitioning theory by Henderson-Hasselbach

At pH 6.8, 1 % of lansoprazole will be ionised and at pH 8.6, 40 % will be ionised. This will impact the absorption as permeation is favoured by non-ionised drugs.

5.4 Penetration enhancers in buccal delivery

The buccal transmembrane route is highly desirable due to the by-passing of first pass metabolism and capacity for increased bioavailability and pharmacological activity. The barrier function of the mucosa limits the penetration and slows the diffusion of drugs; however this can be overcome by enhancers. A clinical accepted enhancer (chemical or physical) must have reproducible actions, minimise membrane irritation, damage and toxicity as well as being able to significantly enhance permeability. It should also allow the membrane to return back to its original properties to maintain barrier functioning and integrity.

Some enhancers can interact with the membrane increasing the fluidisation of the membrane Examples include surfactants such as sodium dodecyl sulphate (SDS), which acts on the lipid components of the membrane to increase fluidisation and bile salts, such as sodium cholate, which can reduce barrier functioning by extracting lipids from the mucosal surface. The effects can be concentration dependent (below critical micelle concentration) but are specific to the different pathways for each permeant used. Fatty acids such as lauric acid and alcohols such as ethanol can disrupt intercellular lipid organisation and packing.

Other enhancers may interact with the drug and dosage form to modulate increases in partitioning (K), diffusivity (D) and concentrations at the mucosal surface (donor) of the drug which will lead to increased permeability. Propylene glycol (PG) has the ability to act as a drug vehicle and permeates across the membrane whilst carrying the drug (Santos *et al*., 2009).

5.4.1 Cyclodextrins as penetration enhancers

Cyclodextrins (CDs) have been reported to increase penetration and also the enhance stability of actives (see section 3.4.6). Studies involve increasing absorption and drug bioavailability of hydrophobic drugs by enhancing the drug's solubility in the complexed form (Davis and

Brewster, 2004 and Brewster and Loftsson, 2007). A drug that is poorly soluble in saliva will have a lower concentration gradient compared to a drug-cyclodextrin complex with a corresponding higher solubility; this will facilitate absorption. Complexation with CDs (β and γ- CD) enhanced sublingual bioavailability of testosterone, the rapid dissolution and reversibility of complex formation allowed efficient absorption. Both CDs improved dissolution with the γ- CD being superior with a 9 fold increase in solubility compared to testosterone alone (Pitha *et al*., 1987).

Cyclodextrins have been proposed as penetration enhancers for omeprazole delivery *via* the buccal mucosa. Methylated β-cyclodextrin (Mβ- CD) was found to be more effective with a 1.7 fold increase in permeation compared to a 1.1 fold with β-cyclodextrin (Figueiras *et al*., 2009). The increased solubility observed with Mβ- CD complexation was suggested to contribute to increasing the drug flux due to super-saturation of the drug in the solution (Santos *et al*., 2009). The presence of L-arginine with beta-cyclodextrin further increased the solubility and stability of omeprazole, although an increase in solubility was not observed with the methylated derivative. It was suggested that L-arginine forced a significant desolvation of omeprazole by establishing hydrogen bonds with the drug molecules (Figueiras *et al*., 2010).

The formation of inclusion complexes can increase the solubility of drugs (Rajewski and Stella, 1996 and Sohi *et al*., 2010). Complex formation is dependent on the drug properties with non-covalent hydrogen bonding and Van de Waals forces forming the inclusion complex. The specific cavity size of the cyclodextrin and the free drug fraction will influence the increase in apparent solubility of the drug and hence the bioavailability (Rajewski and Stella, 1996).

Cyclodextrins can increase permeability due to modification of the mucosal membrane (Carrier *et al*., 2007); free cyclodextrin may disrupt membrane components to increase the transport properties and facilitate absorption thorough the membrane (see section 3.4.6). It has been suggested that cyclodextrins can fluidise the membrane by solubilising specific lipid components of the membrane and was the mechanism for enhancement of absorption of testosterone across the sublingual membrane (Rajewski and Stella, 1996).

Other benefits include increased stability and protection of the drug in the inclusion complex; which could protect the drug from mucosal metabolic barrier (proteases and peptidases) during penetration or enzymatic degradation in the presence of saliva.

5.5 Determination of buccal absorption of drugs

The bioavailability of drugs following buccal delivery can be studied using a variety of methods:

5.5.1 *In vivo* human testing

The most common method for *in vivo* human studies is the buccal absorption test, which involves swirling a sample in the mouth for 15 minutes. The absorbed content is calculated from the missing fraction after expulsion. Some limitations include the inability to specify the absorption site accurately, individual variability, accidental swallowing and dilution with saliva. However analysis of plasma levels after the test can provide a direct indication of bioavailability. Other examples involve drug circulated perfusion chambers which can be attached directly to the site of interest (Kokate *et al*., 2009).

5.5.2 *In vitro* testing

Permeability models can determine the barrier nature of biological tissues in a controlled environment to predict absorption. There are two main types of static permeability apparatus: the horizontal and the vertical set up. The permeable membrane is sandwiched between two cells, the donor and the receiver/receptor compartments with the sampling port (Figure 5.5). The donor cell contains the saturated drug, whereas the receptor cell represents systemic circulation after absorption of the drug.

Both apparatus offer a controlled environment and can be used for prediction of permeability parameters on isolated tissues, including the actual amount of drug diffused and also the rate of drug diffusion (Patel *et al*., 2012). Advantages of *in vitro* testing include the reduced costs compared to *in vivo.* Usually only a small amount of tissue is needed due to the small surface areas exposed for penetration. Also relevant factors can be explored including the effects of pH on permeability. Disadvantages include the time consuming methods and possible variability between replicates. A large number of samples may be required to overcome any variations and to provide an accurate estimation of transmembrane flux.

The isolated tissue placed in the *in vitro* set up should be as fresh as possible to maintain integrity and viability. The Ussing chamber has a system which circulates carbogen gas (95 % O_2 and 5% CO_2) to maintain tissue viability and voltage.

Benefits of the horizontal system include the ability to stir both chambers to ensure limited unstirred water layers. Both compartments are the same size and shape and a large volume of medium is needed to cover the membrane completely on both sides of the cells.

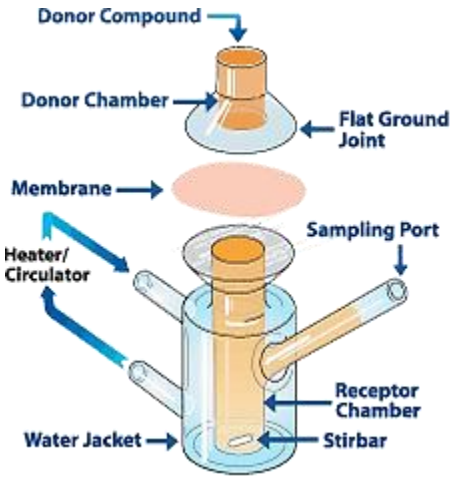


Figure 5.5 Vertical Franz cell apparatus

(http://www.permegear.com/franz.htm)

A specific advantage is the effect of gravity due to the apparatus being in a vertical position however a limitation can arise with the formation of an unstirred water layer in the donor compartment. The effects of this unstirred water layer involve interactions with the buccal interface and this may result in an additional barrier for penetration or act as a concentrated drug depot type effect to increase movement *via* increased concentration gradient (Loftsson and Brewster, 1996). To limit this effect an effective stirring rate is required which will be specific to the set up apparatus.

The EpiOral™ is a human cell culture model and has shown good biochemical and histological correlations with human buccal epithelium. The tissue model consists of cultured human derived buccal epithelial cells forming a differentiated 3D multi layered model with an organised basal cell layer (Agarwal *et al*., 2005). The tissue has relevant protein expressions, lipid content and structure (including cytokeratin's and anti-microbial peptides) (Walle *et al*., 2006). The tissue has high reproducibility and provides an easy to handle effective alternative to animal tissue.

A vertical type Franz cell was used in this work with a diffusional surface area of 2.77cm^2, a donor volume of 10 mL and a receptor volume of 30 mL to maintain sink conditions. The maximum agitation or stirring rate of the receptor compartment was set at 450 rpm to minimise boundary layer effects.

5.5.2.1 Selection of buccal membrane

The use of animal buccal epithelium as a model for predicting transbuccal absorption in humans is acceptable due to similarity in lipid composition, thickness and degree of keratinisation (Patel *et al*., 2012).

Table 5.2 Buccal mucosae comparisons from different species of mammals

Models	**Tissue structure**	**Buccal membrane thickness (µm) (mean ± s.d)**	**Permeability constant for tritiated water (×10^7 cm/min) (mean ± s.d)**
Human	Non-keratinised	580 ± 90	579 ± 122
Dog	Non-keratinised	126 ± 20	1045 ± 37
Hamster	Keratinised	115.3 ± 11.5	Not available
Pig	Non-keratinised	772 ± 150	634 ± 60
Rabbit	Partially keratinised	600	Not available
Rat	Keratinised	Not available	Not available
Monkey	Non-keratinised	271 ± 50	1025 ± 154

(adapted from Patel *et al*., 2012)

The biological differences in tissues can result in unreliability with regards to permeability predictions in humans and careful consideration should be given to tissue selection from animal species to ensure integrity and viability of data. The buccal tissue of pigs, dogs and monkeys has been used extensively due to its non-keratinised nature, although the tissue from dogs and monkeys is thinner and therefore presents a reduced diffusion path length (Patel *et al.*, 2012) (Table 5.2).

Table 5.3 Human and porcine buccal constituents

Lipid type	Pig (% w/w)	Human (% w/w)
Cholesterol	2.04	23.7
Cholesterol esters	0.41	7.4
Cholesteryl sulfate	0.61	---
Fatty acids	1.08	9.2
Ceramides	0.08	0.4
Glycosylceramides	1.59	13.8
Triglyceride	0.47	----
Sphingomyelin	1.63	7.7
Phosphatidylcholine	1.75	18.4
Phosphatidylserine	1.13	0.8
Phosphatidylinositol	0.6	1.8
Phosphatidylethanolamine	1.66	16.8
Phospholipids (total)	13	45.5

(

adapted from Patel *et al*., 2012)

Porcine tissue resembles human buccal mucosa in terms of structure and composition (Table 5.3). Similar permeability constants were found between pigs and humans due to the non-keratinised nature and similar thicknesses of the tissues (Table 5.2). Porcine buccal tissue is the most frequently used animal model and has been extensively used for *in vitro* experiments due to the low cost and accessibility (Kokate *et al*., 2009; Patel *et al*., 2012).

5.6 Method

5.6.1 Preparation and dissection of porcine tissue

Fresh porcine tissue (Hampshire cross large white, weighing between 90 – 100 kg being approximately 6 months old) was obtained from the abattoir (L Wood & Sons, Huddersfield) directly after slaughtering and experiments were conducted within 24 hours. The buccal tissue was dissected from the pigs head after the middle cheek section was cut away from the rest of the head with a knife. The mucosal membrane was removed from the connective tissue using a surgical scalpel and scissors to a thickness of 500 ± 50 µm corresponding to the rate limiting penetration barrier in the upper third segment of the tissue (Kulkarni *et al*., 2010). The method and region of buccal tissue was kept consistent to maintain reproducibility.

A significant increase in permeability was reported using tissues stored at - 20 °C by Kulkarni *et al*., (2010). This was attributed to the freezing process at - 20 °C; ice crystals are formed in an uncontrolled manner within the tissue and caused permanent damage (observed upon histological visual examinations) (Lee *et al*., 2002). Due to the damaging effects of freezing and thawing on tissue integrity, experiments were conducted within 2 hours of killing or within 20 hours of killing after being stored at 4 °C overnight (due to the nature of experiments and availability of fresh tissue). Studies have shown that porcine buccal mucosa retained its integrity at 4 °C for up to 24 hours compared to other conditions such as storage at - 20 °C (Lee *et al*., 2002; Kulkarni *et al*., 2010).

5.6.2 Determination of membrane thickness

Membrane thickness was measured using two spacers of known thickness used to sandwich the tissue between and digital callipers (Whitworth: LIN 6747113) (total thickness of spacers= 0.83 mm).

Membrane thickness = total sandwiched thickness – thickness of spacers

To validate this method, the thickness of silicone membrane (0.13 mm) was measured. Two different batches of silicone membrane (Silatos silicone sheeting Ref 7458 Lot: 1003090) were used to validate the reproducibility and accuracy of the method. The thickness of the membrane was measured and compared with manufacturer specifications.

As thickness is inversely proportional to permeability (Equation 5.4) and to ensure that the membrane contained the rate limiting penetration barrier in the upper third section, thickness of porcine buccal tissue (dissected from three different pigs) was measured and five replicate measurements were taken from each tissue sample (n=5).

5.6.3 Partition coefficient of lansoprazole into porcine buccal tissue

The effect of pH and complexation on partitioning of lansoprazole from saturated solutions into porcine buccal tissue was studied. The method was based on a method by Nair *et al*., (1997) and Ungphaiboon and Maitani, (2001).

Saturated solutions of pure lansoprazole and complexed lansoprazole (1:1 with Mβ-CD) were prepared by adding an excess of drug to artificial saliva at pH 6.8 (mean physiological pH in oral cavity) and pH 8.65 (pH value after 30 minutes *in vitro* release of buffering excipient from gums) before filtering (0.45 µm) prior to use. Buccal tissue portions of similar surface area, thickness and weight were taken from two pigs' cheeks and were added to 25 mL of the saturated solutions.

Duplicate samples were placed in a shaking water bath at 37 °C at 275 shakes per minute (Grants GLS Aqua 12 plus). Amber vials were used to protect against possible photo degradation. A control sample containing 25 mL saturated solution with no tissue was also analysed to monitor the stability of the saturated solution. Samples were collected after 2 hours to correspond with the relatively short *in vitro* release experiments (30 minute) and *in vivo* chewing times. The samples were diluted with mobile phase (1:1) before being filtered through a 0.45 µm filter and quantified using the HPLC method described in Chapter 2.

The partition coefficient (Log P) was calculated using Equation 5.6

$$K = \frac{C_b - C_a}{C_a}$$

Equation 5.6 Partition coefficient (Log P)

Where,

C_b = concentration before (control)

C_a = concentration after exposure to buccal tissue

(Ungphaiboon and Maitani, 2001)

5.6.4 Buccal permeability studies

Permeability through porcine buccal mucosa using vertical Franz cells was studied in artificial saliva at pH 6.8 and pH 8.6, using saturated solutions of:

- Lansoprazole
- Complexed lansoprazole (1:1 with Mβ-CD)

The method was based on methods by Kokate *et al.,* (2009) and Kulkarni *et al.,* (2010). Artificial saliva was prepared (as described in Chapter 2) to mimic *in vivo* conditions and was used in both compartments to minimise diffusion controlled by osmosis. The saturated solutions were prepared by adding an excess of drug/ complex to 100 mL of artificial saliva at the required pH, stirred at ambient temperature for a minimum of six hours and filtered (0.45 µm) before use. Artificial saliva (30 mL) at pH 7.4 (simulating the pH of systemic circulation) was added to each of the receptor cells and the temperature was maintained at 37 ± 0.5°C using heated water jackets. The stirring speed was set to 450 rpm which was the maximum speed to minimise effects of any unstirred water layers. Once the tissue was mounted with the exterior surface (epithelium) facing towards the donor compartment and the connective tissue side facing towards the receptor, it was left to equilibrate for 45 minutes, before 10 mL of filtered saturated solution (finite dose) was added to each of the donor compartments. All cells were carefully observed for visible leaks and air bubbles which would affect permeability results. The tissue was visually inspected at the end to ensure there were no holes or lesions in the membrane also. Samples (2 mL) were taken at 0, 15, 30, 45, 60, 75, 90 and 120 minutes from the receptor compartment and replaced with equal volumes of artificial saliva at pH 7.4, pre warmed at 37 °C, dilutions were accounted for using Equation 2.6. The samples were diluted with mobile phase (1:1) before being filtered through a 0.45 µm filter and quantified using the stability-indicating HPLC method described in Chapter 2. Sink conditions were maintained throughout experiments as 10 mL of saturated solution were added to each donor compartment; the volume in the receptor compartment was 30 mL and was continually diluted after sample replacement.

The steady state flux (J_{ss}) was calculated using Fick's first law of diffusion by measuring the slope of the linear portion of the graph of cumulative drug diffusion (µg/cm²) over time (minutes) once a steady state was achieved (Dias *et al*., 2007). Typically the first and the last

points (0 and 120 minutes) were disregarded to calculate the linear section from the remaining time points (15 - 90 minutes) this excluded the initial lag phase. Lag/burst times were estimated from the intercept value from the straight line equation. The permeability coefficient (K_p) was calculated by dividing the flux by donor concentration (Korinth *et al*., 2004). Statistical testing was calculated using SPSS (Chicago, U.S) and used Independent-Samples Mann-Whitney T Tests (see Appendix B).

5.7 Results

The mean thickness of Sample 1 of silicone membrane sheeting was 0.14 ± 0.01 mm (n = 5; mean ± s.d). The mean thickness of Sample 2 of silicone membrane sheeting was 0.12 ± 0.01 mm (n = 5; mean ± s.d). The overall mean thickness of the two silicone membrane sheets was 0.13 ± 0.01 mm (n = 10; mean ± s.d), which agreed with the manufacturers specifications of a thickness of 0.13 mm (Table 5.4). This showed that the method was accurate and could be used to determine the thickness of porcine buccal membranes.

Table 5.4 Buccal tissue thickness measurements

	Sample 1	**Sample 2**	**Sample 3**
	0.55	0.48	0.54
	0.49	0.55	0.45
	0.46	0.53	0.49
	0.48	0.52	0.51
	0.52	0.47	0.47
Thickness in mm (n =5; mean ± s.d)	**0.50 ± 0.03**	**0.51 ± 0.03**	**0.49 ± 0.04**

The overall mean thickness of porcine buccal tissue from three pig samples was 0.50 ± 0.03 mm or 501 µm (n=15; mean ± s.d) (Table 5.4). The dissected buccal membrane contained the rate limiting mucosal epithelium barrier/ functioning layer (in the upper third portion (500 ± 50 µm) (Figure 5.2) and would provide suitable tissue samples to allow the determination of permeability (Kulkarni *et al*., 2010).

Table 5.5 Partition coefficient of lansoprazole in artificial saliva and porcine buccal mucosae

Test conditions	Concentration of lansoprazole (µg/mL)	Partition coefficient (Log P)
Lansoprazole at pH 6.8	15	0.32
Complexed lansoprazole at pH 6.8	133	0.12
Lansoprazole at pH 8.6	38	0.24
Complexed lansoprazole at pH 8.6	255	0.13

All sample tissues weighed between 1.40 – 1.53 grams and were paired to normalise results to minimise variations between the duplicates. The drug was stable at all conditions with less than 3 % breakdown products observed, however different peaks were seen compared to *in vitro* release data, which suggested this may have been due to the buccal tissue. The highest partition coefficient was for free lansoprazole at pH 6.8 (Table 5.5). The drug had a lower ionised fraction at pH 6.8 (1 %) (see section 5.4.7 and Figure 5.4). The partition coefficient halved with complexed lansoprazole at the same pH, therefore complexation reduced partitioning into the buccal membrane due to the increase in solubility (hence reduced its lipophilicity) along with the accompanying increase in molecular size and weight of the inclusion complex. Complexed lansoprazole did not enhance the partitioning of lansoprazole in porcine buccal mucosae.

At pH 8.6, there was a decrease in the partition coefficient with free lansoprazole; this was because at the higher pH the drug is more ionised (40 %) which is known to decrease the partitioning of the drug due to decreasing its lipid solubility. Complexed lansoprazole had a similar partition coefficient at both pHs suggesting that effects of ionisation were limited due to molecular shielding in the inclusion complex.

The saturated solutions showed similar concentrations as seen previously (see section 3.6.2), solubility increased with increasing pH and complexed lansoprazole had the highest

solubility. However increasing aqueous solubility is inversely proportional to permeability due to decreasing the intrinsic lipophilicity of lansoprazole (Beig *et al*., 2013).

5.7.1 Buccal permeability of lansoprazole

Table 5.6 Permeability of lansoprazole through porcine buccal mucosa

Time after sacrifice (hours)	n	R^2	Concentration (µg/mL)	Flux (J_{ss}) (µg.cm^2/minute)	(K_p) (cm/minute)
2	7	0.989	13.50	0.268	0.0198
20	8	0.994	13.24	0.259	0.0196

The permeability of free lansoprazole at pH 6.8 was studied using excised tissue from the same pig. The drug flux and permeability coefficient was similar at both time points studied (Table 5.6). This suggested that the tissue maintained its integrity and viability with regards to permeability over both the time points after sacrifice. This agreed with previous data where porcine buccal tissue kept its integrity over 24 hours (Lee *et al*., 2002 and Kulkarni *et al*., 2010). There was no significant difference in drug flux and permeability coefficient between experiments preformed after 2 hours and 20 hours after sacrifice ($P > 0.05$).

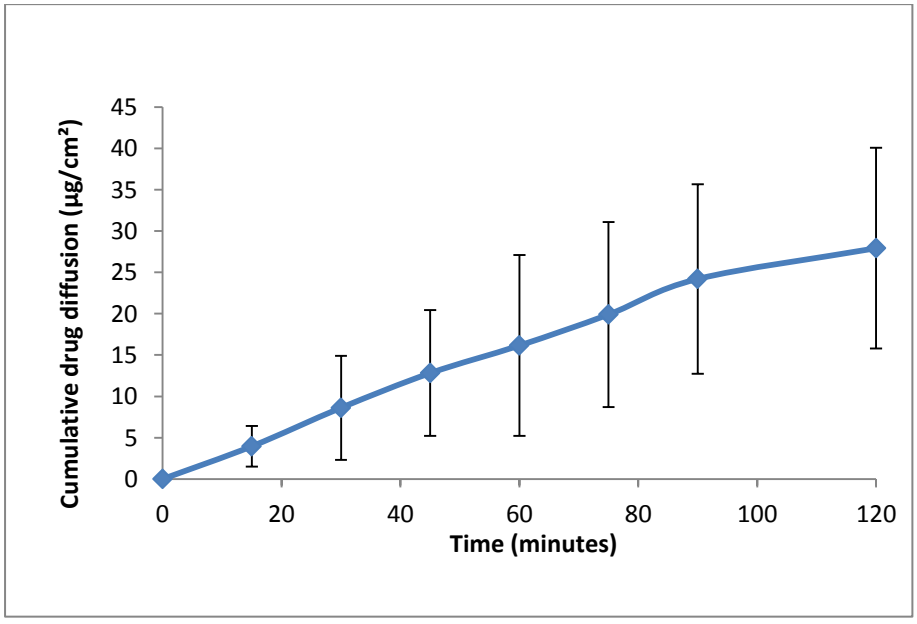


Figure 5.6 Cumulative drug diffusion of lansoprazole at pH 6.8 (n=15; mean ± s.d)

The diffusion of lansoprazole at pH 6.8 displayed a small burst effect at 0.4 minutes and reached a maximum cumulative drug diffusion of 27.9 µg/cm^2 after 120 minutes (Figure 5.6).

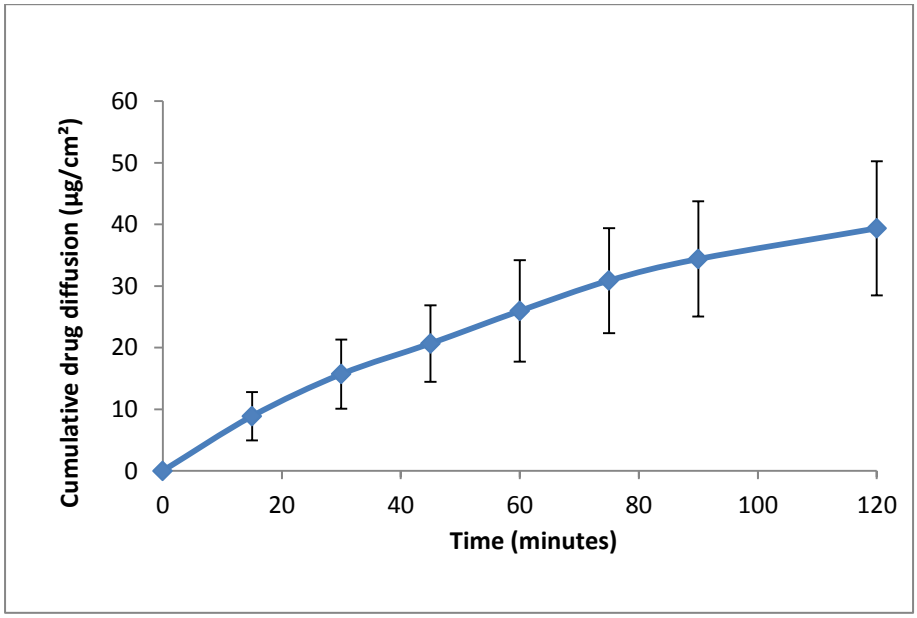


Figure 5.7 Cumulative drug diffusion of complexed lansoprazole at pH 6.8 (n=15; mean ± s.d)

The diffusion of complexed lansoprazole at pH 6.8 displayed a burst effect up to 4.9 minutes (Figure 5.7). The maximum cumulative drug diffusion increased to 39.4 µg/cm^2 after 120 minutes when compared to free lansoprazole.

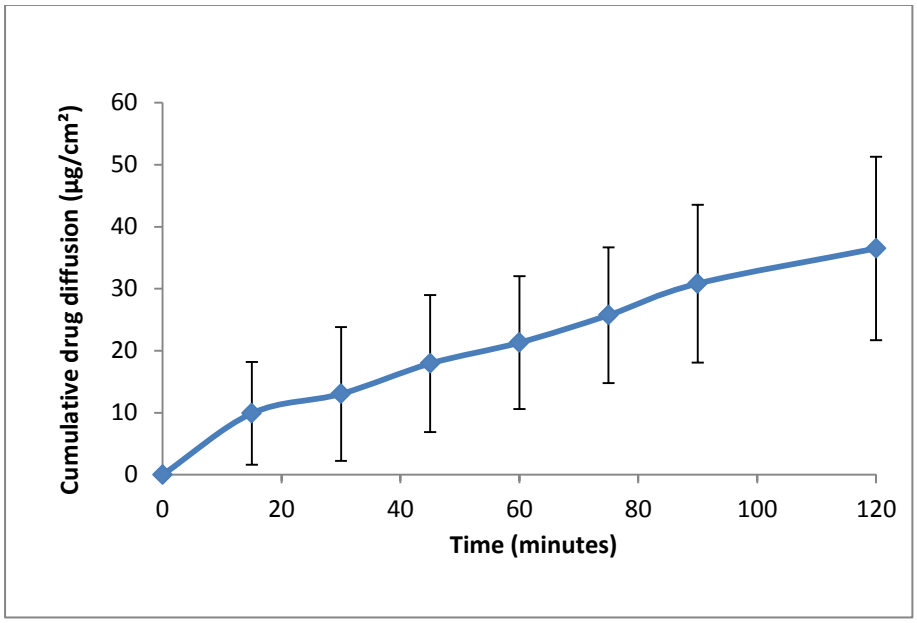


Figure 5.8 Cumulative drug diffusion of lansoprazole at pH 8.6 (n=15; mean ± s.d)

The diffusion of lansoprazole at pH 8.6 displayed a burst effect at 5.2 minutes (Figure 5.8). The maximum cumulative drug diffusion was 36.5 µg/cm^2 after 120 minutes which was higher than that at the lower pH.

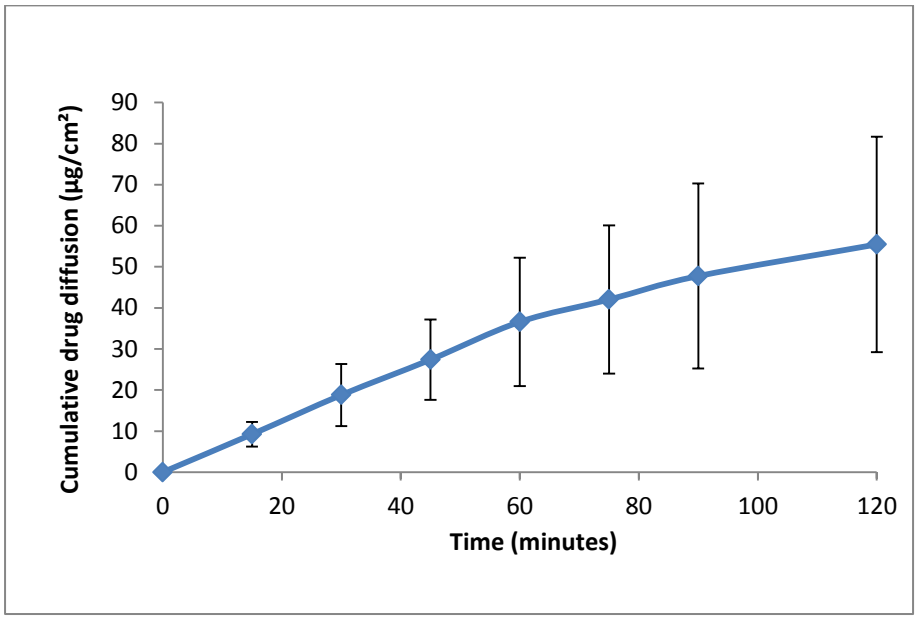


Figure 5.9 Cumulative drug diffusion of complexed lansoprazole at pH 8.6 (n=15; mean ± s.d)

The diffusion of complexed lansoprazole at pH 8.6 displayed a burst effect at 3.2 minutes (Figure 5.9). The overall highest cumulative drug diffusion was observed; 55.5 µg/cm^2 after 120 minutes.

Table 5.7 Summary of lansoprazole buccal permeability results

Test conditions	Flux (J_{ss}) (µg.cm^2/min)	Permeability coefficient (K_p) (cm/min)	Lag/burst release time (minutes)
Lansoprazole at pH 6.8 (n=15)	0.264	0.020	0.44
Complexed lansoprazole at pH 6.8 (n=17)	0.340	0.010	4.91
Lansoprazole at pH 8.6 (n=15)	0.278	0.008	5.19
Complexed lansoprazole at pH 8.6 (n=15)	0.517	0.009	3.16

5.7.1.1 Lag times and burst effects

The regression coefficient (R^2) values for all experiments ranged from 0.988 to 0.998 and the intercept was taken as a consideration of lag times (Table 5.7). The lag time is the time taken for drug concentration to become uniform throughout the membrane before diffusion can begin, the majority of the drug diffusion curves showed short lag times and a burst effect of permeability ranging from 0.44 – 5.19 minutes due to a combination of drug related factors including lipophilicity and also the barrier related properties of the buccal membrane. The first 30 minutes of permeation is of high interest due to the short chewing time of the gum by the patient; therefore lag times will need to be short to facilitate maximum possible diffusion over the chewing cycle, future work would involve evaluating the first initial phases of permeability during 0 - 30 minutes.

5.7.1.2 Flux (J_{ss}) for lansoprazole and complexed lansoprazole

Drug flux was significantly different between free lansoprazole and complexed lansoprazole ($P = 0.03$). The flux increased with complexed lansoprazole compared to free lansoprazole at both pHs; this was due to the increased concentration gradient (9 fold increase in solubility with complexed lansoprazole) which is a driving force during passive diffusion. The highest flux was seen with complexed lansoprazole at pH 8.6 (Table 5.8), this correlated with the highest solubility and therefore highest concentration gradient. Similar results were reported for caffeine, whereby an increase in solubility related to an increase in drug flux through human skin. The permeation of caffeine was dependent on the solvent vehicle chosen with decanol and octanol promoting higher flux, it was suggested that the solvent vehicle altered the fluidisation of the skin (Dias *et al*., 2007).

The flux for free lansoprazole remained constant under varying pHs, whereas the flux for complexed lansoprazole increased with the higher pH, possibly due to solubility and concentration gradient increases at the accompanying pHs and also possible protection from ionisation of the free drug in the inclusion complex.

5.7.1.3 Permeability coefficient for lansoprazole and complexed lansoprazole

The permeability coefficient (K_p) is a function of drug flux divided by the donor concentration. The highest permeability coefficient was observed with free lansoprazole at pH 6.8 and this halved when comparing complexed lansoprazole at the same pH. The data agreed with the partition coefficient data previously seen, which showed that free lansoprazole at pH 6.8 had the highest partition coefficient (Log P) value compared to other forms. The permeability coefficient was also observed to half when comparing the permeability of the lipophilic drug progesterone with complexed forms (Hβ-CD, 1:1) across Caco-2 cells (Beig *et al*., 2013).

Complexation was shown to increase solubility but also increased molecular size and weight (inversely proportional to partioning and permeability) as the drug is incorporated into the inclusion complex form. This contributed to an overall negative effect on permeability through the transcellular pathway following passive diffusion. This has been seen previously with other complexed drugs such as progesterone complexed with HP-βCD (Carrier *et al*., 2007). There were no significant differences in the permeability coefficients of free and complexed lansoprazole ($P > 0.05$).

Increasing pH decreased the permeability coefficient of free lansoprazole due to increasing the ionised fraction of the drug from 1 % at pH 6.8 to 40 % at pH 8.6. This showed that the trade-off between increasing solubility and the concentration gradient was negligible compared to the increased in the ionised fraction of the drug causing reduced lipophilicity, as ionised particles will have reduced penetration *via* the transcellular pathway.

When comparing complexed lansoprazole at the two pHs the permeability coefficient was similar suggesting that cyclodextrins may shield against the effects of ionisation.

5.7.2 Discussion

Previous studies have reported similar effects with a decrease in permeability after complexation with Hβ-CD to improve solubility of the lipophilic drug progesterone (Dahan and Miller, 2012), this paradoxical effect was due to a decreased free drug fraction available for permeability. A dynamic equilibrium exists with complexed lansoprazole (bound within inclusion complexes) and free lansoprazole through self-association. The association and

dissociation of drug from CDs is considered to be rapid in comparison to dissolution and permeability (Beig *et al*., 2013). The mechanism behind the decreased permeability can be explained by the microencapsulation of drug at molecular level by the inclusion complex formation. This will directly decrease the drug free fraction which translates to a lower concentration gradient and driving force for membrane permeability (Dahan and Miller, 2012 and Beig *et al.*, 2013). A solubility-permeability balance is needed to maximise absorption and that the use of solubility increasing techniques is factored against the possible loss in permeability. A mass transport model has been proposed to predict the effective intestinal permeability based on the concentration of cyclodextrin. The mathematical model considers other contributing factors such as the unstirred water layer (UWL) (an aqueous boundary layer), however it does not consider interactions between free cyclodextrin and membrane (Dahan *et al*., 2010). The authors found that permeability decreased with increasing cyclodextrin concentration (decreasing free drug). The role of the UWL can be investigated by assessing differences in rotation speed, (Beig *et al.*, 2013).

Variability's in data sets increased over the duration of time and may have occurred due to the tissue losing its integrity over time other variations included biological inter individual variability between the animals. Pigs have a tendency to damage buccal linings due to their chewing behaviour and this can cause the composition of the buccal membrane to change (scarring) which would have affected permeability. To ensure integrity, the tissue can be examined using microscopy to give visual histological assessment and also with the use of a tracer/biomarker such as fluorescein to ensure the barrier functioning of the epithelium (Kristl, 2009).

There is a lack of standardised methods and guidance in evaluating delivery system IVIVCs prior to clinical evaluations (Patel *et al.*, 2012). Sources of variability may have arisen from the dissecting methods. Any underlying connective tissue would have caused an additional stagnant layer which would decrease permeability. The technique required practice using surgical scissors and was difficult and time consuming. The use of a dermatome to section an area of uniform thickness may improve reproducibility.

Further work involves testing other relevant apparatus possibly a flow-through Franz cell apparatus, this would ensure there is no accumulation of the drug in the receptor cell. The use of an Ussing chamber would explore permeability with increased tissue integrity due to the

circulation of carbogen gas and possibly provide more information on the role of the unstirred water layer. Also as the process of absorption of drug after release from chewing gum is dynamic, increasing the sampling times over the initial 30 minute period i.e. every 5 minutes for first 30 minutes would identify and represent relevant short term absorption pathways.

In vivo studies would allow the possible combined absorption effects of different regions e.g sublingual and would provide a realistic evaluation of absorption. Also the combination of chewing factors (e.g. parking strategy) can be employed to improve localisation of the gum to the cheek which would also improve absorption. Relevant pharmacokinetic and pharmacodynamic data on absorption, distribution, metabolism and elimination of the drug can be gathered with regards to the novel buccal absorption mechanism of the drug.

5.8 Conclusion

The buccal mucosa is the target site of delivery during release from chewing gum formulations. The partitioning of lansoprazole between artificial saliva and porcine buccal mucosae resulted in free lansoprazole at pH 6.8 having the highest partitioning coefficient. This was due to a lower ionised fraction at the lower pH and also a lower molecular weight compared to complexed lansoprazole. Complexed lansoprazole had the highest drug flux through porcine buccal membrane but also had the paradoxical effect of decreasing the partition coefficient and the permeability coefficient. Careful consideration must be taken when optimising a formulation. Increases in solubility will cause adjustments to other important parameters and an overall detailed picture must be gathered to ensure successful formulations. An optimal balance should be found between increased solubility and permeability to maximise absorption on a case-by-case basis. An ideal formulation would release the drug at a rate and extent to facilitate buccal absorption.

Chapter Six:

General conclusions and future work

6.1 General Conclusion

There is an increased demand for convenient drug delivery systems to fit into modern active lifestyles and the potential to manage and improve on various treatments using medicated chewing gums presents great opportunities. An innovative drug delivery system can significantly impact success by providing product distinctiveness in the market. The concept of chewing gum for medical purposes provides discrete, convenient administration, the potential for buccal absorption and the avoidance of first pass or GI degradation. Lansoprazole was chosen as a model drug due to its characteristics including poor solubility and instability (under acidic conditions). It has also been identified as being in need of reformulation for the paediatric market, so a chewing gum formulation may be of particular benefit for this API to increase compliance and also avoid degradation in the GI tract.

Chewing gums were developed incorporating lansoprazole for potential targeted absorption across the buccal mucosa and investigated the use of Revolymer's® hydrophilic polymer Rev7, the effect of buffering excipients and the use of β-cyclodextrin complexation to assess the effects on release of the active.

The solubility and stability of lansoprazole in artificial saliva was found to be dependent on the pH of the solution, an increase in pH caused an increase in solubility with a significant increase between pH 9 and 10. At the lower pHs, concentrations decreased over time confirming the acid instability of lansoprazole. The use of cyclodextrins as solubilisers and stabilisers for lansoprazole were investigated; complexed lansoprazole (with Mβ-CD, 1:1) resulted in a 9 fold increase in solubility compared to free lansoprazole and remained stable at low pHs. Solid state characterisation of Mβ-CD complexes confirmed that interactions (benzimidazole region of the drug was included in the CD cavity) were taking place between the guest (lansoprazole) and the host (CD); giving further evidence to support the formation of inclusion complexes through molecular encapsulation of the drug.

In vitro release of lansoprazole from gums was evaluated using the EP approved masticator and utilised a variety of discriminatory test settings. Significant differences in release after 30 minutes *in vitro* mastication were found from gums containing Rev7 and potassium carbonate (L,8,K and C,8,K). Significant differences were also observed in hardness in the same gums due to physical interactions causing a plasticising effect on the gum base resulting in softer,

less cohesive gums. A combination of mechanisms controlling release from gums containing Rev7 and potassium carbonate were proposed including textural changes due to physical interactions in the gum base elastomer, the hydrophilic capacity of the gum and the solubility of the buffering excipient, which may have led to an increase in the rate and extent of micropore formation in the gum matrix upon contact with saliva when compared to the other formulations.

To ensure success of the formulation the active must be released at a rate and extent to facilitate buccal absorption. The study assessed the buccal absorption of free lansoprazole and complexed lansoprazole (with Mβ-CD, 1:1) using porcine buccal mucosae. The highest partitioning coefficient was observed for free lansoprazole at pH 6.8 due to a lower ionised fraction in combination with a lower molecular weight. Complexed lansoprazole had the highest drug flux but also had the paradoxical effect of decreasing the permeability coefficient.

Overall the study contributed to increasing the understanding of factors governing the release of a poorly soluble and unstable API, lansoprazole, from a medicated chewing gum formulation. Significant differences in hardness and release after 30 minutes *in vitro* mastication were found from gums containing Rev7 and potassium carbonate (L,8,K and C,8,K). The highest partitioning coefficient was observed for free lansoprazole at pH 6.8, whereas complexed lansoprazole (Mβ-CD: lansoprazole, 1:1) had the highest drug flux but also had the paradoxical effect of decreasing the permeability coefficient.

6.2 Future Work

The complexity of release mechanisms from chewing gum formulations are still not fully understood and require further work to increase this understanding. Further experiments exploring the drugs affinity for different gum bases and excipients could be conducted utilising partition coefficient (cLog P) experiments. Further refinements to represent *in vitro/in vivo* conditions would involve incorporating artificial saliva containing specific combinations of polyols and buffering excipients (representative in the gum formulation), which are also readily released alongside the drug. The use of a pycnometer may provide information into the density and relative surface porosity of gums which would provide further evidence of any physical interactions between excipients in the gum to increase the

understanding of release mechanisms and to further discriminate between formulations. Further formulation considerations would involve exploring the effect of other buffering excipients, e.g. magnesium carbonate and evaluating physical interactions and the effects on release.

Further evaluations on *in vitro* release methods involve exploring apparatus B to compare release, the use of different biorelevant media, chew rates (less than 60 chews per minute) and increasing the duration of mastication. *In vitro* testing using a flow through system would eliminate the need for sample replacement. During the mastication process, the gum texture changes due to the gum contacting saliva and the leeching out of soluble excipients. Texture analysis of masticated gums would provide an insight into this phenomenon. Characterising gums after mastication would allow a complete picture including mass changes due to the loss of soluble excipients resulting in harder gums.

In vivo studies provide the most informative evaluations of buccal delivery systems in human volunteers, including the extent of the washing out effect (due to swallowing) and the total bioavailability after absorption in the oral cavity, allowing a full comparison to current formulations. *In vitro/ in vivo* correlations would involve chew out studies and measurements of residual gum contents to indicate *in vivo* release performance. I*n vivo* studies would also allow the combined absorption effects of different regions of the oral cavity e.g sublingual. Also the use of chewing instructions/factors (e.g. parking strategy) can be employed to improve localisation of the gum to the cheek which would also affect absorption. Relevant pharmacokinetic data on absorption and distribution of the drug could be gathered with regards to the novel buccal absorption route. Sensory characteristics can also be evaluated including overall mouth feel and taste to evaluate compliance.

Appendices

Appendix A: Experimental raw data

Table A1 *In vitro* release from commercial 2 mg Nicorette® gums (n=9; mean ± s.d)

Time (minutes)	Nicotine released (%)
0	0 ± 0.1
5	37.7 ± 5.1
10	64.3 ± 7.7
15	78.3 ± 9.2
20	88.3 ± 8.2
25	97.9 ± 7.4
30	101.2 ± 7.1

HPLC example chromatograms of lansoprazole

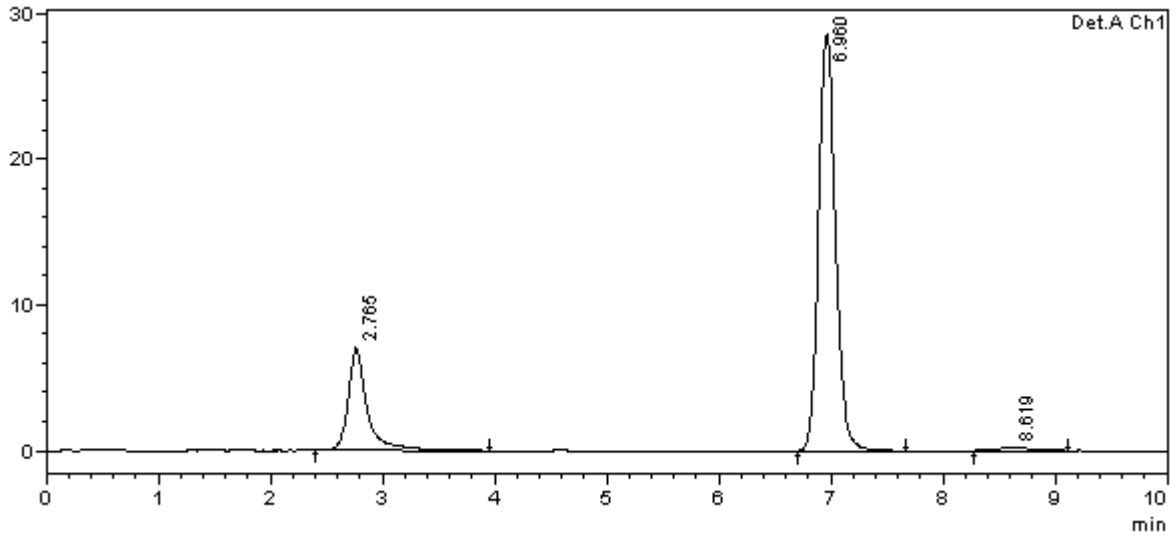


Figure A1 *In vitro* release from gums in artificial saliva pH 6.7 at 30 minutes

The following peaks were identified solvent front at 2.77 minutes, lansoprazole at 6.96 minutes and degradation breakdown products at 8.62 minutes (1.17 %).

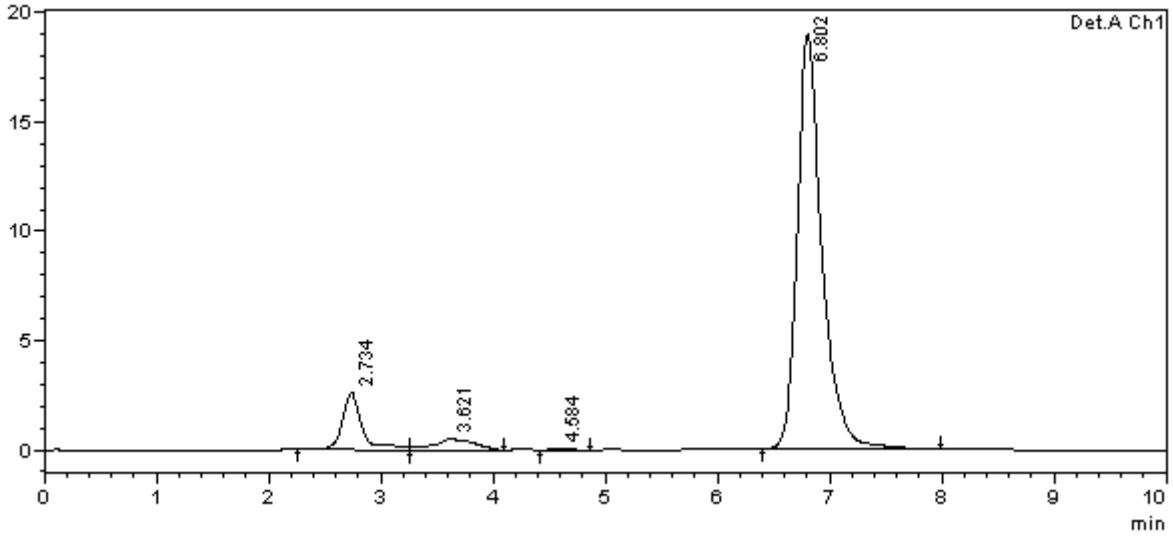


Figure A2 *In vitro* buccal permeability (pH 6.7) at 2 hours sampling time

The following peaks were identified solvent front at 2.73 minutes, lansoprazole peak at 6.8 minutes and degradation products/ buccal by products at 3.62 minutes (2.5 %) and 4.58 minutes (0.32 %).

Table A2: The effect of pH on solubility of lansoprazole (n = 3; mean ± s.d)

8.5 hours	Mean concentration of lansoprazole (µg/mL)
pH 6	14.87 ± 1.39
pH 7	14.58 ± 2.20
pH 8	16.35 ± 1.44
pH 9	29.94 ± 0.56
pH 10	89.70 ± 0.68

15.5 hours	Mean concentration of lansoprazole (µg/mL)
pH 6	10.94 ± 0.68
pH 7	12.91 ± 0.86
pH 8	15.34 ± 3.06
pH 9	26.05 ± 1.78
pH 10	91.65 ± 1.58

Table A3: The solubility of complexed lansoprazole (n= 3; mean ± s.d)

pH 6, 8.5 hours	Mean concentration of lansoprazole (µg/mL)
1:1 βCD	49.34 ± 3.30
1:1 MβCD	133.94 ± 4.38
3:1 βCD	65.34 ± 2.44
3:1 MβCD	64.97 ± 1.70
Lansoprazole	14.33 ± 2.19

pH 8, 8.5 hours	Mean concentration of lansoprazole (µg/mL)
1:1 βCD	278.87 ± 5.45
1:1 MβCD	288.80 ± 7.65
3:1 βCD	64.97 ± 3.56
3:1 MβCD	168.72 ± 4.98
Lansoprazole	25.06 ± 2.50

pH 6, 15.5 hours	Mean concentration of lansoprazole (µg/mL)
1:1 βCD	47.26 ± 3.23
1:1 MβCD	134.36 ± 4.65
3:1 βCD	29.32 ± 2.85
3:1 MβCD	19.78 ± 1.40
Lansoprazole	8.33 ± 2.80

pH 8, 15.5 hours	Mean concentration of lansoprazole (µg/mL)
1:1 βCD	228.91 ± 4.98
1:1 MβCD	287.39 ± 8.23
3:1 βCD	48.69 ± 4.89
3:1 MβCD	145.28 ± 3.65
Lansoprazole	23.06 ± 2.30

Table A4: *In vitro* release from L gums and H gums (n= 3; mean ± s.d)

H gums pH 6

Time (minutes)	Lansoprazole released (%)
0	0 ± 0.00
5	1.04 ± 0.16
10	2.60 ± 0.13
15	4.67 ± 0.25
20	5.96 ± 0.18
25	7.48 ± 0.39
30	8.95 ± 0.71

H gums pH 8

Time (minutes)	Lansoprazole released (%)
0	0 ± 0.01
5	2.06 ± 0.39
10	4.54 ± 0.56
15	7.11 ± 0.81
20	9.01 ± 1.17
25	10.84 ± 0.89
30	12.29 ± 1.23

L gums pH 6

Time (minutes)	Lansoprazole released (%)
0	0 ± 0.01
5.0	2.64 ± 0.30
10.0	5.56 ± 0.34
15.0	7.79 ± 0.45
20.0	9.23 ± 0.40
25.0	10.53 ± 0.34
30.0	12.11 ± 0.62

L gums pH 8

Time (minutes)	Lansoprazole released (%)
0	0 ± 0.01
5	4.06 ± 1.61
10	6.06 ± 0.26
15	8.74 ± 0.27
20	10.50 ± 0.61
25	12.51 ± 0.69
30	13.97 ± 0.44

Table A5: Diffusion from surfaces of formulated gums (n= 2)

Time (minutes)	L,8,Na	L,8,K	C,8,K	C,8,Na	L,0,Na	L,0,K	C,0,K	C,0,Na	Mean
0	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
5	0.08	0.63	0.34	0.06	0.15	0.04	0.04	0.15	0.19
10	0.39	0.88	0.50	0.64	0.21	0.25	0.23	0.25	0.42
20	0.60	1.02	1.01	0.87	0.27	0.35	0.40	0.37	0.61
30	0.76	1.24	1.34	0.92	0.61	0.56	0.55	0.57	0.82
60	0.86	1.88	2.07	0.95	0.64	0.80	0.79	0.79	1.10
1440	11.33	11.21	13.00	11.64	8.08	9.87	9.94	8.65	10.46

Table A6: *In vitro* release from L gum batches (n=3; mean ± s.d)

Coding key	Drug form	Rev7 (%)	Buffering excipients	Lansoprazole released after 30 minutes (%)				
				Phosphate buffer pH 6.0	Artificial saliva pH 6.7	20 mL replace pH 6.7	20 mL replace pH 8	20 mL replace pH 8 83 chews
L,8,Na	lansoprazole	8	Na_2CO_3	12.24 ± 0.04	17.6 ± 0.45	12.2 ± 1.64	9.16 ± 0.33	20.98 ± 0.63
L,8,K	lansoprazole	8	K_2CO_3	25.38 ± 2.02	35.62 ± 3.77	54.1 ± 1.87	65.24 ± 1.68	67.46 ± 0.94
C,8,Na	complex	8	Na_2CO_3	12.26 ± 0.82	21.27 ± 1.66	18.69 ± 0.60	20.12 ± 0.28	29.49 ± 1.22
C,8,K	complex	8	K_2CO_3	21.00 ± 0.49	35.08 ± 1.61	44.8 ± 1.89	63.33 ± 6.17	61.21 ± 2.37
L,0,Na	lansoprazole	0	Na_2CO_3	11.40 ± 0.04	24.11 ± 3.85	18.82 ± 2.24	12.8 ± 0.16	33.44 ± 2.25
L,0,K	lansoprazole	0	K_2CO_3	11.96 ± 0.56	22.24 ± 1.50	20.27 ± 2.83	17.44 ± 0.98	35.97 ± 1.17
C,0,Na	complex	0	Na_2CO_3	10.98 ± 1.38	29.06 ± 2.50	26.03 ± 2.11	31.8 ± 1.43	44.21 ± 1.84
C,0,K	complex	0	K_2CO_3	12.57 ± 0.43	20.73 ± 2.70	22.74 ± 1.58	32.76 ± 1.75	40.72 ± 7.92
			Total release (%)	14.99 ± 5.36	25.71 ± 6.80	27.20 ± 14.07	31.58 ± 21.14	41.68 ± 15.37

** All experiments were performed with artificial saliva, 2 mL replacement and 60 chews per minute unless stated otherwise.

Appendix B: Statistical analysis

Normality testing

Tests of Normality

Gums	Shapiro-Wilk		
	Statistic	df	Sig.
L,8,Na	0.750	3	0.762
L,8,K	0.990	3	0.809
C,8,K	0.750	3	0.0607
C,8,Na	0.893	3	0.363
L,0,Na	0.850	3	0.842
L,0,K	0.995	3	0.862
C,0,K	0.818	3	0.157
C,0,Na	1.000	3	0.964

a. Lilliefors Significance Correction

When looking at release after 30 minutes *in vitro* mastication in phosphate buffer, the significance was above 0.05 from all gums using the Shapiro-Wilk test for normality. Parametric testing ANOVA (uni-variate interaction with post hoc LSD tests) and t tests were used to calculate significance differences, with release after 30 minutes *in vitro* mastication between formulations and experimental *in vitro* conditions. The following assumptions were considered; all formulations were independent, equal in variance and contained sample groups of equal sizes.

ANOVA testing: Impact of gum base content on *in vitro* release from lansoprazole gum formulations (H gums and L gums)

Tests of Between-Subjects Effects

Dependent Variable: Release

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	49.827[a]	3	16.609	15.927	0.000
Intercept	1878.362	1	1878.362	1801.256	0.000
composition	18.278	1	18.278	17.528	0.002
pH	21.140	1	21.140	20.272	0.001
composition * pH	1.445	1	1.445	1.385	0.266
Error	10.428	10	1.043		
Total	1903.714	14			
Corrected Total	60.255	13			

a. R Squared = .827 (Adjusted R Squared = .775)

Significant differences in release after 30 minutes were found between H and L gums as a result of changing pH of dissolution medium and gum base composition ($P < 0.01$).

ANOVA with LSD post hoc testing with optimised 41 % w/w percentage gums: *In vitro* release in phosphate buffer pH 6.0

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	588.512	7	84.073	79.703	0.000
Within Groups	14.768	14	1.055		
Total	603.279	21			

Significant differences in release of active after 30 minutes in phosphate buffer pH 6.0 were found between gums ($P < 0.01$).

Post Hoc Tests Multiple Comparisons

LSD

(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	L,8,K	-13.13667*	.93756	.000	-15.1475	-11.1258
	C,8,K	-8.75667*	.93756	.000	-10.7675	-6.7458
	C,8,Na	-.02000	.93756	.983	-2.0309	1.9909
L,8,Na	L,0,Na	.84500	1.02705	.424	-1.3578	3.0478
	L,0,K	.27667	.93756	.772	-1.7342	2.2875
	C,0,K	-.33000	.93756	.730	-2.3409	1.6809
	C,0,Na	1.26000	.93756	.200	-.7509	3.2709
	L,8,Na	13.13667*	.93756	.000	11.1258	15.1475
	C,8,K	4.38000*	.83858	.000	2.5814	6.1786
	C,8,Na	13.11667*	.83858	.000	11.3181	14.9152
L,8,K	L,0,Na	13.98167*	.93756	.000	11.9708	15.9925
	L,0,K	13.41333*	.83858	.000	11.6148	15.2119
	C,0,K	12.80667*	.83858	.000	11.0081	14.6052
	C,0,Na	14.39667*	.83858	.000	12.5981	16.1952
	L,8,Na	8.75667*	.93756	.000	6.7458	10.7675
	L,8,K	-4.38000*	.83858	.000	-6.1786	-2.5814
	C,8,Na	8.73667*	.83858	.000	6.9381	10.5352
C,8,K	L,0,Na	9.60167*	.93756	.000	7.5908	11.6125
	L,0,K	9.03333*	.83858	.000	7.2348	10.8319
	C,0,K	8.42667*	.83858	.000	6.6281	10.2252
	C,0,Na	10.01667*	.83858	.000	8.2181	11.8152
	L,8,Na	.02000	.93756	.983	-1.9909	2.0309
	L,8,K	-13.11667*	.83858	.000	-14.9152	-11.3181
	C,8,K	-8.73667*	.83858	.000	-10.5352	-6.9381
C,8,Na	L,0,Na	.86500	.93756	.372	-1.1459	2.8759
	L,0,K	.29667	.83858	.729	-1.5019	2.0952
	C,0,K	-.31000	.83858	.717	-2.1086	1.4886

	C,0,Na	1.28000	.83858	.149	-.5186	3.0786
	L,8,Na	-.84500	1.02705	.424	-3.0478	1.3578
	L,8,K	-13.98167*	.93756	.000	-15.9925	-11.9708
	C,8,K	-9.60167*	.93756	.000	-11.6125	-7.5908
L,0,Na	C,8,Na	-.86500	.93756	.372	-2.8759	1.1459
	L,0,K	-.56833	.93756	.554	-2.5792	1.4425
	C,0,K	-1.17500	.93756	.231	-3.1859	.8359
	C,0,Na	.41500	.93756	.665	-1.5959	2.4259
	L,8,Na	-.27667	.93756	.772	-2.2875	1.7342
	L,8,K	-13.41333*	.83858	.000	-15.2119	-11.6148
	C,8,K	-9.03333*	.83858	.000	-10.8319	-7.2348
L,0,K	C,8,Na	-.29667	.83858	.729	-2.0952	1.5019
	L,0,Na	.56833	.93756	.554	-1.4425	2.5792
	C,0,K	-.60667	.83858	.481	-2.4052	1.1919
	C,0,Na	.98333	.83858	.261	-.8152	2.7819
	L,8,Na	.33000	.93756	.730	-1.6809	2.3409
	L,8,K	-12.80667*	.83858	.000	-14.6052	-11.0081
	C,8,K	-8.42667*	.83858	.000	-10.2252	-6.6281
C,0,K	C,8,Na	.31000	.83858	.717	-1.4886	2.1086
	L,0,Na	1.17500	.93756	.231	-.8359	3.1859
	L,0,K	.60667	.83858	.481	-1.1919	2.4052
	C,0,Na	1.59000	.83858	.079	-.2086	3.3886
	L,8,Na	-1.26000	.93756	.200	-3.2709	.7509
	L,8,K	-14.39667*	.83858	.000	-16.1952	-12.5981
	C,8,K	-10.01667*	.83858	.000	-11.8152	-8.2181
C,0,Na	C,8,Na	-1.28000	.83858	.149	-3.0786	.5186
	L,0,Na	-.41500	.93756	.665	-2.4259	1.5959
	L,0,K	-.98333	.83858	.261	-2.7819	.8152
	C,0,K	-1.59000	.83858	.079	-3.3886	.2086

*. The mean difference is significant at the 0.05 level.

In vitro **release in artificial saliva pH 6.7**

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	644.053	7	92.008	14.622	0.001
Within Groups	50.340	8	6.293		
Total	694.393	15			

Significant differences in release of active in artificial saliva pH 6.7 (2 mL replacement) were found between gums ($P < 0.01$).

Post Hoc Tests Multiple Comparisons

LSD

(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	L,8,K	-18.01500*	2.50849	.000	-23.7996	-12.2304
	C,8,K	-17.48000*	2.50849	.000	-23.2646	-11.6954
	C,8,Na	-3.66500	2.50849	.182	-9.4496	2.1196
L,8,Na	L,0,Na	-6.51000*	2.50849	.032	-12.2946	-.7254
	L,0,K	-4.64000	2.50849	.102	-10.4246	1.1446
	C,0,K	-3.13000	2.50849	.247	-8.9146	2.6546
	C,0,Na	-11.45500*	2.50849	.002	-17.2396	-5.6704
	L,8,Na	18.01500*	2.50849	.000	12.2304	23.7996
	C,8,K	.53500	2.50849	.836	-5.2496	6.3196
	C,8,Na	14.35000*	2.50849	.000	8.5654	20.1346
L,8,K	L,0,Na	11.50500*	2.50849	.002	5.7204	17.2896
	L,0,K	13.37500*	2.50849	.001	7.5904	19.1596
	C,0,K	14.88500*	2.50849	.000	9.1004	20.6696
	C,0,Na	6.56000*	2.50849	.031	.7754	12.3446
	L,8,Na	17.48000*	2.50849	.000	11.6954	23.2646
	L,8,K	-.53500	2.50849	.836	-6.3196	5.2496
	C,8,Na	13.81500*	2.50849	.001	8.0304	19.5996
C,8,K	L,0,Na	10.97000*	2.50849	.002	5.1854	16.7546
	L,0,K	12.84000*	2.50849	.001	7.0554	18.6246
	C,0,K	14.35000*	2.50849	.000	8.5654	20.1346
	C,0,Na	6.02500*	2.50849	.043	.2404	11.8096
	L,8,Na	3.66500	2.50849	.182	-2.1196	9.4496
	L,8,K	-14.35000*	2.50849	.000	-20.1346	-8.5654
	C,8,K	-13.81500*	2.50849	.001	-19.5996	-8.0304
C,8,Na	L,0,Na	-2.84500	2.50849	.290	-8.6296	2.9396
	L,0,K	-.97500	2.50849	.708	-6.7596	4.8096
	C,0,K	.53500	2.50849	.836	-5.2496	6.3196
	C,0,Na	-7.79000*	2.50849	.015	-13.5746	-2.0054
	L,8,Na	6.51000*	2.50849	.032	.7254	12.2946
	L,8,K	-11.50500*	2.50849	.002	-17.2896	-5.7204
	C,8,K	-10.97000*	2.50849	.002	-16.7546	-5.1854
L,0,Na	C,8,Na	2.84500	2.50849	.290	-2.9396	8.6296
	L,0,K	1.87000	2.50849	.477	-3.9146	7.6546
	C,0,K	3.38000	2.50849	.215	-2.4046	9.1646
	C,0,Na	-4.94500	2.50849	.084	-10.7296	.8396
	L,8,Na	4.64000	2.50849	.102	-1.1446	10.4246
L,0,K	L,8,K	-13.37500*	2.50849	.001	-19.1596	-7.5904

	C,8,K	-12.84000*	2.50849	.001	-18.6246	-7.0554
	C,8,Na	.97500	2.50849	.708	-4.8096	6.7596
	L,0,Na	-1.87000	2.50849	.477	-7.6546	3.9146
	C,0,K	1.51000	2.50849	.564	-4.2746	7.2946
	C,0,Na	-6.81500*	2.50849	.026	-12.5996	-1.0304
	L,8,Na	3.13000	2.50849	.247	-2.6546	8.9146
	L,8,K	-14.88500*	2.50849	.000	-20.6696	-9.1004
	C,8,K	-14.35000*	2.50849	.000	-20.1346	-8.5654
C,0,K	C,8,Na	-.53500	2.50849	.836	-6.3196	5.2496
	L,0,Na	-3.38000	2.50849	.215	-9.1646	2.4046
	L,0,K	-1.51000	2.50849	.564	-7.2946	4.2746
	C,0,Na	-8.32500*	2.50849	.011	-14.1096	-2.5404
	L,8,Na	11.45500*	2.50849	.002	5.6704	17.2396
	L,8,K	-6.56000*	2.50849	.031	-12.3446	-.7754
	C,8,K	-6.02500*	2.50849	.043	-11.8096	-.2404
C,0,Na	C,8,Na	7.79000*	2.50849	.015	2.0054	13.5746
	L,0,Na	4.94500	2.50849	.084	-.8396	10.7296
	L,0,K	6.81500*	2.50849	.026	1.0304	12.5996
	C,0,K	8.32500*	2.50849	.011	2.5404	14.1096

*. The mean difference is significant at the 0.05 level.

In vitro **release in artificial saliva pH 6.7 with 20 mL sample replacement**

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2940.137	7	420.020	111.710	0.000
Within Groups	30.079	8	3.760		
Total	2970.216	15			

Significant differences in release of active in artificial saliva pH 6.7 (20 mL replacement) were found between gums ($P < 0.01$).

Post Hoc Tests Multiple Comparisons

LSD

(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	L,8,K	-41.89500*	1.93905	.000	-46.3665	-37.4235
	C,8,K	-32.59500*	1.93905	.000	-37.0665	-28.1235
L,8,Na	C,8,Na	-6.48500*	1.93905	.010	-10.9565	-2.0135
	L,0,Na	-6.61500*	1.93905	.009	-11.0865	-2.1435
	L,0,K	-8.07000*	1.93905	.003	-12.5415	-3.5985

	C,0,K	-10.53500*	1.93905	.001	-15.0065	-6.0635
	C,0,Na	-13.83000*	1.93905	.000	-18.3015	-9.3585
	L,8,Na	41.89500*	1.93905	.000	37.4235	46.3665
	C,8,K	9.30000*	1.93905	.001	4.8285	13.7715
	C,8,Na	35.41000*	1.93905	.000	30.9385	39.8815
L,8,K	L,0,Na	35.28000*	1.93905	.000	30.8085	39.7515
	L,0,K	33.82500*	1.93905	.000	29.3535	38.2965
	C,0,K	31.36000*	1.93905	.000	26.8885	35.8315
	C,0,Na	28.06500*	1.93905	.000	23.5935	32.5365
	L,8,Na	32.59500*	1.93905	.000	28.1235	37.0665
	L,8,K	-9.30000*	1.93905	.001	-13.7715	-4.8285
	C,8,Na	26.11000*	1.93905	.000	21.6385	30.5815
C,8,K	L,0,Na	25.98000*	1.93905	.000	21.5085	30.4515
	L,0,K	24.52500*	1.93905	.000	20.0535	28.9965
	C,0,K	22.06000*	1.93905	.000	17.5885	26.5315
	C,0,Na	18.76500*	1.93905	.000	14.2935	23.2365
	L,8,Na	6.48500*	1.93905	.010	2.0135	10.9565
	L,8,K	-35.41000*	1.93905	.000	-39.8815	-30.9385
	C,8,K	-26.11000*	1.93905	.000	-30.5815	-21.6385
C,8,Na	L,0,Na	-.13000	1.93905	.948	-4.6015	4.3415
	L,0,K	-1.58500	1.93905	.437	-6.0565	2.8865
	C,0,K	-4.05000	1.93905	.070	-8.5215	.4215
	C,0,Na	-7.34500*	1.93905	.005	-11.8165	-2.8735
	L,8,Na	6.61500*	1.93905	.009	2.1435	11.0865
	L,8,K	-35.28000*	1.93905	.000	-39.7515	-30.8085
	C,8,K	-25.98000*	1.93905	.000	-30.4515	-21.5085
L,0,Na	C,8,Na	.13000	1.93905	.948	-4.3415	4.6015
	L,0,K	-1.45500	1.93905	.475	-5.9265	3.0165
	C,0,K	-3.92000	1.93905	.078	-8.3915	.5515
	C,0,Na	-7.21500*	1.93905	.006	-11.6865	-2.7435
	L,8,Na	8.07000*	1.93905	.003	3.5985	12.5415
	L,8,K	-33.82500*	1.93905	.000	-38.2965	-29.3535
	C,8,K	-24.52500*	1.93905	.000	-28.9965	-20.0535
L,0,K	C,8,Na	1.58500	1.93905	.437	-2.8865	6.0565
	L,0,Na	1.45500	1.93905	.475	-3.0165	5.9265
	C,0,K	-2.46500	1.93905	.239	-6.9365	2.0065
	C,0,Na	-5.76000*	1.93905	.018	-10.2315	-1.2885
	L,8,Na	10.53500*	1.93905	.001	6.0635	15.0065
	L,8,K	-31.36000*	1.93905	.000	-35.8315	-26.8885
C,0,K	C,8,K	-22.06000*	1.93905	.000	-26.5315	-17.5885
	C,8,Na	4.05000	1.93905	.070	-.4215	8.5215
	L,0,Na	3.92000	1.93905	.078	-.5515	8.3915

	L,0,K	2.46500	1.93905	.239	-2.0065	6.9365
	C,0,Na	-3.29500	1.93905	.128	-7.7665	1.1765
	L,8,Na	13.83000*	1.93905	.000	9.3585	18.3015
	L,8,K	-28.06500*	1.93905	.000	-32.5365	-23.5935
	C,8,K	-18.76500*	1.93905	.000	-23.2365	-14.2935
C,0,Na	C,8,Na	7.34500*	1.93905	.005	2.8735	11.8165
	L,0,Na	7.21500*	1.93905	.006	2.7435	11.6865
	L,0,K	5.76000*	1.93905	.018	1.2885	10.2315
	C,0,K	3.29500	1.93905	.128	-1.1765	7.7665

*. The mean difference is significant at the 0.05 level.

In vitro **release in artificial saliva pH 8.0 with 20 mL sample replacement**

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6657.557	7	951.080	161.524	0.000
Within Groups	47.105	8	5.888		
Total	6704.662	15			

Significant differences in release of active in artificial saliva pH 8.0 (20 mL replacement) were found between gums ($P < 0.01$).

Post Hoc Tests Multiple Comparisons

LSD

(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	L,8,K	-56.07500*	2.42655	.000	-61.6706	-50.4794
	C,8,K	-54.17000*	2.42655	.000	-59.7656	-48.5744
	C,8,Na	-10.96000*	2.42655	.002	-16.5556	-5.3644
L,8,Na	L,0,Na	-3.64000	2.42655	.172	-9.2356	1.9556
	L,0,K	-8.28000*	2.42655	.009	-13.8756	-2.6844
	C,0,K	-23.60000*	2.42655	.000	-29.1956	-18.0044
	C,0,Na	-22.64000*	2.42655	.000	-28.2356	-17.0444
	L,8,Na	56.07500*	2.42655	.000	50.4794	61.6706
	C,8,K	1.90500	2.42655	.455	-3.6906	7.5006
	C,8,Na	45.11500*	2.42655	.000	39.5194	50.7106
L,8,K	L,0,Na	52.43500*	2.42655	.000	46.8394	58.0306
	L,0,K	47.79500*	2.42655	.000	42.1994	53.3906
	C,0,K	32.47500*	2.42655	.000	26.8794	38.0706
	C,0,Na	33.43500*	2.42655	.000	27.8394	39.0306
C,8,K	L,8,Na	54.17000*	2.42655	.000	48.5744	59.7656

	L,8,K	-1.90500	2.42655	.455	-7.5006	3.6906
	C,8,Na	43.21000*	2.42655	.000	37.6144	48.8056
	L,0,Na	50.53000*	2.42655	.000	44.9344	56.1256
	L,0,K	45.89000*	2.42655	.000	40.2944	51.4856
	C,0,K	30.57000*	2.42655	.000	24.9744	36.1656
	C,0,Na	31.53000*	2.42655	.000	25.9344	37.1256
	L,8,Na	10.96000*	2.42655	.002	5.3644	16.5556
	L,8,K	-45.11500*	2.42655	.000	-50.7106	-39.5194
	C,8,K	-43.21000*	2.42655	.000	-48.8056	-37.6144
C,8,Na	L,0,Na	7.32000*	2.42655	.017	1.7244	12.9156
	L,0,K	2.68000	2.42655	.302	-2.9156	8.2756
	C,0,K	-12.64000*	2.42655	.001	-18.2356	-7.0444
	C,0,Na	-11.68000*	2.42655	.001	-17.2756	-6.0844
	L,8,Na	3.64000	2.42655	.172	-1.9556	9.2356
	L,8,K	-52.43500*	2.42655	.000	-58.0306	-46.8394
	C,8,K	-50.53000*	2.42655	.000	-56.1256	-44.9344
L,0,Na	C,8,Na	-7.32000*	2.42655	.017	-12.9156	-1.7244
	L,0,K	-4.64000	2.42655	.092	-10.2356	.9556
	C,0,K	-19.96000*	2.42655	.000	-25.5556	-14.3644
	C,0,Na	-19.00000*	2.42655	.000	-24.5956	-13.4044
	L,8,Na	8.28000*	2.42655	.009	2.6844	13.8756
	L,8,K	-47.79500*	2.42655	.000	-53.3906	-42.1994
	C,8,K	-45.89000*	2.42655	.000	-51.4856	-40.2944
L,0,K	C,8,Na	-2.68000	2.42655	.302	-8.2756	2.9156
	L,0,Na	4.64000	2.42655	.092	-.9556	10.2356
	C,0,K	-15.32000*	2.42655	.000	-20.9156	-9.7244
	C,0,Na	-14.36000*	2.42655	.000	-19.9556	-8.7644
	L,8,Na	23.60000*	2.42655	.000	18.0044	29.1956
	L,8,K	-32.47500*	2.42655	.000	-38.0706	-26.8794
	C,8,K	-30.57000*	2.42655	.000	-36.1656	-24.9744
C,0,K	C,8,Na	12.64000*	2.42655	.001	7.0444	18.2356
	L,0,Na	19.96000*	2.42655	.000	14.3644	25.5556
	L,0,K	15.32000*	2.42655	.000	9.7244	20.9156
	C,0,Na	.96000	2.42655	.703	-4.6356	6.5556
	L,8,Na	22.64000*	2.42655	.000	17.0444	28.2356
	L,8,K	-33.43500*	2.42655	.000	-39.0306	-27.8394
	C,8,K	-31.53000*	2.42655	.000	-37.1256	-25.9344
C,0,Na	C,8,Na	11.68000*	2.42655	.001	6.0844	17.2756
	L,0,Na	19.00000*	2.42655	.000	13.4044	24.5956
	L,0,K	14.36000*	2.42655	.000	8.7644	19.9556
	C,0,K	-.96000	2.42655	.703	-6.5556	4.6356

* The mean difference is significant at the 0.05 level.

In vitro release in artificial saliva pH 8.0 with 20 mL sample replacement and 83 chews per minute

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3461.745	7	494.535	48.900	0.000
Within Groups	80.906	8	10.113		
Total	3542.651	15			

Significant differences in release of active in artificial saliva pH 8.0 (20 mL replacement) and 83 chews per minute were found between gums ($P < 0.01$).

Post Hoc Tests Multiple Comparisons

LSD

(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
L,8,Na	L,8,K	-46.48000*	3.18013	.000	-53.8134	-39.1466
	C,8,K	-40.23000*	3.18013	.000	-47.5634	-32.8966
	C,8,Na	-8.51000*	3.18013	.028	-15.8434	-1.1766
	L,0,Na	-12.46500*	3.18013	.004	-19.7984	-5.1316
	L,0,K	-14.99000*	3.18013	.002	-22.3234	-7.6566
	C,0,K	-19.74500*	3.18013	.000	-27.0784	-12.4116
	C,0,Na	-23.23500*	3.18013	.000	-30.5684	-15.9016
L,8,K	L,8,Na	46.48000*	3.18013	.000	39.1466	53.8134
	C,8,K	6.25000	3.18013	.085	-1.0834	13.5834
	C,8,Na	37.97000*	3.18013	.000	30.6366	45.3034
	L,0,Na	34.01500*	3.18013	.000	26.6816	41.3484
	L,0,K	31.49000*	3.18013	.000	24.1566	38.8234
	C,0,K	26.73500*	3.18013	.000	19.4016	34.0684
	C,0,Na	23.24500*	3.18013	.000	15.9116	30.5784
C,8,K	L,8,Na	40.23000*	3.18013	.000	32.8966	47.5634
	L,8,K	-6.25000	3.18013	.085	-13.5834	1.0834
	C,8,Na	31.72000*	3.18013	.000	24.3866	39.0534
	L,0,Na	27.76500*	3.18013	.000	20.4316	35.0984
	L,0,K	25.24000*	3.18013	.000	17.9066	32.5734
	C,0,K	20.48500*	3.18013	.000	13.1516	27.8184
	C,0,Na	16.99500*	3.18013	.001	9.6616	24.3284
C,8,Na	L,8,Na	8.51000*	3.18013	.028	1.1766	15.8434
	L,8,K	-37.97000*	3.18013	.000	-45.3034	-30.6366
	C,8,K	-31.72000*	3.18013	.000	-39.0534	-24.3866
	L,0,Na	-3.95500	3.18013	.249	-11.2884	3.3784

	L,0,K	-6.48000	3.18013	.076	-13.8134	.8534
	C,0,K	-11.23500*	3.18013	.008	-18.5684	-3.9016
	C,0,Na	-14.72500*	3.18013	.002	-22.0584	-7.3916
	L,8,Na	12.46500*	3.18013	.004	5.1316	19.7984
	L,8,K	-34.01500*	3.18013	.000	-41.3484	-26.6816
	C,8,K	-27.76500*	3.18013	.000	-35.0984	-20.4316
L,0,Na	C,8,Na	3.95500	3.18013	.249	-3.3784	11.2884
	L,0,K	-2.52500	3.18013	.450	-9.8584	4.8084
	C,0,K	-7.28000	3.18013	.051	-14.6134	.0534
	C,0,Na	-10.77000*	3.18013	.010	-18.1034	-3.4366
	L,8,Na	14.99000*	3.18013	.002	7.6566	22.3234
	L,8,K	-31.49000*	3.18013	.000	-38.8234	-24.1566
	C,8,K	-25.24000*	3.18013	.000	-32.5734	-17.9066
L,0,K	C,8,Na	6.48000	3.18013	.076	-.8534	13.8134
	L,0,Na	2.52500	3.18013	.450	-4.8084	9.8584
	C,0,K	-4.75500	3.18013	.173	-12.0884	2.5784
	C,0,Na	-8.24500*	3.18013	.032	-15.5784	-.9116
	L,8,Na	19.74500*	3.18013	.000	12.4116	27.0784
	L,8,K	-26.73500*	3.18013	.000	-34.0684	-19.4016
	C,8,K	-20.48500*	3.18013	.000	-27.8184	-13.1516
C,0,K	C,8,Na	11.23500*	3.18013	.008	3.9016	18.5684
	L,0,Na	7.28000	3.18013	.051	-.0534	14.6134
	L,0,K	4.75500	3.18013	.173	-2.5784	12.0884
	C,0,Na	-3.49000	3.18013	.304	-10.8234	3.8434
	L,8,Na	23.23500*	3.18013	.000	15.9016	30.5684
	L,8,K	-23.24500*	3.18013	.000	-30.5784	-15.9116
	C,8,K	-16.99500*	3.18013	.001	-24.3284	-9.6616
C,0,Na	C,8,Na	14.72500*	3.18013	.002	7.3916	22.0584
	L,0,Na	10.77000*	3.18013	.010	3.4366	18.1034
	L,0,K	8.24500*	3.18013	.032	.9116	15.5784
	C,0,K	3.49000	3.18013	.304	-3.8434	10.8234

* The mean difference is significant at the 0.05 level.

Interaction between experimental *in vitro* conditions - t tests

The effect of dissolution medium phosphate buffer pH 6.0 *vs* saliva pH 6.7

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Release	Equal variances assumed	1.815	.186	5.437	36	**0.000**	10.72506	1.97266	6.72432	14.72579
	Equal variances not assumed			5.234	27.582	**0.000**	10.72506	2.04917	6.52465	14.92546

Significant differences in release from gums were found between phosphate buffer pH 6.0 and saliva pH 6.7 ($P < 0.01$).

ANOVA with LSD post hoc testing on optimised 41 % w/w percentage gums

Texture profile analysis

Hardness

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2822781.935	7	403254.562	26.153	0.000
Within Groups	246703.386	16	15418.962		
Total	3069485.322	23			

Significant differences in hardness were found between gums ($P < 0.01$).

Post hoc Multiple Comparisons

LSD

(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	L,8,K	470.70700*	101.38692	.000	255.7763	685.6377
	C,8,K	405.41733*	101.38692	.001	190.4867	620.3480
	C,8,Na	-214.90500	101.38692	.050	-429.8357	.0257
L,8,Na	L,0,Na	-364.97900*	101.38692	.002	-579.9097	-150.0483
	L,0,K	-463.80600*	101.38692	.000	-678.7367	-248.8753
	C,0,K	-400.88133*	101.38692	.001	-615.8120	-185.9507
	C,0,Na	-310.35433*	101.38692	.007	-525.2850	-95.4237

	L,8,Na	-470.70700*	101.38692	.000	-685.6377	-255.7763
	C,8,K	-65.28967	101.38692	.529	-280.2203	149.6410
	C,8,Na	-685.61200*	101.38692	.000	-900.5427	-470.6813
L,8,K	L,0,Na	-835.68600*	101.38692	.000	-1050.6167	-620.7553
	L,0,K	-934.51300*	101.38692	.000	-1149.4437	-719.5823
	C,0,K	-871.58833*	101.38692	.000	-1086.5190	-656.6577
	C,0,Na	-781.06133*	101.38692	.000	-995.9920	-566.1307
	L,8,Na	-405.41733*	101.38692	.001	-620.3480	-190.4867
	L,8,K	65.28967	101.38692	.529	-149.6410	280.2203
	C,8,Na	-620.32233*	101.38692	.000	-835.2530	-405.3917
C,8,K	L,0,Na	-770.39633*	101.38692	.000	-985.3270	-555.4657
	L,0,K	-869.22333*	101.38692	.000	-1084.1540	-654.2927
	C,0,K	-806.29867*	101.38692	.000	-1021.2293	-591.3680
	C,0,Na	-715.77167*	101.38692	.000	-930.7023	-500.8410
	L,8,Na	214.90500	101.38692	.050	-.0257	429.8357
	L,8,K	685.61200*	101.38692	.000	470.6813	900.5427
	C,8,K	620.32233*	101.38692	.000	405.3917	835.2530
C,8,Na	L,0,Na	-150.07400	101.38692	.158	-365.0047	64.8567
	L,0,K	-248.90100*	101.38692	.026	-463.8317	-33.9703
	C,0,K	-185.97633	101.38692	.085	-400.9070	28.9543
	C,0,Na	-95.44933	101.38692	.360	-310.3800	119.4813
	L,8,Na	364.97900*	101.38692	.002	150.0483	579.9097
	L,8,K	835.68600*	101.38692	.000	620.7553	1050.6167
	C,8,K	770.39633*	101.38692	.000	555.4657	985.3270
L,0,Na	C,8,Na	150.07400	101.38692	.158	-64.8567	365.0047
	L,0,K	-98.82700	101.38692	.344	-313.7577	116.1037
	C,0,K	-35.90233	101.38692	.728	-250.8330	179.0283
	C,0,Na	54.62467	101.38692	.597	-160.3060	269.5553
	L,8,Na	463.80600*	101.38692	.000	248.8753	678.7367
	L,8,K	934.51300*	101.38692	.000	719.5823	1149.4437
	C,8,K	869.22333*	101.38692	.000	654.2927	1084.1540
L,0,K	C,8,Na	248.90100*	101.38692	.026	33.9703	463.8317
	L,0,Na	98.82700	101.38692	.344	-116.1037	313.7577
	C,0,K	62.92467	101.38692	.544	-152.0060	277.8553
	C,0,Na	153.45167	101.38692	.150	-61.4790	368.3823
	L,8,Na	400.88133*	101.38692	.001	185.9507	615.8120
	L,8,K	871.58833*	101.38692	.000	656.6577	1086.5190
	C,8,K	806.29867*	101.38692	.000	591.3680	1021.2293
C,0,K	C,8,Na	185.97633	101.38692	.085	-28.9543	400.9070
	L,0,Na	35.90233	101.38692	.728	-179.0283	250.8330
	L,0,K	-62.92467	101.38692	.544	-277.8553	152.0060
	C,0,Na	90.52700	101.38692	.385	-124.4037	305.4577
C,0,Na	L,8,Na	310.35433*	101.38692	.007	95.4237	525.2850

L,8,K	781.06133*	101.38692	.000	566.1307	995.9920
C,8,K	715.77167*	101.38692	.000	500.8410	930.7023
C,8,Na	95.44933	101.38692	.360	-119.4813	310.3800
L,0,Na	-54.62467	101.38692	.597	-269.5553	160.3060
L,0,K	-153.45167	101.38692	.150	-368.3823	61.4790
C,0,K	-90.52700	101.38692	.385	-305.4577	124.4037

*. The mean difference is significant at the 0.05 level.

Cohesion

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20538333.365	7	2934047.624	33.022	0.000
Within Groups	1421607.406	16	88850.463		
Total	21959940.771	23			

Significant differences in cohesion were found between gums ($P < 0.01$).

Post hoc Multiple Comparisons

LSD

(I) batch number	(J) batch number	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	L,8,K	950.33233*	243.37963	.001	434.3906	1466.2741
	C,8,K	866.97700*	243.37963	.003	351.0352	1382.9188
	C,8,Na	-835.33067*	243.37963	.003	-1351.2724	-319.3889
L,8,Na	L,0,Na	-1084.30633*	243.37963	.000	-1600.2481	-568.3646
	L,0,K	-1534.01400*	243.37963	.000	-2049.9558	-1018.0722
	C,0,K	-1400.35433*	243.37963	.000	-1916.2961	-884.4126
	C,0,Na	-1039.50300*	243.37963	.001	-1555.4448	-523.5612
	L,8,Na	-950.33233*	243.37963	.001	-1466.2741	-434.3906
	C,8,K	-83.35533	243.37963	.736	-599.2971	432.5864
	C,8,Na	-1785.66300*	243.37963	.000	-2301.6048	-1269.7212
L,8,K	L,0,Na	-2034.63867*	243.37963	.000	-2550.5804	-1518.6969
	L,0,K	-2484.34633*	243.37963	.000	-3000.2881	-1968.4046
	C,0,K	-2350.68667*	243.37963	.000	-2866.6284	-1834.7449
	C,0,Na	-1989.83533*	243.37963	.000	-2505.7771	-1473.8936
	L,8,Na	-866.97700*	243.37963	.003	-1382.9188	-351.0352
	L,8,K	83.35533	243.37963	.736	-432.5864	599.2971
C,8,K	C,8,Na	-1702.30767*	243.37963	.000	-2218.2494	-1186.3659
	L,0,Na	-1951.28333*	243.37963	.000	-2467.2251	-1435.3416
	L,0,K	-2400.99100*	243.37963	.000	-2916.9328	-1885.0492

	C,0,K	-2267.33133*	243.37963	.000	-2783.2731	-1751.3896
	C,0,Na	-1906.48000*	243.37963	.000	-2422.4218	-1390.5382
	L,8,Na	835.33067*	243.37963	.003	319.3889	1351.2724
	L,8,K	1785.66300*	243.37963	.000	1269.7212	2301.6048
	C,8,K	1702.30767*	243.37963	.000	1186.3659	2218.2494
C,8,Na	L,0,Na	-248.97567	243.37963	.322	-764.9174	266.9661
	L,0,K	-698.68333*	243.37963	.011	-1214.6251	-182.7416
	C,0,K	-565.02367*	243.37963	.034	-1080.9654	-49.0819
	C,0,Na	-204.17233	243.37963	.414	-720.1141	311.7694
	L,8,Na	1084.30633*	243.37963	.000	568.3646	1600.2481
	L,8,K	2034.63867*	243.37963	.000	1518.6969	2550.5804
	C,8,K	1951.28333*	243.37963	.000	1435.3416	2467.2251
L,0,Na	C,8,Na	248.97567	243.37963	.322	-266.9661	764.9174
	L,0,K	-449.70767	243.37963	.083	-965.6494	66.2341
	C,0,K	-316.04800	243.37963	.212	-831.9898	199.8938
	C,0,Na	44.80333	243.37963	.856	-471.1384	560.7451
	L,8,Na	1534.01400*	243.37963	.000	1018.0722	2049.9558
	L,8,K	2484.34633*	243.37963	.000	1968.4046	3000.2881
	C,8,K	2400.99100*	243.37963	.000	1885.0492	2916.9328
L,0,K	C,8,Na	698.68333*	243.37963	.011	182.7416	1214.6251
	L,0,Na	449.70767	243.37963	.083	-66.2341	965.6494
	C,0,K	133.65967	243.37963	.590	-382.2821	649.6014
	C,0,Na	494.51100	243.37963	.059	-21.4308	1010.4528
	L,8,Na	1400.35433*	243.37963	.000	884.4126	1916.2961
	L,8,K	2350.68667*	243.37963	.000	1834.7449	2866.6284
	C,8,K	2267.33133*	243.37963	.000	1751.3896	2783.2731
C,0,K	C,8,Na	565.02367*	243.37963	.034	49.0819	1080.9654
	L,0,Na	316.04800	243.37963	.212	-199.8938	831.9898
	L,0,K	-133.65967	243.37963	.590	-649.6014	382.2821
	C,0,Na	360.85133	243.37963	.158	-155.0904	876.7931
	L,8,Na	1039.50300*	243.37963	.001	523.5612	1555.4448
	L,8,K	1989.83533*	243.37963	.000	1473.8936	2505.7771
	C,8,K	1906.48000*	243.37963	.000	1390.5382	2422.4218
C,0,Na	C,8,Na	204.17233	243.37963	.414	-311.7694	720.1141
	L,0,Na	-44.80333	243.37963	.856	-560.7451	471.1384
	L,0,K	-494.51100	243.37963	.059	-1010.4528	21.4308
	C,0,K	-360.85133	243.37963	.158	-876.7931	155.0904

*. The mean difference is significant at the 0.05 level.

Buccal mucosal permeability t tests (Mann-Whitney)

Comparison of free lansoprazole *versus* complexed lansoprazole (mean ± s.d)			
Parameter	**Lansoprazole**	**Complexed lansoprazole**	**P value**
Flux (μg/mL/cm^2)	0.55 ± 0.12	0.85 ± 0.33	0.030
Permeability coefficient (K_P) (μg/mL)	0.025 ± 0.014	0.019 ± 0.004	0.792

Significant differences in flux were found between free lansoprazole and complexed lansoprazole ($P < 0.05$).

Comparison of Killing Time (mean ± s.d)			
Parameter	**2 hours**	**20 hours**	**P value**
Flux (μg/mL/cm^2)	0.75 ± 0.16	0.69 ± 0.38	0.177
Permeability coefficient (K_P) (μg/mL)	0.024 ± 0.010	0.020 ± 0.010	0.247

No significant differences in permeability were found between porcine tissues after time of sacrifice ($P > 0.05$).

Comparison of pH conditions (mean ± s.d)			
Parameter	**pH 6.8**	**pH 8.6**	**P value**
Flux (μg/mL/cm^2)	0.63 ± 0.13	0.79 ± 0.38	0.662
Permeability coefficient (K_P) (μg/mL)	0.028 ± 0.011	0.016 ± 0.004	0.052

No significant differences in permeability were found between pH conditions ($P > 0.05$).

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