FORMULATION AND EVALUATION OF MUCOADHESIVE POLYMERIC FILMS FOR BUCCAL DELIVERY OF DIDANOSINE

by

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Master of Pharmacy (Pharmaceutics) in the Discipline of Pharmaceutical Sciences
of the School of Health Sciences at the University of KwaZulu-Natal

Supervisor: Professor Thirumala Govender
Co-supervisor: Mrs Elizabeth Ojewole

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“I have been impressed with the urgency of doing. Knowing is not enough; we must apply. Being willing is not enough; we must do.”

- Leonardo da Vinci -

~ This thesis is dedicated to my parents
for their love, endless support
and encouragement. ~

DECLARATION 1 - PLAGIARISM

I, Ms Elsabé Jones, declare that

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DECLARATION 2 - PUBLICATIONS

Details of contribution to publications that form part and/or include research presented in this thesis:

1. Jones, E., Ojewole, E., Pillay, V., Kumar, P., Rambharose, S., Govender, T., 2013. Monolayered multipolymeric buccal films with drug and polymers of opposing solubilities for ARV therapy: Physico-mechanical evaluation and molecular mechanics modelling. ***International Journal of Pharmaceutics***, 455, 197-212.

   Ms E. Jones contributed to the design of the project, modification and optimisation of methods and preparation and characterisation of all polymeric films in terms of assay, *in vitro* drug release, *in vitro* permeations, transepithelial electrical resistance measurements, mucoadhesivity, mechanical strength and surface pH as well as interpretation of the data and writing of the paper. Mr S. Rambharose assisted Ms Jones with the LM/TEM histological evaluation section. Mr P. Kumar and Professor V. Pillay were collaborators and performed the molecular modelling studies. The remaining authors served as supervisor and co-supervisor.

2. Jones, E., Ojewole, E., Kalhapure, R., Govender, T., 2013. *In vitro* comparative evaluation of monolayered multipolymeric films embedded with didanosine-loaded solid lipid nanoparticles: A potential buccal drug delivery system for ARV therapy. ***Drug Development and Industrial Pharmac****y, SUBMITTED MANUSCRIPT. Reference Number: LDDI-2013-0624.*

   Ms E. Jones contributed to the design of the project and developed suitable methods for preparation of didanosine solid lipid nanoparticles for the 1st time and undertook their characterisation. Furthermore, she was responsible for the preparation and characterisation of conventional and nano-enabled polymeric films in terms of assay, *in vitro* drug release, *in vitro* permeations, mucoadhesivity, mechanical strength, interpretation of the data and writing of the overall manuscript. The remaining authors served as supervisor (T. Govender), co-supervisor (E. Ojewole) and postdoctoral advisor (R. Kalhapure).

DECLARATION 3 – ANIMAL ETHICS

Ethical approval and subsequent renewal was obtained from the University of KwaZulu-Natal Animal Ethics Research Committee, for harvesting and preparation of porcine buccal mucosa used in experimental studies reported in this dissertation (Reference No: 011/12/Animal & 041/13/Animal).

* The ethical clearance letters can be found in Appendix A.

RESEARCH OUTPUT FROM THE DISSERTATION

1. PUBLICATIONS

The following paper was published in an international ISI journal from data generated during this study:

- Jones, E., Ojewole, E., Pillay, V., Kumar, P., Rambharose, S., Govender, T., 2013. Monolayered multipolymeric buccal films with drug and polymers of opposing solubilities for ARV therapy: Physico-mechanical evaluation and molecular mechanics modelling. ***International Journal of Pharmaceutics,*** 455, 197-212.

* The published article can be found in Appendix B.

2. SUBMITTED MANUSCRIPT

The following manuscript was submitted to an international ISI journal from data generated during this study:

- Jones, E., Ojewole, E., Kalhapure, R., Govender, T., 2013. In vitro comparative evaluation of monolayered multipolymeric films embedded with didanosine-loaded solid lipid nanoparticles: A potential buccal drug delivery system for ARV therapy. ***Drug Development and Industrial Pharmac****y, SUBMITTED MANUSCRIPT. Reference Number: LDDI-2013-0624.*

* The manuscript submission cover page from the journal can be found in Appendix C.

3. CONFERENCE PRESENTATIONS

The following conference presentations were produced from data generated during this study:

International:

- Jones, E., Ojewole, E., Govender, T. Mucoadhesivity and mechanical evaluation of Hydroxypropyl methylcellulose and Eudragit RS100® copolymeric films for buccal delivery of Didanosine. ***European Polymer Congress***, Pisa, Italy, 16-21 June 2013.

- Jones, E., Ojewole, E., Pillay, V., Kumar, P., Govender, T. Static lattice atomistic simulations for co-blended-co-plasticized multipolymeric films for buccal drug delivery. ***European Polymer Congress***, Pisa, Italy, 16-21 June 2013.

- Jones, E., Ojewole, E., Govender, T. Formulation and characterization of Hydroxypropyl methylcellulose and Eudragit RS100® co-blended polymeric films for transbuccal delivery of Didanosine. ***2nd International Conference & Exhibition on Pharmaceutical, Nutraceutical and Cosmeceutical Technology***, Kuala Lumpur, Malaysia, 21-22 November 2012.

Local:

- Jones, E., Ojewole, E., Govender, T. Formulation and characterization of Hydroxypropyl methylcellulose and Eudragit RS100® co-blended polymeric films for transbuccal delivery of Didanosine. ***33rd Annual Conference of the Academy of Pharmaceutical Sciences***, Rhodes University, Grahamstown, SA, 12-15 September 2012.

- Jones, E., Ojewole, E., Govender, T. Development of an assay method for Didanosine quantification in buccal polymeric films. ***6th International Conference on Pharmaceutical and Pharmacological Sciences***, Coastlands Hotel, Umhlanga, Durban, SA, 25-27 September 2011.

* The posters can be found in Appendix D & E.

ABSTRACT

The development of new chemical entities, novel drug delivery systems and alternative routes to deliver antiretrovirals (ARVs) are being explored to overcome the numerous limitations associated with HIV & AIDS drug therapy. Drug delivery via the buccal route has recently emerged as a promising alternative to delivery via the oral route. Drugs can directly enter the systemic circulation, bypass gastrointestinal degradation and first-pass hepatic metabolism, thereby increasing bioavailability. Although buccal permeation investigations with ARV drug solutions have confirmed their trans-buccal delivery potential, studies on their formulation into delivery systems are lacking. Rapid drug degradation of didanosine (DDI) in the gastrointestinal tract due to acid hydrolysis, together with the need for repetitive dosing, its short half-life, low oral bioavailability and dose-related toxicity, make DDI a suitable model ARV drug for buccal delivery. The aim of this study was therefore to design, evaluate and optimize the preparation of novel polymeric films for buccal delivery of DDI as a model ARV drug.

Multipolymeric monolayered films (MMFs) with drugs and polymers of opposing solubilities will offer several advantages for the controlled release delivery of DDI via the buccal route. The first aim of this study was therefore to prepare DDI loaded films with polymers of opposing solubilities and to undertake extensive physico-chemical/mechanical and molecular modelling characterisations. MMFs were prepared via a simplified solvent casting/evaporation method and characterised in terms of drug content uniformity, *in vitro* drug release, *in vitro* permeation, histomorphology, mucoadhesivity, mechanical properties and surface pH. Uniform drug content (91–105 %) with low variability was obtained for all films. Co-blending of DDI, Hydroxypropyl methylcellulose (HPMC) and Eudragit®RS 100 (EUD) (1:1:10) was required to achieve controlled drug release. The buccal permeability potential of DDI from the MMFs was successfully demonstrated with a permeability coefficient of $0.72 \pm 0.14 \times 10^{-2}$cm/h and a steady state flux of 71.63 ± 13.54 µg/cm^{2}h. Films had acceptable mucoadhesivity (2184 mN), mechanical strength (0.698 N/mm^2) and surface pH (6.63). The co-blending-co-plasticization technique for preparation of MMFs containing EUD and HPMC was justified via static lattice molecular mechanics simulations (SLAS). The mechanism inherent to the mucoadhesive and drug release profile performance of the MMFs was also elucidated via SLAS wherein a close corroboration among the in *vitro–in silico* (IVIS) data was observed. These extensive physico-mechanical and molecular atomistic studies confirmed the use of MMFs containing DDI, HPMC and EUD as a buccal delivery system.

A large portion of ARV limitations are related to inadequate drug concentrations reaching the site of action and low oral bioavailability. Recent developments in the field of buccal drug delivery show an increased interest towards nano-enabled drug delivery. The advantages of buccal drug delivery can be combined with that of the nanoparticulate delivery systems to provide a superior delivery system in terms of enhanced bioavailability and drug targeting. The second aim of the study was therefore to design, evaluate and optimize the preparation of novel nano-enabled polymeric films for buccal delivery of DDI as a model ARV drug. Solid lipid nanoparticles (SLNs) were prepared via a hot homogenization technique followed by ultrasonication and were characterized in terms of size, surface charge, morphology and drug entrapment efficiency (EE). Optimal parameters for preparation of the DDI loaded SLNs were identified before preparing and comparing the physico-mechanical properties of nano-enabled multipolymeric monolayered films (MMFs) to conventionally prepared MMFs. Glyceryl tripalmitate in combination with Poloxamer 188 as a surfactant was identified as being most suitable for preparation of DDI-loaded SLNs. Optimized particles exhibited a desired particle size (201 nm), polydispersity index (0.168), zeta potential (-18.8 mV) and formulation pH (5.5). Conventional and SLN entrapped MMFs were prepared via solvent casting/evaporation using EUD and HPMC in combination and characterised in terms of drug content, drug release, permeation, mucoadhesion and mechanical properties. Drug release from the nano-enabled films was higher, with 56 % released in the 1st hour as opposed to 20 % for the conventionally loaded MMFs. DDI was released from the buccal film and permeated across the mucosa as evidenced by steady state

flux values of 71.63 ± 13.54 µg/cm^{2}h and 74.39 ± 15.95 µg/cm^{2}h, for the conventional and nano-enabled MMFs, respectively. SLNs did not adversely affect the flux and confirms the potential of DDI being delivered via the buccal route using nano-enabled MMFs. Conventional MMFs exhibited higher mucoadhesion (1425.00 ± 77.15 mN) and mechanical strength (0.6976 ± 0.064 N/mm^2) than nano-enabled MMFs (914.33 ± 68.09 mN and 0.4930 ± 0.003 N/mm^2). These physico-mechanical studies confirm the potential use of nano-enabled MMFs containing DDI-loaded SLNs as a buccal delivery system and serves as a platform for future formulation optimisation studies.

These results confirm the feasibility of preparing films for buccal delivery of DDI as a model ARV drug that may ultimately lead to optimized drug therapy for HIV & AIDS patients.

Key words: Antiretrovirals, Didanosine, Buccal delivery, Films, Co-blended polymers, Permeation, Physico-mechanical properties, Solid lipid nanoparticles.

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LIST OF ABBREVIATIONS

3D	Three-dimensional	NaCl	Sodium chloride
AIDS	Acquired Immunodeficiency Syndrome	Na_2HPO_4	Disodium hydrogen phosphate
AMBER	Assisted Model Building and Energy Refinements	NaOH	Sodium hydroxide
ANOVA	Analysis of variance	NDDS	Novel drug delivery system
ARV	Antiretroviral	NLC	Nanostructured lipid carriers
AUC	Area under the curve	NNRTI	Non-nucleoside reverse transcriptase inhibitors
AZT	Zidovudine	NRTI	Nucleoside reverse transcriptase inhibitor
CCR5	Chemokine receptor 5	PBS	Phosphate buffered saline
CV	Coefficient of variation	PCS	Photon correlation spectroscopy
DDI	Didanosine	PDI	Polydispersity index
DLS	Dynamic light scattering	PEG	Polyethylene glycol
DNA	Deoxyribonucleic acid	PS	Particle size
DODAB	Dioctadecyldimethyl ammonium bromide	PSD	Particle size distribution
DSC	Differential scanning calorimetry	PVP	Polyvinylpyrrolidone
EE	Entrapment efficiency	RNA	Ribonucleic acid
EFV	Efavirenz	rpm	Revolutions per minute
EtOH	Ethanol	SA	Stearic acid
EUD	Eudragit	SD	Standard deviation
FDA	Food and Drug Administration	SDC	Sodium deoxycholate
FE-SEM	Freeze fracture electron microscopy	SEM	Scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy	SLAS	Static lattice atomistic simulations
GIT	Gastrointestinal tract	SLN	Solid lipid nanoparticle
GLY	Glycerol	SLS	Sodium lauryl sulphate
H&E	Hematoxylin and Eosin	SMT	Silicone molded tray
HAART	Highly active antiretroviral therapy	$t_{½}$	Half-life
HCl	Hydrochloric acid	TEER	Transepithelial electrical resistance
HIV	Human Immunodeficiency Virus	TEC	Triethyl citrate
HPMC	Hydroxypropyl methylcellulose	TEM	Transmission electron microscopy
IVIS	*In vitro – in silico*	TGA	Thermogravimetric analysis
KH_2PO_4	Potassium dihydrogen phosphate	TPA	Textural profile analysis
LM	Light microscopy	UNAIDS	Joint United Nations Programme on HIV/AIDS
MDF	Maximum detachment force	USA	United States of America
MeOH	Methanol	UV	Ultraviolet
MMF	Multipolymeric monolayered film	WHO	World Health Organization
MP	Melting point	XRD	X-ray diffractometry
MUC	Mucin	Z-ave	Mean particle size
MW	Molecular weight	ZP	Zeta potential

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CHAPTER ONE
INTRODUCTION

1.1 INTRODUCTION

This chapter provides an introduction and summarizes the background to the study. It outlines the challenges encountered with current antiretroviral therapy and buccal drug delivery systems and explores the rationale for and novelty of the study. It also covers the aim and objectives of the study.

1.2 BACKGROUND TO THE STUDY

Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS), commonly referred to as HIV & AIDS, have remained one of the leading causes of death worldwide, and is a major cause of mortality in sub-Saharan Africa (Merson et al., 2008, WHO, 2013). While antiretrovirals (ARVs) have proven to be useful in the treatment and management of HIV & AIDS, several disadvantages, including extensive first pass metabolism, gastrointestinal degradation, low bioavailability and short half-lives (Li and Chan, 1999), limit their efficacy. Large doses, complex dosing regimens and multiple drugs contribute to reduced patient compliance (Chandwani et al., 2012). Poor drug solubility and limited membrane permeability also present formulation difficulties (Sharma and Garg, 2010), resulting in more effective treatment strategies needing to be developed.

The development of new chemical entities, novel drug delivery systems (NDDS) and alternative routes to deliver ARVs (Ojewole et al., 2008) are being explored to overcome these limitations. Novel drug delivery systems for ARVs receiving increased attention include sustained release matrix tablets (Sánchez-Lafuente et al., 2002b) ceramic implants (Benghuzzi, 2000), liposomes (Dubey et al., 2010) and nanoparticles (Kuo and Chung, 2011b). Alternate routes for ARV delivery under investigation include: transdermal (Gerber et al., 2008), nasal (Carvalho et al., 2013), vaginal (Johnson et al., 2010) and the buccal route (Ojewole et al., 2012, Xiang et al., 2002).

Drug delivery via the buccal route has recently emerged as a promising alternative to delivery via the oral route. Drugs can directly enter the systemic circulation, bypass gastrointestinal degradation and first-pass hepatic metabolism, thereby increasing

bioavailability (Hoogstraate and Wertz, 1998). The buccal mucosa is easily accessible and more permeable than skin (Squier and Hall, 1985), making this a suitable route for drug delivery in pediatrics and geriatrics. Formulating a drug into a controlled release, mucoadhesive buccal dosage form may further improve drug delivery and patient compliance (Morales and McConville, 2011). Several ARV drugs may therefore benefit from delivery via the buccal route.

To date, studies reporting on the delivery of ARVs via the buccal route remain limited. The majority of work thus far has focussed on *in vitro* drug permeability studies using only drug solutions of zalcitabine (Shojaei et al., 1999, Xiang et al., 2002), didanosine (Ojewole et al., 2012, Rambharose et al., 2013) and tenofovir (Rambharose et al., 2013). Furthermore, the only available research paper on buccal polymeric dosage forms of ARVs is of zidovudine polymeric patches recently produced by Reddy et al. (2012). Characterization studies were limited and do not include critical parameters such *as in vitro* permeation or mechanical properties. ARV buccal drug delivery systems have not been comprehensively investigated or characterised, and a clear need exists for formulation optimization in this field.

Various mucoadhesive buccal dosage forms being investigated for different classes of drugs, include adhesive tablets (Cappello et al., 2006), gels (Ayensu et al., 2012b), ointments (Petelin et al., 2004), patches (Vasantha et al., 2011), and more recently, films (Abruzzo et al., 2012, Sievens-Figueroa et al., 2012). Polymeric films are flexible and comfortable, and can circumvent the relatively short residence time of oral gels on the mucosa (Ahn et al., 2001, Okamoto et al., 2001). Polymeric films formulated for controlled drug release could also decrease dose related side effects and improve patient compliance. Therefore, buccal films for delivery of ARVs would be ideal due to their numerous advantages over other buccal dosage forms.

A polymer for buccal films ought to adhere easily and sufficiently to the buccal mucosa, must have sufficient mechanical strength, should demonstrate penetration enhancement and provide for controlled release of the drug. Single polymers often fail to demonstrate all the ideal characteristics. To overcome this problem, researchers have been focusing on blending polymers with similar solubilities (Abruzzo et al., 2012, Dubolazov et al., 2006, Juliano et al., 2008). For controlled drug release, good mucoadhesion and suitable mechanical strength, polymers and drugs of opposing solubilities may often be required. While multipolymeric multilayered films and wafers

have been prepared with drugs and polymers of opposing solubilities (Ding et al., 2012, Perugini et al., 2003), monolayered multipolymeric films (MMFs) offer more advantages i.e. lower production costs, improved drug release, mucoadhesivity and size (Perugini et al., 2003). Reports on formulation and characterization studies on MMFs with polymers and drugs of opposing solubilities are limited. Furthermore, the methods used to produce the aforementioned MMFs require carcinogenic solvents (Perugini et al., 2003), involve the combination of two separate mixtures under high shear rates (Pendekal and Tegginamat, 2012), require emulsification below room temperature (Perumal et al., 2008b), or need multiple solvents with additional emulsifiers (Vasantha et al., 2011).

Didanosine (DDI) is a nucleoside reverse transcriptase inhibitor (NRTI), acts by competitive inhibition of HIV-1 reverse transcriptase, and can also be incorporated into the growing viral DNA chain to cause chain termination (Katzung et al., 2003). DDI is currently faced with many limitations. Rapid drug degradation of DDI in the gastrointestinal tract due to acid hydrolysis, together with the need for repetitive dosing, its short half-life, low oral bioavailability and dose-related toxicity, make DDI a suitable model ARV drug for incorporation into a novel buccal delivery system.

DDI is currently not used as first-line therapy of HIV & AIDS due to its numerous limitations (Katzung et al., 2003, Rossiter, 2012). It should be noted that internationally, the trend is for scientists to reformulate old/disused drugs into superior delivery systems to improve efficacy and overcome limitations. This eliminates the high cost of developing new chemical entities and provides a cost effective alternative for optimization of drug delivery (Langer, 1990). For example, DDI is being reported as a drug suitable for development into novel drug delivery systems, such as enteric coated bioadhesive matrix tablets (Deshmukh et al., 2003), polymeric nanoparticles (Al-Ghananeem et al., 2010), and transdermal delivery systems (Kim and Chien, 1996). The formulation of DDI into a buccal NDDS has not been reported in the literature, therefore DDI is an ideal model ARV for investigation in this study.

Recent developments in the field of buccal drug delivery show an increased interest in nano-enabled buccal drug delivery systems. The advantages of buccal drug delivery can be combined with that of the nanoparticulate drug delivery systems, as discussed further in chapter 2. A very limited number of studies have been reported to date in this emerging field and antiretrovirals remain to be investigated.

The following important points have therefore been identified in this study:

- More effective treatment strategies for HIV & AIDS are urgently required.
- ARV drugs such as DDI currently face numerous limitations and may benefit from being formulated into a NDDS.
- Although buccal drug delivery offers many advantages, formulations for this route have not been investigated in depth for ARVs.
- Formulation and characterization studies on MMFs with polymers and drugs of opposing solubilities are limited and the preparation methods needs careful consideration.
- Emerging trends indicate nano-enabled buccal films could offer more benefits compared to conventional films and ARVs remain to be investigated in this emerging field.

To date, no studies have been done to establish if it is possible to design, evaluate and optimize the preparation of novel polymeric films for buccal delivery of DDI as a model ARV drug. This project therefore focused on developing a novel drug delivery system (controlled release polymeric films) to deliver DDI as a model ARV via an alternative route (transbuccal) to improve drug delivery.

1.3 AIM AND OBJECTIVES OF STUDY

The aim of the study was to design, evaluate and optimize the preparation of novel polymeric films for buccal delivery of DDI as a model ARV drug.

In order to accomplish this aim, the objectives of the study were to:

1. Identify optimal process and formulation variables for the preparation of monolayered multipolymeric films containing DDI.
2. Evaluate the films in terms of of drug content uniformity, drug release, permeability, mucoadhesivity, mechanical properties and surface pH.
3. Perform static lattice atomistic simulations (SLAS) to identify the suitability of the polymeric blend for buccal film formulations and to identify correlations between *in vitro* and *in silico* (IVIS) results.
4. Undertake preliminary formulation studies on nano-enabled polymeric films for buccal delivery of ARVs.

1.4 SIGNIFICANCE OF THE STUDY

The formulation of DDI-loaded multipolymeric mucoadhesive films offers a novel and promising concept for enhanced drug therapy via the buccal route. The potential benefits of formulating a drug delivery system proposed in this study may include the following:

- In the absence of any antiretroviral buccal delivery systems commercially available in South Africa or internationally, a successful system could be of considerable value to HIV & AIDS patients worldwide. Cost-effective dosage forms could be developed that could lead to a reduction in healthcare costs in South Africa.

- The development of this technology and polymeric system could also lend itself to the formulation of mucoadhesive systems for other routes (vaginal, rectal, and ocular), and for a wide range of disease conditions significantly affecting South Africa and other countries globally, e.g. diabetes, hypertension and communicable diseases such as tuberculosis.

- The multipolymeric films proposed in this study could facilitate the loading of multiple drugs, which may be insoluble or incompatible with conventional monopolymeric systems.

1.5 NOVELTY OF STUDY

Monolayered multipolymeric films (MMFs) with drug and polymers of opposing solubilities for buccal drug delivery have been formulated previously in our laboratory (Perumal et al., 2008b), however, the MMFs reported in this study is novel for a number of reasons.

- Although buccal permeation studies with antiretroviral drug solutions have confirmed their delivery potential via this route in the literature, detailed studies on their subsequent formulation into a delivery system are lacking. While the buccal permeation properties of DDI solutions have been reported, this study is the first to report on its incorporation into a buccal delivery system and subsequent detailed characterization including essential parameters such as permeation from the dosage form.

- While several studies on monolayered films with drugs and polymers of similar solubilities are reported in the literature, there are very few studies on these films with drugs and polymers of opposing solubilities. The advantages of the latter for providing multifunctional properties have been highlighted in the literature. This study reports for the first time on a simplified method compared to those in the literature for preparation of these MMFs. The method used in this study eliminates the need for carcinogenic or multiple solvents and emulsifiers, can be done at room temperature, and does not require special equipment such as a homogenizer. In addition, detailed physico-mechanical evaluations, essential for optimisation of these MMFs, are lacking in the current literature.

- To the best of my knowledge, this is the first time that molecular modelling on buccal polymeric film formulations has been done to identify the mechanism of interaction between these two polymers (EUD & HPMC) and their suitability for combined use. This led to a mechanistic understanding of film formation as well as mucoadhesivity and drug release properties.

- This study reports on the first nano-enabled buccal MMF using SLNs for delivery of an ARV. This technology may serve as a platform for developing future nano-enabled buccal MMFs for other antiretrovirals as well as other disease conditions.

1.6 OVERVIEW OF DISSERTATION

Chapter 1 provides an introduction and summarizes the background to the study. It outlines the challenges encountered with current antiretroviral therapy and buccal drug delivery systems and explores the rationale for and novelty of the study. It also covers the aim and objectives of the study.

Chapter 2 is a literature review, focusing on HIV & AIDS drug therapy and strategies to address its current limitations. The chapter particularly focuses on buccal drug delivery and polymeric films as a drug delivery strategy. An overview of buccal drug delivery is presented and different types of buccal drug delivery systems are outlined. Furthermore, various preparation methods and characterization techniques of buccal polymeric films are elucidated before highlighting emerging work in the field of nano-enabled buccal drug delivery. Finally, DDI as a model ARV is elaborated upon.

Chapter 3 (publication) is a first-author article reporting on novel work published in an ISI international journal. The chapter is presented in the required format of the journal and is the final revised accepted version. It describes the development of novel monolayered multipolymeric buccal films with drug and polymers of opposing solubilities using DDI as model ARV drug.

Chapter 4 (manuscript) is a first-author manuscript submitted to an ISI international journal. The chapter is presented in the required format of the journal and is the final version submitted for review. It explores the use of nano-enabled polymeric films. More specifically, it summarizes work done using solid lipid nanoparticles entrapped into films for buccal delivery of DDI.

Chapter 5 describes the conclusions and future recommendations from the study to optimise the buccal delivery system.

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CHAPTER TWO
HIV & AIDS AND BUCCAL DRUG DELIVERY

2.1 INTRODUCTION

This chapter presents a review of the literature of the theoretical concepts on HIV & AIDS and buccal drug delivery. The chapter focuses on HIV & AIDS drug therapy and strategies to address its current limitations. An overview of buccal drug delivery is presented and different types of buccal drug delivery systems are outlined. Furthermore, various preparation methods and characterization techniques of buccal polymeric films are elucidated, before highlighting emerging work in the field of nano-enabled buccal drug delivery. Finally, didanosine as a model ARV is elaborated upon.

2.2 INTRODUCTION TO HIV & AIDS

Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS), commonly referred to as HIV & AIDS, have remained one of the leading causes of death worldwide, and is a major cause of mortality in sub-Saharan Africa (Merson et al., 2008, WHO, 2013). While antiretrovirals (ARVs) have proven to be useful in the treatment and management of HIV & AIDS, several disadvantages currently exist with respect to drug therapy (Carpenter et al., 2000).

According to estimates from the UNAIDS (2012) Report on the Global AIDS Epidemic, approximately 34 million people worldwide were living with HIV by the end of 2011, 2.5 million new infections and 1.7 million AIDS-related deaths were reported in 2011 alone. When compared to global statistics, sub-Saharan Africa is the most affected region, with nearly one in every 20 adults living with HIV. More than two-thirds (69 %) of all people worldwide who are infected with HIV live in sub-Saharan Africa. It is estimated that more than 90% of all children newly infected with HIV in 2011 live in sub-Saharan Africa (UNAIDS, 2012). Despite the increased global and local interventions, such as patient counselling, increased awareness, education and improved drug supply, much remains to be accomplished, as the number of new infections remains disproportionately high. HIV is most commonly transmitted via vaginal or anal sexual intercourse. Other possible means of infection include sharing of contaminated needles among drug users, transfusion of contaminated blood products and transmission from mother-to-child during pregnancy, labour or breastfeeding (das Neves et al., 2010).

From the two known species of HIV, HIV-1 is globally more prevalent than HIV-2 (Lever, 2009). HIV-2 is associated with slower progression to immunodeficiency and is more prevalent in West Africa (das Neves et al., 2010). HIV is a retrovirus known for its ability to use its reverse transcriptase enzyme to convert the ribonucleic acid (RNA) genome to double-stranded deoxyribonucleic acid (DNA). The DNA is then integrated into the chromosomes of the infected host cells where it is termed a provirus (Lever, 2009). The structure of the HIV viron is shown in Figure 2.1. The viral genome contains three structural genes i.e. *gag*, *pol* and *env*. Respectively, these genes code for important antigens (*gag gene*); viral enzymes such as reverse transcriptase, integrase & protease (*pol gene*), gp120 and gp41 glycoproteins, responsible for recognizing the CD4+ receptor and the CCR5 or CXCR4 chemokine receptors of the host cell membrane; and for virus/cell fusion (*env gene*) (das Neves et al., 2010, Lever and Jeang, 2006, Lever, 2009). The virus mostly infects T-helper lymphocytes (CD4+) but may also infect macrophages. The defining characteristic of AIDS is the depletion of CD4+ cells (T-helper lymphocytes). The ensuing immunosuppression may result in opportunistic infections such as *Mycobacterium tuberculosis* (Stoddart and Reyes, 2006).

An understanding of the processes involved in the HIV lifecycle is important for developing innovative therapeutic strategies to suppress or eliminate the virus.

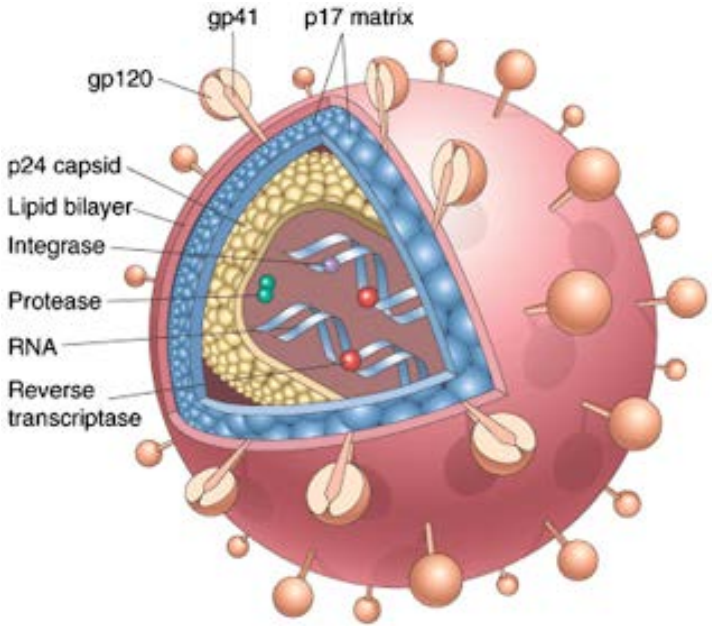

Figure 2.1: Structure of HIV viron (MBBS Medicine (Humanity First), 2013).

2.3 HIV & AIDS DRUG THERAPY AND ITS CURRENT LIMITATIONS

The goal of treating an established HIV infection with antiretroviral therapy is to achieve durable suppression of viral replication (i.e. an undetectable viral load). This is generally achieved using a combination of three or more antiretrovirals, known as highly active antiretroviral therapy (HAART) (Rossiter, 2012). Presently, there are five major classes of antiretroviral drugs used to treat people infected with HIV, namely reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, integrase inhibitors and more recently, CCR5 antagonists which is also known as entry inhibitors. Figure 2.2 shows the site of action of the major classes of ARVs during the HIV lifecycle.

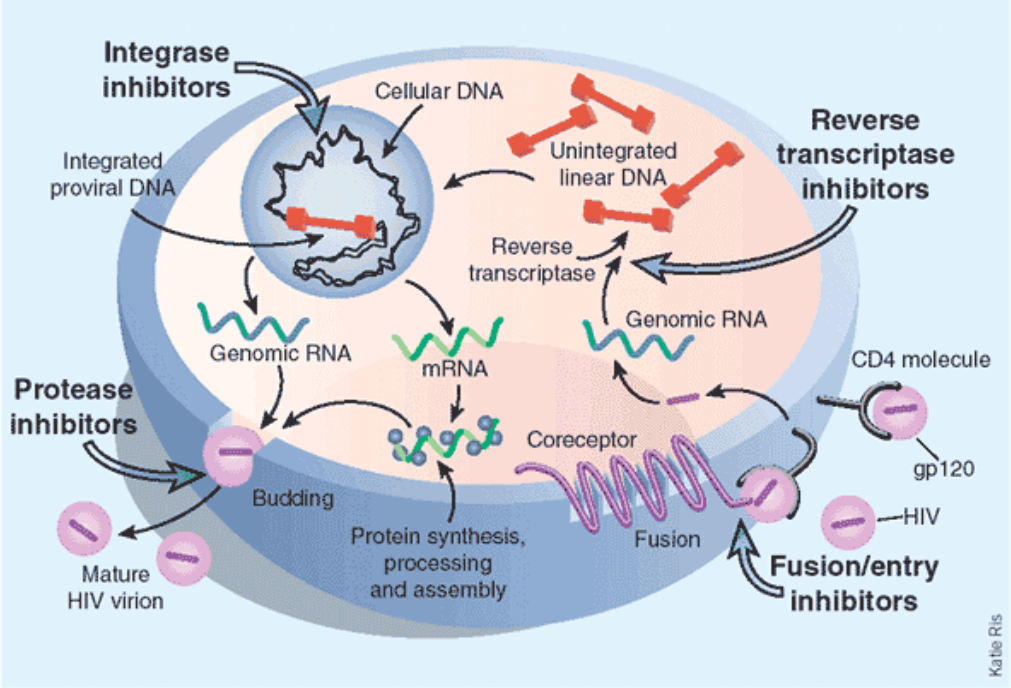

Figure 2.2: Sites of drug action during HIV lifecycle (University of Arizona, 2013).

The currently available drugs suppress the virus, even to undetectable levels. Hence, people with HIV need to continuously take antiretroviral drugs (National Institute of Allergy and Infectious Diseases, 2013). Antiretrovirals may be used to prevent infection following accidental exposure, to prevent transmission from mother to child, or to treat established HIV infection. The goal of treating established HIV infection is to achieve durable suppression of viral replication. As HIV reproduces itself, variants of the virus

emerge, including some that are resistant to antiretroviral drugs. Therefore, guidelines recommend that people infected with HIV take a combination of antiretroviral drugs known as HAART. This strategy, which typically combines drugs from at least two different classes of antiretroviral drugs, has been shown to effectively suppress the virus when used properly. HAART has revolutionised how people infected with HIV are treated, and works by suppressing the virus and decreasing the rate of opportunistic infections (National Institute of Allergy and Infectious Diseases, 2013).

Fixed-dose combination tablets have emerged in an effort to facilitate combining two or more ARV drugs of a HAART regimen, and to improve patient compliance by reducing the total daily pill burden (Zolopa, 2010). However, fixed-dose combinations should not be prescribed for patients requiring dose adjustments, such as in the case of hepatic or renal impairment or in young children. The fixed dose combination containing emtricitabine (FTC), tenofovir (TDF) and efavirenz (EFV), which is a single pill on a once-a-day regimen marketed as Atripla, and has been welcomed as an expanded effort to combat the HIV & AIDS crisis (Zolopa, 2010).

Drugs currently available for the treatment of HIV & AIDS vary significantly in their pharmacokinetic properties, as highlighted in Table 2.1. The ARVs with low oral bioavailability and a short half-life are set to benefit from being reformulated into novel drug delivery systems. Although more than twenty ARVs have been approved for treating HIV, a need still exists to develop new chemical entities, due to ever increasing drug resistance and unavoidable side effects. Among the newer drugs under investigation are PRO 140 (EI), TNX-355 (EI), BMS-663068 (EI), Cenicriviroc (EI), Dolutegravir (II), Lersivirine (NNRTI), KP-1461 (NRTI), Elvucitabine (NRTI), Racivir (NRTI) and Festinavir (NRTI) undergoing phase II clinical trials at the Food and Drug Administration (FDA) in the USA. Elvitegravir (II) and Apricitabine (NRTI) are showing promising results in phase III trials. Vivecon is currently the only candidate in a potentially new class of ARVs called maturation inhibitors, and is undergoing phase III clinical trials (Avert, 2013).

Although ARV drug therapy has contributed significantly to improved disease management, annual mortality rates due to HIV & AIDS are still alarmingly high, with approximately two million deaths reported globally. Of concern is that as recently as the end of 2011, nearly seven million people eligible for HIV treatment still did not have access to suitable and affordable drug therapy (UNAIDS, 2012).

Whilst ARVs have proven to be useful in the treatment and management of HIV & AIDS, several disadvantages and limitations currently exists. Many of the ARVs undergo extensive first pass hepatic metabolism and gastrointestinal degradation, which leads to reduced bioavailability (Table 2.1). The short half-lives of several ARVs (Table 2.1) necessitates frequent administration of doses, thereby leading to reduced patient compliance (Li and Chan, 1999).

There are concerns regarding adverse effects associated with long-term usage of HAART, such as HIV associated lipodystrophy, central adiposity, dyslipidaemia, hyperlipidaemia, hyperglycaemia and insulin resistance (Behrens et al., 2000, Vigouroux et al., 1999). The major contributing factor to ARV related side effects can be attributed to the inadequate drug concentrations reaching the site of action, and the low bioavailability of several ARV drugs, necessitating the use of large doses to achieve a therapeutic effect. Many of the currently available tablet formulations (Table 2.1) are very large and pose swallowing difficulties, especially for geriatric and paediatric patients. High doses, complex HAART dosing regimens, physical size limitations and side effects from multiple drugs all contribute to reduced patient compliance (Chandwani et al., 2012).

Poor drug solubility and limited membrane permeability also pose formulation difficulties (Sharma and Garg, 2010). HIV, being localised to inaccessible compartments in the human body, such as the lymphatic system, central nervous system and within macrophages, results in yet another treatment challenge. Therapeutic drug concentrations cannot be achieved in these compartments by the majority of ARVs, and the necessary plasma drug concentrations fail to be maintained at the site of HIV localisation for the required extent of time (Vyas et al., 2006a).

Table 2.1: Available ARV drugs, their classes, dosage forms and pharmacokinetic properties.

Antiretroviral	Half-life (h)	Tmax (h)	Oral Bioavailability (%)	Dosage Form
Nucleoside/Nucleotide reverse transcriptase inhibitors (NRTI)				
Zidovudine (AZT)	1.1	0.5-1.5	60	Capsule, tablet, syrup, injection
Lamivudine (3TC)	3-6	0.9	86	Tablet, oral solution
Emtricitabine (FTC)	10	1-2	93	Tablet
Abacavir (ABC)	1-2	1-1.5	83-100	Tablet, oral solution
Tenofovir DF (TDF)	17	1-2	25-39	Tablet
Didanosine (DDI)	1.3-1.6	0.6-1	30-40	Tablet, capsule (EC), powder for reconstitution
Stavudine (D4T)	1-1.6	0.5-0.75	80	Capsule, powder for reconstitution
*Zalcitabine (DDC)	1-3	0.8-1.5	85	Tablet
Non-nucleoside reverse transcriptase inhibitors (NNRTI)				
Rilpivirine (RPV)	50	4-5	Unknown	Tablet
Etravirine (ETR)	30-40	2.5-4	Unknown	Tablet
Delavirdine (DLV)	5.8	1.2	85	Tablet
Efavirenz (EFV)	40-50	5	42-80	Tablet, capsule
Nevirapine (NVP)	25-30	1.5	>90	Tablet, suspension
Protease Inhibitors (PI)				
Tipranavir (TPV)	6	3	No data	Capsule, oral solution
Indinavir (IDV)	1.2	0.8	65	Capsule
Saquinavir (SQV)	1.5-2	> 1 fasting, 3 with food	Erratic, 4	Tablet, capsule
Lopinavir/ritonavir (LPV/r)	5-6 (LPV)	No data	No data	Tablet, oral solution
**Amprenavir	7-10	1-2	No data	Liquid-filled capsule
***Fosamprenavir (FPV)	7.7	1.5-4	No data	Tablet, suspension
Ritonavir (RTV)	3-5	3.4	65	Tablet, capsule, oral solution
Darunavir (DRV)	15	2.5-4	37	Tablet, suspension
Atazanavir (ATV)	7	2.5	No data	Capsules
Nelfinavir (NFV)	3.5-5	3.4-4	20-80	Tablet, oral powder
Fusion & Entry Inhibitors (FI & EI)				
Enfuvirtide (ENF)	3.8	8	84	Injection
Maraviroc	14-18	No data	23-33	Tablets
Integrase Inhibitors (II)				
Raltegravir	9	3	No data	Tablet

*Zalcitabine was discontinued in 2006. **Amprenavir was discontinued in 2004; a prodrug version (***fosamprenavir) is currently available. Data obtained from: (Ojewole et al., 2008, Li and Chan, 1999, Rossiter, 2012, drugs.com, 2013)

2.4 STRATEGIES TO ADDRESS LIMITATIONS OF ARV DRUGS

The identification of new drugs and chemical modification of existing ARV drugs (Hartman and Buckheit, 2012), the design and development of novel drug delivery systems (Benghuzzi, 2000, Dutta et al., 2007, Saravanakumar et al., 2010) and investigation of alternative routes to deliver ARVs (Carvalho et al., 2013, Patel et al., 2012, Rambharose et al., 2013) are being explored to overcome the current limitations associated with ARV therapy. Novel drug delivery systems (NDDS) have been identified as a useful tool by formulation scientists to enhance drug delivery, and have contributed significantly in the past decade to augment various classes of drugs including ARVs. Table 2.2 provides a summary of some of the formulation studies exploring NDDS for delivery of ARVs.

The rationale for developing these NDDS clearly shows that formulation modification serves as an effective strategy to overcome current limitations. It can also be seen from Table 2.2 that a wide range of ARVs have been receiving increased attention, specifically in the last decade. For these ARVs, numerous NDDS have been explored to overcome the specific ARV's limitations. Examples of novel drug delivery systems that have been explored include sustained release matrix tablets (Sánchez-Lafuente et al., 2002b) ceramic implants (Benghuzzi, 2000), liposomes (Dubey et al., 2010) and nanoparticles (Kuo and Chung, 2011b).

Along with developing NDDS for ARVs, researchers have also explored various alternate routes to improve drug delivery of ARVs other than the conventional oral route. Alternate routes for delivery under investigation include: transdermal (Gerber et al., 2008), nasal (Carvalho et al., 2013), vaginal (Johnson et al., 2010) and buccal delivery (Ojewole et al., 2012, Xiang et al., 2002). Table 2.3 provides an extensive summary of formulation studies exploring alternate routes for delivery of ARVs. The rationale for selecting the route, as well as different formulations and ARVs being investigated per route are summarized. It can be seen that the focus to date has been mainly on the delivery of single ARVs via these routes. More research into multi-ARV delivery systems is required. From Table 2.3 it can also be seen that the majority of studies have been focusing on the transdermal and nasal routes of administration of ARVs. This may be due to the higher levels of patient compliance associated with these routes. Conversely, the rectal route appears to have received no attention in recent studies, possibly due to poor patient compliance associated with this route.

Many ARVs have been delivered via alternate routes, with zidovudine having received the most interest and being investigated for delivery via all the alternative routes.

For the buccal route, studies are limited compared to the transdermal route, and have focused mostly on permeability studies with ARV drug solutions rather than formulation studies. To date, studies reporting on the delivery of ARVs via the buccal route remain limited. The majority of work thus far has focussed on *in vitro* drug permeability studies using only drug solutions of zalcitabine (Shojaei et al., 1999, Xiang et al., 2002), didanosine (Ojewole et al., 2012, Rambharose et al., 2013) and tenofovir (Rambharose et al., 2013). The only available published paper on buccal polymeric dosage forms containing ARVs is of zidovudine polymeric patches recently produced by Reddy et al. (2012). Characterization studies were limited and did not include critical parameters such *as in vitro* permeation or mechanical properties. ARV buccal drug delivery systems have not been comprehensively investigated or characterised, and a clear need exists for formulation optimization in this field. This study focused on the development of a NDDS for delivery of an ARV via the buccal route. The following sections therefore provide an overview on buccal drug delivery and buccal films.

Table 2.2: Summary of formulation studies exploring novel drug delivery systems (NDDS) for delivery of ARVs.

NDDS	Antiretroviral	Rationale for Development	Reference
Enteric coated bioadhesive matrix tablets	Didanosine	Enteric-coating prevents acid-induced degradation of didanosine, whilst sustained-release and bioadhesive properties may further improve the drug's low oral bioavailability.	Deshmukh et al. (2003)
Extended release matrix tablets	Stavudine	Once daily, sustained release formulations reduce the frequency of administration and improve patient compliance.	Saravanakumar et al. (2010)
Suspensions	Indinavir	Subcutaneously administered lipid–drug complexes in suspension form can accumulate in lymph nodes at much higher levels than the soluble form of the drug where HIV localizes.	Kinman et al. (2003)
Gastroretentive tablet	Zidovudine	Improve the drugs low oral bioavaibility and provide sustained action through continuously releasing the drug.	Dalavi and Patil (2009)
Ceramic implants	Zidovudine	The sustained delivery of AZT from ceramic implantable capsules could be achieved and oral as well as intravenous side effects of AZT would be minimised.	Benghuzzi (2000)
Ethanolic liposomes	Indinavir	Penetration-enhancing quality of ethanol is well known. Ethanolic liposomes can transport drugs more effectively through the stratum corneum into the deeper layers of the skin than conventional liposomes.	Dubey et al. (2010)
Micelles / Microemulsions	Saquinavir	Saquinavir is lipophilic, poorly water-soluble and undergoes extensive first-pass metabolism. By formulating it as a lipid formulation that targets intestinal lymphatic transport, oral bioavailability may be improved.	Griffin and O'Driscoll (2006)
Niosomes	Tenofovir	Niosomes offers greater stability than liposomes and are more cost-effective. Colloidal drug carrier system would be cleared by the mononuclear phagocytes system where HIV localizes. No pediatric liquid formulation of tenofovir is available.	Zidan et al. (2011)
Polymeric micelles	Efavirenz	Liquid pediatric formulation of EFV not available. Polymeric micelles would improve the aqueous solubility and the oral bioavailability of the drug.	Chiappetta et al. (2009)
Dendrimers	Efavirenz	Due to their highly branched, synthetic, monodispersed nature they can be useful for targeted drug delivery of ARVs.	Dutta et al. (2007)
Nanopowders	Saquinavir	The dissolution rates of poorly soluble drugs can be enhanced by milling thereby increasing GIT absorption and/or membrane permeation.	Branham et al. (2012)

Table 2.3: Summary of formulation studies exploring alternate routes for delivery of ARVs.

Route of Administration	Formulation	Antiretroviral	Reference	Rationale of Route Selected
Transdermal	Suspension	Zidovudine	Jin et al. (2000)	Drugs exhibit dose dependent toxic side effects. Controlled drug delivery systems are preferred for long-term treatment of HIV/AIDS. Noninvasive zero-order delivery via the transdermal route would be desirable.
	Liposomes	Lamivudine	Pai and Devi (2009)	
	Pheroid™	Stavudine	Holmes et al. (2010)	
	Alcoholic solutions	Zalcitabine	Kim and Chien (1995)	
	Gel	Zidovudine	Pokharkar et al. (2010)	
	Niosomal gel	Lopinavir	Patel et al. (2012)	
Rectal	Sustained-release suppository	Zidovudine	Kawaguchi et al. (1991)	Avoidance of hepatic first pass metabolism and extensive GIT degradation thereby increasing drug bioavailability.
	Solution	Didanosine*	Wintergerst et al. (1999)	
	Solution	Zidovudine	Wintergerst et al. (1997)	
Vaginal	Polyurethane intravaginal ring	Dapivirine & Tenofovir	Johnson et al. (2010)	Anti-HIV microbicide can block transmission of HIV at the vaginal mucosal epithelium. This route is not used to achieve systemic drug concentrations.
	Intravaginal bioadhesive polymeric device	Zidovudine	Ndesendo et al. (2011)	
	Gel	Tenofovir	Abdool Karim et al. (2010)	
Nasal	Suspension	Zidovudine	Seki et al. (1994)	Allows for painless, minimally invasive, self-administration of drugs and can also bypass the blood–brain barrier when used together with nanoparticles. Avoiding first-pass metabolism and oral administration side effects. Rapid absorption can be achieved due to the highly vascularized nature.
	Micelles	Efavirenz	Chiappetta et al. (2013)	
	Liquid crystal precursor	Zidovudine	Carvalho et al. (2013)	
	Polymeric Nanoparticles	Efavirenz	Seremeta et al. (2013)	
	Polymeric Nanoparticles	Didanosine	Al-Ghananeem et al. (2010)	
Buccal	Solution	Didanosine	Ojewole et al. (2012)	Bypasses hepatic first pass metabolism and GIT degradation, resulting in increased drug bioavailability. It has higher permeability the skin, has a relatively large surface area and good accessibility.
	Solution	Zalcitabine	Xiang et al. (2002)	
	Solutions	Tenofovir or Didanosine	Rambharose et al. (2013)	
	Patches	Zidovudine	Reddy et al. (2012)	

* This study demonstrated suitable rectal absorption of didanosine cannot be achieved.

2.5 BUCCAL DRUG DELIVERY

Drug delivery via the buccal route has recently received increased interest in the literature as an alternative to oral and other conventional routes of administration, due to its numerous advantages over these routes. A number of reviews have been published on the structure of the oral cavity (Squier and Kremer, 2001), mucoadhesion mechanisms (Smart, 2005a), drug delivery via the buccal route (Patel et al., 2011, Shojaei, 1998, Hoogstraate and Wertz, 1998), buccal dosage forms (Sudhakar et al., 2006, Nair et al., 2013, Madhav et al., 2009, Morales and McConville, 2011) and buccal permeation enhancement (Şenel and Hıncal, 2001, Nicolazzo et al., 2005, Hassan et al., 2010). This section therefore serves only as an overview of the relevant elements essential to this study.

2.5.1 Overview of the Oral Mucosa

The oral cavity is lined with the oral mucosa, and includes the buccal, sublingual, gingival and palatal mucosa. The uppermost layers of the oral mucosa are comprised of closely compacted epithelial cells (Figure 2.3) the function of which is to protect the underlying tissues from damage and fluid loss (Patel et al., 2011). Below the epithelial layer are the basement membrane, lamina propria and submucosa. This epithelium is comparable to stratified squamous epithelium found in other areas of the body. It has a mitotically active basal cell layer (basement membrane) (Figure 2.3) that gives rise to a number of intermediate differentiating cell layers, with the outermost layers being sloughed off (Haas and Lehr, 2002). The buccal mucosal epithelium consist of approximately 40–50 cell layers, whereas the sublingual epithelium consist of comparatively fewer layers (Madhav et al., 2009).

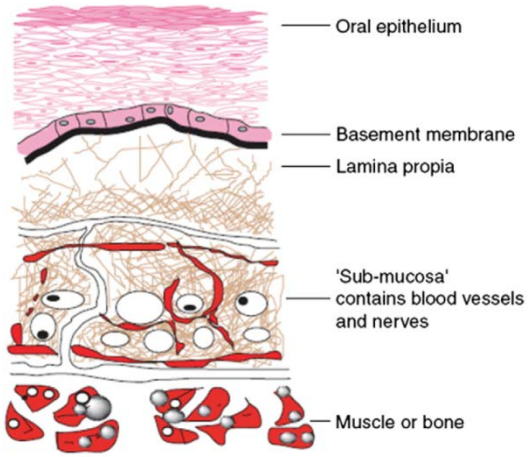

Figure 2.3: Schematic representation of buccal mucosa (Smart, 2005b).

There are three categories of drug delivery within the oral cavity (i.e. sublingual, buccal and localized drug delivery). Selecting one over another is mainly based on anatomical and permeability differences that exist among the various oral mucosal sites (Xiang et al., 2002). The buccal and sublingual mucosa is non-keratinized with relatively good permeability (Table 2.4), making them potential candidates for systemic delivery of drugs via an alternate route. As the main aim of this study was the preparation of novel polymeric films for buccal delivery of didanosine, this route would be reviewed in depth.

Table 2.4: Characteristics of the oral mucosal delivery sites.

Site	Structure	Thickness (μm)	Turnover Time (days)	Surface Area (cm^2±SD)	Permeability	Residence Time	Blood Flow*
Buccal	NK	500-600	5-7	50.2 ± 2.9	Intermediate	Intermediate	20.3
Sublingual	NK	100-200	20	26.5 ± 4.2	Very good	Poor	12.2
Gingival	K	200	-	-	Poor	Intermediate	19.5
Palatal	K	250	24	20.1 ± 1.9	Poor	Very good	7.0

Adapted from (Patel et al., 2011) [NK = non-keratinized, K = Keratinized, * In rhesus monkeys (mL/min/100g tissue)]

Buccal transportation mainly occurs via passive diffusion across lipid membranes, either through paracellular or transcellular pathways (Figure 2.4). This makes buccal drug delivery suitable for transporting both hydrophilic and lipophilic drugs (Patel et al., 2011). Hydrophilic drugs would be limited to the hydrophilic regions of the paracellular spaces and cytoplasm. Likewise, lipophilic drugs would favour penetration through the lipophilic cell membrane of one cell directly into the next until the systemic circulation is reached (Shojaei, 1998). While a drug can make use of both pathways simultaneously, one route would be predominant depending on the balance of the drug's physico-chemical properties.

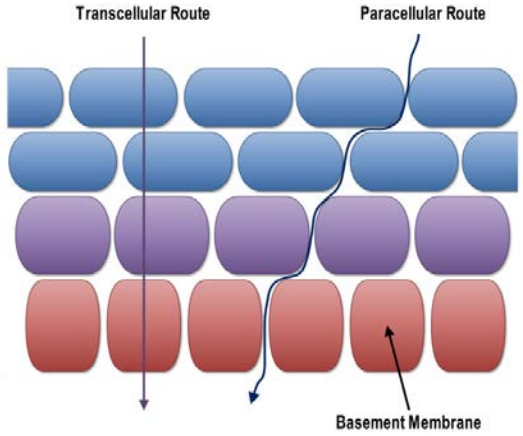

Figure 2.4: Schematic representation of different routes of drug permeation. Adapted from (Patel et al., 2011).

2.5.2 Advantages of Drug Delivery via the Oral Mucosa

Drug delivery via the buccal route can be considered as a favourable alternative to oral and other conventional routes of administration for the following reasons:

- Drugs that are absorbed through the buccal mucosa directly enter the systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism in the liver leading to improved bioavailability (Madhav et al., 2009).
- The buccal mucosa is relatively permeable and robust in comparison to other mucosal tissues (Patel et al., 2011).
- The buccal mucosa has a smooth and relatively immobile surface, which is easily accessible, and makes self-application and removal of the delivery system easy (Madhav et al., 2009).
- The permeability of the buccal mucosa is higher than that of skin (Squier and Hall, 1985). Hence, a lower loading dose in a buccal device could provide the same therapeutic effect as a transdermal patch.
- Buccal delivery is also a potential attractive delivery system for pediatrics as well as for patients with swallowing difficulties.
- The advantages of a buccal delivery system can further be increased by formulating the drug into a controlled release dosage form. This will lead to a reduction of dose related side effects and improved patient compliance.

2.5.3 Disadvantages of Drug Delivery via the Oral Mucosa

There are some disadvantages of using the buccal route for drug delivery, which includes low mucosal permeability of certain drugs, a continuous secretion of saliva leading to dilution of drug and the need for formulation approaches to promote retention on the mucosae (Patel et al., 2011). Nonetheless, the distinct advantages render the disadvantages of this route much less significant in comparison to its therapeutic benefits.

2.5.4 Candidate Drugs and Disease States

Buccal mucoadhesive dosage forms have been developed for numerous types of drugs and disease conditions. Drugs with short half-lives requiring prolonged effects, or having low membrane permeability, with sensitivity to enzymatic or acidic degradation in the GIT and poor solubility may be successfully delivered via mucoadhesive oral delivery systems (Ahuja et al., 1997).

Treatment of systemic disease conditions in addition to local oral diseases may be achieved using buccal drug administration. Fluconazole (Yehia et al., 2009) and metronizazole (El-Kamel et al., 2007) are a few of the drugs being investigated for enhanced local effects by incorporating them into mucoadhesive buccal dosage forms. Drugs that undergo gastrointestinal degradation may benefit from buccal delivery. Several peptides, including insulin (Giovino et al., 2012) and lysozyme (Morales et al., 2013), have been reported to successfully be delivered via the buccal route, thereby avoiding their GIT degradation.

Drugs such as propranolol (Abruzzo et al., 2012) and carvedilol (Rana and Murthy, 2013), which are used in hypertension, undergo significant first past metabolism and may benefit considerably from being delivered via this route. Examples of other drugs and disease states potentially benefitting from being delivered bucally include: salbutamol sulphate used in asthma (Vasantha et al., 2011), glibenclamide for diabetes mellitus (Muzib and Kumari, 2011), griseofulvin (Meng et al., 2011) as systemic antimicrobial and ARVs such as zalcitabine (Xiang et al., 2002) or didanosine (Ojewole et al., 2012) for HIV & AIDS treatment. Thus, both non-communicable and communicable diseases may benefit from using this route of drug administration.

2.5.5 Development of Buccal Dosage Forms

Several conventional and novel buccal dosage forms have been developed in the past two decades. They include solutions (Ungphaiboon and Maitani, 2001), sprays, ointments (Petelin et al., 2004), gels (Martin et al., 2003), lozenges (Codd and Deasy, 1998), tablets (Boyapally et al., 2010, Cappello et al., 2006), powders, chewing gums, patches (Cavallari et al., 2013, Perioli et al., 2004), wafers (Ayensu et al., 2012a) and films (Abruzzo et al., 2012, El-Kamel et al., 2007, Prodduturi et al., 2005). They are categorized into three types namely: liquid, semi-solid or solid formulations (Sudhakar et al., 2006).

Solid buccal formulations, such as tablets and lozenges, are produced commercially more often than liquids, semi-solids or even buccal films. Only a few buccal films or patches have successfully entered the pharmaceutical market, with most being designed to release drugs rapidly in order to produce a fast onset of action. A prime example is fentanyl buccal films, marketed as Onsolis® used in cancer breakthrough pain management (Twycross et al., 2012). Limitations associated with buccal films, including uncontrolled swallowing of released drug and difficulties maintaining the film at the absorption site, prevent it from being used more widely (Patel et al., 2011). More research is required to address these limitations, especially by using mucoadhesive systems, before more buccal films become commercially viable.

2.5.6 Characteristics of a Buccal Delivery System

It is important to take cognizance of the desired characteristics required during the development of a novel drug delivery system. Important factors to consider when developing a buccal drug delivery system therefore include the following (Hearnden et al., 2012):

- Disturbances to taste and speech will decrease patient acceptability. Films should be thin, flexible and smooth.
- Self-administration of films should be easy to apply and remove if adverse effects occur.
- The drug release and penetration across the oral mucosa's epithelium are critical factors. Controlled delivery may be advantageous to avoid repeated administration of doses especially in chronic conditions.
- The nature of the drug is vital. Lipophilic, non-ionised species and low molecular weight substances are best suited for buccal delivery.

2.5.7 Types of Buccal Delivery Systems

Numerous types of buccal delivery systems currently exists, with a majority of studies focussing on buccal mucoadhesive tablets, ointments, gels, patches, wafers and films (Hearnden et al., 2012, Madhav et al., 2009, Morales and McConville, 2011, Patel et al., 2011, Sudhakar et al., 2006).

2.5.7.1 Buccal Mucoadhesive Tablets

Tablets utilise the whole absorptive surface of the oral cavity after the drug is dissolved in the saliva. The use of conventional solid preparations, such as tablets and lozenges are restricted due to variability in saliva production and sucking intensity, accidental swallowing of the system and relatively short exposure times (Madhav et al., 2009). To overcome these limitations, research has been focussing on developing a range of mucoadhesive tablet formulations (Figure 2.5) (Boyapally et al., 2010, Cappello et al., 2006, Şenel et al., 1998, Taylan et al., 1996). These tablets are superior, as they adhere to the mucosa, thereby increasing exposure time and drug absorption.

Buccal tablets are prepared by compressing powder mixes that can dissolve or adhere, depending on the type of excipients used. Simple matrix tablets (Figure 2.5a), composed of drug and bioadhesive polymers, produce multidirectional drug release into the oral cavity. Alternatively, a water impermeable backing layer can be incorporated (Figure 2.5b,c,f) to ensure unidirectional drug release. Formulation incompatibilities or additional requirements for controlled drug delivery might necessitate the use of other inert excipients (Figure 2.5e) or a non-bioadhesive controlled release matrix (Figure 2.5d,f) (Patel et al., 2011, Rossi et al., 2005).

Major limitations associated with the use of buccal tablets include their physical size and thickness, which would influence patient acceptability should it cause irritation. Children and the elderly are more prone to possible discomfort, and a possibility exist of the dosage form being swallowed if not adhered properly (Patel et al., 2011, Smart, 2005b).

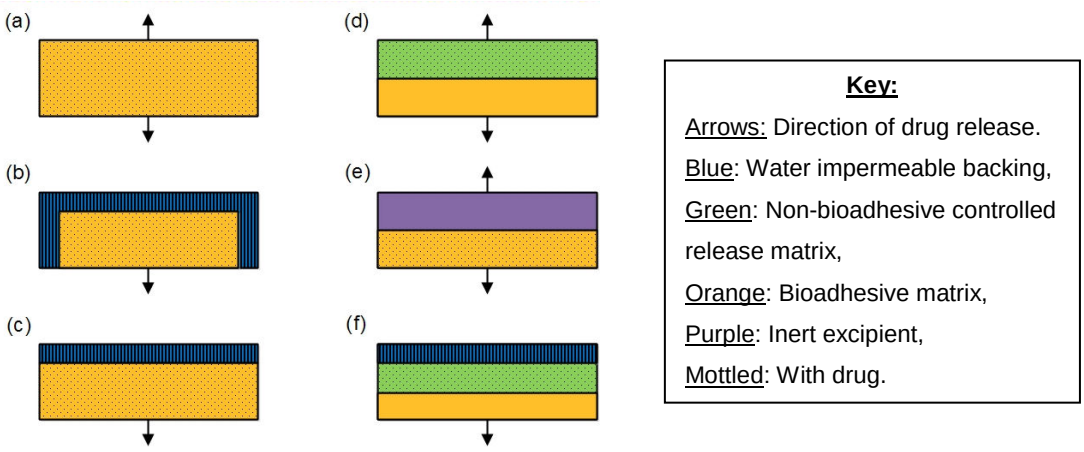

Figure 2.5: Schematic representation of different types of matrix tablets intended for buccal drug delivery. Adapted from (Rossi et al., 2005).

2.5.7.2 Ointments and Gels

Semi-solid oral dosage forms, including ointments and gels, are applied topically onto the oral mucosa surface, and can be used to achieve either local or systemic effects. They are formulated to contain a polymer(s), the drug and other required excipients, either dissolved or suspended as a fine powder in a suitable base. Hydrogels are produced using polymers, which are hydrated in an aqueous environment without dissolving. They act as a controlled release drug delivery system by physically entrapping drug molecules, which are slowly released by diffusion or erosion after gel hydration (Martin et al., 2003). Bioadhesive polymers can be incorporated to prolong the adherence to mucosal surfaces or modulate the rate of drug release (Sudhakar et al., 2006). Semi-solid preparations are applied using a finger or applicator to the target region, and have higher patient acceptability in terms of mouth feel compared to solid dosage forms (Patel et al., 2011). Other advantages include the intimate contact being attained with the mucosal membrane and the rapid drug release at the absorption site. Gels may not deliver an accurately measured dose of drug in comparison with a unit dosage form (Squier and Kremer, 2001), making them less suitable for drugs, with a narrow therapeutic window. Another shortcoming of semi-solid buccal preparations is the poor retention at the site of application, requiring the incorporation of a bioadhesive polymer (Patel et al., 2011).

2.5.7.3 Powders

Limited work has been reported on powders intended for administration into the oral cavity. Powders prepared as bioadhesive microparticles allow for intimate contact with the oral mucosa as a result of their unique physical properties. Due to their reduced size, they are less likely to cause local irritation at the site of adhesion compared to buccal tablets (Sudhakar et al., 2006).

2.5.7.4 Solutions and Sprays

Liquid dosage forms include solutions, suspensions and sprays, which are produced by dissolving or suspending the drug in a suitable vehicle. Their use is predominantly aimed at exerting local action in the oral cavity. Several commercially available antimicrobial preparations are available (Hearnden et al., 2012) containing actives such as chlorhexidine gluconate. Major drawbacks associated with such liquid dosage forms are that they have reduced retention times and precise dosing of drugs are difficult to achieve (Smart, 2005b).

2.5.7.5 Patches, Wafers and Films

Patches, wafers and films are solid dosage forms intended for drug administration into the oral cavity. They can be used to achieve localized or systemic effects. Patches and films are most often prepared by casting a solution of the polymer, drug and other required excipients onto a substrate and allowing it to dry. The size of the patches can vary (≤ 10-15 cm^2), but are most frequently 1-3 cm^2 (Smart, 2005b). Similar to buccal tablets, patches can also be prepared for multidirectional or unidirectional drug release by incorporating an impermeable backing layer (Patel et al., 2011). Wafers are thin strips of polymeric films, containing up to 20 mg of drug, which dissolves rapidly on the tongue in less than 30 seconds. Wafers deliver drugs (which are able to cross the permeability barrier) directly into the blood supply for quick treatment of conditions such as migraines, pain relief and nausea (Hearnden et al., 2012).

Films, patches and wafers share many of the advantages and disadvantages of buccal tablets, but by being thin and flexible, they cause less irritation and therefore will have higher patient acceptability. A drawback is the relative thinness of the films, which may result in overhydration and loss of the adhesive properties (Squier and Kremer, 2001). This can however be overcome by the incorporation of appropriate mucoadhesive polymers. Current literature indicates that research is more focused towards mucoadhesive films and patches. Inclusion of various mucoadhesive agents can be used to extend the residence time of dosage forms at the site of application and facilitate drug absorption (Patel et al., 2011).

Table 2.5 shows a summary of several drugs investigated for buccal films in the literature. The main excipients used, preparation methods and characterizations were extracted from these papers and are presented. It can be clearly seen that buccal films are being investigated for various classes of drugs, confirming its wide applicability. In addition, although excipients vary from study to study, the most common method of film preparation remains the casting and solvent evaporation technique. Characterization methods have advanced over the last few years and have evolved from simple drug release studies to detailed characterization of all relevant aspects involved in formulation development. Similarly to the other NDDS summarised in Table 2.2 and Table 2.3, it can be clearly seen from Table 2.5 that studies into buccal polymeric films have mainly been limited to incorporation of single drugs. Clearly, the possibility to deliver multiple drugs exists and researchers should focus on this in the future.

Table 2.5: Summary of investigated buccal films.

Active Ingredients	Main Excipients	Preparation Methods	Characterizations	References
Lidocaine	Hydroxypropyl cellulose, glycyrrhizic acid	Casting/solvent evaporation	*In vitro* permeation, dissolution studies, DSC.	Okamoto et al. (2001)
Salmon calcitonin	Polycarbophil, Eudragit S100	Modified Casting/solvent evaporation	*In vitro* drug release, *in vivo* drug release studies.	Cui and Mumper (2002)
Ipriflavone	Poly(d,l-lactide-co-glycolide), chitosan	Emulsification/ casting/solvent evaporation	Drug content, morphology (SEM), swelling, film degradation (3 months), *in vitro* drug release.	Perugini et al. (2003)
Ibuprofen	Polyvinylpyrrolidone, Carboxymethyl cellulose Na^+ salt	Casting/solvent evaporation	Swelling, erosion, mucoadhesion, organoleptic characteristics, *in vitro* drug release, *in vivo* drug release.	Perioli et al. (2004)
Clotrimazole	Poly(ethylene oxide)	Hot-melt extrusion	Drug content, bioadhesion, DSC, TGA, mechanical properties, *in vitro* drug release, stability studies, XRD.	Prodduturi et al. (2005)
Lidocaine	Hydroxypropyl cellulose, HPMC	Hot-melt extrusion	Drug content, bioadhesion, dissolution studies DSC, wide angle XRD.	Repka et al. (2005)
Fentanyl	Polyvinylpyrrolidone (PVP) K30 & PVP K90	Casting/solvent evaporation	*In vitro* drug release, drug permeability studies.	Diaz del Consuelo et al. (2007)
Triamcinolone acetonide	Carbopol, poloxamer, HPMC in various ratios	Casting/solvent evaporation	FTIR, swelling, mucoadhesion, tensile strength, *in vitro* drug release.	Kim et al. (2007)
Chlorhexidine	Sodium alginate, HPMC, chitosan	Casting/solvent evaporation	Drug content, film morphology, *in vitro* drug release, swelling, preliminary *in vivo* studies.	Juliano et al. (2008)
Drug Free	Silk-fibroin, HPMC, PEG 400	Casting/solvent evaporation	Thickness, weight, mechanical properties, swelling, bioadhesion, *in vitro* stability, FTIR.	Kundu et al. (2008)
Propranolol HCl	Chitosan, Eudragit RS100	Casting/solvent evaporation	Drug content, thickness, *in vitro* drug release, mucoadhesivity, swelling and erosion, surface pH, morphology (SEM), mechanical properties.	Perumal et al. (2008b)
Atenolol	Ethylcellulose, Polyvinyl alcohol, HPMC	Casting/solvent evaporation	Swelling, bioadhesion, *in vitro* drug release, phase solubility studies, DSC, FTIR.	Jug et al. (2009)
Salbutamol sulphate	Sodium carboxymethyl cellulose, Carbopol 940P	Casting/solvent evaporation	Bioadhesion, drug release, thickness, weight, folding endurance, drug content, surface pH, swelling, mechanical properties, *in vivo* efficacy.	Singh et al. (2010)
Griseofulvin	HPMC, Polyvinylpyrrolidone	Anti-solvent precipitation + Casting/solvent evaporation	Particle surface morphology, particle size, zeta potential, DSC, mechanical properties, *in vitro* drug release.	Meng et al. (2011)
Glibenclamide	Different grades of HPMC	Casting/solvent evaporation	Weight, thickness, surface pH, swelling, folding endurance, drug content, *in vitro* drug release, *ex vivo* permeation, FTIR	Muzib and Kumari (2011)
Propranolol HCl	Chitosan, gelatin	Casting/solvent evaporation	Thickness, weight, drug content, morphology, FTIR, TGA, DSC, swelling, *in vivo* residence time, *in vitro* drug release, drug permeation, antimicrobial activity assay.	Abruzzo et al. (2012)
Rizatriptan benzoate	Tamarind seed xyloglucan, carbopol 934P	Casting/solvent evaporation	Tensile strength, bioadhesion force, drug release, DSC, swelling, surface pH, folding endurance, thickness, weight, *ex vivo* permeation.	Avachat et al. (2013)

Abbreviations: DSC: Differential scanning calorimetry HPMC: Hydroxypropyl methylcellulose
TGA: Thermogravimetric analysis SEM: Scanning electron microscopy
FTIR: Fourier transform infrared spectroscopy XRD: X-ray diffractometry

2.6 FILMS FOR BUCCAL DRUG DELIVERY OF ARVS

A thorough literature search revealed hundreds of reported studies on various classes of drugs formulated into buccal films for systemic delivery. It became evident that a gap existed in incorporating ARVs in buccal polymeric films (Morales and McConville, 2011, Ojewole et al., 2008, Sudhakar et al., 2006). To the best of the researcher's knowledge, the only published paper thus far on buccal polymeric dosage forms of ARVs is of zidovudine polymeric patches recently produced by Reddy et al. (2012). Characterization studies were limited and did not include critical parameters such as *in vitro* permeation or mechanical properties. ARV buccal delivery systems have not been comprehensively investigated or characterised, which is clearly essential for formulation optimization. A detailed overview of buccal films are presented in the following section.

2.6.1 Methods of Film Preparation

There are two major methods commonly reported for the preparation of polymeric buccal films namely, the solvent evaporation method, and the other that is solvent free, called the hot-melt extrusion method (Morales and McConville, 2011).

2.6.1.1 Film Casting and Solvent Evaporation

The most widely reported method used to manufacture buccal polymeric films is the solvent evaporation method. This is largely due to the relatively simple process and low costs incurred at the laboratory scale (Morales and McConville, 2011). The method entails dissolving the drug and appropriate polymer(s), with or without plasticizers, in a suitable solvent or solvent mixture. This solution is then cast onto a suitable substrate or into a mold, and the solvent(s) are allowed to evaporate, leaving behind a solid polymeric film that contains the drug (Patel et al., 2011).

A recent review by Morales and McConville (2011) highlighted some limitations associated with this method of film preparation:

- Air bubbles introduced into the polymeric solution during the manufacturing process can result in films with an uneven surface and uneven thickness, therefore removal of air during preparation is a vital step for homogeneity reasons (Dixit and Puthli, 2009).

- The use of organic solvents during film preparation is another pressing concern. The problems concerning solvent collection, residual solvents in the films, and biological hazards to the environment and human health (Jones et al., 2013) should always be borne in mind when developing a buccal polymeric film.

- Complex manufacturing methods using expensive equipment or emulsification below room temperature (Perumal et al., 2008b) have been reported. While producing a dosage form, efforts should be taken to simplify production methods and production costs must be kept to a minimum.

- The problems with content uniformity was highlighted in a recent review paper (Morales and McConville, 2011) and have been addressed in our laboratory. Specially developed silicone-molded trays, with individual wells for film casting, resulting in improved uniformity of drug content, uniformity of mucoadhesive properties, *in vitro* drug release and thickness uniformity, were designed in our laboratory to address this problem and was reported by Perumal et al. (2008a).

2.6.1.2 Hot-melt Extrusion

The other method sometimes being used for buccal film preparation is hot-melt extrusion. In this technique of film preparation, a blend of suitable polymers, the active ingredient, and other excipients required for processing or formulation performance is molten and then forced through an orifice containing a die to yield uniformly dispersed granules, tablets or films (Morales and McConville, 2011).

Cilurzo and co-workers (2008) prepared fast-disintegrating oral films using both the solvent casting/evaporation and hot-melt extrusion techniques. The casting method proved to be more advantageous, as the films resulted in the highest patient compliance and faster *in vitro* and *in vivo* disintegration times to achieve the desired drug release. A limited number of studies have been published where hot-melt extrusion was used to prepare mucoadhesive buccal films, with the majority being undertaken by a specific research unit (Prodduturi et al., 2005, Repka et al., 2005, Repka et al., 2006, Repka et al., 2003).

2.6.2 Types of Buccal Film Preparations

Mucoadhesive buccal polymeric films can either be designed to deliver drugs systemically or to act only locally on the oral mucosa (Patel et al., 2011).

There are three main types of buccal films (Hearnden et al., 2012):

1. Films with a dissolvable matrix of polymers and drugs for administration to the buccal mucosa. These films can produce sustained drug release for treating conditions such as oral candidiasis and mucositis (Madhav et al., 2009). They dissolve slowly and completely, leaving no remains in the oral cavity.
2. Films with an impermeable backing layer normally used for systemic drug delivery. They provide controlled drug release but can only deliver to a restricted area of the mucosa, thereby limiting the available dose. In addition, the impermeable layer remains behind which the patient has to remove.
3. Films with a dissolvable impermeable backing layer, where the complete film eventually dissolves. They have the same controlled delivery as above, without the need to remove the film after the drug was released (Madhav et al., 2009).

For controlled drug release, good mucoadhesion and suitable mechanical strength, polymers and drugs of opposing solubilities may often be required. While multipolymeric *multilayered* films and wafers have been prepared with drugs and polymers of opposing solubilities (Ding et al., 2012, Perugini et al., 2003), *monolayered* multipolymeric films (MMFs) offer more advantages, i.e. lower production costs, improved drug release, mucoadhesivity and size (Perugini et al., 2003). Limited formulation and characterization studies on MMFs with polymers and drugs of opposing solubilities have been reported. Furthermore, the methods employed to produce the aforementioned MMFs require carcinogenic solvents (Perugini et al., 2003), involve the combination of two separate mixtures under high shear rates (Pendekal and Tegginamat, 2012), require emulsification below room temperature (Perumal et al., 2008b) or need multiple solvents with additional emulsifiers (Vasantha et al., 2011). In this study, a new technique, whereby drugs and polymers of opposing solubilities can be co-blended using a co-solvent to produce buccal MMFs, is reported. This method is simple, eliminates the need for emulsifiers, can be done at room temperature and requires minimal equipment.

2.6.3 Characterisation of Buccal Films

Buccal films should be thin, flexible, sufficiently elastic yet resist breakage due to handling or oral application, display good mucoadhesive properties to ensure prolonged retention, and have predictable drug release. These characteristics need to be carefully evaluated during formulation development for optimization, regulatory approval and commercialization. Several techniques are reported in the literature to characterize and evaluate buccal polymeric films. These techniques investigate the physical properties of the films through mucoadhesive characteristics, *in vitro* permeation to *in vivo* absorption in humans (Nair et al., 2013).

A review of the literature indicates that the main properties being evaluated in studies on buccal films are dosage form uniformity, mechanical properties, buccal performance, morphology, compatibility and drug release characteristics. The specific tests being undertaken by researchers to address these properties were identified from various experimental papers, and are summarised in the schematic below (Figure 2.6). Details on methods for the evaluation of films by these techniques can be found in the literature (Morales and McConville, 2011, Nair et al., 2013, Sudhakar et al., 2006).

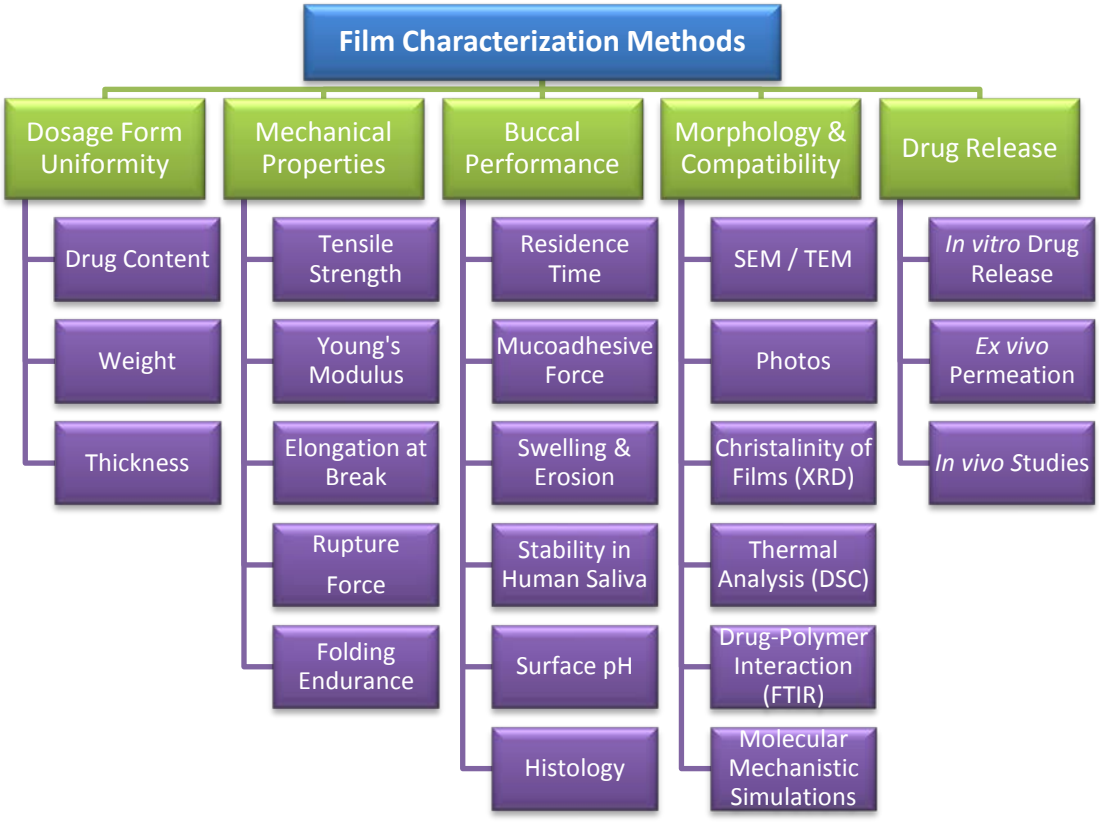

Figure 2.6: Film characterization methods.

2.7 EMERGING WORK ON NANO-ENABLED BUCCAL DRUG DELIVERY

Recent developments in the field of buccal drug delivery show an increased interest towards nano-enabled buccal drug delivery systems (Giovino et al., 2012, Morales et al., 2013, Silva et al., 2012). The advantages of buccal drug delivery can be combined with that of the nanoparticulate drug delivery systems to provide a superior drug delivery system in terms of enhanced bioavailability and drug targeting. Table 2.6 gives an overview of all studies reported to date on these emerging nano-enabled buccal films. It can be seen that a very limited number of studies have been done to date in this emerging field, and antiretrovirals have yet to be investigated.

The use of nanotechnology in HIV & AIDS therapy is warranted by benefits such as its versatility, nearly all types of drugs may be incorporated, relatively non-toxic biocompatible excipients can be used, drug-release modification is possible, the production costs are relative low, ease of producing nanoparticles, and the possibility for scale-up exists. Furthermore, nanoparticulate systems for ARVs may be of particular interest to achieve targeted delivery to HIV reservoirs (Shahiwala and Amiji, 2007, Vyas et al., 2006b).

A nanoparticulate system of particular interest is solid lipid nanoparticles (SLNs). SLNs are prepared from lipids, which are solid at room temperature, and surfactants or stabilizers (Shegokar et al., 2011), in the nanometer size (< 1000 nm) range. Advantages of SLNs over other nanoparticulate systems include: increased stability (Shegokar et al., 2011), controlled drug release (Kuo and Chen, 2009), targeted drug delivery (Aji Alex et al., 2011, Chiappetta et al., 2013) and the incorporation of both hydrophilic (Ghadiri et al., 2012) and lipophilic (Kumar et al., 2007) drugs. Furthermore, SLNs lipids are biocompatible and organic solvents can be avoided during manufacturing processes (Mehnert and Mäder, 2012).

As evident from Table 2.6, SLNs incorporated into buccal monolayered multipolymeric films (MMFs) have not been explored in the literature for any drug. There is a clear need to explore the use of SLNs and buccal polymeric films. Furthermore, DDI SLNs have not been successfully prepared (Table 2.7). As illustrated in Table 2.7, a wide range of ARVs have been successfully incorporated into SLNs, which are suitable for delivery via the oral or parenteral route.

By incorporating the drug in the form of nanoparticles into the buccal film, a reduction in dose-dependent side effects can be expected, as drug targeting to the required site of action can be achieved using a smaller dose. The additional reduced cost could make DDI more therapeutically useful once more. Incorporating multiple ARVs into nanoparticles can be accomplished to achieve multi-drug HAART regimens (Shibata et al., 2013).

This study also explored the incorporation of DDI SLNs into the reported MMFs to investigate for potential of buccal drug delivery of an ARV using nano-enabled films. As a result, this study can be considered as a platform that opens up numerous possibilities for future development and formulation optimization studies for nano-enabled buccal ARV films and DDI SLN formulations.

Table 2.6: Emerging Nano-enabled buccal films.

Active Ingredients	Film Type & Preparation Methods	Nanoparticulate System & Preparation Methods	References
Phenylephrine	HPMC and Carbopol 934P Multi-layered patch with a microtablet containing the dry nanosuspension	Nanosuspension prepared by wet stirred media milling or by high-pressure homogenization	Rao et al. (2011)
Insulin (Protein)	Chitosan films prepared by solvent casting / evaporation	PEG-b-PLA copolymeric nanoparticles prepared by double emulsion solvent evaporation	Giovino et al. (2012)
Naproxen, Fenofibrate or Griseofulvin	HPMC films prepared by solvent casting / evaporation	Nanosuspensions prepared by wet stirred media milling	Sievens-Figueroa et al. (2012)
Risperidone	Semi-solid hydrogel using Carbomer 2001	Glyceryl monostearate solid lipid nanoparticles prepared via homogenization/ultrasonication	Silva et al. (2012)
Lysozyme (Protein)	Polymethacrylates and HPMC films prepared by solvent casting / evaporation	Lysozyme-loaded-$_{D,L}$-valine protein-coated nanoparticles prepared by antisolvent co-precipitation	Morales et al. (2013)
Carvedilol	Tri-layered films containing HPMC, Carbopol 934P and ethyl cellulose	Polymeric nanosuspension prepared by precipitation-ultrasonication	Rana and Murthy (2013)

Table 2.7: Overview of studies reporting on SLNs incorporating ARVs.

ARV	Excipients	Preparation Methods	Routes of Delivery	Characterizations	References
Zidovudine prodrug	Trilaurin, Dipalitoylphos-phatidylcholine, Dimyristoylphod phatidylglycerol	Hot high pressure homogenization	Parenteral	Encapsulation efficiency, particle size, zeta potential, morphology - Freeze fracture electron microscopy (FE-SEM), drug release.	Heiati et al. (1997)
Zidovudine prodrug	Trilaurin, Dipalitoylphos-phatidylcholine, Dimyristoylphod phatidylglycerol	Hot high pressure homogenization	Parenteral	Particle size, zeta potential, drug retention by gel permeation chromatography, biodistribution studies.	Heiati et al. (1998)
Atazanavir	Stearic acid, Pluronic F68	Thin film hydration	Parenteral (Brain)	Encapsulation efficiency, particle size, morphology, zeta potential, drug release and cell viability.	Chattopadhyay et al. (2008)
Saquinavir	Stearylamine, Compritol 888 ATO, Cacao butter, DODAB*, Polysorbate 80,	Microemulsion method	Parenteral	Entrapment efficiency, particle size, zeta potential, morphology (FE-SEM), Nuclear magnetic resonance analysis, *in vitro* drug release.	Kuo and Chen (2009)
Lopinavir	Compritol 888 ATO	Hot Homogenization followed by Ultrasonication	Oral	Encapsulation efficiency, particle size, zeta potential, DSC, Wide angle X-ray scattering, Atomic force microscopy, *in vitro* drug release*, in vivo* studies, stability studies.	Aji Alex et al. (2011)
Tenofovir	Softisan 100	Modified phase-inversion technique	Vaginal microbicide	Encapsulation efficiency particle size, zeta potential, morphology (TEM), cytotoxicity studies.	Alukda et al. (2011)
Saquinavir	Stearic acid, Poloxamer 407, Tween 80	Hot high pressure homogenization	Oral	Encapsulation efficiency, particle size, zeta potential, DSC, drug release, XRD, morphology (TEM), *in vivo* studies.	Dodiya et al. (2011)
Stavudine Delavirdine Saquinavir	Compritol 888 ATO, Tripalmitin, Cacao butter	Hot Homogenization	No data	Entrapment efficiency, particle size distribution, morphology (FE-SEM), stability, drug release.	Kuo and Chung (2011a)
Nevirapine	Stearic acid, Compritol 888 ATO, Tween 80	Microemulsion method	Parenteral	Particle size, zeta potential, morphology (FE-SEM), DSC, *in vitro* drug release*,* cytotoxicity.	Kuo and Chung (2011b)
Stavudine	Trimyristin, Solutol HS 15, Poloxamer 188, Tween 80	Hot high pressure homogenization	Parenteral	Particle size, polydispersity index, zeta potential, long-term stability measurements.	Shegokar et al. (2011)
Lopinavir	Stearic acid, Poloxamer, Polyethylene glycol	Hot self nano-emulsification	Oral	Entrapment efficiency, drug loading, particle size, zeta potential, DSC, XRD, morphology (TEM), Atomic force microscopy, *in vitro* release, *in vivo* studies.	Negi et al. (2013)

***DODAB = dioctadecyldimethyl ammonium bromide

2.8 DIDANOSINE AS A MODEL ARV FOR BUCCAL DELIVERY

Didanosine (DDI) is a synthetic analogue of deoxyadenosine (Figure 2.7), and is commonly referred to as 2′,3′-Dideoxyinosine, while its systematic structural name (9 -((2R,5S) -5- (hydroxymethyl) tetrahydrofuran -2-yl) -3H-purin-6(9H)-one) is used seldom. DDI has a molecular formula of $C_{10}H_{12}N_4O$ and a molecular weight of 236.23 g/mol (Moffat et al., 2004).

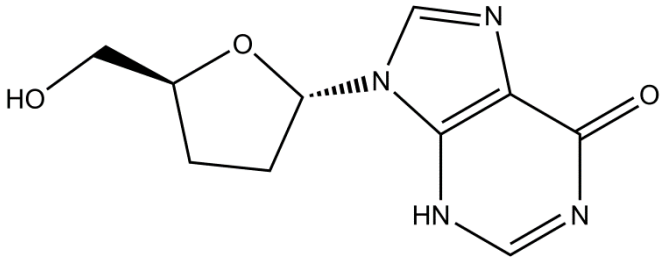

Figure 2.7: Chemical structure of didanosine.

Many factors need consideration during development of a NDDS. Parameters related to the drug delivery system is of great importance and equally critical to consider is the physico-chemical and pharmacological properties of the drug.

Solubility: DDI is sparingly soluble in water, freely soluble in dimethyl sulfoxide, slightly soluble in methanol and in 96 % ethanol (BP 2009). The solubility of DDI in water is pH-dependent and reported as 20.29 mg/mL (Sánchez-Lafuente et al., 2002a).

Dissociation constant: DDI is an amphoteric compound that has a weakly acidic hydrogen atom on the hypoxanthine moiety and a number of basic nitrogen atoms (Figure 2.7). The apparent *pKa* of DDI in water, has been reported as 9.12 (Moffat et al., 2004), representing the basic properties of the molecule. An unionized form of a drug is more likely to interact with lipid membranes than a drug in the ionized form. By maintaining a pH range of between 6-7, amphoteric nucleoside analogues such as DDI may be kept in their unionized forms. Formulations are thus best prepared at pH 6-7 to readily promote absorption.

Partition coefficient: The octanol/water partition coefficient [Log *P*(octanol/water)], as determined by the traditional shake-flask method, has been reported as -1.24 (Moffat et al., 2004), thereby suggesting DDI is hydrophilic.

Melting point/Thermal stability: DDI melts between 160 °C and 163 °C (Moffat et al., 2004). Kasongo and co-workers (2011) determined the thermal stability of DDI to ensure no degradation product would form while manufacturing nanostructured lipid carriers (NLC) at high temperatures. DDI was proven to be thermostable beyond 80°C. Temperatures used to produce proposed buccal polymeric films (43°C, 24 hours) and solid lipid nanoparticles (80°C, 20 min) in this study would not lead to thermal degradation of DDI.

Ultraviolet absorption: DDI's maximum wavelength of absorption have been reported for numerous media: aqueous acid (pH 2) 248 nm; (ethanol) 250 nm; aqueous alkali (pH 12) 254 nm (Moffat et al., 2004). A typical ultraviolet (UV) absorption spectrum of DDI generated in this study is shown in Figure 2.8. A wavelength of 250 nm was used for *in vitro* analyses of DDI containing samples during formulation development and optimization studies.

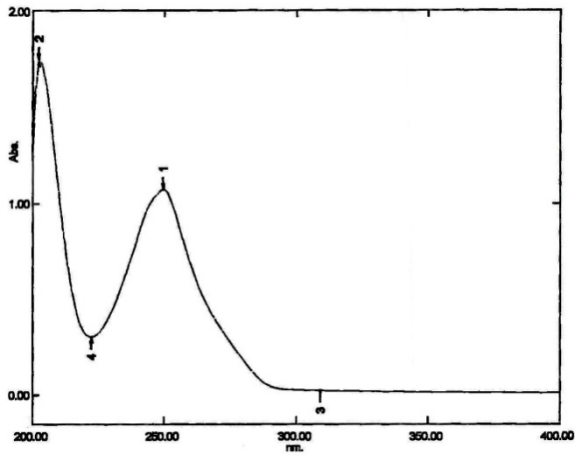

Figure 2.8: A typical UV spectrum produced during evaluation of didanosine buccal films prepared in this study.

Pharmacological properties: DDI is a nucleoside reverse transcriptase inhibitor (NRTI), acts by competitive inhibition of HIV-1 reverse transcriptase and can also be incorporated into the growing viral DNA chain to cause termination (Katzung et al., 2003). The severe side effects associated with long term use of NRTIs include lactic acidemia and severe hepatomegaly with steatosis. Side effects associated with DDI include dose-dependent pancreatitis, peripheral distal neuropathy, diarrhea, hepatitis, esophageal ulceration, cardiomyopathy, and central nervous system toxicity (Katzung et al., 2003, Rossiter, 2012).

The oral bioavailability of DDI ranges from 30 to 40 %, depending on the formulation being administered. The oral bioavailability is reduced by up to 55 % if ingested within two hours after a meal. Maximum plasma concentrations are achieved within approximately one hour after oral administration (Tmax). The plasma elimination half-life ($t_{½}$) is reported to be only 1.3 to 1.6 hours (Sweetman, 2009). The low oral bioavailability combined with the short half-life necessitates frequent administration of large doses, leading to dose-dependent toxicities such as pancreatitis.

At acidic pH, hydrolysis of the glycosidic bond between the sugar and the base moieties of DDI will inactivate the drug (Katzung et al., 2003). Originally, a buffered powder formulation of DDI was available that was subsequently replaced by chewable and dispersible buffered tablets with greater oral bioavailability (30–40 %). As the chewable tablets contain both phenylalanine (36.5 mg) and sodium (1380 mg), caution should be exercised in patients with phenylketonuria and those taking sodium-restricted diets (Katzung et al., 2003). A new enteric-coated formulation was developed that further improved patient convenience and tolerability (Deshmukh et al., 2003), and is currently marketed as Videx EC® (Bristol-Myers Squibb, 2013), but challenges with using DDI in its current oral formulations still exists.

Rapid degradation of DDI in the GIT due to acidic hydrolysis, together with the need for repetitive dosing, its short elimination half-life, dose-related toxicity and relatively low daily dosage (250–400 mg), make this drug a suitable candidate for incorporating into novel buccal polymeric films. Moreover, DDI's favourable physico-chemical properties, such as adequate water solubility, octanol/water partition coefficient and thermal stability, further suggest that it is suitable for developing buccal polymeric films.

2.9 CONCLUSION

This chapter highlighted the current status of HIV & AIDS, its current drug therapy and the limitations associated with therapy. Strategies, including buccal polymeric films, aimed at addressing current limitations of ARV drugs were examined. This literature review showed that although buccal permeation investigations with antiretroviral drug solutions have confirmed their transbuccal delivery potential, studies on their formulation into delivery systems are lacking. Although multipolymeric monolayered films (MMFs) with drugs and polymers of opposing solubilities offer several advantages for the controlled release of drugs via the buccal route, more research still needs to be done in the area. Didanosine was identified as a model ARV due to its extensive first pass metabolism and short half-life, making it an ideal candidate for controlled release buccal delivery.

It was also identified that emerging research have been exploring the use of nano-enabled buccal drug delivery. Through this, a considerable scope for future developments into novel nano-enabled buccal drug delivery systems has been identified.

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CHAPTER THREE
PUBLISHED PAPER

3.1 INTRODUCTION

The following paper was published in an international peer reviewed journal and reports on original research:

Jones, E., Ojewole, E., Pillay, V., Kumar, P., Rambharose, S., Govender, T., 2013. Monolayered multipolymeric buccal films with drug and polymers of opposing solubilities for ARV therapy: Physico-mechanical evaluation and molecular mechanics modelling. ***International Journal of Pharmaceutics***, 455, 197-212.

Ms E. Jones contributed to the design of the project, modification and optimisation of methods and preparation and characterisation of all polymeric films in terms of assay, *in vitro* drug release, *in vitro* permeations, transepithelial electrical resistance measurements, mucoadhesivity, mechanical strength and surface pH as well as interpretation of the data and writing of the paper. Mr S. Rambharose assisted Ms Jones with the LM/TEM histological evaluation section. Mr P. Kumar and Professor V. Pillay were collaborators and performed the molecular modelling studies. The remaining authors served as supervisor and co-supervisor.

This chapter is presented in the required format of the journal and is the final revised accepted version. The published article (doi:10.1016/j.ijpharm.2013.07.037) can be found in Appendix B.

3.2 PUBLISHED PAPER

Monolayered multipolymeric buccal films with drug and polymers of opposing solubilities for ARV therapy: Physico-mechanical evaluation and molecular mechanics modelling

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ABSTRACT

Although buccal permeation investigations with antiretroviral drug solutions have confirmed their transbuccal delivery potential, studies on their formulation into delivery systems are lacking. Multipolymeric monolayered films (MMFs) with drugs and polymers of opposing solubilities will offer several advantages for the controlled release delivery of didanosine (DDI) via the buccal route. The aim of this study was to employ a co-blending-co-plasticization technique for preparation of MMFs containing Eudragit®RS100 (EUD) and Hydroxypropyl methylcellulose (HPMC) and to undertake molecular modelling and *in vitro* characterizations. Uniform drug content (91%-105%) with low variability was obtained for all films. Co-blending of DDI:HPMC:EUD (1:1:10) was required to achieve controlled drug release. The buccal permeability potential of DDI from the MMFs was successfully demonstrated with a permeability coefficient of $0.72\pm0.14x10^{-2}$ cm/h and a steady state flux of 71.63±13.54 µg/cm^{2}h. Films had acceptable mucoadhesivity (2184 mN), mechanical strength (0.698 N/mm^2) and surface pH (6.63). The mechanism inherent to the mucoadhesive and drug release profile performance of the MMFs was elucidated via static lattice molecular mechanics simulations wherein a close corroboration among the *in vitro–in silico* (IVIS) data was observed. These extensive physico-mechanical and molecular atomistic studies have confirmed the use of MMFs containing DDI, HPMC and EUD as a buccal delivery system.

KEYWORDS:

Didanosine, Buccal, Films, Co-blended polymers, Physico-mechanical properties, Static lattice atomistic simulations.

1.0 INTRODUCTION

Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS) commonly referred to as HIV & AIDS, have emerged as the leading cause of mortality worldwide and is the main cause of death in sub-Saharan Africa (Merson et al., 2008). While antiretrovirals (ARVs) have proven to be useful in the treatment and management of HIV & AIDS, several disadvantages including extensive first pass metabolism, gastrointestinal degradation, low bioavailability and short half-lives (Li and Chan, 1999) limit their efficacy. Large doses, complex dosing regimens and multiple drugs contribute to reduced patient compliance (Chandwani et al., 2012). Poor drug solubility and limited membrane permeability also pose formulation difficulties (Sharma and Garg, 2010).

The development of new chemical entities, novel drug delivery systems and alternative routes to deliver ARVs (Ojewole et al., 2008) are being explored to overcome these limitations. Novel drug delivery systems receiving increased attention include sustained release matrix tablets (Sánchez-Lafuente et al., 2002b), ceramic implants (Benghuzzi, 2000), liposomes (Dubey et al., 2010) and nanoparticles (Kuo and Chung, 2011b). Alternate routes for delivery under investigation include: transdermal (Gerber et al., 2008), nasal (Carvalho et al., 2013), vaginal (Johnson et al., 2010) and buccal delivery (Ojewole et al., 2012, Xiang et al., 2002).

Drug delivery via the buccal route has recently emerged as a lucrative alternative to the oral route. Drugs can directly enter the systemic circulation and bypasses gastrointestinal degradation and first-pass hepatic metabolism, thereby improving bioavailability (Hoogstraate and Wertz, 1998). The buccal mucosa is easily accessible and more permeable than skin (Squier and Hall, 1985). Formulating the drug into a controlled release mucoadhesive dosage form may further improve drug delivery and patient compliance (Morales and McConville, 2011). Buccal transportation mainly occurs via passive diffusion across lipid membranes either via paracellular or transcellular pathways making this route suitable for both hydrophilic and lipophillic drugs (Patel et al., 2011). This is relevant considering HIV & AIDS is treated with multiple-drug regimens. The disadvantages associated with the buccal route of drug delivery are its low mucosal permeability, continuous secretion of saliva leading to dilution of drug and the need for formulation approaches to promote retention on the mucosae (Patel et al., 2011).

To date, reports regarding buccal permeability of antiretrovirals remain limited. *In vitro* drug permeability studies with solutions of zalcitabine (Shojaei et al., 1999, Xiang et al., 2002), didanosine (Ojewole et al., 2012) and tenofovir (Rambharose et al., 2013) have been reported. To the best of our knowledge, the only report thus far on buccal polymeric dosage forms of ARVs is of zidovudine polymeric patches recently produced by Reddy et al. (2012). Characterization studies were limited and did not include critical parameters such as *in vitro* permeation or mechanical properties. ARV buccal delivery systems have not been comprehensively investigated or characterised and it is clearly essential for formulation optimization.

Various mucoadhesive buccal dosage forms are being investigated for different classes of drugs, which include adhesive tablets (Cappello et al., 2006), gels (Ayensu et al., 2012b), ointments (Petelin et al., 2004), patches (Vasantha et al., 2011), and more recently films (Sievens-Figueroa et al., 2012, Abruzzo et al., 2012). Films may be preferred over tablets in terms of flexibility and comfort. They can circumvent the relatively short residence time of oral gels on the mucosa, which is easily washed away by saliva (Ahn et al., 2001, Okamoto et al., 2001). Polymeric films formulated for controlled drug release could also decrease dose-related side effects and improve patient compliance. A polymer for buccal films should adhere easily and sufficiently to the buccal mucosa, should have sufficient mechanical strength, should demonstrate penetration enhancement and provide for controlled release of the drug. Single polymers often fail to demonstrate all the ideal characteristics. To overcome this problem, researchers have been focusing on blending of polymers with similar solubilities (Abruzzo et al., 2012, Dubolazov et al., 2006, Juliano et al., 2008).

For controlled drug release, good mucoadhesion and suitable mechanical strength, polymers and drugs of opposing solubilities may often be required. While multipolymeric multilayered films and wafers have been prepared with drugs and polymers of opposing solubilities (Ding et al., 2012, Perugini et al., 2003), monolayered multipolymeric films (MMFs) offer more advantages i.e. lower production costs, improved drug release, mucoadhesivity and size (Perugini et al., 2003). Limited formulation and characterization studies on MMFs with polymers and drugs of opposing solubilities have been reported. Further, the methods employed to produce the aforementioned MMFs require carcinogenic solvents (Perugini et al., 2003), involve the combination of two separate mixtures under high shear rates (Pendekal and Tegginamat, 2012), require emulsification below room temperature (Perumal et al.,

2008b) or need multiple solvents with additional emulsifiers (Vasantha et al., 2011). In this paper a new technique whereby drugs and polymers of opposing solubilities can be co-blended using a co-solvent to produce buccal MMFs is reported. This method is simple, eliminates the need for emulsifiers, can be done at room temperature and requires minimal equipment. Limited studies on Eudragit® RS 100 (EUD) in combination with hydroxypropyl methylcellulose (HPMC) for buccal films have been reported (Koland et al., 2010, Mishra et al., 2012). These studies involved complex preparation methods and lacked evaluation of critical physico-mechanical properties. Molecular modelling to identify the mechanism of interaction between these two polymers and their suitability for combined use and indeed for any other buccal delivery system has not been previously reported. Therefore such physico-mechanical evaluation and molecular modelling of MMFs is essential for formulation optimization and facilitating a mechanistic understanding of MMFs.

Didanosine was selected as a model ARV due to its extensive first pass metabolism and short half-life making it an ideal candidate for controlled buccal delivery. The aim of this study was, therefore, to use a simplified method to prepare and characterize monolayered mucoadhesive films comprising of various ratios of co-blended EUD and HPMC for buccal delivery of didanosine. Films prepared by the solvent casting/evaporation technique were evaluated in terms of drug content uniformity, drug release, permeability, mucoadhesivity, mechanical properties and surface pH. Static lattice atomistic simulations (SLAS) were performed to identify the suitability of the polymeric blend for buccal film formulations and to identify correlations between *in vitro* and *in silico* results (IVIS).

2.0 MATERIALS AND METHODS

2.1 MATERIALS

Didanosine (DDI) was purchased from Ruland Chemistry Co., Ltd. (Nanjing, China) and used as received. Hydroxypropyl methylcellulose (HPMC), triethyl citrate (TEC) and mucin (Sigma–Aldrich, UK) were purchased and used as received. Eudragit® RS 100 (EUD) (Evonik Rohm GMBH, Germany) was donated by Degussa Africa (Pty) Ltd. All other reagents used [NaCl, Na_2HPO_4, KH_2PO_4, NaOH, HCl, MeOH, EtOH and Glycerol (GLY)] were of analytical reagent grade. Purified water used throughout the studies was produced in the laboratory with a Milli-Q purification system (Millipore Corp., USA).

Phosphate buffered saline (PBS) used for *in vitro* drug release, permeation and mucoadhesion studies had the following composition per litre of distilled water: 2.38 g $Na_2HPO_4 \cdot 10H_2O$, 0.19 g KH_2PO_4, 8 g NaCl and adjusted to pH 6.8 or pH 7.4 with hydrochloric acid or sodium hydroxide as required (Peh and Wong, 1999).

2.2 METHODS

2.2.1 Preparation of Films via Co-blending

Films were prepared using the solvent casting and evaporation method. For this study silicone moulded trays (SMTs) with individual wells of 6 cm^2 were used instead of conventional film casting trays, since it has been shown in our previous publication that SMTs enhance drug content uniformity, and reduce the variability in mucoadhesivity as well as drug release (Perumal et al., 2008a).

Multipolymeric films comprising of DDI, HPMC and EUD in various ratios were prepared as shown in Table 3.1. Specified quantities of EUD and TEC as its plasticizer together with HPMC and GLY as its plasticizer were dissolved in 40 mL methanol in a 100 mL volumetric flask. DDI and 40 mL water was added to this and sonicated until the drug has been dissolved. The mixture was made up to volume with 50 % methanol in water and agitated by hand at room temperature until a homogenous solution resulted. Preformulation studies informed the specific formulation variables to use. The plasticizer content for both polymers was kept constant at 30 % (w/w) of polymer weight for all ratios prepared.

Thereafter 2 mL of each polymeric solution containing 20 mg of DDI was syringed into each 6 cm^2 well of the SMT containing Teflon coated Perspex inserts. The drug–

polymeric mixture was allowed to dry in an oven (Series 2000, Scientific, SA) at 43 °C for approximately 24 h, until the solvent had evaporated and constant film weight was achieved. Films were removed from the moulds and stored using wax paper and foil in a desiccator at room temperature (23 °C) up to a maximum of three months until further use.

Table 3.1: Composition of the buccal film formulations (DDI:HPMC:EUD).

Ingredients (% w/v)	Effect of HPMC				Effect of EUD				
	1:0.25:10	1:0.5:10	1:0.75:10	1:1:10	1:0.5:5	1:0.5:7.5	1:0.5:10	1:0.5:15	1:0.5:20
DDI	1	1	1	1	1	1	1	1	1
HPMC	0.25	0.5	0.75	1	0.5	0.5	0.5	0.5	0.5
GLY	0.075	0.15	0.225	0.3	0.15	0.15	0.15	0.15	0.15
EUD	10	10	10	10	5	7.5	10	15	20
TEC	3	3	3	3	1.5	2.25	3	4.5	6

2.2.2 Characterization of Films

2.2.2.1 Weight and Thickness Uniformity

For weight uniformity three films per batch were randomly selected and individually weighed on an electronic balance (Metller Toledo AB204-S., Switzerland). The thicknesses of the films were measured using a digital micrometer (Mitutoyo Co., Japan) with an accuracy of 0.001 mm. Thicknesses were measured in five different locations (centre and four corners) of the films. Results are represented as a mean and standard deviation of the replicate determinations.

2.2.2.2 Assay of Films

The assay solvent consisted of 80 % ethanol in water. A 6 cm^2 film as a unit from the SMT was dissolved in approximately 40 mL of the assay solvent in a 100 mL volumetric flask before making up to volume with the same assay solvent. Following appropriate dilution (1 in 10), the drug content in the samples was quantified using a UV spectrophotometer (Shimadzu 1650 PC, Japan) at a wavelength of 250 nm. All assays were performed in triplicate.

A calibration curve of DDI concentration versus absorbance was plotted across a concentration range from 0.1 to 50 µg/mL and a linear response was found (r^2 = 0.9997). The UV methodology was also successfully validated in terms of specificity, linearity, precision, accuracy and robustness (data not shown).

2.2.2.3 In Vitro Drug Release

A modified BP2009 Type II paddle dissolution test apparatus (Erweka DTR-6., Germany) was employed to determine *in vitro* drug release of the films. The dissolution studies were carried out in 900 mL PBS adjusted to pH 6.8 and maintained at 37 ± 0.5 °C; with a stirring speed of 50 rpm. The film size required for dose delivery (6 cm^2) was used. The film was placed into a stainless steel wire mesh basket and dropped into the dissolution vessel at the start of the experiment. A wire mesh basket was used, instead of attaching a film to a glass slide with adhesives as commonly reported (Nair et al., 2013), in an attempt to limit interference with drug release. Aliquots of 6 mL samples from the dissolution medium were collected at predetermined time intervals of 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min using a syringe and in line filtration (0.45 µm). An equal volume (6 mL) of fresh PBS was replaced into each dissolution vessel, to ensure that a constant volume of dissolution medium was maintained throughout the duration of the study. The filtered samples were quantified for drug using a UV spectrophotometer (Shimadzu 1650 PC, Japan) at a wavelength of 250 nm. The results are represented as the average of three films.

2.2.2.4 In Vitro Permeation

In vitro permeation experiments were performed on the 1:0.5:10 formulation to confirm the permeability potential of DDI incorporated into multipolymeric films. Porcine buccal mucosa was used as a biological membrane for these experiments due to the many similarities to the human buccal mucosa as highlighted by Shojaei (1998) and Sudhakar et al. (2006).

Porcine buccal mucosa was excised from domestic pigs (30-40 kg) immediately upon euthanasia at the university's biomedical research unit after obtaining necessary ethical clearance (011/12/Animal). Excess adipose and connective tissue were cut away from the mucosal specimens leaving the mucosa with an average thickness of (665 ± 72 µm). Samples were wrapped in foil before being snap-frozen in liquid nitrogen and stored at -85 °C in a biofreezer for up to 3 months (Van Der Bijl, 1998).

In vitro permeation experiments on DDI films were performed similar to *in vitro* permeability studies of DDI solutions recently reported (Ojewole et al., 2012). On the day of the experiments, frozen buccal mucosal specimens were allowed to thaw and equilibrate in PBS pH 7.4 to regain elasticity temporarily lost while frozen. Franz diffusion cells (PermeGear, Inc., USA) each with a diffusional area of 0.786 cm^2 were

used for the *in vitro* permeation experiments. The buccal mucosa and polymeric film were mounted between the donor and receptor compartments using the two membrane holders. Two millilitres PBS at pH 6.8, simulating human saliva (Peh and Wong, 1999), was placed on the film in the donor compartment while the receptor compartment contained 27 mL PBS pH 7.4 maintained at 37 °C (by means of a surrounding jacket) and stirred constantly.

At predetermined time intervals over 360 min, samples (27 mL) were taken from the receptor compartments and replaced by drug-free PBS. Similar to dissolution studies samples were immediately filtered through a 0.45 µm membrane filter and the drug content was quantified using a UV spectrophotometer (Shimadzu 1650 PC, Japan) at a wavelength of 250 nm. A minimum of three replicates were performed.

The viability of the mucosa was assessed by transepithelial electrical resistance (TEER) measurements using a Millicell ERS meter (Millipore, USA) connected to a pair of chopstick electrodes (STX01). TEER measurements were taken across the mucosa before and at the end of, the permeation experiment (Dezani et al., 2013) and thereafter following exposure to fresh PBS pH 6.8 for 60 min (Chen et al., 2009).

The cumulative amount of DDI permeated per unit surface area was plotted versus time. The steady state flux (J_{ss}) across the mucosal membrane was determined from the linear portion of the permeation graph by linear regression analysis (Microsoft Excel 2010). The permeability coefficient (P) was calculated using the following equation (Shojaei et al., 1999):

$$P = \frac{(dQ/dt)}{A \times C_d} = \frac{J_{ss}}{C_d}$$

Where dQ/dt is the cumulative amount (Q) of DDI which permeated into the receptor compartment per unit time (t), A the active cross-sectional area (0.786 cm^2) available for diffusion and C_d is the drug concentration in the donor compartment.

2.2.2.5 Histological Evaluation

Histological studies were performed to evaluate for pathological changes occurring in cell morphology and tissue organization. Directly after excision of mucosa, untreated buccal mucosa was transferred from normal saline into 10 % buffered formalin without any equilibration in PBS and served as the control. Treated samples comprised of buccal mucosae that were exposed to PBS only, or a placebo film or drug loaded film

(1:0.5:10). Permeation experiments were performed as described previously in Section 2.2.2.4, without drug quantification (Rambharose et al., 2013). At the end of the experiment the buccal mucosa was cut into cross sections. The samples for light microscopy were fixed in 10 % buffered formalin for 7 days, washed in water, dehydrated in graded ethanol and, after permeation in xylene, embedded in paraffin using standard procedures. Samples were cut into sections (1 µm thick) on a microtome and stained with hematoxylin and eosin (H&E). Sections were examined using a light microscope (Nikon 80i, Japan), and bright field images were digitally captured using NIS Elements D software and a camera (Nikon U2, Japan). Samples for transmission electron microscopy (TEM) were collected under the same conditions. They were fixed for 24 hours (4 °C) using Karnovsky's fixative buffered to pH 7.2, embedded in epoxy resin, cut into ultrathin section (90 nm) and contrasted with uranyl acetate and lead citrate using standard protocols before viewing with a transmission electron microscope (JEOL 1010, Japan). All experiments were performed using a minimum of three replicates.

2.2.2.6 Mucoadhesivity of Films

The effects of the different polymeric ratios on the mucoadhesive properties were studied using methods adapted from (Ayensu et al., 2012a) and (Perumal et al., 2008b). A TA.XT2*i* Texture Analyser (Stable Micro Systems, UK) equipped with a 5 kg load cell in tension mode, removable 2 cm x 3 cm aluminium probes, and Texture Expert™ software were used for this purpose.

Film samples (*n=3*), 30 mm long x 20 mm wide, and free from physical imperfections were individually attached to probes using double sided adhesive tape. The probes were attached to the upper movable arm of the TA.XT2*i.* A Petri-dish containing solidified 10% (w/v) gelatine gel, simulating buccal mucosa, was clamped into place on the stationary platform of the TA.XT2*i* (Ayensu et al., 2012a). Two millilitres of 30% (w/v) mucin at 37 °C was spread on the surface of the gelatin immediately prior to testing (Perumal et al., 2008b). The film, securely attached to the probe, was allowed to hydrate for 120 seconds in PBS pH 6.8 before being brought into contact with the mucin covered gelatin. The film was held in place with a force of 100 grams for 60 seconds before the mobile arm was raised. Parameters used were pre-test speed: 0.5 mm/s; test speed: 0.5 mm/s and post-test speed: 1 mm/s. The mucoadhesive performance of the samples was determined by measuring the Maximum Detachment Force (MDF) (*mN*) and work (*mJ*). The MDF represents the maximum force required to

detach the film from the mucin covered gelatin. The area under the force/distance curve was also determined to represent the work required for detachment of the two systems (mucin/polymeric film) (Eouani et al., 2001). A minimum of 9 replicate determinations were performed.

2.2.2.7 Mechanical Testing

Mechanical properties of the films were studied as a function of various polymer ratios prepared. A TA.XT2*i* Texture Analyser (Stable Micro Systems, UK) equipped with a 5 kg load cell, TA-96 grips and Texture Expert™ software were utilized for this purpose.

Individual film samples (*n=5*), 30 mm long by 20 mm wide with varying thickness (Table 3.2), and free from physical imperfections were held between the grips (TA-96). The grip separation was set at 15 mm. A sheet of Teflon was attached to the surface of the grips via double-sided tape to prevent the film being cut by the grooves of the grips. During measurement, the film was pulled by the top grip at a rate of 1 mm/s to a distance of 150 mm before returning to the starting point. Data acquisition was terminated when the film ruptured completely. The data of the film samples that failed at, and not between, the grips were not utilized in the evaluation of the mechanical properties. The force and elongation were measured when the films broke.

The tensile strength, percent elongation, and Young's modulus were used as indicators of the mechanical properties of the films. Mechanical properties of the films were evaluated using the following equations (Heng et al., 2003):

$$\text{Tensile strength (N/mm}^2\text{)} = \frac{\text{force at break (N)}}{\text{width (mm)} \times \text{thickness of film (mm)}}$$

$$\text{Elongation at break (\%)} = \frac{\text{increase in length}}{\text{original length}} \times 100$$

Young's modulus was determined from the slope of the initial linear portion of the stress-strain plots generated with the Texture Expert™ software.

A rupture test was also performed to assess the mechanical film properties. A film support rig with an exposed area of 0.786 cm^2 was attached to the heavy duty platform of the TA.XT2*i* Texture Analyser. Individual film samples (*n=3*) were clamped between the film support rig before passing a 5 mm stainless steel ball probe through a sample at 1 mm/s in compression mode. The force (*N*) required to rupture the film was measured (Sievens-Figueroa et al., 2012).

2.2.2.8 Surface pH

Saliva has a natural buffering capacity (Bardow et al., 2000) and its pH ranges from 5.6 to 7 (Sudhakar et al., 2006). Buccal formulations should be within this range to avoid causing mucosal irritation. The surface pH of films was determined using methods adapted from (Cavallari et al., 2013) to assess for any potential buccal mucosa irritation. The film was allowed to swell in 15 mL PBS as simulated saliva at pH 6.8 and the pH was measured at predetermined time intervals over 6 hours. The film was carefully removed from the PBS, pH paper (Hydrion MicroFine, Micro Essential Laboratory, USA) was placed on its surface and the pH was measured. Results are represented by the mean of three measurements.

2.2.3 Establishment of the Polymeric Complexation Profile and Potential Impact on Mucoadhesion and Drug Release via SLAS

All modelling procedures and computations, including energy minimizations in Molecular Mechanics, were performed using HyperChem™ 8.0.8 Molecular Modelling Software (Hypercube Inc., Gainesville, FL, USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, Cambridge, UK). The 3D structure of EUD was archetyped using ChemBio3D Ultra in its syndiotactic stereochemistry as a 3D model, whereas the structure of HPMC (4 saccharide units) was built from standard bond lengths and angles using the Sugar Builder Module on HyperChem 8.0.8. The structures of GLY and TEC were constructed with natural bond angles. The structure of the glycosylated mucopeptide analogue (MUC) mucin was generated using the sequence editor module on HyperChem 8.0.8. The glycosylation was performed at the threonine amino acid residues. The models were primarily energy-minimized using the MM+ Force Field algorithm and the resulting structures were once again energy-minimized using the AMBER 3 (Assisted Model Building and Energy Refinements) Force Field algorithm. The conformer having the lowest energy was used to develop the polymer-polymer; polymer-plasticizer; and polymer-mucin complexes. A complex of one polymer molecule with another was assembled by parallel disposition and the energy-minimization was repeated to generate the final models: HPMC-GLY, EUD-TEC, HPMC-EUD, HPMC-GLY/EUD-TEC, HPMC-MUC, EUD-MUC, and HPMC-MUC-EUD. Full geometrical optimization was conducted in vacuum employing the Polak–Ribiere Conjugate Gradient method until an RMS gradient of 0.001 kcal/mol was reached (Kumar et al., 2012).

2.2.4 Statistical Analysis

All calculations were undertaken with Microsoft Excel® (Microsoft Office 2010, USA). A minimum of three replicates were performed and results are expressed as mean ± SD. Statistical analysis of data were performed using GraphPad Prism, Version 5 (GraphPad Software., Inc., USA). One-way ANOVA followed by Dunnett's multiple comparisons test was used to determine statistical significance. *p*-Values of $p < 0.05$ were considered significant.

3.0 RESULTS AND DISCUSSION

3.1 PREPARATION OF FILMS VIA CO-BLENDING

During preliminary studies, monopolymeric films containing DDI and either HPMC, as a hydrophilic polymer, or EUD, as a hydrophobic polymer, were prepared. However these films were deemed unsuitable for drug delivery due to unfavourable physico-mechanical film properties (data not shown). Monopolymeric films exhibited undesired drug release kinetics, had irregular surfaces and unsuitable mechanical strength. Monolayered multipolymeric co-blended films (MMFs) were prepared thereafter with HPMC and EUD to improve film characteristics. Instead of using carcinogenic solvents (Perugini et al., 2003), complex mixing and emulsification methods (Pendekal and Tegginamat, 2012, Perumal et al., 2008b) or multiple solvents with additional emulsifiers (Vasantha et al., 2011) as previously reported, our group used a simple method that eliminated the need for homogenization and cooling as well as the use of complex or carcinogenic solvents and additional emulsifiers, to produce MMFs. We simply used methanol as the co-solvent in which the hydrophobic EUD as well as the hydrophilic DDI could dissolve. Methanol is miscible with water and allowed for sufficient swelling of HPMC in the aqueous medium. Multipolymeric monolayered films (MMFs), containing polymers of opposing solubilities and DDI, were successfully prepared using this simplified co-blending technique. SLAS results described under Section 3.8.2 indicate that the two polymers and two plasticizers form a stable quadra-molecular system with the total energy of stabilization being six times higher than that of only the polymers in combination. It thereby supported the choice of polymers and plasticizers for the "novel co-blending-co-plasticizing strategy" employed in this study.

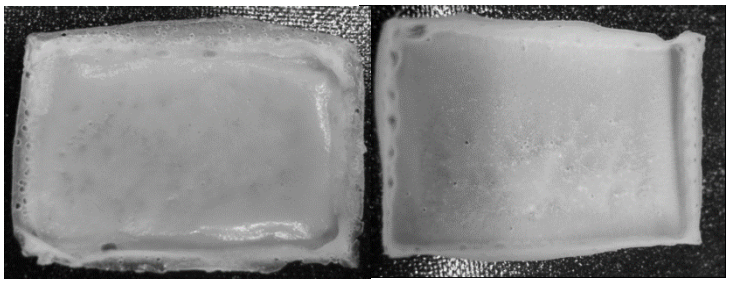


Figure 3.1: Digital photographs of 1:0.5:10 (DDI:HPMC:EUD) monolayered multipolymeric films.

The films generated by this simplified technique were translucent to opaque, thin, flexible and their surface appeared homogenous (Figure 3.1). The drug-polymeric casting solution prepared using the co-blending technique was also completely homogenous and no phase separation occurred upon drying of the films. Drying for 24 h at 43 °C did not pose stability concerns as Kasongo et al. (2011) established didanosine's thermal stability in excess of 85 °C. Limited drug precipitation was also noted. The average thickness and weight of the films ranged from 124 to 666 µm and 159 to 556 mg respectively, increasing proportionally as polymer content increased (Table 3.2). Drug content uniformity across buccal films is a major problem as highlighted in the literature (Morales and McConville, 2011). By using similar silicone moulded trays with individual wells for film casting as previously investigated by our group (Perumal et al., 2008a) we were able to overcome problems with drug content uniformity (Table 3.2). Drug content values ranged from 91 % to 105 % with low CV values of less than 6 % indicating good drug content uniformity. All ratios prepared of monolayered multipolymeric films were homogenous, had limited drug precipitate and acceptable drug content uniformity.

Table 3.2: Effect of polymer ratios on drug content uniformity, thickness and film weight. (Mean ± SD values; *n* = 3).

FORMULATION		Assay (%) *n=3*		Thickness (µm) *n=3*		Weight (mg) *n=3*	
DDI:HPMC:EUD		MEAN ± SD	CV%	MEAN ± SD	CV%	MEAN ± SD	CV%
EFFECT OF HPMC	1:0.25:10	91.17 ± 0.85	0.93	209.40 ± 17.40	8.31	243.53 ± 10.51	4.32
	1:0.5:10	95.62 ± 5.41	5.66	299.60 ± 25.00	8.34	293.60 ± 1.50	0.51
	1:0.75:10	91.69 ± 2.11	2.30	308.07 ± 26.97	8.75	306.10 ± 2.52	0.82
	1:1:10	91.64 ± 2.48	2.70	319.73 ± 3.19	1.00	318.60 ± 2.56	0.80
EFFECT OF EUD	1:0.5:5	96.79 ± 0.36	0.37	124.13 ± 2.64	2.13	159.03 ± 0.96	0.60
	1:0.5:7.5	98.06 ± 2.36	2.41	205.20 ± 3.60	1.75	228.67 ± 0.72	0.32
	1:0.5:10	95.62 ± 5.41	5.66	299.60 ± 25.00	8.34	293.60 ± 1.50	0.51
	1:0.5:15	102.83 ± 3.06	2.98	434.33 ± 24.23	5.58	420.90 ± 10.19	2.42
	1:0.5:20	105.24 ± 1.69	1.60	666.47 ± 11.60	1.74	556.13 ± 4.92	0.88

3.2 IN VITRO DRUG RELEASE

The influence of HPMC and EUD on the drug release of DDI MMFs were studied. Figure 3.2a shows the drug release profiles of DDI films prepared using increasing amounts of HPMC. An increase in HPMC led to increase in drug release while still maintaining controlled release profiles with no significant dose dumping. This increased drug release could be attributed to the hydrophilic nature of HPMC, which can erode more readily (Morales and McConville, 2011), thereby releasing the drug into the dissolution medium (33% within the 1st hour for 1:1:10). In addition to polymer solubility, molecular mechanistic simulations (Section 3.8.3) also showed that comparatively higher concentrations of HPMC-GLY will make the quadra-molecular architecture less stable. This leads to an increase in hydrophillicity and chain relaxation or degradation which causes increased drug release. Although drug release increased with increasing HPMC, the controlled drug release seen with all four profiles (Figure 3.2a) was due to the incorporation of EUD, a hydrophobic polymer, into the multipolymeric films.

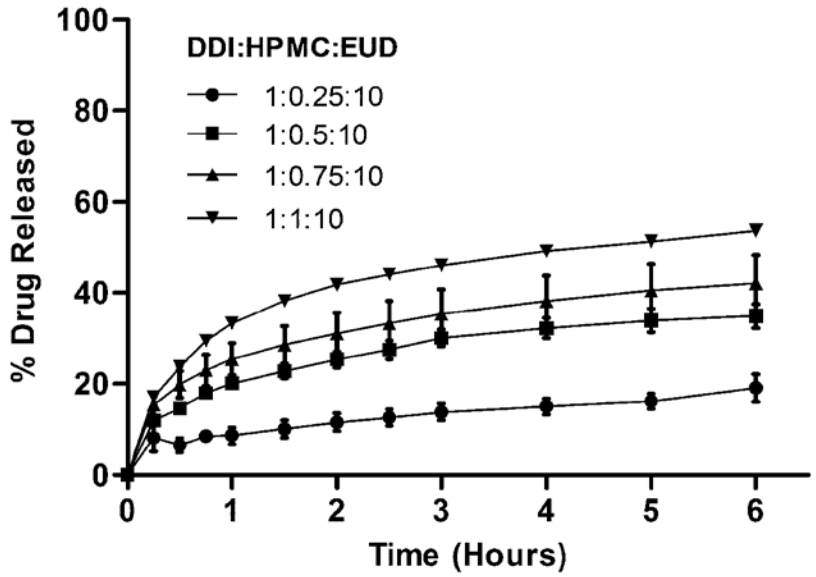


Figure 3.2 (a): Effect of HPMC on DDI release from multipolymeric films.

The effect on drug release upon altering the EUD content of the multipolymeric films was also investigated. Figure 3.2b shows the drug release profiles of DDI films prepared using increasing amounts of EUD and constant amounts of HPMC. 1:0.5:5 and 1:0.5:7.5 showed very rapid drug release. 80 % of the loaded drug was release from 1:0.5:5 within the first 15 minutes. Rapid drug release systems would be unfavourable for the delivery of DDI, as frequent drug administration would lead to

decreased patient compliance. Further increase in EUD content in the films led to decreased rates of drug release. It is possible to achieve controlled drug release with changing the ratios of polymers used in the formulation. Drug release retardation could be attributed to the hydrophobic nature of the EUD and the resultant lower solubility in the aqueous dissolution medium and slower rate of film erosion (Magdy I. Mohamed et al., 2011). The low aqueous solubility of EUD prevented free and deep water penetration into the film, thereby only allowing the DDI that was near the external surface of the film to be initially released into the dissolution medium (Perumal et al., 2008b). The molecular mechanistic model employed in this study (Section 3.8.3) indicated that the presence of the stable EUD-TEC complex increase the stability of the films leading to slower drug release since there would be less tendency of the stabilized system to undergo a change in terms of chain relaxation or film degradation.

Blending of EUD and HPMC polymers were necessary to obtain a desired controlled release profile of DDI. Molecular mechanistic simulations discussed in detail in Section 3.8.3 provided additional supportive information to understand drug release profiles when blending polymers of opposing solubilities.

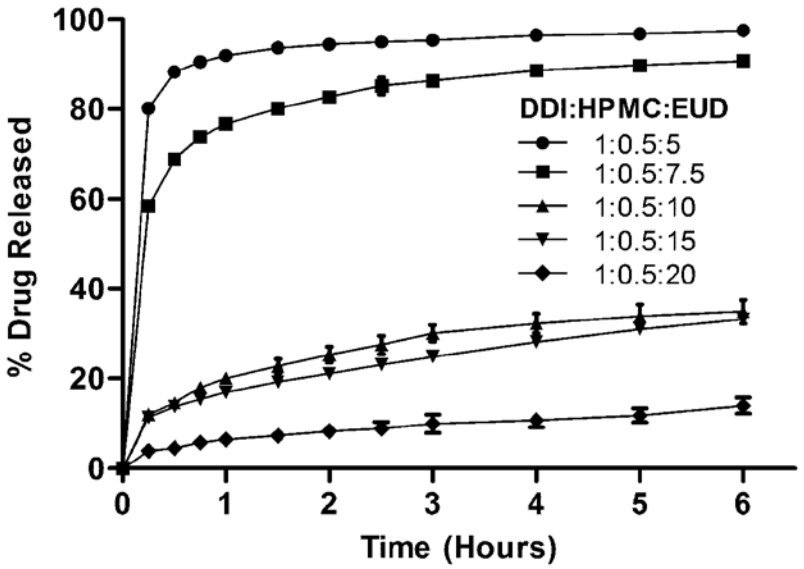


Figure 3.2 (b): Effect of EUD on DDI release from multipolymeric films.

3.3 IN VITRO PERMEATION

It is recognized that the bioavailability of drugs administered via the buccal route can be greatly influenced by the permeation rate through the buccal mucosal membrane (Morales and McConville, 2011). Limited work has been published regarding buccal permeability of antiretrovirals. Thus far the buccal permeability potential of only drug solutions containing zalcitabine (Shojaei et al., 1999, Xiang et al., 2002), didanosine (Ojewole et al., 2012) or tenofovir (Rambharose et al., 2013) have been published. In terms of drug delivery systems for the buccal route, buccal patches for zidovudine has been reported on but, permeability of drug across the mucosa was not reported. The buccal permeability potential of DDI from 1:0.5:10 films were investigated due to their potentially suitable mucoadhesion and drug release (Figure 3.3). The non-linear portion of the plot was considered as the lag time and it was the time required for steady state permeation to be achieved. A lag time of 45 minutes in this case is acceptable since the formulation under investigation will be for controlled release. In this study, we show that the drug can be released from the buccal film and can permeate across the mucosa as evidenced by a permeability coefficient of 0.72 ± 0.14 $x10^{-2}$ cm/h and a steady state flux (*Jss*) value of 71.63 ± 13.54 µg/cm^2h. The slope of the linear portion (R^2 = 0.9932) of the plot was used to determine the *Jss*. The flux value (71.63 ± 13.54 µg/cm^2h) obtained in this study compares favourably to that achieved in permeation studies performed on DDI solutions only as recently reported by (Ojewole et al., 2012). Therefore these experiments indicate that the flux was not adversely affected by the formulation of DDI into a film with polymeric film components. The data thus confirms the potential of DDI being delivered transbuccaly via multipolymeric films and can be used for improving HIV and AIDS drug therapy. DDI is a hydrophilic drug, and passive diffusion should have preference towards the paracellular pathway (Hassan et al., 2010, Sandri et al., 2006). Several other classes of drugs incorporated into buccal polymeric films exhibited similar or lower flux values (Diaz del Consuelo et al., 2007, Pendekal and Tegginamat, 2012) and were considered as having potential for buccal delivery.

Transepithelial electrical resistance (TEER) measurements can be used as an indicator of epithelial viability for mucosal permeation experiments (Holm et al., 2013, Muendoerfer et al., 2010). There are currently limited reported TEER studies with buccal permeation experiments specifically and therefore standardization remains to be developed. The reported values in these limited available studies vary widely (136 ± 17

to 950 ± 392 Ω/cm^2) (Holm et al., 2013, Nielsen and Rassing, 2002). The TEER value across the buccal mucosa prior to the permeation experiment in this study was found to be 144 ± 12 Ω/cm^2. After 6 hours of permeation this value decreased to 109 ± 21 Ω/cm^2 (24 % reduction). 60 min after removal of the DDI films and exposure to fresh PBS, the TEER increased again to 123 ± 12 Ω/cm^2, which is a 14.5 % difference of the baseline value. This signified a return towards the initial measured integrity. The TEER values obtained in this study appear to be within the reported range and the overall percentage change also indicates that mucosal integrity was not irreversibly affected (Kowapradit et al., 2010). In addition, the extent of TEER changes before and after the permeation experiments also compares favourably to reported studies using rat intestinal segments (Dezani et al., 2013) and porcine nasal mucosa (Sintov et al., 2010) for drug permeation assessments. The TEER values also correlate with the histomorphological studies (Section 3.4) which further confirmed that integrity and viability of the tissue was maintained.

The buccal permeation potential of DDI demonstrated in this study therefore warrants the need for future studies with excipients to enhance permeation.

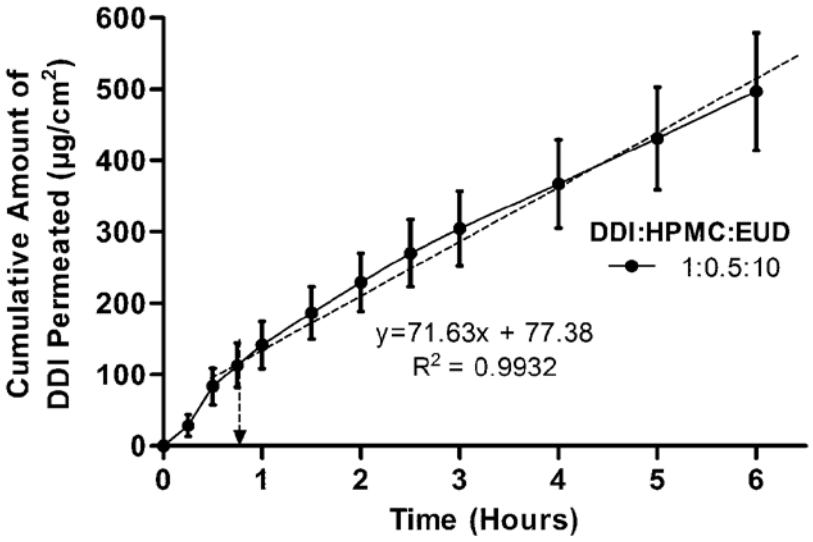


Figure 3.3: Cumulative amount of DDI permeated per unit surface area versus time from films. (Mean ± SD values; $n \geq 3$)

3.4 HISTOMORPHOLOGICAL EVALUATIONS

While buccal permeation studies on drug solution are extensively reported, reports on the effect of polymeric films on buccal mucosa morphology remain limited. Buccal films are often designed for prolonged retention on the mucosa and therefore assessment of histological effects of the drug and the polymeric film on the mucosa is essential. Histomorphological effects of the control/untreated and the treated porcine buccal mucosae (PBS alone, PBS + Placebo Film and PBS + Drug Loaded Film) were assessed. The morphology of porcine buccal mucosa and similarities between it and human buccal mucosa has been described in detail previously (Madhav et al., 2009, Shojaei, 1998, Sudhakar et al., 2006).

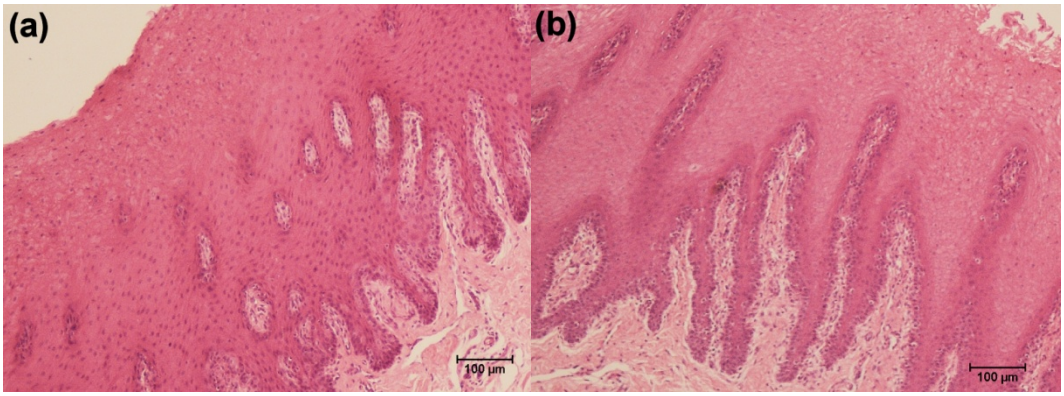
The mucosa lining the buccal cavity is stratified squamous epithelium with a high recovery rate (Squier and Hall, 1985). Since this mucosa is multilayered the cell structure differs as the cells transcends from the basal lamina to the mucosal surface, with cells becoming more flattened in appearance and more closely packed at the surface as compared to basal cells that appear more cuboidal in shape with more distinguishable intercellular spaces. This epithelium remains unkeratinized as an adaptation to its main functions which is to withstand abrasion due to mastication and also at the same time remain lubricated to protect against mechanical abrasion (Shojaei, 1998). Any particle that is able to permeate this mucosal lining has to travel either via transcellular or intracellular pathways to the basal membrane and enter the circulation present in the lamina propria.

The light microscopy (LM) micrograph of the control slides (Figure 3.4a) closely resembled the above description with cells progressively getting flatter and more closely packed at the surface and basal cells being more distinguishable from each other. The PBS treated samples also appeared very similar to the controls, which correlate with other similar studies (Ojewole et al., 2012). Both the placebo film (Figure 3.4b) and drug treated film (Figure 3.4c) mucosal treatments following 6 hours of permeation studies displayed no noticeable histomorphological changes that were indicative of tissue damage. The basal cell layer (Figure 3.4d) appeared intact and darkly stained in H&E reflecting their greater mitotic activity characteristic of these basal cells, therefore suggesting that any changes from DDI or polymers would not be permanent.

These observations were then further confirmed using TEM. TEM images allow a deeper investigation at a cellular level to assess any destruction of the cellular membranes, of individual cells, as well as to the cellular organelles that ensure proper functioning of these cells. Damage to the cellular membrane, destruction of the nuclear membrane and the nucleus, as well as cytoplasmic blebbing are all markers of necrosis (Zong and Thompson, 2006). TEM images can also allow for the evaluation of tight-junctions or similar interconnections between adjacent cells, as well as the evaluation of intercellular spaces that can be used as a route of paracellular transport. The control (Figure 3.5a) displayed cells that are characteristic of normal healthy cells, with no signs of either apoptosis or necrosis. These images also displayed very small intercellular spaces and relatively closely packed cells. The placebo film treatment (Figure 3.5b) displayed cellular morphology similar to those of the controls, with slightly enlarged intercellular spaces as compared to the control samples. This increase in intercellular spaces could be attributed to the polymers that were incorporated into the design of the film.

The drug loaded film treatment showed a further increase in these intercellular spaces, which possibly aided in the transport of the drug via the paracellular route through the buccal mucosa. Although there was an increase in the size of the intercellular spaces in the drug loaded film, the tight junctions were still intact which is indicative that the changes caused by the drug treatment are not permanent. Apart from the observed changes mentioned above, no other detrimental changes to the buccal mucosa due to the placebo/drug treatment were observed.

Both the LM and TEM studies confirmed that there was no tissue damage or distress due to either placebo or drug loaded film treatment. These studies further identified a possible route of transport for the DDI loaded film across the buccal mucosa and also showed evidence that the changes observed were temporary.



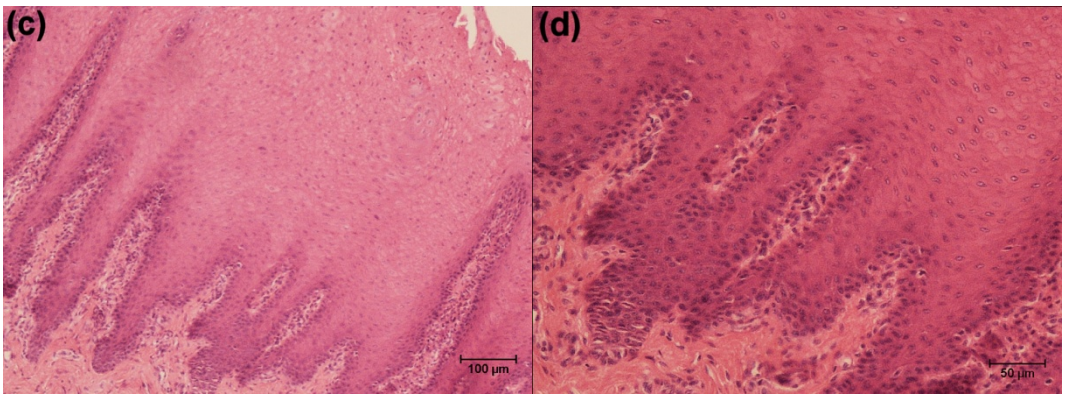


Figure 3.4: Microphotographs of the control and treated ultra-thin buccal mucosal sections for light microscopy (LM): (a) untreated control, (b) placebo film, (c) drug film, (d) basal cells of drug film.

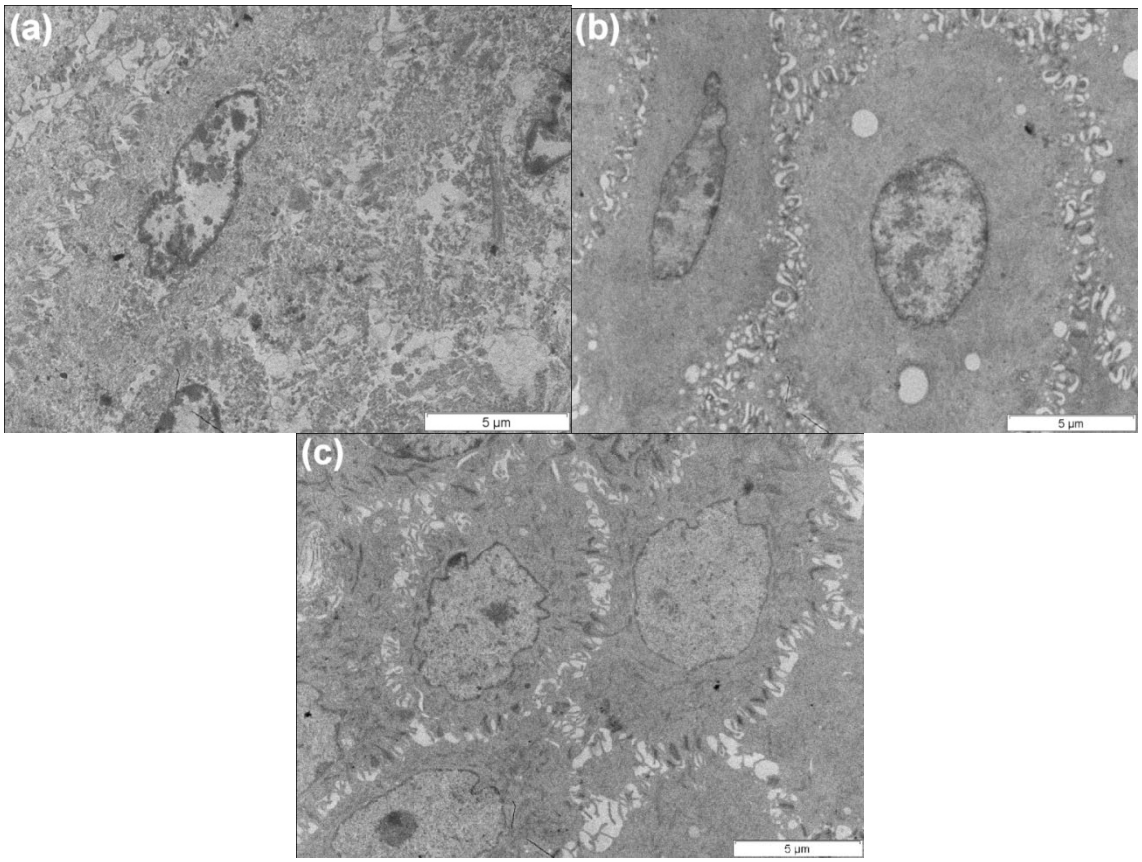


Figure 3.5: Microphotographs of the control and treated ultra-thin buccal mucosal sections for transmission electron (TEM): (a) untreated control, (b) placebo film, (c) drug film.

3.5 MUCOADHESIVITY OF FILMS

The majority of mucoadhesive polymers investigated for buccal films are hydrophilic (Morales and McConville, 2011). Conversely hydrophobic Eudragit® can also demonstrate mucoadhesiveness when used separately or together with other hydrophilic polymers such as chitosan (Pendekal and Tegginamat, 2012, Perumal et al., 2008b). Prepared formulations were subsequently tested for their mucoadhesivity, since a prerequisite for buccal controlled drug delivery systems is adhesion on the oral mucosa (Eouani et al., 2001). A maximum detachment force (MDF) of 2184 mN per film (Figure 3.6) seen with 1:0.25:10 is comparable to optimized buccal films (Ayensu et al., 2012b, Eouani et al., 2001) and buccal tablets (Boyapally et al., 2010, Cappello et al., 2006) published previously. This indicates that the films prepared in this study show potential for retention at the site of drug absorption for prolonged time periods.

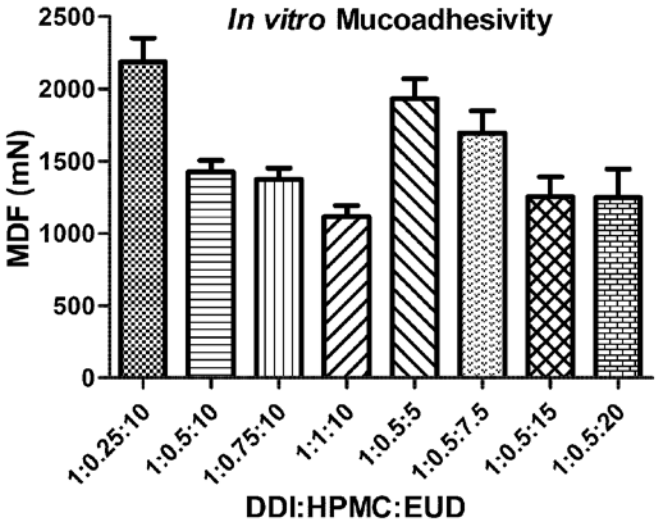


Figure 3.6: Effect of HPMC and EUD on *in vitro* mucoadhesivity.

The effect of HPMC content on mucoadhesivity was investigated (Table 3.3). The decrease in MDF observed as HPMC content increased could be attributed to the increased intermolecular interactions possibly between the higher levels of plasticizers and polymers in the subsequent formulations. HPMC was selected as the hydrophilic polymer for the formulation based on preliminary drug-polymer interaction studies. Molecular mechanistic simulations as discussed later under Section 3.8.4 indicated that HPMC showed few electrostatic interactions with –COOH and $–NH_2$ groups of the mucin potentially explaining the decrease in MDF observed as the HPMC content increased from 0.25 % to 1 % (Table 3.3).

The effect of EUD content on mucoadhesivity was also investigated (Figure 3.6). A decrease in MDF was also noted as EUD content increased. EUD, a cationic polymer, is positively charged and could interact to some extent with the negatively charged mucin glycoproteins. Molecular simulations supported the level of mucoadhesivity observed. The quaternary ammonium groups of EUD seemed to form the much needed electrostatic interaction to impart mucoadhesivity to the buccal films, but the hydrophobic nature of EUD may have caused destabilization of H-bonding. The mucoadhesive interaction was reduced (1930.2 ± 137.9 to 1245.7 ± 196.1 mN) as the EUD content increased from 5 % to 20 % respectively. EUD being a hydrophobic polymer restricted the free entry of water causing less efficient chain mobility and physical entanglement with mucus (Perumal et al., 2008b). Work of adhesion is the area under the force/distance curve generated by the TA-XT2*i*. High coefficient of variance (CV) percentages for work of adhesion reflects the difficulty in accurately measuring the area under the curve (AUC) of the narrow peaks generated on the stress-strain curves. Work of adhesion and MDF values followed similar trends throughout. Statistical significant ($p < 0.05$) differences between mucoadhesion (MDF) values were observed between the identified 1:0.5:10 formulation and other ratios prepared (Table 3.3). Films had acceptable mucoadhesivity but increased polymeric content affected mucoadhesion negatively in the multipolymeric films. A balance needs to be achieved between acceptable mucoadhesivity and desired drug release.

Table 3.3: Effect of polymer concentration on mucoadhesivity of films.

FORMULATION (DDI:HPMC:EUD)		Maximum Detachment Force (mN) *n=9*		Work of Adhesion (mJ) *n=9*	
		MEAN ± SD	CV %	MEAN ± SD	CV %
EFFECT OF HPMC	1:0.25:10	2184.56 ± 164.28***	7.52	1.42 ± 0.42	29.63
	1:0.5:10	1425.00 ± 77.15$^{N/A}$	5.41	1.21 ± 0.32	26.18
	1:0.75:10	1371.33 ± 79.62^{NS}	5.81	1.02 ± 0.13	12.47
	1:1:10	1116.44 ± 75.08***	6.72	0.90 ± 0.18	20.48
EFFECT OF EUD	1:0.5:5	1930.22 ± 137.87***	7.14	1.31 ± 0.23	17.17
	1:0.5:7.5	1695.78 ± 151.96***	8.96	1.25 ± 0.15	12.00
	1:0.5:10	1425.00 ± 77.15$^{N/A}$	5.41	1.21 ± 0.32	26.18
	1:0.5:15	1253.67 ± 134.62*	10.74	0.99 ± 0.17	16.82
	1:0.5:20	1245.67 ± 196.05*	15.74	1.04 ± 0.33	31.88

Statistical significance compared to 1:0.5:10 - *** $p < 0.001$; ** p 0.001 to 0.01; * p 0.01 to 0.05; NS $p > 0.05$; N/A – Non-applicable.

3.6 MECHANICAL TESTING

The mechanical strength of films reflects their ability to withstand mechanical damage during production, handling and application (Yoo et al., 2006). It was therefore necessary to assess the mechanical properties of the DDI monolayered multipolymeric films (Table 3.4). The investigated mechanical properties together represent film abrasion resistance, ductility and stiffness or elasticity. Increases in HPMC concentration resulted in increased tensile strength, Young's modulus and force required to rupture the films (Table 3.4). This would lead to improved abrasion resistance making films less prone to breakage and more durable to handle. The effect of EUD on mechanical properties was investigated. Overall improved mechanical properties were noted for films as the EUD content increased from 5 % to 20 %. In this series film elongation increased up until 1:0.5:10 (69.54 ± 7.77 %) then decreased as polymer content further increased in 1:0.5:15 and 1:0.5:20 (54.68 ± 0.49 % and 45.36 ± 6.47 %). This decrease could be attributed to the increased stiffness and thickness resulting in tougher films. These films were rigid and could cause discomfort when being administered into the buccal cavity. Interestingly, the tensile strength increased for films with increasing amounts of HPMC, whereas the opposite was noted for films containing increasing amounts of EUD. A tensile strength of 0.698 N/mm^2 compares favourably to previously produced buccal formulations (El-Kamel et al., 2007, Shidhaye et al., 2008, Sievens-Figueroa et al., 2012). Polymers selected and the concentration in the buccal formulations affect all mechanical properties. It is clear that films should have sufficient tensile strength to withstand necessary handling yet, be flexible enough to ensure patient comfort. It is for this reason that optimal polymeric blends need to be identified.

Table 3.4: Effect of polymer concentration on mechanical properties of films.

FORMULATION (DDI:HPMC:EUD)		Rupture Force (N) *n=3*	Tensile Strength (N/mm^2) *n=5*	Young's Modulus (N/mm) *n=5*	Elongation (%) *n=5*	Toughness (N.mm) *n=5*
		MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD
EFFEFT OF HPMC	1:0.25:10	2.62 ± 0.60	0.2733 ±0.051***	0.10 ± 0.04	53.75 ± 15.61	41.27 ± 3.89
	1:0.5:10	6.69 ± 0.39	0.6976 ± 0.064$^{N/A}$	0.23 ± 0.05	69.54 ± 7.77	111.70 ± 12.98
	1:0.75:10	11.90 ± 0.40	0.7288 ± 0.057^{NS}	0.36 ± 0.03	57.41 ± 2.18	99.46 ± 17.26
	1:1:10	19.47 ± 0.48	1.1301 ± 0.054***	0.59 ± 0.08	56.00 ± 6.37	102.91 ± 10.64
EFFECT OF EUD	1:0.5:5	5.46 ± 0.68	2.5545 ± 0.144***	1.13 ± 0.17	25.20 ± 2.90	42.71 ± 7.74
	1:0.5:7.5	5.42 ± 0.86	1.1387 ± 0.097***	0.53 ± 0.04	38.02 ± 4.75	57.66 ± 13.04
	1:0.5:10	6.69 ± 0.39	0.6976 ± 0.064$^{N/A}$	0.23 ± 0.05	69.54 ± 7.77	111.70 ± 12.98
	1:0.5:15	25.43 ± 0.66	0.6315 ± 0.029^{NS}	0.43 ± 0.04	54.68 ± 0.49	187.58 ± 15.54
	1:0.5:20	27.75 ± 1.25	0.4358 ± 0.007***	0.50 ± 0.09	45.36 ± 6.47	279.89 ± 33.32

Statistical significance compared to 1:0.5:10 - *** p < 0.001; ** p 0.001 to 0.01; * p 0.01 to 0.05; NS p > 0.05; N/A – Non-applicable.

3.7 SURFACE PH

The surface pH of buccal polymeric films is an important characteristic to evaluate. *In vivo* studies by Bottenberg et al. (1991) demonstrated that fluctuations in pH beyond the normal range of saliva (pH 5.8-7) may cause local irritation to the buccal mucosa. A minimal decrease of 0.17 in pH, from pH 6.8-6.63 was measured *in vitro* over 24 hours for 1:1:10 films. The slight decreases in pH for films over time can be attributed to the availability of polymer that can ionise at PBS pH. As the film swells more polymer from the inner areas of the film become available to ionise. *In vivo* this does not pose a problem since the buccal environment is an open system, with a continuous production and flow of saliva (Cavallari et al., 2013). The pH values for all the formulations remained within a suitable range indicating that buccal mucosal irritation is unlikely to occur. These results indicate that the multipolymeric buccal films were suitable for buccal application owing to the acceptable pH measurements.

3.8 ESTABLISHMENT OF THE POLYMERIC COMPLEXATION PROFILE AND POTENTIAL IMPACT ON MUCOADHESION AND DRUG RELEASE VIA SLAS

3.8.1 Molecular Mechanics Assisted Model Building and Energy Refinements

Molecular mechanics energy relationship (MMER), a method for analytico-mathematical representation of potential energy surfaces, was used to provide information about the contributions of valence terms, non-covalent Coulombic terms, and non-covalent van der Waals interactions for polymer/plasticizer/mucin interactions. The MMER model for potential energy factor in various molecular complexes can be written as:

$$\mathbf{E_{molecule/complex}} = V_{\sum} = V_b + V_\theta + V_\varphi + V_{ij} + V_{hb} + V_{el} \quad ...(1)$$

where, $V_{\sum}$ is related to total steric energy for an optimized structure, V_b corresponds to bond stretching contributions (reference values were assigned to all of a structure's bond lengths), V_θ denotes bond angle contributions (reference values were assigned to all of a structure's bond angles), V_φ represents torsional contribution arising from deviations from optimum dihedral angles, V_{ij} incorporates van der Waals interactions due to non-bonded interatomic distances, V_{hb} symbolizes hydrogen-bond energy function and V_{el} stands for electrostatic energy.

In addition, the total potential energy deviation, ΔE_{Total}, was calculated as the difference between the total potential energy of the complex system and the sum of the potential energies of isolated individual molecules, as follows:

$$\Delta E_{Total(A/B)} = E_{Total(A/B)} - [E_{Total(A)} + E_{Total(B)}] \quad ...(2)$$

The molecular stability can then be estimated by comparing the total potential energies of the isolated and complexed systems. If the total potential energy of complex is smaller than the sum of the potential energies of isolated individual molecules in the same conformation, the complexed form is more stable and its formation is favoured (Yu et al., 2008).

Table 3.5: Inherent energy attributes representing the molecular assemblies modelled using static lattice atomistic simulations in vacuum.

Molecular complex	ΔE^a $(V_\Sigma)^b$	ΔE $(V_b)^c$	ΔE $(V_\theta)^d$	ΔE $(V_\varphi)^e$	ΔE $(V_{ij})^f$	ΔE $(V_{hb})^g$	ΔE $(V_{el})^h$
HPMC-GLY	-13.259 [i]	0.202 [j]	1.330	5.852	-18.225	-2.414	0.000
EUD-TEC	-21.886	0.267	3.766	0.219	-25.849	-0.268	-0.019
HPMC-EUD	-9.025	-0.463	-0.720	13.059	-19.207	-0.271	-1.420
EUD-TEC/HPMC-GLY	-52.943	0.294	5.181	7.838	-62.297	-3.504	-0.45
HPMC-MUC	-31.073	0.075	0.534	6.798	-35.727	-0.502	-2.249
EUD-MUC	-80.275	-0.177	3.011	0.842	-25.097	0.180	-59.033
HPMC-MUC-EUD	-65.011	-1.054	-6.788	24.282	-45.795	-0.658	-34.994

[a] $\Delta E_{(A/B)} = E_{(A/B)} - [E_{(A)} + E_{(B)}]$.
[b] Total steric energy for an optimized structure.
[c] Bond stretching contributions.
[d] Bond angle contributions.
[e] Torsional contribution arising from deviations from optimum dihedral angles.
[f] Van der Waals interactions.
[g] Hydrogen-bond energy function.
[h] Electrostatic energy.
[i] Values inked green depicts the structure stabilizing contribution.
[j] Values inked red depicts the structure destabilizing contribution.

3.8.2 Effect of the Incorporation of Plasticizer on the Individual Polymer's Performance

$$E_{HPMC} = 49.713V_\Sigma = 2.089V_b + 18.821V_\theta + 22.360V_\varphi + 6.776V_{ij} - 0.335V_{hb} \quad ...(3)$$

$$E_{GLY} = 2.863V_\Sigma = 0.046V_b + 0.269V_\theta + 2.123V_\varphi + 0.437V_{ij} - 0.013V_{hb} \quad ...(4)$$

$$E_{HPMC\text{-}GLY} = 39.317V_\Sigma = 2.337V_b + 20.420V_\theta + 30.335V_\varphi - 11.012V_{ij} - 2.762V_{hb} \quad ...(5)$$

$$E_{EUD} = 43.885V_\Sigma = 5.002V_b + 21.451V_\theta + 8.255V_\varphi + 2.398V_{ij} + 6.777V_{el} \quad ...(6)$$

$$E_{TEC} = 3.913V_\Sigma = 0.497V_b + 2.524V_\theta + 1.206V_\varphi - 0.105V_{ij} - 0.209V_{hb} \quad ...(7)$$

$$E_{EUD\text{-}TEC} = 25.912V_\Sigma = 5.766V_b + 27.741V_\theta + 9.680V_\varphi - 23.556V_{ij} - 0.477V_{hb} + 6.758V_{el} \quad ...(8)$$

The plasticization and filler effects of GLY and TEC w.r.t. HPMC and EUD are depicted in equations 3-5 and equations 6-8, respectively. Both the plasticized complexes, HPMC-GLY and EUD-TEC, were energetically stable with negative energy of formation (ΔE) values of ≈13kcal/mol and ≈22kcal/mol, respectively, justifying the selection of plasticizers for the respective polymers for the film formation and performance.

In case of HPMC-GLY, glycerine formed a well-connected H-bonded molecular complex with HPMC as shown in Figure 3.7a with a space-filling ability to accommodate within the van der Waals space of HPMC molecule which is strengthened by the stabilization of V_{ij} and V_{hb} (Table 3.5). With a closer look at the HPMC-GLY complex, one can assume that one glycerine molecule can additionally form an “intermolecular-bridge” between two adjacent HPMC molecules inducing an “adjacent chain-sliding phenomenon” resulting in an increase in elasticity. Interestingly, introduction of glycerine to the HPMC led to an increase (destabilization) in V_{φ} due to the torsional constraints experienced by the polymer which in turn was due to the filling of the intramolecular-space providing the much needed alignment and distribution of polysaccharide side-chains. On another note, the H-bonding may however decrease the accessibility of HPMC functional groups as discussed later in this paper.

Unlike HPMC-GLY, EUD-TEC was characterized by the absence of H-bonding at all modelling poses tested (data not shown), which may be due to different polarities of the complexing molecules. Additionally, the space-filling appeared more intermolecular because of the size of the TEC molecule w.r.t. EUD modelled which is evident from more stabilized V_{ij}. Furthermore, as the filling was intermolecular; the torsional strain was much reduced as compared to HPMC-GLY (V_{φ} values in Table 3.5). These two reasons made EUD-TEC more stable than HPMC-GLY. As evident from Figure 3.7b, the EUD-EUD interpolymeric interaction appeared less likely because of the presence of plasticizer molecules between the adjacent polymeric chains which may further lead to increased elasticity or decreased rigidity of the EUD-component of the films (Gutiérrez-Rocca and McGinity, 1994).

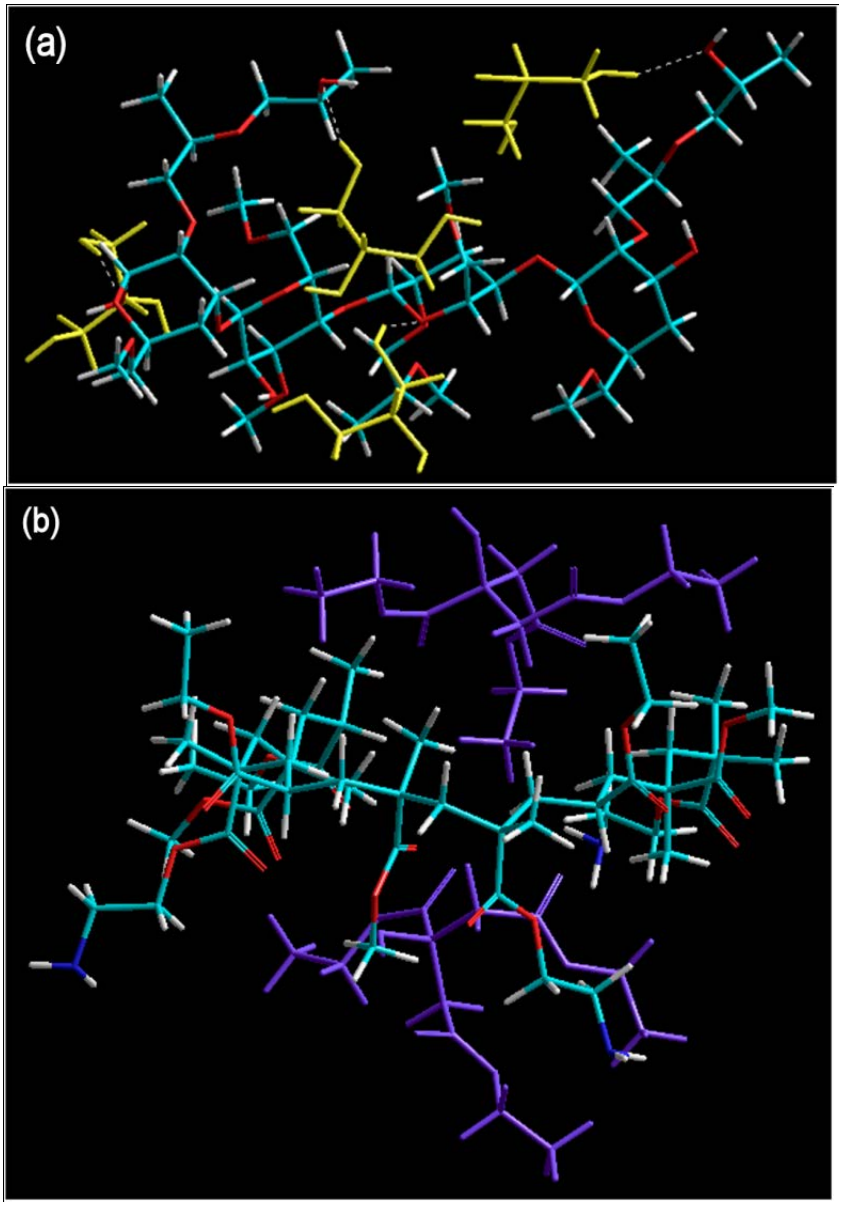


Figure 3.7: Visualization of geometrical preferences of (a) HPMC-GLY: HPMC (standard colours) in molecular complexation with GLY (yellow rendering); and (b) EUD-TEC: EUD (standard colours) in molecular complexation with TEC (violet rendering), after molecular simulations in vacuum. Colour codes for HPMC and EUD tube rendering: C (cyan), O (red), H (white), and P (yellow).

3.8.3 HPMC-EUD Co-blending and Co-plasticization

$$E_{HPMC\text{-}EUD} = 84.573V_{\sum} = 6.628V_b + 39.552V_\theta + 43.674V_\varphi - 10.033V_{ij} - 0.606V_{hb} + 5.357V_{el} \quad \ldots(9)$$

$$E_{EUD\text{-}TEC/HPMC\text{-}GLY} = 47.431V_{\sum} = 7.928V_b + 48.246V_\theta + 41.782V_\varphi - 52.791V_{ij} - 4.061V_{hb} + 6.327V_{el} \quad \ldots(10)$$

The films were fabricated using a unique blend of two polymers, with their respective plasticizers, in a binary-solvent system. To simulate the fabrication conditions; the polymers were modelled together along with their respective plasticizers forming a quadra-molecular system with HPMC-GLY/EUD-TEC: co-blended-co-plasticized polymeric architecture (BPPA). A binary blend system sans plasticizers was also modelled to elucidate the compatibility of the two polymers.

From equation 9 and Table 3.5; it can be deduced that the polymers formed a stabilized geometrically and energetically stable system with all the bonding (V_b and V_θ) and non-bonding (V_{ij},V_{hb}, and V_{el}) interaction energies except V_φ. The HPMC-EUD molecular complex failed to demonstrate an H-bonded system with all the conformation poses tried (Figure 3.8a and 3.8b). Furthermore, the total energy of stabilization, $\Delta E(V_e)$ recorded as ≈-9kcal/mol, was too low to justify an efficient blending. However, an intramolecular EUD H-bonding was reported in the binary mixture which may be due to the torsional constraints caused by the presence of HPMC which was further proven by the instability caused by V_φ (Figure 3.8b).

The HPMC-GLY/EUD-TEC quadra-molecular system is depicted in Figure 3.8c where a well-connected intermolecular architecture is evident by the presence of H-bonding involving HPMC-GLY-EUD linked structure. Additionally; the TEC molecule was well-fitted into the intramolecular space of EUD against the intermolecular space in case of EUD-TEC discussed in the previous section. Now this well-fitted and well-connected structure presented the “standard pattern of energy stabilization” wherein all the bonding interactions (V_b, V_φ, and V_θ) were destabilized and the non-bonding (V_{ij},V_{hb}, and V_{el}) ones were stabilized. Numerically, the total energy of stabilization, $\Delta E(V_e)$ = -52.943, for HPMC-GLY/EUD-TEC was ≈6 times that of HPMC-EUD justifying the “novel co-blending-co-plasticizing strategy” of preparing buccal films.

The drug release profile of the developed buccal films in this study can be explained molecular mechanistically taking the geometrical stabilization of the components in consideration. As explained previously under Section 3.2; an increase and decrease in drug release was observed with an increase in concentration of HPMC and EUD,

respectively. We hereby hypothesize that the component stabilizing a vacuum system (non-aqueous system) and an aqueous system would lead to a respective increase in hydrophobicity and hydrophilicity of the matrix. Convincingly, the presence of EUD-TEC increased the stability of the films (Equations 5 and 8; Table 3.5) leading to slower release of the drug as there would be less tendency of the stabilized system to undergo a change in terms of chain relaxation (release via diffusion) or degradation (release via erosion). Correspondingly, the comparatively higher concentration of HPMC-GLY will make the quadra-molecular architecture less stable (Equations 5 and 8; Table 3.5) in vacuum leading to an increase in hydrophilicity and chain relaxation or degradation – causing an increase in drug release.

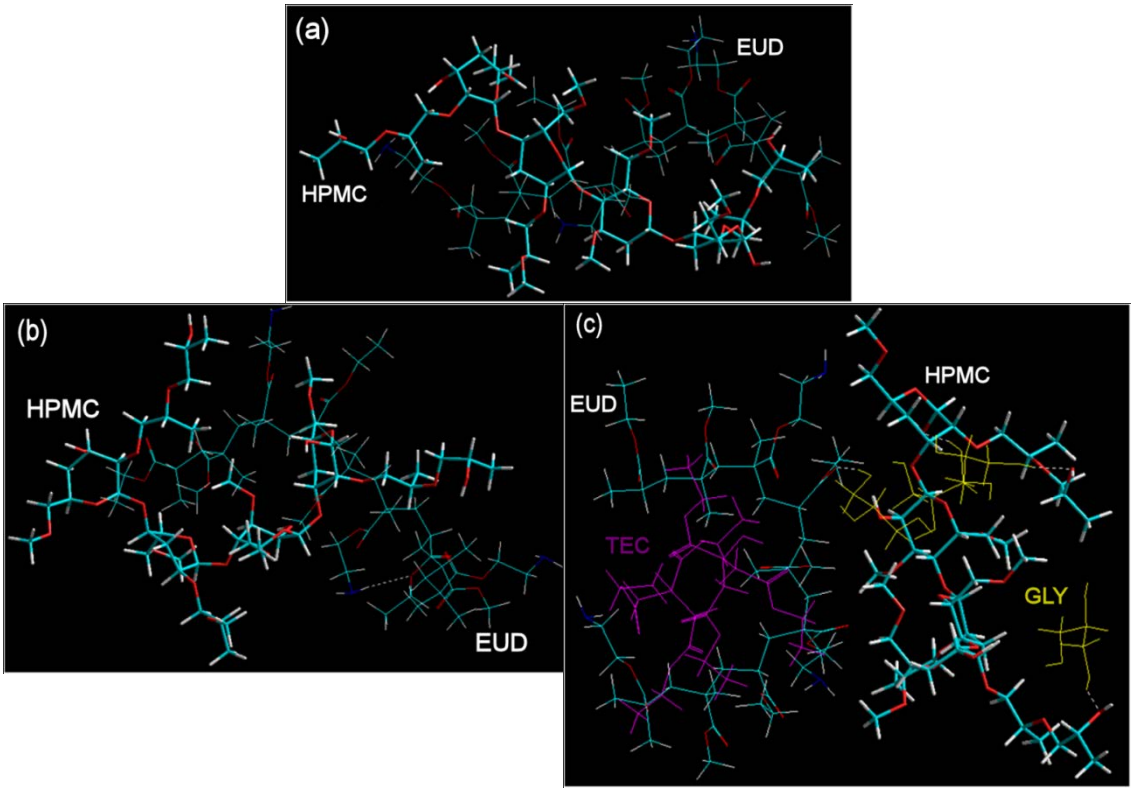


Figure 3.8: Visualization of geometrical preferences of (a) HPMC-EUD: HPMC (tube rendering) in molecular complexation with EUD (stick rendering) with no H-bonding; and (b) HPMC-EUD: HPMC (tube rendering) in molecular complexation with EUD (stick rendering) with intramolecular H-bonding; and (c) HPMC-GLY/EUD-TEC: HPMC-GLY in molecular complexation with EUD-TEC, after molecular simulations in vacuum. Colour codes for HPMC and EUD: C (cyan), O (red), H (white), and P (yellow). GLY and TEC molecules are shown in yellow and purple colour coding, respectively.

3.8.4 Effect of Component Polymers on the Mucoadhesivity of Buccal Films

$E_{MUC} = -166.812V_{\sum} = 5.474V_b + 70.351V_\theta + 55.173V_\varphi - 29.066V_{ij} - 7.096V_{hb} - 261.649V_{el}$ …(11)

$E_{HPMC\text{-}MUC} = -148.172V_{\sum} = 7.638V_b + 89.706V_\theta + 84.331V_\varphi - 58.017V_{ij} - 7.933V_{hb} - 263.898V_{el}$ …(12)

$E_{EUD\text{-}MUC} = -203.202V_{\sum} = 10.299V_b + 94.813V_\theta + 64.270V_\varphi - 51.765V_{ij} - 6.916V_{hb} - 313.905V_{el}$ …(13)

$E_{HPMC\text{-}MUC\text{-}EUD} = -138.225V_{\sum} = 11.511V_b + 103.835V_\theta + 110.07V_\varphi - 65.687V_{ij} - 8.089V_{hb} - 289.866V_{el}$ …(14)

In the present molecular mechanics simulations; a standard muco-platform was employed wherein glycosylated mucopeptide was modelled with EUD and HPMC individually and in combination (Murphy et al., 2012, Ndesendo et al., 2011). HPMC, though hydrophilic, is a non-ionic polymer with hydroxyl- and carboxymethyl-functionalities in the structure whereas EUD is a cationic polymer with quaternary ammonium groups capable of forming electrostatic interactions with the mucin. Referring to ∆E values in Table 3.5; HPMC-MUC and EUD-MUC were stabilized by ≈-31kcal/mol and ≈-80kcal/mol, respectively, with non-bonding interaction playing the major part. The neutral nature of HPMC showed few electrostatic interactions with close proximity of –OH groups of HPMC to the –COOH and $–NH_2$ groups of MUC. However, the HPMC fitted well in the steric environment created by the van der Waals radii of MUC resulting in a geometrical stabilization [$\Delta E(V_{ij})$≈-25kcal/mol] (Figure 3.9a). The quaternary ammonium groups of EUD seemed to form the much needed electrostatic interactions to impart mucoadhesivity to the buccal films. This electrostatic stabilization [$\Delta E(V_{el})$≈-59kcal/mol] was affected by the interaction of $–NH_3^+$ functionality of EUD with the –COOH functionality of MUC (Figure 3.9b). The hydrophobic nature of EUD may have caused the destabilization of H-bonding energy by 0.18kcal/mol. In case of HPMC-MUC-EUD; the value of ΔE of stabilization for HPMC-MUC-EUD [$\Delta E(V_{\sum})$≈-65kcal/mol] lied between that of HPMC-MUC and EUD-MUC validating the molecular modelling approach employed in this study (Table 3.5). Interestingly, the stabilized and destabilized energy terms were alike in case of HPMC-EUD and HPMC-MUC-EUD with all the bonding and non-bonding interaction terms except V_φ were lowered (and stabilized) during the formation of bimolecular and trimolecular assemblies, further validating the accuracy and appropriateness of the computational method applied. A high numerical decrease (and hence stabilization) in the van der Waals forces represented by V_{ij} [$\Delta E(V_{ij})$≈-45kcal/mol] confirms the better fit of HPMC-EUD combination with MUC as compared to the individual polymers – hence justifying the use of HPMC-EUD as a blend for the formulation of the buccal films. Predictably;

an increase in HPMC concentration may lead to a comparative increase in HPMC:EUD ratio which may further lead to a decrease in mucoadhesion as mentioned in the experimental finding under Section 3.5.

The above molecular mechanistic studies therefore provide useful quantitative information to simultaneously predict the stability of polymeric and plasticizer blends film formulation and to also identify the potential mechanisms for the observed drug release and mucoadhesion shown previously in Sections 3.2 and 3.5.

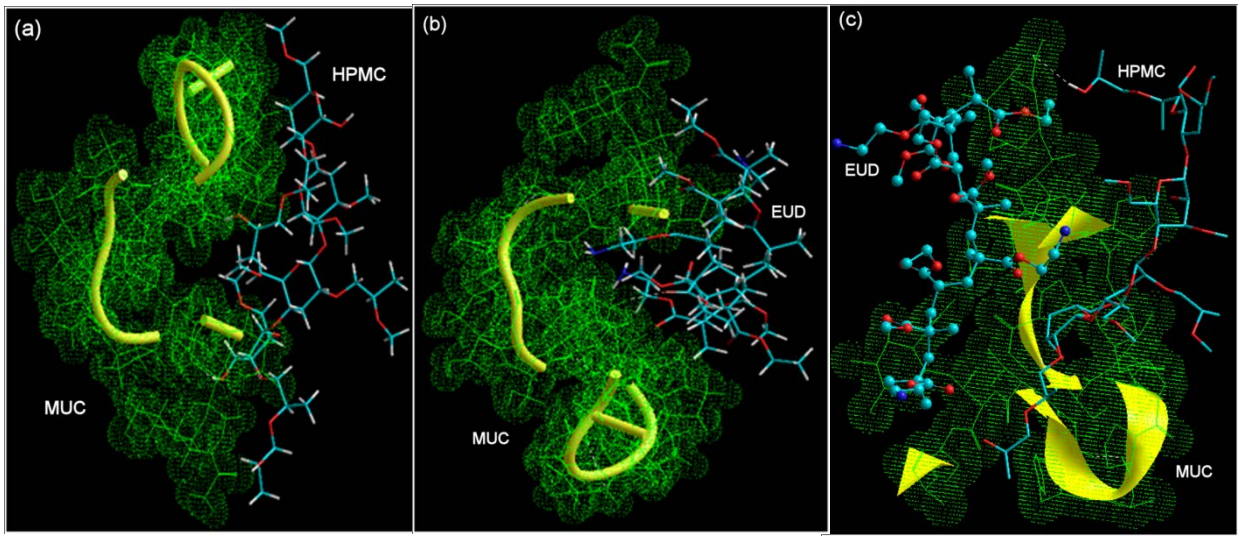


Figure 3.9: Visualization of geometrical preferences of (a) HPMC-MUC: HPMC (tube rendering) in molecular complexation with MUC (dot rendering); (b) EUD-MUC: EUD (tube rendering) in molecular complexation with MUC (dot rendering); and (c) HPMC-MUC-EUD: MUC (dot rendering) in molecular complexation with HPMC (tube rendering) and EUD (ball-and-tube rendering), after molecular simulations in vacuum. Colour codes for HPMC and EUD: C (cyan), O (red), H (white), and P (yellow). Secondary structure of MUC is shown in yellow tube or ribbon rendering.

4.0 CONCLUSIONS

The aim of this study was to formulate and characterize monolayered mucoadhesive multipolymeric films comprising of various ratios of co-blended polymers for buccal delivery of DDI. Films containing DDI were successfully prepared with HPMC and EUD by a simplified solvent casting/evaporation technique that eliminated the need for homogenization and cooling, carcinogenic solvents and additional emulsifiers. Drug content was uniform and within required specifications. Controlled release of DDI from MMFs could be obtained by modifying the ratios of HPMC and EUD. Formulations exhibiting desired controlled drug release and drug content uniformity had acceptable mechanical strength and mucoadhesivity. The buccal permeability potential of DDI from polymeric films was successfully demonstrated for the first time and histomorphological studies confirmed no buccal tissue damage or distress due to drug loaded MMFs. Static lattice atomistic simulations (SLAS) provided a mechanistic understanding of the molecular interactions involved in film formation and confirmed the corroboration of the *in silico* and *in vitro* mucoadhesive and drug release experimental data. SLAS further justified the "novel co-blending-co-plasticizing strategy" of preparing buccal films. The data obtained in the study demonstrated for the first time the potential of buccal polymeric films to serve as platforms for delivery of DDI. These extensive physico-mechanical and molecular atomistic studies have confirmed the use of MMFs containing DDI, HPMC and EUD as a potential buccal drug delivery system to enhance patient therapy. They further serve as a platform for future studies to statistically optimize the formulations for simultaneous enhancement of drug release, permeation, mucoadhesion and mechanical strength.

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CHAPTER FOUR
SUBMITTED MANUSCRIPT

4.1 INTRODUCTION

The following manuscript was submitted to an international ISI journal from data generated during this study:

Jones, E., Ojewole, E., Kalhapure, R., Govender, T., 2013. *In vitro* comparative evaluation of monolayered multipolymeric films embedded with didanosine-loaded solid lipid nanoparticles: A potential buccal drug delivery system for ARV therapy. ***Drug Development and Industrial Pharmac****y, SUBMITTED MANUSCRIPT. Reference Number: LDDI-2013-0624.*

Ms E. Jones contributed to the design of the project and developed suitable methods for preparation of DDI SLNs for the 1st time and undertook their characterisation. Furthermore, she was responsible for the preparation and characterisation of conventional and nano-enabled polymeric films in terms of assay, *in vitro* drug release, *in vitro* permeations, mucoadhesivity, mechanical strength, interpretation of the data and writing of the overall manuscript. The remaining authors served as supervisor (T. Govender), co-supervisor (E. Ojewole) and postdoctoral advisor (R. Kalhapure).

This chapter is presented in the required format of the journal and is the final version submitted for review and the manuscript submission cover page can be found in Appendix C.

4.2 SUBMITTED MANUSCRIPT

In vitro comparative evaluation of monolayered multipolymeric films embedded with didanosine-loaded solid lipid nanoparticles: A potential buccal drug delivery system for ARV therapy

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Running Head: Nano-enabled films for buccal delivery of didanosine

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ABSTRACT

Drug delivery via the buccal route has emerged as a promising alternative to oral drug delivery. Didanosine (DDI) undergoes rapid degradation in the gastrointestinal tract, has a short half-life and low oral bioavailability, making DDI a suitable candidate for buccal delivery. Recent developments in buccal drug delivery show an increased interest towards nano-enabled delivery systems. The advantages of buccal drug delivery can be combined with that of nanoparticulate delivery systems to provide a superior delivery system. The aim of the study was to design and evaluate the preparation of novel nano-enabled films for buccal delivery of DDI. Solid lipid nanoparticles (SLNs) were prepared via hot homogenization followed by ultrasonication and were characterized before being incorporated into nano-enabled multipolymeric monolayered films (MMFs). Glyceryl tripalmitate with Poloxamer 188 was identified as most suitable for preparation of DDI-loaded SLNs. SLNs with desired particle size (201nm), PDI (0.168) and zeta potential (-18.8mV) were incorporated into MMFs and characterised. Conventional and nano-enabled MMFs were prepared via solvent casting/evaporation using Eudragit RS100 and hydroxypropyl methylcellulose. Drug release from the nano-enabled films was found to be faster (56% vs 20% in 1st hour). Conventional MMFs exhibited higher mucoadhesion and mechanical strength than nano-enabled MMFs. SLNs did not adversely affect the steady state flux (71.63±13.54 µg/cm^{2}h vs 74.39±15.95 µg/cm^{2}h) thereby confirming the potential transbuccal delivery of DDI using nano-enabled MMFs. Nano-enabled buccal films for delivery of DDI can be successfully prepared and these physico-mechanical studies serve as a platform for future formulation optimisation work in this emerging field.

KEYWORDS:

Antiretrovirals, Entrapment efficiency, Hydrophilic drug, Mucoadhesion, Permeation, Transmucosal

1.0 INTRODUCTION

Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS) have remained as one of the leading causes of death worldwide, and is a major cause of mortality in sub-Saharan Africa[1,2]. While antiretrovirals (ARVs) have proven to be useful in the treatment and management of HIV & AIDS, several disadvantages and limitations currently exist with respect to drug therapy[3]. Many of the ARVs undergo extensive first pass hepatic metabolism and gastrointestinal degradation, which leads to reduced bioavailability. The short half-lives of several ARVs necessitates frequent administration of doses, thereby leading to reduced patient compliance[4]. There are concerns regarding adverse effects associated with long-term usage of highly active antiretroviral therapy (HAART), such as HIV associated lipodystrophy, central adiposity, dyslipidaemia, hyperlipidaemia, hyperglycaemia and insulin resistance[5,6]. The major contributing factor to ARV related side effects can be attributed to the inadequate drug concentrations reaching the site of action, and the low bioavailability of several ARV drugs, necessitating the use of large doses to achieve a therapeutic effect. Many of the currently available tablet formulations are very large and pose swallowing difficulties, especially for geriatric and paediatric patients. High doses, complex HAART dosing regimens, physical size limitations and side effects from multiple drugs all contribute to reduced patient compliance[7]. Poor drug solubility and limited membrane permeability also pose formulation difficulties[8]. HIV, being localised to inaccessible compartments in the human body, such as the lymphatic system, central nervous system and within macrophages, results in yet another treatment challenge. Therapeutic drug concentrations cannot be achieved in these compartments by the majority of ARVs, and the necessary plasma drug concentrations fail to be maintained at the site of HIV localisation for the required extent of time[9].

The identification of new drugs and chemical modification of existing ARV drugs[10], the design and development of novel drug delivery systems[11-13] and investigation of alternative routes to deliver ARVs[14-16] are being explored to overcome the current limitations associated with ARV therapy. Novel drug delivery systems (NDDS) have been identified as a useful tool by formulation scientists to enhance drug delivery and have contributed significantly in the past decade to augment various classes of drugs including ARVs[17]. Examples of novel drug delivery systems that have been explored in the past for ARVs include sustained release matrix tablets[18] and ceramic implants[11], while more recently liposomes[19] and nanoparticles[20] are receiving increased interest.

Along with developing NDDS for ARVs, researchers have also explored various alternate routes to improve drug delivery of ARVs other than the conventional oral route[14,21-23].

Drug delivery via the buccal route has recently emerged as a promising alternative to delivery via the oral route. Drugs can directly enter the systemic circulation, bypass gastrointestinal degradation and first-pass hepatic metabolism, thereby increasing bioavailability[24]. The buccal mucosa is easily accessible and more permeable than skin[25], making this a suitable route for drug delivery in pediatrics and geriatrics. Formulating a drug into a controlled release, mucoadhesive buccal dosage form may further improve drug delivery and patient compliance[26]. Several ARV drugs may therefore benefit from delivery via the buccal route.

For the buccal route, studies investigating the delivery of ARVs are limited compared to the transdermal route, and have focused mostly on permeability studies with ARV drug solutions rather than formulation studies. The majority of work thus far has focussed on *in vitro* drug permeability studies using only drug solutions of zalcitabine[23,27], didanosine[16,28] and tenofovir[16]. The only available published papers on buccal polymeric dosage forms containing ARVs are of zidovudine polymeric patches produced by Reddy et al.[29] and of didanosine monolayered multipolymeric films (MMFs) recently reported by our group[30]. ARV buccal drug delivery systems have not been comprehensively investigated or characterised, and a clear need still exists for formulation optimization in this field. Didanosine (DDI) is a nucleoside reverse transcriptase inhibitor (NRTI), acts by competitive inhibition of HIV-1 reverse transcriptase, and can also be incorporated into the growing viral DNA chain to cause chain termination[31]. DDI is currently faced with many limitations. Rapid drug degradation of DDI in the gastrointestinal tract due to acid hydrolysis, together with the need for repetitive dosing, its short half-life, low oral bioavailability and dose-related toxicity, make DDI a suitable model ARV drug for incorporation into a novel buccal delivery system.

Recent developments in the field of buccal drug delivery show an increased interest towards nano-enabled buccal drug delivery systems[32-34]. The advantages of buccal drug delivery can be combined with that of the nanoparticulate drug delivery systems to provide a superior drug delivery system in terms of enhanced bioavailability[35] and drug targeting depending on the nanoparticulate system involved[8,36]. A very limited number

of studies have been done to date in this emerging field with a majority of these studies focussing on using hydrogels[34] or multilayered polymeric patches[35,37] as delivery vehicles, and delivery of nanosized drug particles[38] or therapeutic proteins[32,33]. Antiretrovirals in a nano-enabled film for buccal drug delivery remain to be investigated in this emerging field. Nanoparticulate systems that have been studied for transmucosal drug delivery include: drug nanosuspensions prepared by wet stirred media milling of the drug[37,38], PEG-b-PLA copolymeric nanoparticles prepared by double emulsion solvent evaporation[32] and protein-coated D,L-valine nanoparticles prepared by antisolvent co-precipitation[33,39]. A nanoparticulate system of particular interest is solid lipid nanoparticles (SLNs). SLNs are prepared from lipids, which are solid at room temperature and surfactants or stabilizers[40], in the nanometer size (< 1000 nm) range. Advantages of SLNs over other nanoparticulate systems include: increased stability[40], controlled drug release[41], targeted drug delivery[36,42] and the incorporation of both hydrophilic[43] and lipophilic[44] drugs. Furthermore, SLNs lipids are biocompatible and organic solvents can be avoided during manufacturing processes[45]. SLNs incorporated into buccal MMFs have not been explored in the literature for any drug. There is therefore a clear need to explore the use of SLNs and buccal polymeric films to potentiate the delivery of an ARV drug via the buccal route. Furthermore, the incorporation of DDI into SLNs has not been reported in the literature for any application or route to the best of our knowledge. Thus the identification of a formulation will lend itself to its application as a nanoparticulate system for numerous delivery routes. By incorporating the drug in the form of nanoparticles into the buccal film, a reduction in dose-dependent side effects can be expected, as drug targeting to the required site of action can be achieved using a smaller dose. The additional reduced cost could make DDI more therapeutically useful once more. Incorporating multiple ARVs into nanoparticles can be accomplished to achieve multi-drug HAART regimens[46].

The aim of the study was to design and evaluate novel nano-enabled polymeric films for buccal delivery of DDI as a model ARV drug. In the present study, the potential of SLNs entrapped into MMFs for buccal delivery of DDI has been specifically examined. The objectives were to identify optimal parameters for preparation of DDI loaded SLNs and to prepare and compare the physico-mechanical properties of nano-enabled DDI films with conventionally prepared DDI films.

2.0 MATERIALS AND METHODS

2.1 MATERIALS

Didanosine (DDI) was purchased from Ruland Chemistry Co., Ltd (Nanjing, China) and used as received. Stearic acid, glyceryl tripalmitate (GTP), yellow beeswax, theobroma oil, glycerol monostearate, sodium lauryl sulphate, Poloxamer 188 (P188), Tween 80, Span 85, Solutol® HS 15, sodium deoxycholate, hydroxypropyl methylcellulose (HPMC), triethyl citrate (TEC) and mucin were purchased from Sigma-Aldrich (UK) and used as received. Compritol® 888 ATO was obtained as a gift sample from Gattefossé, France. Crodamol® MM and Crodamol® CP were obtained as a gift samples from Croda (Pty) Ltd, South Africa. Eudragit® RS 100 (EUD) (Evonik Rohm GMBH, Germany) was donated by Degussa Africa (Pty) Ltd. All other reagents used (NaCl, Na_2HPO_4, KH_2PO_4, NaOH, HCl, MeOH, EtOH and Glycerol) were of analytical reagent grade. Purified water used throughout the studies was produced in the laboratory with a Milli-Q purification system (Millipore Corp., USA).

The formula for phosphate buffered saline (PBS) used for *in vitro* drug release, permeation and mucoadhesion studies has been reported previously[30,47].

2.2 METHODS

2.2.1 Lipid Screening Study

The lipid solubility of DDI in different lipids was estimated using adapted methods[48,49]. The drug was mixed individually with eight different lipids: Stearic acid, glyceryl tripalmitate, yellow beeswax, theobroma oil, Crodamol® MM (myristyl myristate), Crodamol® CP (cetyl palmitate), Compritol® 888 ATO (glyceryl behenate) and glycerol monostearate in concentrations ranging from 0.0625 to 1 % w/w. The physical mixtures of lipid and DDI were heated at 95 °C and agitated for 3 hours using a thermostatically controlled shaking water bath with a mechanical shaker platform onto which a bottle holder plate was positioned. The melts obtained were visually examined for undissolved drug. The samples were left at room temperature (25 °C) until solidification. Full solubilisation was deemed when clear molten liquids were obtained and no precipitation upon solidification was noted.

Further lipid screening studies consisted of preparation of drug-free preformulations using methods described under preparation of SLNs, and measuring the pH of the resulting mixture to evaluate for possible incompatibilities with DDI due to the acid-labile nature of the drug. Results were represented by the mean ± SD of three replicate measurements.

2.2.2 Preparation of SLNs

SLNs were prepared using a hot homogenization process followed by ultrasonication[42,48]. Briefly, the solid lipid (5 % w/v) was heated 5-10 °C above its melting point to 80 °C, and then added to a mixture of surfactants (1.6 % w/v) and water, previously heated at the same temperature. A pre-emulsion was obtained upon homogenization at 12000 rpm, for 4 min, using a high-speed homogenizer (Ultra Turrax T25, IKA, Germany) whilst maintaining the temperature at 80 °C during the homogenization step. The resulting pre-emulsion (25 mL) was ultrasonified using a probe sonicator (Omni Sonic Ruptor 400, Omni Inc., USA) with a 4 mm diameter probe and by applying a 40 % amplitude for 10 min, which lead to droplet breakage by acoustic cavitation, and subsequent formation of nanoparticles[50]. The obtained o/w nanoemulsion was cooled in an ice bath to room temperature to form SLNs. For drug-loaded SLNs, the drug (0.4 to 2 % w/v) was added to the aqueous phase before heating and addition to the lipid phase. Samples were stored at 4 °C for further analyses.

2.2.3 Characterisation of SLNs

2.2.3.1 Particle Size and Zeta Potential Measurements

The mean particle size diameter (Z-average) and polydispersity index (PDI) as a measure of the width of particle size distribution, were measured via photon correlation spectroscopy (PCS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Prior to particle size analysis the SLN dispersions were diluted with purified water (1:30) to obtain the required opalescence and count rates (50-200 Kcps)[44]. All measurements were performed in triplicate and results are reported as the mean and standard deviation of the three replicates.

The surface charge was measured by determining the zeta potential (ZP) of SLN dispersions after samples were suitably diluted with purified water, the conductivity of which was adjusted to 50 µS/cm.

2.2.3.2 Morphology of SLNs

The shape of SLN formulations were observed under a transmission electron microscope (TEM) to study the morphology of the resulting SLNs. SLN dispersions were diluted with purified water (1:3), mounted on copper grids and air dried at room temperature. The samples were then stained for 30 seconds using 4 % uranyl acetate before viewing under a TEM (JOEL1010, Japan).

2.2.3.3 Entrapment Efficiency

SLN dispersions (± 25 mL) were made up to 100 mL with purified water in a volumetric flask prior to determination of drug entrapment efficiency (EE). A volume of 1 mL of per SLN formulation was ultrafiltered using Amicon® Ultra 4 centrifugal filter units (Merck Millipore, Germany) equipped with a 10 kDa membrane filter in a centrifuge at 5000 *g* for 15 min. The EE was calculated by comparing the amount of unencapsulated DDI in the ultrafiltrate versus, the total amount of drug added to the formulation. Samples of the ultra filtrate were appropriately diluted with purified water before quantification via a validated UV method at a wavelength of 250 nm (Shimadzu 1650 PC, Japan). All determinations were performed in triplicate.

The amount of encapsulated DDI was calculated using the equation below. The free amount of DDI remaining in the aqueous phase following separation via ultrafiltration was subtracted from the total amount of DDI used to prepare the formulation[51].

$$\text{EE}\,\% = \frac{(\text{Total amount of DDI}) - (\text{Free amount of DDI})}{(\text{Total amount of DDI})} \times 100$$

2.2.2 Preparation of SLN Loaded Buccal Films

Buccal films were prepared as previously described[30]. Briefly, monolayered multipolymeric films (MMFs) comprising of HPMC and EUD were prepared by dissolving specified quantities (Table 4.1) of EUD and TEC as its plasticizer together with HPMC and glycerol (GLY) as its plasticizer in 50 mL methanol. To this polymeric mixture 25 mL of purified water and either 25 mL of DDI in purified water for conventional drug-loaded films (CD) or 25 mL DDI SLN nano-emulsion for nano-enabled drug-loaded films (ND) was added and mixed until homogenous suspensions formed.

An amount of the polymeric casting mixture, equivalent to 20 mg of DDI, was syringed into each 6 cm^2 well of the silicone moulded tray (SMT) containing Teflon coated Perspex inserts[52]. The mixtures were allowed to dry in an oven (Series 2000, Scientific, SA) at 43 °C for approximately 24 h, until the solvent had evaporated and constant film weight was achieved. Films were removed from the moulds and stored using wax paper and foil in a desiccator at room temperature (23 °C) up to a maximum of three months until further use.

Conventional drug free films (Conventional Placebo / CP) and drug free SLN films (Nano-enabled Placebo / NP) were prepared as described above by omitting the drug from the 25 mL purified water or during preparation of SLNs, respectively.

Table 4.1: Composition of the buccal film formulations.

Type of Buccal Film		EUD (% w/w)	TEC (% w/w)	HPMC (% w/w)	GLY (% w/w)	SLN (mL)	Water / Methanol up to (mL)	Volume per film (mL)	DDI per film (mg)
CP	Conventional Drug Free	10	3	0.5	0.15	-	100	2	-
CD	Conventional Drug Loaded	10	3	0.5	0.15	-	100	2	20
NP	Nano-enabled Drug Free	5	1.5	0.25	0.075	25	100	4	-
ND	Nano-enabled Drug Loaded	5	1.5	0.25	0.075	25	100	4	20*

*Include entrapped and unentrapped drug.

2.2.3 Characterisation of Films

2.2.3.1 Weight and Thickness Uniformity

Three films per batch were randomly selected and individually weighed using an electronic balance (Metller Toledo AB204-S., Switzerland) and measured in five different locations (centre and four corners) using a digital micrometer (Mitutoyo Co., Japan), respectively.

2.2.3.2 Assay of Films

The assay solvent consisted of 80 % ethanol in water. A 6 cm^2 film as a unit from the SMT was dissolved in approximately 40 mL of the assay solvent in a 100 mL volumetric flask before making up to volume with the same assay solvent. Following appropriate dilution (1 in 10), the drug content in the samples was quantified using a validated UV spectrophotometric method at a wavelength of 250 nm (Shimadzu 1650 PC, Japan). All assays were performed in triplicate.

2.2.3.3 In Vitro Drug Release

A modified BP2009 Type II paddle dissolution test apparatus (Erweka DTR-6., Germany) was employed to determine *in vitro* drug release of the buccal films[30,53]. The dissolution studies were carried out in 900 mL PBS adjusted to pH 6.8 and maintained at 37 ± 0.5 °C; with a stirring speed of 50 rpm. The film size required for dose delivery (6 cm^2) was used. The film was placed into a stainless steel wire mesh basket and dropped into the dissolution vessel at the start of the experiment. A wire mesh basket was used, instead of attaching a film to a glass slide with adhesives as commonly reported[54], in an attempt to limit interference with drug release. Aliquots of 6 mL samples from the dissolution medium were collected at predetermined time intervals of 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 minutes using a syringe and in line filtration (0.45 µm). An equal volume (6 mL) of fresh PBS was replaced into each dissolution vessel, to ensure that a constant volume of dissolution medium was maintained throughout the duration of the study. The filtered samples were quantified for drug using a UV spectrophotometer (Shimadzu 1650 PC, Japan) at a wavelength of 250 nm. The results are represented as the average of three films.

2.2.3.4 In Vitro Permeation

In vitro permeation experiments were performed on the prepared MMFs as reported[28,55,56]. Porcine buccal mucosa was used as a biological membrane for these experiments due to the many similarities to the human buccal mucosa as highlighted by Shojaei[57] and Sudhakar et al.[58].

Briefly, porcine buccal mucosa was excised from domestic pigs (30-40 kg), the excess adipose and connective tissue was removed using surgical scissors, and samples were wrapped in foil before being snap-frozen in liquid nitrogen and stored at – 85 °C in a biofreezer for up to 3 months[59].

On the day of the experiments, frozen buccal mucosal specimens were allowed to thaw and equilibrate in PBS pH 7.4 to regain elasticity temporarily lost while frozen. Franz diffusion cells (PermeGear, Inc, USA) each with a diffusional area of 0.786 cm^2 were used for the *in vitro* permeation experiments. The buccal mucosa and polymeric film were mounted between the donor and receptor compartments using two membrane holders. Two millilitres PBS at pH 6.8, simulating human saliva[47], was placed on the

film in the donor compartment while the receptor compartment contained 27 mL PBS pH 7.4 maintained at 37 °C (by means of a surrounding jacket) and stirred constantly.

At predetermined time intervals over 360 minutes, samples (27 mL) were taken from the receptor compartments and replaced by drug-free PBS. Similar to dissolution studies; samples were immediately filtered through a 0.45 µm membrane filter and the drug content was quantified using a UV spectrophotometer (Shimadzu 1650 PC, Japan) at a wavelength of 250 nm. A minimum of three replicates were performed.

The cumulative amount of DDI permeated per unit surface area was plotted versus time. The steady state flux (J_{ss}) across the mucosal membrane was determined from the linear portion of the permeation graph by linear regression analysis (Microsoft Excel 2010). The permeability coefficient (*P*) was calculated using the following equation[27]: $P = (dQ/dt)/A \times C_d = J_{ss}/C_d$. Where *dQ/dt* is the cumulative amount (*Q*) of DDI which permeated into the receptor compartment per unit time (*t*), *A* is the active cross-sectional area (0.786 cm^2) available for diffusion and C_d is the drug concentration in the donor compartment.

2.2.3.5 Mucoadhesivity of Films

The mucoadhesive properties were studied using methods adapted from Ayensu et al.[60] and Perumal et al.[61] as previously reported by us[30]. Briefly, film samples (*n=3*), free from physical imperfections were individually attached to removable 2x3 cm aluminium probes using double sided adhesive tape. The probes were attached to the upper movable arm of the TA.XT2*i* Texture Analyser (Stable Micro Systems, UK). A Petri-dish containing 10 % w/v solidified gelatin gel, simulating the buccal mucosa, was clamped into place on the stationary platform of the TA.XT2*i*[60] and 2 mL of 30 % w/v mucin at 37 °C was spread on the surface of the gelatin immediately prior to testing[61]. The film was allowed to hydrate for 120 seconds in PBS pH 6.8 before being brought into contact with the mucin covered gelatin. The film was held in place with a force of 100 grams for 60 seconds before the mobile arm was raised. The mucoadhesive performance of the samples was determined by measuring the Maximum Detachment Force (MDF) (*mN*) and Work (*mJ*). The MDF represents the maximum force required to detach the film from the mucin covered gelatin. The area under the force/distance curve was also determined to represent the work required for detachment of the two systems (mucin/polymeric film)[62]. A minimum of nine replicate determinations were performed.

2.2.3.6 Mechanical Testing

Mechanical properties of the films were studied using methods previously reported by us[30], with the aid of a TA.XT2*i* Texture Analyser (Stable Micro Systems, UK). Briefly, individual film samples (*n=5*), were held between the grips (TA-96). A sheet of Teflon was attached to the surface of the grips via double-sided tape to prevent the film being cut by the grooves of the grips. During measurement, the film was pulled by the top grip at a rate of 1 mm/s to a distance of 150 mm before returning to the starting point. Data acquisition was terminated when the film ruptured completely. The force and elongation were measured when the films broke.

The tensile strength, percent elongation, film toughness and Young's modulus were used as indicators of the mechanical properties of the films. Mechanical properties of the films were evaluated using the following equations[63]:

$$\text{Tensile strength (N/mm}^2\text{)} = \frac{\text{Force at break (N)}}{\text{Width (mm)} \times \text{Thickness of film (mm)}}$$

$$\text{Elongation at break (\%)} = \frac{\text{Increase in length}}{\text{Original length}} \times 100$$

Young's modulus was determined from the slope of the initial linear portion of the stress-strain plots generated with the Texture Expert™ software. The area under the force-time plots were used as an indication of film toughness[32].

A rupture test was also performed to assess the mechanical film properties. A film support rig with an exposed area of 0.786 cm^2 was attached to the heavy duty platform of the TA.XT2*i* Texture Analyser. Individual film samples (*n=3*) were clamped between the film support rig before passing a 5 mm stainless steel ball probe through a sample at 1 mm/s in compression mode. The force (*N*) required to rupture the film was measured[38].

2.2.4 Statistical Analysis

All calculations were undertaken with Microsoft Excel ® (Microsoft Office 2010, USA). A minimum of three replicates were performed and results are expressed as mean ± SD. Statistical analysis of data were performed using GraphPad Prism, Version 5 (GraphPad Software., Inc, USA). One-way ANOVA followed by Dunnett's multiple comparisons test or a two-tailed unpaired *t*-test was used to determine statistical significance where appropriate. P-values of $p < 0.05$ were considered significant.

3.0 RESULTS AND DISCUSSION

3.1 LIPID SCREENING STUDY

The solubility of the drug in melted lipid is a critical factor that determines the degree of drug loading in the solid lipid[64]. For the purpose of this study freely available lipids, commonly used in the preparation of SLNs, were screened for suitability for use with DDI (Table 4.2). The results of the lipid solubility studies indicated that DDI had the best solubility in stearic acid (SA) and appeared to be most suitable for the preparation of DDI-loaded SLNs. However, DDI is an acid labile drug and undergoes rapid degradation in the GIT. At acidic pH, hydrolysis of the glycosidic bond between the sugar and the base moieties of DDI will inactivate the drug[31]. For this reason formulations should ideally be prepared at neutral or at a slightly alkaline pH to ensure that drug stability is maintained.

The measurements of preformulation pH values showed a substantially lower pH (4.03 ± 0.058) with stearic acid when compared to the other investigated lipids. Even though stearic acid showed the greatest potential for preparation of DDI SLNs in terms of drug solubility, the resulting low pH would preclude its use, and alternative lipids should be used. One cannot only select a suitable lipid for SLNs based on lipid solubility studies alone, physico-chemical properties and stability of the drug needs to also be considered. Neutral lipids such as glyceryl tripalmitate may be more suitable for an acid labile drug such as DDI. Studies by Teeranachaideekul and co-workers have highlighted the importance of examining the chemical stability of the drug in the lipid matrix during preformulation studies and concluded that the chemical stability of the drug is heavily dependent on the lipid type[65].

Table 4.2: Solubility of DDI in various molten solid lipids and pH of preformulations.

Lipid Type	Melting Point (°C)	1 % (w/w)	0.5 % (w/w)	0.25 % (w/w)	0.125 % (w/w)	0.0625 % (w/w)	pH
Stearic acid	67-72	I	I	I or PS	PS	NS	4.03 ± 0.058
Theobroma oil	34-38	I	I	I	I	I or PS	5.47 ± 0.058
Yellow beeswax	62-64	I	I	I	I or PS	PS	4.27 ± 0.115
Glyceryl tripalmitate	66-68	I	I	I	I	I or PS	5.93 ± 0.115
Myristyl myristate	39-43	I	I	I	I	I	4.80 ± 0.000
Cetyl palmitate	54-55	I	I	I	I	I or PS	4.73 ± 0.058
Compritol® 888 ATO	65-77	I	I	I	I	I	5.53 ± 0.058
Glycerol monostearate	55-60	I	I	I	I	I	5.23 ± 0.058

Key: I = Insoluble, PS = Partially soluble, NS = Nearly soluble.

3.2 PREPARATION OF SLNS

SLNs offer numerous advantages over other nanoparticulate systems such as prolonged stability, controlled drug release and the ability to incorporate both hydrophilic and lipophilic drugs[45]. Although DDI have been incorporated into various nanoparticulate systems[49,66-68], to date the preparation of DDI SLNs have not been reported. Kasongo et al., reported DDI-loaded nanostructured lipid carriers (NLC) which are prepared by mixing solid lipids with liquid lipids rather than solid lipids only[49,51]. The current study reports a hot homogenization process followed by ultrasonication technique for preparation of DDI SLNs and investigates the effect of different lipids, surfactants and drug loading on SLN characteristics.

3.3 CHARACTERISATION OF SLNS

3.3.1 Effect of Lipid Type

The effect of different lipids on the particle size (PS), polydispersity index (PDI), zeta potential (ZP) and drug entrapment efficiency (EE) were evaluated (Table 4.3). The ZP, which is a measure of the surface charge of the particles, ranged between -16.2 mV and -27.3 mV, indicating that particle aggregation would be unlikely to occur[20]. Stearic acid showed the highest EE (14.48 %), but was not suitable for further investigation due to the low formulation pH (4.03). Stearic acid is a self-emulsifying lipid able to entrap more hydrophilic drug in the SLNs[43] than lipids with a highly ordered crystalline structure[69] such as cetyl palmitate (1.55 %). Glycerol monostearate displayed an EE of 13.75 %, which is high in comparison to the lipids investigated, but posed formulation difficulties and had an undesirable PS and PDI. From the investigated lipids, glyceryl tripalmitate was identified as the most suitable lipid for preparation of DDI-loaded SLNs. Formulations had a near neutral pH (5.93) essential to ensure drug stability, small PS (198 nm), with a low PDI (0.175) indicating that the particle size distribution was monodisperse.

Although the Poloxamer 188 (P188) is a non-ionic surfactant, the presence of negatively charged lipids in the formulation (e.g. stearic acid), could have contributed to the negative zeta potential values measured. These findings are consistent with the preparation of SLNs also prepared with P188 in a previous study[70].

Table 4.3: Influence of different lipids on characteristics of SLNs.

Lipid Type	PS (nm)	PDI	ZP (mV)	EE (%)	pH
	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD
Myristyl myristate	195.6 ± 13.8	0.234 ± 0.047	-24.39 ± 3.26	6.60 ± 3.16	4.80 ± 0.000
Cetyl palmitate	201.3 ± 5.6	0.229 ± 0.050	-26.09 ± 1.51	1.55 ± 3.00	4.73 ± 0.058
Yellow beeswax	162.5 ± 5.7	0.181 ± 0.011	-26.77 ± 1.93	11.45 ± 6.52	4.27 ± 0.115
Glycerol monostearate	332.7 ± 93.1	0.265 ± 0.133	-20.16 ± 1.99	13.75 ± 4.45	5.23 ± 0.058
Compritol® 888 ATO	159.0 ± 1.3	0.235 ± 0.003	-22.67 ± 0.40	6.90 ± 1.36	5.53 ± 0.058
Theobroma oil	183.3 ± 4.8	0.192 ± 0.017	-18.10 ± 2.06	3.93 ± 3.01	5.47 ± 0.058
Glyceryl tripalmitate	198.3 ± 0.6	0.175 ± 0.010	-16.20 ± 0.27	10.66 ± 0.95	5.93 ± 0.115
Stearic acid	230.1 ± 3.0	0.120 ± 0.030	-27.34 ± 2.48	14.48 ± 2.60	4.03 ± 0.058

1g Lipid + 400 mg P188 + 100mg DDI

3.3.2 Effect of Surfactant Type

The effect of different surfactants on PS, PDI, ZP and EE of the SLNs was evaluated (Table 4.4). Glyceryl tripalmitate as identified from Table 4.3 was used in this study. Surfactant free particles were also prepared and resulted in relatively large particle sizes (406.9 ± 26.2) highlighting the importance of adding adequate amounts of surfactant to cover the surface of the particles. Surfactants can be used to reduce the particle size by decreasing the interfacial tension in the aqueous phase[43]. Poloxamer 188 was identified as the most suitable surfactant due to the low particle size (198 nm), narrow particle size distribution (PDI of 0.175) and comparatively higher EE (10.7 %) from amongst the surfactants investigated. Anionic surfactants such as sodium deoxycholate or sodium lauryl sulphate had an alkaline formulation pH (7.4 and 7.2) which would be ideal for DDI stability and strong negative surface charges (-24.2 mV and -18.7 mV) for formulation stability, yet displayed low EE (6.7 % and 3.8 %). The solubility of DDI in water is pH-dependent with its solubility increasing in the aqueous phase as the pH increases[71]. This pH-dependent solubility could have resulted in DDI being more soluble in the alkaline aqueous phase during preparation of our SLNs with anionic surfactants, leading to lower EE in the lipid matrix. The stability of DDI at different pH conditions has been reported[72,73] and these studies highlighted pronounced drug degradation under acidic conditions. It can therefore be suggested that the SLNs should be prepared at a pH where the drug solubility is limited, yet at which drug degradation would not be increased.

Table 4.4: Influence of different surfactants on characteristics of SLNs.

Surfactant Type	PS (nm)	PDI	ZP (mV)	EE (%)	pH
	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	
Span 85	212.7 ± 0.6	0.326 ± 0.054	-22.53 ± 1.68	6.63 ± 0.07	5.5
Tween 80	224.0 ± 4.2	0.298 ± 0.010	-5.24 ± 0.89	7.99 ± 1.85	5.2
Solutol HS 15	220.7 ± 2.4	0.263 ± 0.004	-3.49 ± 1.45	0.43 ± 0.40	5.5
Poloxamer 188	198.3 ± 0.6	0.175 ± 0.010	-16.20 ± 0.27	10.66 ± 0.95	5.8
Sodium deoxycholate	217.6 ± 0.6	0.257 ± 0.015	-24.20 ± 1.47	6.70 ± 5.95	7.4
Sodium lauryl sulphate	211.0 ± 1.2	0.175 ± 0.003	-18.67 ± 3.96	3.75 ± 0.77	7.2

1g GTP + 400mg surfactant + 100mg DDI

3.3.3 Effect of Drug Loading

The effect of drug amount added to SLN preparations on the particle characteristics was investigated and a maximum drug entrapment efficiency of 18 % was achieved at a drug loading of 1.2 % w/v (Table 4.5). Further increases in drug loading did not improve EE and this could possibly be attributed to saturation of the lipid matrix[74]. Excess drug not entrapped/associated with the SLNs prepared, were still incorporated into MMFs as free drug and allowed for conventional drug release and buccal permeation as also reported previously by another study[34].

In a study on saquinavir stearic acid SLNs, saturation of the lipid matrix occurred at 1 % w/v and further increases in drug loading also did not result in higher EE[74]. A study by Ghadiri and co-workers reported contrasting results concerning increasing the drug amount and the resulting entrapment efficiency[43]. When using a microemulsion technique for preparation of paromomycin (hydrophilic) SLNs, the EE decreased as the drug amount increased. The converse was found when using a solvent diffusion technique for preparation of their SLNs.

Table 4.5: Effect of drug loading on characteristics of SLNs.

Drug Amount (mg - % w/v)	PS (nm)	PDI	ZP (mV)	EE (%)	pH
	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	
Drug Free - 0 % w/v	202.9 ± 1.0	0.183 ± 0.004	-15.10 ± 1.80	-	5.8
100 mg - 0.4 % w/v	198.3 ± 0.6	0.175 ± 0.010	-16.20 ± 0.27	10.66 ± 0.95	5.8
300 mg - 1.2 % w/v	196.8 ± 2.9	0.181 ± 0.018	-18.40 ± 1.40	18.06 ± 1.04	5.5
500 mg - 2 % w/v	201.3 ± 1.9	0.168 ± 0.007	-17.83 ± 0.40	15.79 ± 0.33	5.5

1g GTP + 400mg P188 + varying DDI

Incorporation of hydrophilic drugs, such as DDI, poses difficulties for incorporation into SLNs as demonstrated by the relatively low drug entrapment efficiency found in this study (< 18 %). Reported EE values for hydrophilic drugs incorporated into SLNs vary widely: 42 % for paromomycin[43], 27 % for zidovudine[75] and as low as 13 % for cyclosporine A[76] have been reported. The main aim of this study was not to optimise the drug entrapment efficiency of DDI into SLNs, but the focus was rather on the preparation and evaluation of nano-enabled films for buccal drug delivery of DDI. Future studies can focus on optimizing EE specifically, by considering factors such as ion-pairing, polymer coating of the SLNs or investigating the effect of novel formulation strategies.

3.3.3 Morphology of SLNs

TEM images of DDI SLNs prepared using GTP (Figure 4.1) indicate that the particles were spheriodal in shape with smooth, nonporous surfaces. Particles were non-aggregated, homogenously distributed and the size (± 230 nm) correlated well with size data obtained using photon correlation spectroscopy.

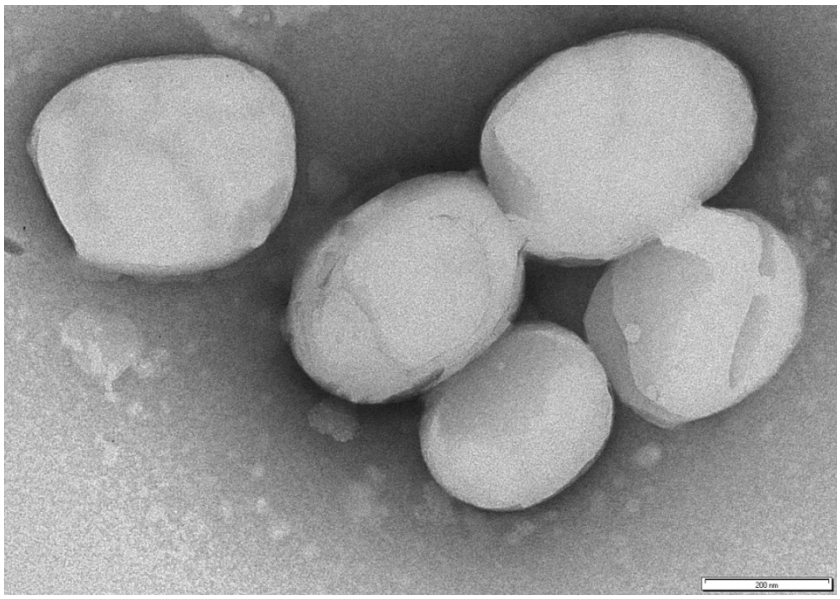

Figure 4.1: TEM micrograph depicting the shape and surface morphology of the DDI solid lipid nanoparticles. The bar represents 200 nm.

3.4 PREPARATION OF SLN LOADED BUCCAL FILMS

Studies previously reported[30] revealed the most suitable formulation variables to use in terms of polymeric ratios for the preparation of monolayered multipolymeric films (MMFs) containing polymers of opposing solubilities and DDI. For the purpose of this comparative study a total of four MMF formulations were prepared either being designated as drug-free or drug-loaded as well as being formulated with conventionally loaded drug or drug entrapped into nano-emulsions (Table 4.1). All films prepared via the different formulations were thin, flexible and their surface appeared to be fairly homogenous with limited entrapped air bubbles (Figure 4.2a-c). Nano-enabled drug loaded films (Figure 4.2d) appeared to have a similar appearance than conventional drug loaded films (Figure 4.2b).

Drug loaded films had the highest opaqueness and interesting to note, the films prepared using drug-free SLNs (Figure 4.2c) were not as transparent as their conventional drug-free counterparts (Figure 4.2a). The polymeric/nano-emulsion mixtures were also completely homogenous prior to film casting and no phase separation occurred upon drying. Drying at 43 °C for 24 h did not pose stability concerns for the drug since didanosine's thermal stability in excess of 85 °C has been previously established[51]. Nano-enabled films with acceptable appearance can therefore be prepared.

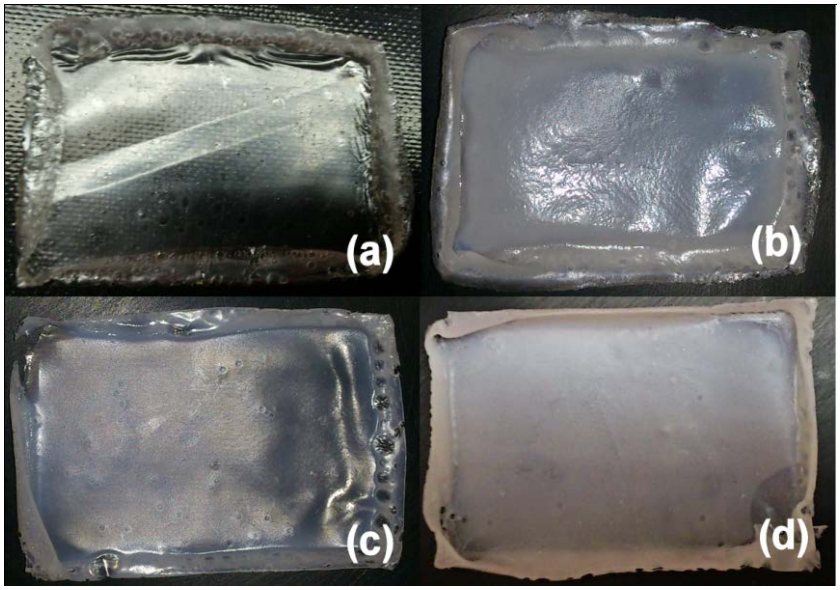

Figure 4.2: Digital photographs of: (a) Conventional drug free film – CP,
(b) Conventional drug loaded film – CD,
(c) Nano-enabled drug free film – NP,
(d) Nano-enabled drug loaded film – ND.

3.4.1 Characterisation of SLN Loaded Buccal Films

Physico-chemical characteristics of the MMFs are depicted in Table 4.6. Film thickness was not influenced by drug loading, whereas the effect of drug loading on film weight was more pronounced. The lipid content of nano-emulsions contributed to the weight. Problems encountered with drug content uniformity in buccal films[26] have been overcome in this study by using similar silicone moulded trays with individual wells for film casting as previously investigated by our group[52]. Drug content of films ranged between 92.6 % and 95.6 % with low CV values (< 5.7 %) signifying good drug content uniformity. All films were homogenous and had acceptable drug content uniformity.

Table 4.6: Drug content uniformity, thickness and film weight of various film formulations. (Mean ± SD values; *n*=3).

Type of Buccal Film		Assay (%)		Thickness (µm)		Weight (mg)	
		MEAN ± SD	CV %	MEAN ± SD	CV %	MEAN ± SD	CV %
CP	Conventional Drug Free	-	-	293.67 ± 28.02	9.54	277.09 ± 2.44	0.88
CD	Conventional Drug Loaded[30]	95.62 ± 5.41	5.66	299.60 ± 25.00	8.34	293.60 ± 1.50	0.51
NP	Nano-enabled Drug Free	-	-	292.47 ± 24.59	8.41	328.50 ± 7.47	2.27
ND	Nano-enabled Drug Loaded	92.57 ± 1.34	1.45	297.67 ± 34.50	11.59	345.40 ± 1.97	0.56

3.5.2 *In Vitro* Drug Release

The *in vitro* drug release of DDI incorporated into films was investigated (Figure 4.3). A polymeric ratio between HPMC and EUD identified in a previous study[30] known to exhibit sustained drug release, was used during preparation of MMFs in the current study. Nano-enabled MMFs released DDI at a substantially faster rate than conventionally loaded MMFs (Figure 4.3). The lower drug release from films prepared with drug solution as opposed to drug incorporated into nanoparticles, has also been observed previously[33]. Morales and co-workers postulated that when drug is added to the film as a solid solution, the drug molecules are completely surrounded by the polymeric film matrix and a higher number of drug-polymer interactions can be achieved, resulting in slower drug release. In our study DDI release from the nano-enabled films was higher, with 56 % drug released in the 1st hour as opposed to 20 % for the conventionally loaded MMF (Figure 4.3). By the end of six hours this increase in drug release is still evident (35 % vs 72 %). This increased drug release rate could also

be attributed to differences in molecular interactions as identified by molecular mechanistic studies previously reported[30]. Introduction of SLNs into the polymeric film matrix could make the previously identified quadra-molecular architecture between the two co-blended polymers and plasticizers, less stable[30]. This could lead to an increase in hydrophilicity and chain relaxation or degradation which results in increased drug release.

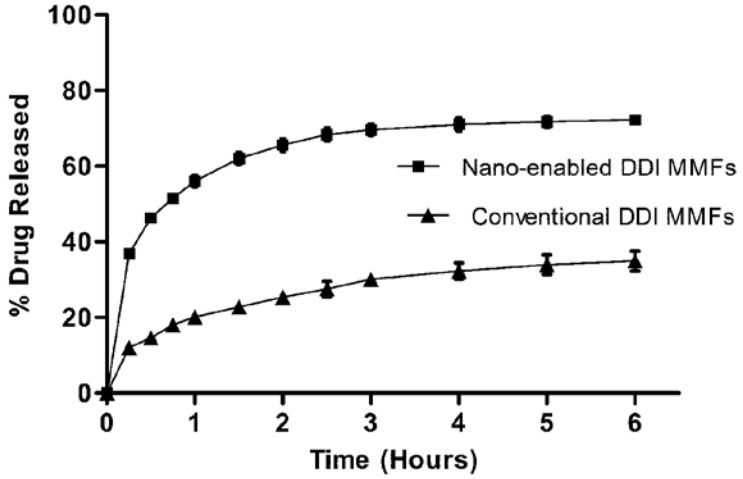

Figure 4.3: Comparison of DDI release from conventional MMFs[30] and nano-enabled MMFs.

Complete drug release from the nano-enabled films was not achieved over the 6 hour testing period (72 %). This could be attributed to incomplete dissolution of the film in the dissolution media, which is related to the low aqueous solubility of EUD. This would prevent free and deep water penetration into the film, thereby only allowing the DDI near the external surface of the film to be released[61]. Incomplete drug release related to EUD solubility is applicable to the conventionally loaded portion of the drug in the film. Another possible theory for incomplete drug release being achieved over the 6 hour period is that approximately 16 % of DDI in the nano-enabled formulation was entrapped inside the SLNs, which would release the drug over a prolonged period of time (days), whilst circulating in the body. In a recent study on nano-enabled films for lysozyme delivery[33] drug release of 50 % over a 4 hour period was achieved, which corresponds with our study. In another study, it was found that 40 % of insulin was released from PEG-b-PLA nanoparticles entrapped in chitosan films, over the initial 6 hour testing period after which this was followed by a constant and complete sustained release over 5 weeks[32]. From our results it can be concluded that the incorporation of SLNs into MMFs intended for buccal drug delivery, alters the rate of drug release achieved with conventional films by possible changes in the film structure.

Since only 16 % of drug is entrapped into the SLNs, the majority of the drug is dispersed in its free form in the film matrix. Although drug entrapment is low, the presence of SLNs does indeed affect the film characteristics. This is evident by the changes in drug release, mucoadhesion and mechanical properties etc. Although faster drug release occurred due to the possible effect of the SLNs on the matrix film structure, drug release from the nano-enabled films could have been further sustained for prolonged periods if higher drug entrapment values are obtained. This aspect could be the focus of subsequent studies.

3.5.3 *In Vitro* Permeation

The buccal permeability of DDI incorporated into films was investigated (Figure 4.4). The non-linear portion of the plot was considered as the time required for steady state permeation (Jss) to be achieved. In both cases, a time of 45 minutes was observed, which is related to the polymeric film matrix entrapping the drug, as well as the mucosal membrane serving as a barrier to drug permeation. Although the buccal mucosa acts as a barrier because of its anatomical structure and thickness, a conventional lag time is not observed in this study (Figure 4.4) and some drug is able to permeate within the first 15 min. This could be due to permeation of DDI (hydrophilic) through the paracellular pathway. Similar lack of lag times during buccal permeation studies with porcine mucosa has been reported previously by Rao et al[37]. They postulated that with the increase in apparent solubility (due to milling of phenylephrine), more dissolved drug is present at the porcine buccal mucosal surface, resulting in a higher concentration gradient across the mucosa. Since DDI permeates through buccal mucosa via passive diffusion[16], the increase in the concentration gradient across the membrane contributed to the observed permeability of DDI and the apparent absence of observed lag time.

Furthermore, DDI is an amphoteric compound that has a weakly acidic hydrogen atom on the hypoxanthine moiety and a number of basic nitrogen atoms[77]. An unionized form of a drug more readily interacts with lipid membranes than a drug in the ionized form. Under buccal permeation conditions (pH 6.8), amphoteric nucleoside analogues such as DDI may be kept in their unionized forms and the permeation of them may be promoted.

In this study, we show that the drug can be released from the buccal film and can permeate across the mucosa as evidenced by permeability coefficients of 0.72 ± 0.14 × 10^{-2}cm/h and 0.74 ± 0.16 × 10^{-2}cm/h, and steady state flux (Jss) values of 71.63 ± 13.54 µg/cm^{2}h and 74.39 ± 15.95 µg/cm^{2}h, for the conventional and nano-enabled MMFs, respectively (Table 4.7). The flux value of the nano-enabled films was only slightly higher (p = 0.775) than that of the conventionally loaded MMFs.

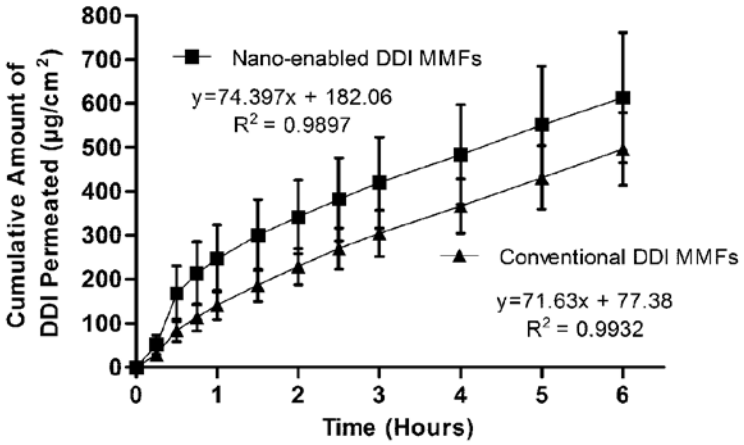

Figure 4.4: Comparison of cumulative amount of DDI permeated per unit surface area versus time from conventional MMFs[30] and nano-enabled MMFs.

Although the steady state flux values (p = 0.775) and permeability coefficients (p = 0.806) did not significantly increase, the cumulative amount of DDI permeated per unit surface area increased by 23.54 % (p = 0.161) by using nano-enabled MMFs. This could be related to the increased drug dissolution as explained in section 3.5.2 seen for nano-enabled MMFs. The relatively low entrapment efficiency (15.8 %) of DDI into SLNs reported in this study could have also contributed to the insignificant increase in buccal permeability as found during the 6 hour *in vitro* permeation study. It is quite possible that DDI entrapped into the SLN would take extended periods of time (> 6 hours) to release entrapped drug in an aqueous environment.

DDI is a hydrophilic drug and has been reported to have an aqueous solubility of 27.3 mg/mL[78]. The solubility properties of the drug ensured that sink conditions were maintained during permeation studies. Solubility facilitated release and permeation of the drug through the mucosa. Drugs with poor aqueous solubility such as saquinavir mesylate[79] or phenylephrine[37] requires modification via ball milling to achieve desired buccal permeation.

However, this study importantly confirmed that the use of SLNs to deliver the drug (DDI) via the buccal mucosa did not adversely affect the flux and confirms the potential

of DDI being delivered via the buccal route using nano-enabled MMFs. To the best of our knowledge this is the first paper examining the buccal permeability of a drug entrapped in a lipid based nanoparticulate system, formulated into a mucoadhesive film; therefore no information is available to validate our hypothesis against.

Table 4.7: Permeability parameters for DDI entrapped in MMFs.

Type of Buccal Film		Steady state flux ($\mu g/cm^2h$)	Permeability coefficient ($x10^{-2}$ cm/h)	Cumulative amount of DDI permeated ($\mu g/cm^2$)
		MEAN ± SD	MEAN ± SD	MEAN ± SD
CD	Conventional films[30]	71.63 ± 13.54	0.72 ± 0.14	496.71 ± 82.45
CP	Nano-enabled films	74.39 ± 15.95^{NS}	0.74 ± 0.16^{NS}	613.63 ± 147.77^{NS}

Statistical significance compared to conventional films - *** $p < 0.001$; ** p 0.001 to 0.01; * p 0.01 to 0.05; NS $p > 0.05$

Holpuch et al. demonstrated the feasibility of oral transmucosal nanoparticle delivery for systemic drug delivery[80]. Their study was done using human oral explants and idarubicin-loaded SLNs and they found that SLNs approximately 200 nm in size and negatively charged can penetrate through the epithelium and basement membrane into the underlying connective tissue, making systemic drug delivery using SLNs feasible via the buccal route. Therefore the possibility for enhanced ARV delivery via the buccal mucosa using SLNs exists and clearly need further exploration and formulation development.

3.5.4 Mucoadhesivity of Films

Conventional MMFs exhibited higher mucoadhesion (1425.00 ± 77.15 mN) than nano-enabled MMFs (914.33 ± 68.09 mN) (Table 4.8). This can be attributed to the higher degree of molecular binding possible between the polymers of the conventional films and the mucin[30] than occurring between the polymers, mucin and SLNs. Nano-enabled films displayed significantly reduced mucoadhesion ($p = 0.007$), a trend which was also apparent in a recent study on nano-enabled films for lysozyme delivery[33].

It was observed that in the presence of DDI the mucoadhesive force required to detach the film, decreased (1992.11 ± 130.04 mN to 1353.67 ± 127.02 mN) and can be attributed to increased molecular interactions possible between the drug and the polymer[30,33]. Reduced mucoadhesion found in this study for drug loaded MMFs compared to placebo MMFs have also been reported for buccal bilayer patches containing pravastatin sodium[81] and buccal tablets containing testosterone[82].

Table 4.8: Mucoadhesive properties of films.

Type of Buccal Film		Maximum Detachment Force (mN) *n=9*		Work of Adhesion (mJ) *n=9*	
		MEAN ± SD	**CV %**	**MEAN ± SD**	**CV %**
CP	Conventional Drug Free	1992.11 ± 130.04	6.53	1.59 ± 0.25	15.95
CD	Conventional Drug Loaded[30]	1425.00 ± 77.15	5.41	1.21 ± 0.32	26.18
NP	Nano-enabled Drug Free	1353.67 ± 127.02	9.38	0.61 ± 0.20	32.26
ND	Nano-enabled Drug Loaded	914.33 ± 68.09	7.45	0.59 ± 0.08	14.29

3.5.5 Mechanical Testing

The mechanical properties of conventional and nano-enabled films were evaluated and the data is presented in Table 4.9. The greater elasticity, tensile strength and toughness demonstrated by conventionally loaded DDI films may be related to the increased degree of cross-linking possible in the film matrix[83,84]. As seen with mucoadhesion studies, the incorporation of SLNs into MMFs also decreased the mechanical properties of the films. This finding differs from a reported study on PEG-b-PLA copolymeric nanoparticles embedded in chitosan films[32]. In their, case molecular interactions or cross-linking between the polymeric nanoparticles and polymeric film may have been increased, resulting in increased tensile strength and Young's modulus for nano-enabled films. However, tensile strength of drug loaded nano-enabled MMFs (0.4930 N/mm^2) compares favourably to nano-enabled films prepared previously[38], indicating that the nano-enabled MMFs in this study have the necessary strength to withstand forces encountered during handling and use.

Table 4.9: Mechanical properties of films.

Type of Buccal Film		Rupture Force (N) *n=3*	Tensile Strength (N/mm^2) *n=5*	Young's Modulus (N/mm) *n=5*	Elongation (%) *n=5*	Toughness (N.mm) *n=5*
		MEAN ± SD	**MEAN ± SD**	**MEAN ± SD**	**MEAN ± SD**	**MEAN ± SD**
CP	Conventional Drug Free	7.38 ± 0.13	0.7150 ± 0.052	0.43 ± 0.05	60.72 ± 3.47	80.65 ± 10.82
CD	Conventional Drug Loaded[30]	6.69 ± 0.39	0.6976 ± 0.064	0.23 ± 0.05	69.54 ± 7.77	111.70 ± 12.98
NP	Nano-enabled Drug Free	4.94 ± 0.27	0.5453 ± 0.019	0.30 ± 0.03	57.30 ± 11.08	58.26 ± 12.53
ND	Nano-enabled Drug Loaded	3.35 ± 0.38	0.4930 ± 0.003	0.24 ± 0.03	72.10 ± 8.06	58.96 ± 19.13

4.0 CONCLUSIONS

The aim of the study was to design and evaluate novel nano-enabled polymeric films for buccal delivery of DDI as a model ARV drug. The objectives were to identify optimal parameters for preparation of DDI loaded SLNs, and to prepare and compare the physico-mechanical properties of nano-enabled MMFs with conventionally prepared MMFs. In this study DDI was incorporated in SLNs for the first time, using a hot homogenization and ultrasonication technique. The optimal DDI SLNs consisted of glyceryl tripalmitate as lipid with Poloxamer 188 as surfactant and exhibited desired particle size (201 nm), PDI (0.168), zeta potential (-18.8 mV) and a formulation pH (5.5). Identified SLN formulations were incorporated in monolayered multipolymeric buccal films and resulting films were evaluated. *In vitro* buccal permeability studies indicates that the use of SLNs to deliver DDI via the buccal mucosa did not adversely affect the flux and confirms the potential of DDI being delivered via the buccal route using nano-enabled MMFs. SLN incorporation into the films decreased the *in vitro* mucoadhesiveness and mechanical properties and could be attributed to decreased molecular interactions between the polymers and mucin upon entrapment of SLNs into the film matrix. *In vitro* drug release of DDI from nano-enabled films was higher as compared to conventionally drug loaded MMFs. The data obtained in the study demonstrated for the first time the potential of DDI SLN preparation and nano-enabled SLN buccal polymeric films to serve as platforms for delivery of an antiretroviral drug, DDI. These physico-mechanical evaluations have confirmed the use of nano-enabled MMFs containing DDI SLNs prepared with glyceryl tripalmitate and Poloxamer 188 entrapped in films consisting of HPMC and EUD, as a potential buccal drug delivery system to enhance patient therapy. These studies further serve as a platform for future investigations to statistically optimize the formulations for simultaneous enhancement of drug entrapment, drug release, permeation, mucoadhesion and mechanical strength.

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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CHAPTER FIVE
GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

5.1 GENERAL CONCLUSIONS

Whilst ARVs have proven to be useful in the treatment and management of HIV & AIDS, several disadvantages limit their efficacy. Novel drug delivery systems and alternative routes to deliver ARVs are being explored to overcome these limitations. To date, studies reporting on the delivery of ARVs via the buccal route remain limited. ARV buccal drug delivery systems have not been comprehensively investigated or characterised, and a clear need exists for formulation optimization in this field.

The aim of the study was therefore to design, evaluate and optimize the preparation of novel polymeric films for buccal delivery of DDI as a model ARV drug. Thus, the objectives of the study were to 1) identify optimal process and formulation variables for the preparation of MMFs containing DDI; 2) evaluate the films in terms of of drug content uniformity, drug release, permeability, mucoadhesivity, mechanical properties and surface pH; 3) perform static lattice atomistic simulations (SLAS) to identify the suitability of the polymeric blend for buccal film formulations and to identify correlations between *in vitro* and *in silico* results (IVIS); and 4) undertake preliminary formulation studies on nano-enabled polymeric films for buccal delivery of DDI. The following conclusions were generated from the various experiments in this study:

- In the first phase of this study, MMFs with co-blended polymers of opposing solubilities and DDI were prepared in various polymeric ratios and extensively characterized. A simplified solvent casting/evaporation technique that eliminated the need for homogenization and cooling, carcinogenic solvents and additional emulsifiers was developed for this purpose. Buccal films containing DDI were successfully prepared via co-blending of HPMC and EUD. Drug content was uniform (CV < 6 %) and within required specifications (91 % to 105 %). Controlled release of DDI from MMFs was obtained by modifying the ratios of HPMC and EUD while an increase in HPMC led to an increase in drug release yet still maintained controlled release profiles with no significant dose dumping (33 % within the 1st hour for 1:1:10). Formulations exhibiting desired

controlled drug release and drug content uniformity had acceptable mechanical strength and mucoadhesivity. The buccal permeability potential of DDI from polymeric films was successfully demonstrated for the first time as evidenced by a permeability coefficient of 0.72 ± 0.14 $x10^{-2}$ cm/h and a steady state flux (*Jss*) value of 71.63 ± 13.54 µg/cm^2h. Histomorphological studies confirmed no buccal tissue damage or distress on exposure to drug loaded MMFs. The transepithelial electrical resistance (TEER) values obtained were within the reported range and the overall percentage change also indicated that mucosal integrity was not irreversibly affected. The data obtained in the first phase of the study demonstrated for the first time the potential of buccal polymeric films to serve as platforms for delivery of DDI.

- SLAS were preformed to identify the suitability of the polymeric blend for buccal film formulations and to identify correlations between *in vitro* and *in silico* results (IVIS) obtained in the first phase above. SLAS provided a mechanistic understanding of the molecular interactions involved in film formation and confirmed the corroboration of the *in silico* and *in vitro* mucoadhesive and drug release experimental data. The SLAS results indicated that HPMC and EUD as polymers in combination with glycerol and trietyl citrate as plasticizers formed a stable quadra-molecular system with the total energy of stabilization being six times higher than that of only the polymers in combination. It thereby supported the choice of polymers and plasticizers for the "novel co-blending-co-plasticizing strategy" employed in this study. Molecular mechanistic simulations also provided additional supportive information to understand drug release profiles and mucoadhesion when blending polymers of opposing solubilities. The SLAS studies therefore provided useful quantitative information to simultaneously predict the stability of polymeric and plasticizer film blends and to also identify the potential mechanisms for the observed drug release and mucoadhesion.

- The final phase of this study involved the preparation and evaluation of novel nano-enabled MMFs for buccal delivery of DDI as a model ARV drug. Optimal parameters for preparation of DDI loaded SLNs were identified before incorporation of the SLNs into nano-enabled MMFs which were subsequently compared to conventionally prepared DDI MMFs. In this study DDI was incorporated into SLNs for the first time, using a hot homogenization and ultrasonication technique. The optimal DDI SLNs consisted of glyceryl

tripalmitate as the lipid with Poloxamer 188 as the surfactant. The highest drug entrapment efficiency (18 %) was achieved with a drug loading of 1.2 % w/v. Identified SLN formulations were incorporated into monolayered multipolymeric buccal films and the resulting films were evaluated similarly as to the first phase of the study. *In vitro* buccal permeability studies indicated that the use of SLNs to deliver DDI via the buccal mucosa did not adversely affect the flux and confirmed the potential of DDI being delivered via the buccal route using nano-enabled MMFs. Future approaches to increase EE may result in significantly increased buccal permeability. SLN incorporation into the films decreased the *in vitro* mucoadhesiveness and mechanical properties. *In vitro* drug release of DDI from nano-enabled films was higher as compared to conventionally drug loaded MMFs, and shows potential for sustained drug release with further modification. The data obtained in the study demonstrated for the first time the potential of nano-enabled buccal polymeric films to serve as platforms for delivery of DDI. These studies further serve as a platform for future investigations to statistically optimize the formulations for simultaneous enhancement of drug entrapment, drug release, permeation, mucoadhesion and mechanical strength.

The findings of this study have contributed significantly to the field of novel drug delivery systems. The formulation strategies developed and the in depth characterisation studies undertaken will be useful to formulation scientists for optimising the development of buccal delivery systems for enhancing drug therapy and patient outcomes. Further studies in this promising filed will require a multidisciplinary approach to achieve the best possible outcomes.

5.2 RECOMMENDATIONS FOR FUTURE STUDIES

This study has laid the groundwork for formulating ARVs into a convenient and effective buccal delivery system. Further studies are essential prior to commercialisation of the buccal films and can be summarized as follows:

- A design of experiments approach must be used for optimising the formulation variables in terms of polymer combinations for the preparation of the MMFs. A design of experiments approach i.e. Response surface modelling will systematically identify the ideal polymeric blends for providing both mucoadhesivity and controlled drug release and also facilitate an understanding of the inter-relationship among and between formulation and process variables for the preparation of DDI loaded films. This understanding will allow the determination of the quantitative influence of polymers and plasticizers on drug delivery rates, mucoadhesion and mechanical properties.

- Permeation studies with enhancers included in the formulation as well as cytotoxicity studies should be considered. Penetration enhancers such as bile salts, surfactants, fatty acids alter the permeability the buccal mucosa thereby improving drug delivery through the buccal mucosa. Drug delivery through the buccal mucosa is limited by the barrier properties of the epithelium and inclusion of permeation enhancers can allow for delivery of therapeutically relevant amounts of drug to the systemic circulation. Cytotoxicity studies on buccal cell lines would provide useful information regarding the safety of use of the films in the human oral cavity.

- Short- and long-term chemical and physical stability studies to assess the stability of the DDI-loaded MMFs generated should be undertaken to confirm the quality of the product as well as to assess alterations in drug stability, drug release and mucoadhesion of the system. Stability studies under ICH guidelines to determine shelf life and suitable storage conditions can be done. This testing should include conditions of accelerated temperatures and cover an appropriate pH range since DDI is known to undergo degradation in acidic conditions, its solubility has been reported to be pH-dependant.

- Additional film characterization can include evaluation using advanced techniques such as XRD, TGA, DSC and FTIR. The physical form of drug molecules inside the MMFs can be easily determined by XDR analysis. This would provide information on whether the drug is present in crystalline or amorphous form in the film and it is of importance since the physico-chemical properties would be influenced. DSC analysis can provide insight into the state of the drug molecules inside the MMFs. Useful information regarding phase transitions, recrystallization or molecular interactions of the drug molecule entrapped inside the MMFs can be obtained. Drug-polymer interaction can be detected via FTIR and can be used to assess the compatibility of drug with the excipients. This would allow for a better understanding of DDI compatibility with polymers identified previously as suitable for combined use via SLAS.

- *In vivo* studies using animals and human subjects should be performed to further test the formulation in terms of retention time of the dosage form on the mucosa. *In vivo* studies using suitable animal models will also provide information on bioavailability and related pharmacokinetic parameters which in turn would provide insight into suitable formulation modifications that would be required to achieve optimal bioavailability. Bioavailability studies may also provide useful comparative information against other currently available per oral formulations of DDI.

- A larger scale production method could be designed for the preparation of the MMFS in order to assess the feasibility of the film preparation method for application in the pharmaceutical industry. A prerequisite for new formulations into the pharmaceutical industry is the availability of a suitable large-scale production method. The method should be qualified, validated and cost-effective. Additionally, the method should yield a preparation that is of acceptable quality that allows registration by the relevant regulatory authorities. Furthermore, large-scale production methods have been established for microparticles, but remain to be established for many nanoparticles.

- For nano-enabled films specifically several future studies can be considered. The drug entrapment efficiency (EE) for hydrophilic drugs remains problematic as also seen in this study and future work should specifically focus on optimizing the EE. Methods to further increase drug entrapment of DDI into

SLNs or incorporation of only separated SLNs into the MMFs may improve buccal permeability if DDI. Stability studies on SLNs and nano-enabled films as well as investigations using techniques such as DSC may provide useful information of the nature of the drug in these kinds of novel preparations. The compatibility and suitability of excipients can also be confirmed. Imaging of nano-enabled films via SEM/TEM to visualize the entrapped nanoparticles can be considered. Molecular mechanistic simulations can be done on the nano-enabled film components to establish a corroboration between the *in silico* and *in vitro* experimental data upon inclusion of SLNs into the MMFs. Future work will also require polymeric modification of the base film components in order to achieve enhanced mucoadhesion and desirable drug release for nano-enabled films for buccal delivery of didanosine.

APPENDIX

Ethical Clearance Letters

UNIVERSITY OF
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Research office
Animal Ethics Research Committee
Govan Mbeki Centre, Westville Campus,
University Road, Chiltern Hills, Westville, 3629, South Africa
Telephone 27 (031) 260-2273/35 Fax (031) 260-2384
Email: Animalethics@ukzn.ac.za

06 December 2011

Reference: 011/12/Animal

Ms. E. Jones
Department of Pharmacy
Block E6 Floor 6 Room 606
Westville Campus

Dear Ms. Jones

Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2011/12 on the following project:

Physicochemical optimization of mucoadhesive polymeric films for buccal delivery of didanosine.

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar, Prof. Jane Meyerowitz
Research Office, Mr N. Moodley
Supervisor, Prof. T. Govender
Head of School, Prof. F. Oosthuisen
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14 December 2012

Reference: 041/13/Animal

Ms E Jones
Discipline of Pharmaceutical
Sciences
School of Health Sciences
WESTVILLE Campus

Dear Ms Jones

RENEWAL: Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for **2013** on the following project:

"Physicochemical optimisation of mucoadhesive polymeric films for buccal delivery of didanosine."

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar – Prof. J Meyerowitz
Research Office – Dr N Singh
Supervisor – Prof. T Govender
Head of School – Prof. S Essack
BRU – Dr S Singh

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Monolayered multipolymeric buccal films with drug and polymers of opposing solubilities for ARV therapy: Physico-mechanical evaluation and molecular mechanics modelling

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ABSTRACT

Although buccal permeation investigations with antiretroviral drug solutions have confirmed their transbuccal delivery potential, studies on their formulation into delivery systems are lacking. Multipolymeric monolayered films (MMFs) with drugs and polymers of opposing solubilities will offer several advantages for the controlled release delivery of didanosine (DDI) via the buccal route. The aim of this study was to employ a co-blending-co-plasticization technique for preparation of MMFs containing Eudragit® RS 100 (EUD) and Hydroxypropyl methylcellulose (HPMC) and to undertake molecular modelling and *in vitro* characterizations. Uniform drug content (91–105%) with low variability was obtained for all films. Co-blending of DDI:HPMC:EUD (1:1:10) was required to achieve controlled drug release. The buccal permeability potential of DDI from the MMFs was successfully demonstrated with a permeability coefficient of $0.72 \pm 0.14 \times 10^{-2}$ cm/h and a steady state flux of 71.63 ± 13.54 μg/cm² h. Films had acceptable mucoadhesivity (2184 mN), mechanical strength (0.698 N/mm²) and surface pH (6.63). The mechanism inherent to the mucoadhesive and drug release profile performance of the MMFs was elucidated via static lattice molecular mechanics simulations wherein a close corroboration among the *in vitro–in silico* (IVIS) data was observed. These extensive physico-mechanical and molecular atomistic studies have confirmed the use of MMFs containing DDI, HPMC and EUD as a buccal delivery system.

1. Introduction

Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS) commonly referred to as HIV & AIDS, have emerged as the leading cause of mortality worldwide and is the main cause of death in sub-Saharan Africa (Merson et al., 2008). While antiretrovirals (ARVs) have proven to be useful in the treatment and management of HIV & AIDS, several disadvantages including extensive first pass metabolism, gastrointestinal degradation, low bioavailability and short half-lives (Li and Chan, 1999) limit their efficacy. Large doses, complex dosing regimens and multiple drugs contribute to reduced patient compliance (Chandwani et al., 2012). Poor drug solubility and limited membrane permeability also pose formulation difficulties (Sharma and Garg, 2010).

The development of new chemical entities, novel drug delivery systems and alternative routes to deliver ARVs (Ojewole et al., 2008) are being explored to overcome these limitations. Novel drug delivery systems receiving increased attention include sustained release matrix tablets (Sánchez-Lafuente et al., 2002), ceramic implants (Benghuzzi, 2000), liposomes (Dubey et al., 2010) and nanoparticles (Kuo and Chung, 2011). Alternate routes for delivery under investigation include: transdermal (Gerber et al., 2008), nasal (Carvalho et al., 2013), vaginal (Johnson et al., 2010) and buccal delivery (Ojewole et al., 2012; Xiang et al., 2002).

Drug delivery via the buccal route has recently emerged as a lucrative alternative to the oral route. Drugs can directly enter the systemic circulation and bypasses gastrointestinal degradation and first-pass hepatic metabolism, thereby improving bioavailability (Hoogstraate and Wertz, 1998). The buccal mucosa is easily accessible and more permeable than skin (Squier and Hall, 1985). Formulating the drug into a controlled release mucoadhesive dosage form may further improve drug delivery and patient compliance (Morales and McConville, 2011). Buccal transportation mainly occurs via passive diffusion across lipid membranes either via

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paracellular or transcellular pathways making this route suitable for both hydrophilic and lipophillic drugs (Patel et al., 2011). This is relevant considering HIV & AIDS is treated with multiple-drug regimens. The disadvantages associated with the buccal route of drug delivery are its low mucosal permeability, continuous secretion of saliva leading to dilution of drug and the need for formulation approaches to promote retention on the mucosae (Patel et al., 2011).

To date, reports regarding buccal permeability of antiretrovirals remain limited. *In vitro* drug permeability studies with solutions of zalcitabine (Shojaei et al., 1999; Xiang et al., 2002), didanosine (Ojewole et al., 2012) and tenofovir (Rambharose et al., 2013) have been reported. To the best of our knowledge, the only report thus far on buccal polymeric dosage forms of ARVs is of zidovudine polymeric patches recently produced by Reddy et al. (2012). Characterization studies were limited and did not include critical parameters such as *in vitro* permeation or mechanical properties. ARV buccal delivery systems have not been comprehensively investigated or characterised and it is clearly essential for formulation optimization.

Various mucoadhesive buccal dosage forms are being investigated for different classes of drugs, which include adhesive tablets (Cappello et al., 2006), gels (Ayensu et al., 2012b), ointments (Petelin et al., 2004), patches (Vasantha et al., 2011), and more recently films (Abruzzo et al., 2012; Sievens-Figueroa et al., 2012). Films may be preferred over tablets in terms of flexibility and comfort. They can circumvent the relatively short residence time of oral gels on the mucosa, which is easily washed away by saliva (Ahn et al., 2001; Okamoto et al., 2001). Polymeric films formulated for controlled drug release could also decrease dose-related side effects and improve patient compliance. A polymer for buccal films should adhere easily and sufficiently to the buccal mucosa, should have sufficient mechanical strength, should demonstrate penetration enhancement and provide for controlled release of the drug. Single polymers often fail to demonstrate all the ideal characteristics. To overcome this problem, researchers have been focusing on blending of polymers with similar solubilities (Abruzzo et al., 2012; Dubolazov et al., 2006; Juliano et al., 2008).

For controlled drug release, good mucoadhesion and suitable mechanical strength, polymers and drugs of opposing solubilities may often be required. While multipolymeric multilayered films and wafers have been prepared with drugs and polymers of opposing solubilities (Ding et al., 2012; Perugini et al., 2003), monolayered multipolymeric films (MMFs) offer more advantages, i.e. lower production costs, improved drug release, mucoadhesivity and size (Perugini et al., 2003). Limited formulation and characterization studies on MMFs with polymers and drugs of opposing solubilities have been reported. Further, the methods employed to produce the aforementioned MMFs require carcinogenic solvents (Perugini et al., 2003), involve the combination of two separate mixtures under high shear rates (Pendekal and Tegginamat, 2012), require emulsification below room temperature (Perumal et al., 2008b) or need multiple solvents with additional emulsifiers (Vasantha et al., 2011). In this paper a new technique whereby drugs and polymers of opposing solubilities can be co-blended using a co-solvent to produce buccal MMFs is reported. This method is simple, eliminates the need for emulsifiers, can be done at room temperature and requires minimal equipment. Limited studies on Eudragit® RS 100 (EUD) in combination with hydroxypropyl methylcellulose (HPMC) for buccal films have been reported (Koland et al., 2010; Mishra et al., 2012). These studies involved complex preparation methods and lacked evaluation of critical physico-mechanical properties. Molecular modelling to identify the mechanism of interaction between these two polymers and their suitability for combined use and indeed for any other buccal delivery system has not been previously reported. Therefore such physico-mechanical evaluation and molecular modelling of MMFs is essential for formulation optimization and facilitating a mechanistic understanding of MMFs.

Didanosine was selected as a model ARV due to its extensive first pass metabolism and short half-life making it an ideal candidate for controlled buccal delivery. The aim of this study was, therefore, to use a simplified method to prepare and characterize monolayered mucoadhesive films comprising of various ratios of co-blended EUD and HPMC for buccal delivery of didanosine. Films prepared by the solvent casting/evaporation technique were evaluated in terms of drug content uniformity, drug release, permeability, mucoadhesivity, mechanical properties and surface pH. Static lattice atomistic simulations (SLAS) were performed to identify the suitability of the polymeric blend for buccal film formulations and to identify correlations between *in vitro* and *in silico* results (IVIS).

2. Materials and methods

2.1. Materials

Didanosine (DDI) was purchased from Ruland Chemistry Co., Ltd. (Nanjing, China) and used as received. Hydroxypropyl methylcellulose (HPMC), triethyl citrate (TEC) and mucin (Sigma–Aldrich, UK) were purchased and used as received. Eudragit® RS 100 (EUD) (Evonik Rohm GMBH, Germany) was donated by Degussa Africa (Pty) Ltd. All other reagents used [NaCl, Na_2HPO_4, KH_2PO_4, NaOH, HCl, MeOH, EtOH and Glycerol (GLY)] were of analytical reagent grade. Purified water used throughout the studies was produced in the laboratory with a Milli-Q purification system (Millipore Corp., USA).

Phosphate buffered saline (PBS) used for *in vitro* drug release, permeation and mucoadhesion studies had the following composition per litre of distilled water: 2.38 g $Na_2HPO_4{\cdot}10H_2O$, 0.19 g KH_2PO_4, 8 g NaCl and adjusted to pH 6.8 or pH 7.4 with hydrochloric acid or sodium hydroxide as required (Peh and Wong, 1999).

2.2. Methods

2.2.1. Preparation of films via co-blending

Films were prepared using the solvent casting and evaporation method. For this study silicone moulded trays (SMT's) with individual wells of 6 cm^2 were used instead of conventional film casting trays, since it has been shown in our previous publication that SMT's enhance drug content uniformity, and reduce the variability in mucoadhesivity as well as drug release (Perumal et al., 2008a).

Multipolymeric films comprising of DDI, HPMC and EUD in various ratios were prepared as follows (Table 1): specified quantities of EUD and TEC as its plasticizer together with HPMC and GLY as its plasticizer were dissolved in 40 ml methanol in a 100 ml volumetric flask. DDI and 40 ml water was added to this and sonicated until the drug has been dissolved. The mixture was made up to volume with 50% methanol in water and agitated by hand at room temperature until a homogenous solution resulted. Preformulation studies informed on formulation variables to use. The plasticizer content for both polymers was kept constant at 30% (w/w) of polymer weight for all ratios prepared.

Thereafter 2 ml of each polymeric solution containing 20 mg of DDI was syringed into each 6 cm^2 well of the SMT containing Teflon coated Perspex inserts. The drug–polymeric mixture was allowed to dry in an oven (Series 2000, Scientific, SA) at 43 °C for approximately 24 h, until the solvent had evaporated and constant film weight was achieved. Films were removed from the moulds and stored using wax paper and foil in a desiccator at room temperature (23 °C) up to a maximum of three months until further use.

Table 1
Composition of the buccal film formulations (DDI:HPMC:EUD).

Ingredients (% w/v)	Effect of HPMC				Effect of EUD				
	1:0.25:10	1:0.5:10	1:0.75:10	1:1:10	1:0.5:5	1:0.5:7.5	1:0.5:10	1:0.5:15	1:0.5:20
DDI	1	1	1	1	1	1	1	1	1
HPMC	0.25	0.5	0.75	1	0.5	0.5	0.5	0.5	0.5
GLY	0.075	0.15	0.225	0.3	0.15	0.15	0.15	0.15	0.15
EUD	10	10	10	10	5	7.5	10	15	20
TEC	3	3	3	3	1.5	2.25	3	4.5	6

2.2.2. Characterization of films

2.2.2.1. Weight and thickness uniformity. For weight uniformity three films per batch were randomly selected and individually weighed on an electronic balance (Metller Toledo AB204-S., Switzerland). The thicknesses of the films were measured using a digital micrometer (Mitutoyo Co., Japan) with an accuracy of 0.001 mm. Thicknesses were measured in five different locations (centre and four corners) of the films. Results are represented as a mean and standard deviation of the replicate determinations.

2.2.2.2. Assay of films. The assay solvent consisted of 80% ethanol in water. A 6 cm^2 film as a unit from the SMT was dissolved in approximately 40 ml of the assay solvent in a 100 ml volumetric flask before making up to volume with the same assay solvent. Following appropriate dilution (1 in 10), the drug content in the samples was quantified using a UV spectrophotometer (Shimadzu 1650 PC, Japan) at a wavelength of 250 nm. All assays were performed in triplicate.

A calibration curve of DDI concentration versus absorbance was plotted across a concentration range from 0.1 to 50 μg/ml and a linear response was found (r^2 = 0.9997). The UV methodology was also successfully validated in terms of specificity, linearity, precision, accuracy and robustness (data not shown).

2.2.2.3. In vitro drug release. A modified BP2009 Type II paddle dissolution test apparatus (Erweka DTR-6., Germany) was employed to determine *in vitro* drug release of the films. The dissolution studies were carried out in 900 ml PBS adjusted to pH 6.8 and maintained at 37 ± 0.5 °C; with a stirring speed of 50 rpm. The film size required for dose delivery (6 cm^2) was used. The film was placed into a stainless steel wire mesh basket and dropped into the dissolution vessel at the start of the experiment. A wire mesh basket was used, instead of attaching a film to a glass slide with adhesives as commonly reported (Nair et al., 2013), in an attempt to limit interference with drug release. Aliquots of 6 ml samples from the dissolution medium were collected at predetermined time intervals of 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min using a syringe and in line filtration (0.45 μm). An equal volume (6 ml) of fresh PBS was replaced into each dissolution vessel, to ensure that a constant volume of dissolution medium was maintained throughout the duration of the study. The filtered samples were quantified for drug using a UV spectrophotometer (Shimadzu 1650 PC, Japan) at a wavelength of 250 nm. The results are represented as the average of three films.

2.2.2.4. In vitro permeation. *In vitro* permeation experiments were performed on the 1:0.5:10 formulation to confirm the permeability potential of DDI incorporated into multipolymeric films. Porcine buccal mucosa was used as a biological membrane for these experiments due to the many similarities to the human buccal mucosa as highlighted by Shojaei (1998) and Sudhakar et al. (2006).

Porcine buccal mucosa was excised from domestic pigs (30–40 kg) immediately upon euthanasia at the university's biomedical research unit after obtaining necessary ethical clearance (011/12/Animal). Excess adipose and connective tissue were cut away from the mucosal specimens leaving the mucosa with an average thickness of (665 ± 72 μm). Samples were wrapped in foil before being snap-frozen in liquid nitrogen and stored at −85 °C in a biofreezer for up to 3 months (Van Der Bijl, 1998).

In vitro permeation experiments on DDI films were performed similar to *in vitro* permeability studies of DDI solutions recently reported (Ojewole et al., 2012). On the day of the experiments, frozen buccal mucosal specimens were allowed to thaw and equilibrate in PBS pH 7.4 to regain elasticity temporarily lost while frozen. Franz diffusion cells (PermeGear, Inc., USA) each with a diffusional area of 0.786 cm^2 were used for the *in vitro* permeation experiments. The buccal mucosa and polymeric film were mounted between the donor and receptor compartments using the two membrane holders. Two millilitres PBS at pH 6.8, simulating human saliva (Peh and Wong, 1999), was placed on the film in the donor compartment while the receptor compartment contained 27 ml PBS pH 7.4 maintained at 37 °C (by means of a surrounding jacket) and stirred constantly.

At predetermined time intervals over 360 min, samples (27 ml) were taken from the receptor compartments and replaced by drug-free PBS. Similar to dissolution studies samples were immediately filtered through a 0.45 μm membrane filter and the drug content was quantified using a UV spectrophotometer (Shimadzu 1650 PC, Japan) at a wavelength of 250 nm. A minimum of three replicates were performed.

The viability of the mucosa was assessed by transepithelial electrical resistance (TEER) measurements using a Millicell ERS meter (Millipore, USA) connected to a pair of chopstick electrodes (STX01). TEER measurements were taken across the mucosa before and at the end of, the permeation experiment (Dezani et al., 2013) and thereafter following exposure to fresh PBS pH 6.8 for 60 min (Chen et al., 2009).

The cumulative amount of DDI permeated per unit surface area was plotted versus time. The steady state flux (J_{ss}) across the mucosal membrane was determined from the linear portion of the permeation graph by linear regression analysis (Microsoft Excel 2010). The permeability coefficient (P) was calculated using the following equation (Shojaei et al., 1999):

$$P = \frac{(dQ/dt)}{A \times C_d} = \frac{J_{ss}}{C_d}$$

where dQ/dt is the cumulative amount (Q) of DDI which permeated into the receptor compartment per unit time (t), A the active cross-sectional area (0.786 cm^2) available for diffusion and C_d is the drug concentration in the donor compartment.

2.2.2.5. Histological evaluation. Histological studies were performed to evaluate for pathological changes occurring in cell morphology and tissue organization. Directly after excision of mucosa, untreated buccal mucosa was transferred from normal saline into 10% buffered formalin without any equilibration in PBS and served as the control. Treated samples comprised of buccal mucosae that were exposed to PBS only, or a placebo film or drug loaded film (1:0.5:10). Permeation experiments were performed as described previously in Section 2.2.2.4, without drug quantification (Rambharose et al., 2013). At the end of the experiment

the buccal mucosa was cut into cross sections. The samples for light microscopy were fixed in 10% buffered formalin for 7 days, washed in water, dehydrated in graded ethanol and, after permeation in xylene, embedded in paraffin using standard procedures. Samples were cut into sections (1 μm thick) on a microtome and stained with hematoxylin and eosin (H&E). Sections were examined using a light microscope (Nikon 80i, Japan), and bright field images were digitally captured using NIS Elements D software and a camera (Nikon U2, Japan). Samples for transmission electron microscopy (TEM) were collected under the same conditions. They were fixed for 24 h (4 °C) using Karnovsky's fixative buffered to pH 7.2, embedded in epoxy resin, cut into ultrathin section (90 nm) and contrasted with uranyl acetate and lead citrate using standard protocols before viewing with a transmission electron microscope (JEOL 1010, Japan). All experiments were performed using a minimum of three replicates.

2.2.2.6. Mucoadhesivity of films. The effects of the different polymeric ratios on the mucoadhesive properties were studied using methods adapted from (Ayensu et al., 2012a) and (Perumal et al., 2008b). A TA.XT2*i* Texture Analyser (Stable Micro Systems, UK) equipped with a 5 kg load cell in tension mode, removable 2 cm × 3 cm aluminium probes, and Texture Expert™ software were used for this purpose.

Film samples ($n = 3$), 30 mm long × 20 mm wide, and free from physical imperfections were individually attached to probes using double sided adhesive tape. The probes were attached to the upper movable arm of the TA.XT2*i*. A Petri-dish containing solidified 10% (w/v) gelatine gel, simulating buccal mucosa, was clamped into place on the stationary platform of the TA.XT2*i* (Ayensu et al., 2012a). Two millilitres of 30% (w/v) mucin at 37 °C was spread on the surface of the gelatin immediately prior to testing (Perumal et al., 2008b). The film, securely attached to the probe, was allowed to hydrate for 120 s in PBS pH 6.8 before being brought into contact with the mucin covered gelatin. The film was held in place with a force of 100 g for 60 s before the mobile arm was raised. Parameters used were pre-test speed: 0.5 mm/s; test speed: 0.5 mm/s and post-test speed: 1 mm/s. The mucoadhesive performance of the samples was determined by measuring the Maximum Detachment Force (MDF) (mN) and work (mJ). The MDF represents the maximum force required to detach the film from the mucin covered gelatin. The area under the force/distance curve was also determined to represent the work required for detachment of the two systems (mucin/polymeric film) (Eouani et al., 2001). A minimum of 9 replicate determinations were performed.

2.2.2.7. Mechanical testing. Mechanical properties of the films were studied as a function of various polymer ratios prepared. A TA.XT2*i* Texture Analyser (Stable Micro Systems, UK) equipped with a 5 kg load cell, TA-96 grips and Texture Expert™ software were utilized for this purpose.

Individual film samples ($n = 5$), 30 mm long by 20 mm wide with varying thickness (Table 2), and free from physical imperfections were held between the grips (TA-96). The grip separation was set at 15 mm. A sheet of Teflon was attached to the surface of the grips via double-sided tape to prevent the film being cut by the grooves of the grips. During measurement, the film was pulled by the top grip at a rate of 1 mm/s to a distance of 150 mm before returning to the starting point. Data acquisition was terminated when the film ruptured completely. The data of the film samples that failed at, and not between, the grips were not utilized in the evaluation of the mechanical properties. The force and elongation were measured when the films broke.

The tensile strength, percent elongation, and Young's modulus were used as indicators of the mechanical properties of the films. Mechanical properties of the films were evaluated using the following equations (Heng et al., 2003):

$$\text{Tensile strength}\,(\text{N/mm}^2) = \frac{\text{force at break}\,(\text{N})}{\text{width}\,(\text{mm}) \times \text{thickness of film}\,(\text{mm})}$$

$$\text{Elongation at break}\,(\%) = \frac{\text{increase in length}}{\text{original length}} \times 100$$

Young's modulus was determined from the slope of the initial linear portion of the stress-strain plots generated with the Texture Expert™ software.

A rupture test was also performed to assess the mechanical film properties. A film support rig with an exposed area of 0.786 cm^2 was attached to the heavy duty platform of the TA.XT2*i* Texture Analyser. Individual film samples ($n = 3$) were clamped between the film support rig before passing a 5 mm stainless steel ball probe through a sample at 1 mm/s in compression mode. The force (N) required to rupture the film was measured (Sievens-Figueroa et al., 2012).

2.2.2.8. Surface pH. Saliva has a natural buffering capacity (Bardow et al., 2000) and its pH ranges from 5.6 to 7 (Sudhakar et al., 2006). Buccal formulations should be within this range to avoid causing mucosal irritation. The surface pH of films was determined using methods adapted from (Cavallari et al., 2013) to assess for any potential buccal mucosa irritation. The film was allowed to swell in 15 ml PBS as simulated saliva at pH 6.8 and the pH was measured at predetermined time intervals over 6 h. The film was carefully removed from the PBS, pH paper (Hydrion MicroFine, Micro Essential Laboratory, USA) was placed on its surface and the pH was measured. Results are represented by the mean of three measurements.

2.2.3. Establishment of the polymeric complexation profile and potential impact on mucoadhesion and drug release via SLAS

All modelling procedures and computations, including energy minimizations in Molecular Mechanics, were performed using HyperChem™ 8.0.8 Molecular Modelling Software (Hypercube Inc., Gainesville, FL, USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, Cambridge, UK). The 3D structure of EUD was archetyped using ChemBio3D Ultra in its syndiotactic stereochemistry as a 3D model, whereas the structure of HPMC (4 saccharide units) was built from standard bond lengths and angles using the Sugar Builder Module on HyperChem 8.0.8. The structures of GLY and TEC were constructed with natural bond angles. The structure of the glycosylated mucopeptide analogue (MUC) mucin was generated using the sequence editor module on HyperChem 8.0.8. The glycosylation was performed at the threonine amino acid residues. The models were primarily energy-minimized using the MM+ Force Field algorithm and the resulting structures were once again energy-minimized using the AMBER 3 (Assisted Model Building and Energy Refinements) Force Field algorithm. The conformer having the lowest energy was used to develop the polymer–polymer; polymer–plasticizer; and polymer–mucin complexes. A complex of one polymer molecule with another was assembled by parallel disposition and the energy-minimization was repeated to generate the final models: HPMC-GLY, EUD-TEC, HPMC-EUD, HPMC-GLY/EUD-TEC, HPMC-MUC, EUD-MUC, and HPMC-MUC-EUD. Full geometrical optimization was conducted in vacuum employing the Polak–Ribiere Conjugate Gradient method until an RMS gradient of 0.001 kcal/mol was reached (Kumar et al., 2012).

2.2.4. Statistical analysis

All calculations were undertaken with Microsoft Excel® (Microsoft Office 2010, USA). A minimum of three replicates were

Table 2
Effect of polymer ratios on drug content uniformity, thickness and film weight. (Mean ± SD values; $n = 3$).

Formulation	Assay (%) $n = 3$		Thickness (μm) $n = 3$		Weight (mg) $n = 3$	
DDI:HPMC:EUD	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%
Effect of HPMC						
1:0.25:10	91.17 ± 0.85	0.93	209.40 ± 17.40	8.31	243.53 ± 10.51	4.32
1:0.5:10	95.62 ± 5.41	5.66	299.60 ± 25.00	8.34	293.60 ± 1.50	0.51
1:0.75:10	91.69 ± 2.11	2.30	308.07 ± 26.97	8.75	306.10 ± 2.52	0.82
1:1:10	91.64 ± 2.48	2.70	319.73 ± 3.19	1.00	318.60 ± 2.56	0.80
Effect of EUD						
1:0.5:5	96.79 ± 0.36	0.37	124.13 ± 2.64	2.13	159.03 ± 0.96	0.60
1:0.5:7.5	98.06 ± 2.36	2.41	205.20 ± 3.60	1.75	228.67 ± 0.72	0.32
1:0.5:10	95.62 ± 5.41	5.66	299.60 ± 25.00	8.34	293.60 ± 1.50	0.51
1:0.5:15	102.83 ± 3.06	2.98	434.33 ± 24.23	5.58	420.90 ± 10.19	2.42
1:0.5:20	105.24 ± 1.69	1.60	666.47 ± 11.60	1.74	556.13 ± 4.92	0.88

performed and results are expressed as mean ± SD. Statistical analysis of data were performed using GraphPad Prism, Version 5 (GraphPad Software., Inc., USA). One-way ANOVA followed by Dunnett's multiple comparisons test was used to determine statistical significance. p-Values of $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Preparation of films via co-blending

During preliminary studies, monopolymeric films containing DDI and either HPMC, as a hydrophilic polymer, or EUD, as a hydrophobic polymer, were prepared. However these films were deemed unsuitable for drug delivery due to unfavourable physico-mechanical film properties (data not shown). Monopolymeric films exhibited undesired drug release kinetics, had irregular surfaces and unsuitable mechanical strength. Monolayered multipolymeric co-blended films (MMFs) were prepared thereafter with HPMC and EUD to improve film characteristics. Instead of using carcinogenic solvents (Perugini et al., 2003), complex mixing and emulsification methods (Pendekal and Tegginamat, 2012; Perumal et al., 2008b) or multiple solvents with additional emulsifiers (Vasantha et al., 2011) as previously reported, our group used a simple method that eliminated the need for homogenization and cooling as well as the use of complex or carcinogenic solvents and additional emulsifiers, to produce MMFs. We simply used methanol as the co-solvent in which the hydrophobic EUD as well as the hydrophilic DDI could dissolve. Methanol is miscible with water and allowed for sufficient swelling of HPMC in the aqueous medium. Multipolymeric monolayered films (MMFs), containing polymers of opposing solubilities and DDI, were successfully prepared using this simplified co-blending technique. SLAS results described under Section 3.8.2 indicate that the two polymers and two plasticizers form a stable quadra-molecular system with the total energy of stabilization being six times higher than that of only the polymers in combination. It thereby supported the choice of polymers and plasticizers for the "novel co-blending-co-plasticizing strategy" employed in this study.

The films generated by this simplified technique were translucent to opaque, thin, flexible and their surface appeared homogenous (Fig. 1). The drug–polymeric casting solution prepared using the co-blending technique was also completely homogenous and no phase separation occurred upon drying of the films. Drying for 24 h at 43 °C did not pose stability concerns as Kasongo et al. (2011) established didanosine's thermal stability in excess of 85 °C. Limited drug precipitation was also noted. The average thickness and weight of the films ranged from 124 to 666 μm and 159 to 556 mg respectively, increasing proportionally as polymer content increased (Table 2). Drug content uniformity across buccal films is a major problem as highlighted in the literature (Morales and McConville, 2011). By using similar silicone moulded trays with individual wells for film casting as previously investigated by our group (Perumal et al., 2008a) we were able to overcome problems with drug content uniformity (Table 2). Drug content values ranged from 91% to 105% with low CV values of less than 6% indicating good drug content uniformity. All ratios prepared of monolayered multipolymeric films were homogenous, had limited drug precipitate and acceptable drug content uniformity.

3.2. In vitro drug release

The influence of HPMC and EUD on the drug release of DDI MMFs were studied. Fig. 2a shows the drug release profiles of DDI films prepared using increasing amounts of HPMC. An increase in HPMC led to increase in drug release while still maintaining controlled release profiles with no significant dose dumping. This increased drug release could be attributed to the hydrophilic nature of HPMC, which can erode more readily (Morales and McConville, 2011) thereby releasing the drug into the dissolution medium (33% within the 1st hour for 1:1:10). In addition to polymer

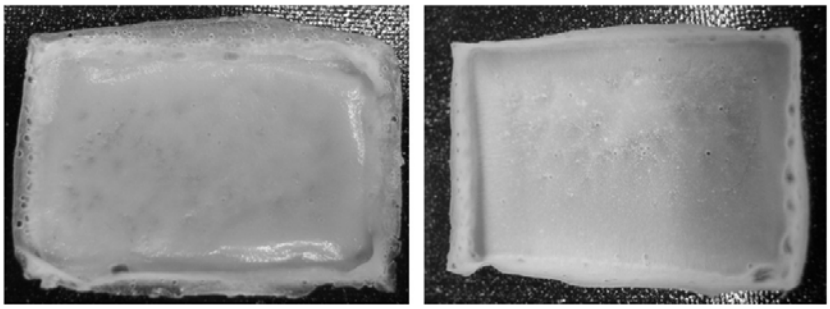

Fig. 1. Digital photographs of 1:0.5:10 (DDI:HPMC:EUD) monolayered multipolymeric films.

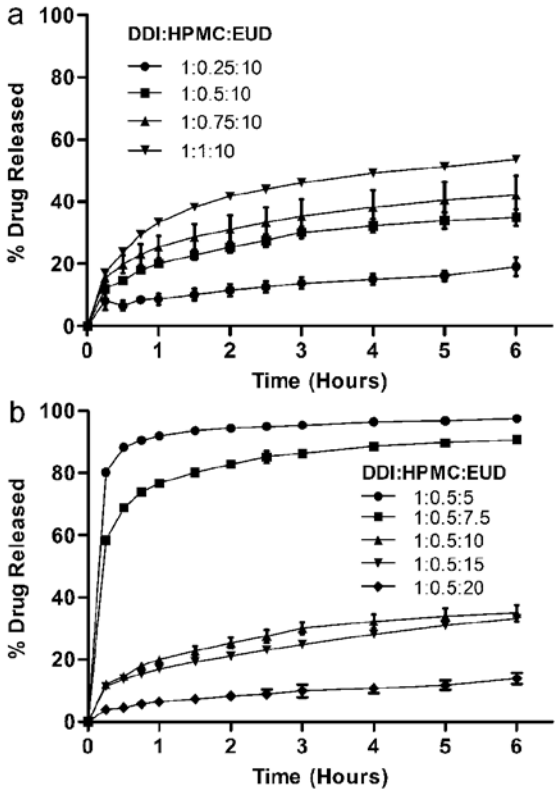

Fig. 2. (a) Effect of HPMC on DDI release from multipolymeric films. (b) Effect of EUD on DDI release from multipolymeric films.

solubility, molecular mechanistic simulations (Section 3.8.3) also showed that comparatively higher concentrations of HPMC-GLY will make the quadra-molecular architecture less stable. This leads to an increase in hydrophillicity and chain relaxation or degradation which causes increased drug release. Although drug release increased with increasing HPMC, the controlled drug release seen with all four profiles (Fig. 2a) was due to the incorporation of EUD, a hydrophobic polymer, into the multipolymeric films.

The effect on drug release upon altering the EUD content of the multipolymeric films was also investigated. Fig. 2b shows the drug release profiles of DDI films prepared using increasing amounts of EUD and constant amounts of HPMC. 1:0.5:5 and 1:0.5:7.5 showed very rapid drug release. 80% of the loaded drug was release from 1:0.5:5 within the first 15 min. Rapid drug release systems would be unfavourable for the delivery of DDI, as frequent drug administration would lead to decreased patient compliance. Further increase in EUD content in the films led to decreased rates of drug release. It is possible to achieve controlled drug release with changing the ratios of polymers used in the formulation. Drug release retardation could be attributed to the hydrophobic nature of the EUD and the resultant lower solubility in the aqueous dissolution medium and slower rate of film erosion (Magdy I. Mohamed et al., 2011). The low aqueous solubility of EUD prevented free and deep water penetration into the film, thereby only allowing the DDI that was near the external surface of the film to be initially released into the dissolution medium (Perumal et al., 2008b). The molecular mechanistic model employed in this study (Section 3.8.3) indicated that the presence of the stable EUD-TEC complex increase the stability of the films leading to slower drug release since there would be less tendency of the stabilized system to undergo a change in terms of chain relaxation or film degradation.

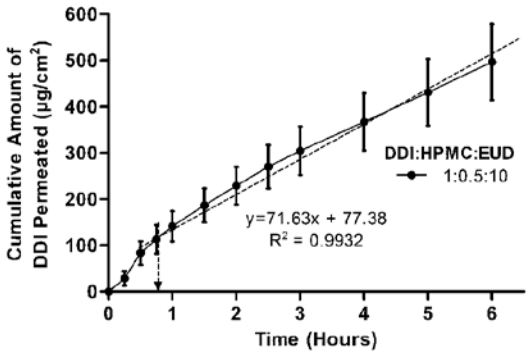

Fig. 3. Cumulative amount of DDI permeated per unit surface area versus time from films. (Mean ± SD values; $n \geq 3$).

Blending of EUD and HPMC polymers were necessary to obtain a desired controlled release profile of DDI. Molecular mechanistic simulations discussed in detail in Section 3.8.3 provided additional supportive information to understand drug release profiles when blending polymers of opposing solubilities.

3.3. *In vitro permeation*

It is recognized that the bioavailability of drugs administered via the buccal route can be greatly influenced by the permeation rate through the buccal mucosal membrane (Morales and McConville, 2011). Limited work has been published regarding buccal permeability of antiretrovirals. Thus far the buccal permeability potential of only drug solutions containing zalcitabine (Shojaei et al., 1999; Xiang et al., 2002), didanosine (Ojewole et al., 2012) or tenofovir (Rambharose et al., 2013) have been published. In terms of drug delivery systems for the buccal route, buccal patches for zidovudine has been reported on but permeability of drug across the mucosa was not reported. The buccal permeability potential of DDI from 1:0.5:10 films were investigated due to their potentially suitable mucoadhesion and drug release (Fig. 3). The non-linear portion of the plot was considered as the lag time and it was the time required for steady state permeation to be achieved. A lag time of 45 min in this case is acceptable since the formulation under investigation will be for controlled release. In this study, we show that the drug can be released from the buccal film and can permeate across the mucosa as evidenced by a permeability coefficient of $0.72 \pm 0.14 \times 10^{-2}$ cm/h and a steady state flux (J_{ss}) value of 71.63 ± 13.54 µg/cm^2 h. The slope of the linear portion ($R^2 = 0.9932$) of the plot was used to determine the J_{ss}. The flux value (71.63 ± 13.54 µg/cm^2 h) obtained in this study compares favourably to that achieved in permeation studies performed on DDI solutions only as recently reported by (Ojewole et al., 2012). Therefore these experiments indicate that the flux was not adversely affected by the formulation of DDI into a film with polymeric film components. The data thus confirms the potential of DDI being delivered transbuccaly via multipolymeric films and can be used for improving HIV and AIDS drug therapy. DDI is a hydrophilic drug, and passive diffusion should have preference towards the paracellular pathway (Hassan et al., 2010; Sandri et al., 2006). Several other classes of drugs incorporated into buccal polymeric films exhibited similar or lower flux values (Diaz del Consuelo et al., 2007; Pendekal and Tegginamat, 2012) and were considered as having potential for buccal delivery.

Transepithelial electrical resistance (TEER) measurements can be used as an indicator of epithelial viability for mucosal permeation experiments (Holm et al., 2013; Muendoerfer et al., 2010). There are currently limited reported TEER studies with buccal permeation experiments specifically and therefore standardization

remains to be developed. The reported values in these limited available studies vary widely (136 ± 17 to 950 ± 392 Ω/cm^2) (Holm et al., 2013; Nielsen and Rassing, 2002). The TEER value across the buccal mucosa prior to the permeation experiment in this study was found to be 144 ± 12 Ω/cm^2. After 6 h of permeation this value decreased to 109 ± 21 Ω/cm^2 (24% reduction). 60 min after removal of the DDI films and exposure to fresh PBS, the TEER increased again to 123 ± 12 Ω/cm^2, which is a 14.5% difference of the baseline value. This signified a return towards the initial measured integrity. The TEER values obtained in this study appear to be within the reported range and the overall percentage change also indicates that mucosal integrity was not irreversibly affected (Kowapradit et al., 2010). In addition, the extent of TEER changes before and after the permeation experiments also compares favourably to reported studies using rat intestinal segments (Dezani et al., 2013) and porcine nasal mucosa (Sintov et al., 2010) for drug permeation assessments. The TEER values also correlate with the histomorphological studies (Section 3.4) which further confirmed that integrity and viability of the tissue was maintained.

The buccal permeation potential of DDI demonstrated in this study therefore warrants the need for future studies with excipients to enhance permeation.

3.4. Histomorphological evaluations

While buccal permeation studies on drug solution are extensively reported, reports on the effect of polymeric films on buccal mucosa morphology remain limited. Buccal films are often designed for prolonged retention on the mucosa and therefore assessment of histological effects of the drug and the polymeric film on the mucosa is essential. Histomorphological effects of the control/untreated and the treated porcine buccal mucosae (PBS alone, PBS + placebo film and PBS + drug loaded film) were assessed. The morphology of porcine buccal mucosa and similarities between it and human buccal mucosa has been described in detail previously (Madhav et al., 2009; Shojaei, 1998; Sudhakar et al., 2006).

The mucosa lining the buccal cavity is stratified squamous epithelium with a high recovery rate (Squier and Hall, 1985). Since this mucosa is multilayered the cell structure differs as the cells transcends from the basal lamina to the mucosal surface, with cells becoming more flattened in appearance and more closely packed at the surface as compared to basal cells that appear more cuboidal in shape with more distinguishable intercellular spaces. This epithelium remains unkeratinized as an adaptation to its main functions which is to withstand abrasion due to mastication and also at the same time remain lubricated to protect against mechanical abrasion (Shojaei, 1998). Any particle that is able to permeate this mucosal lining has to travel either via transcellular or intracellular pathways to the basal membrane and enter the circulation present in the lamina propria.

The light microscopy (LM) micrograph of the control slides (Fig. 4a) closely resembled the above description with cells progressively getting flatter and more closely packed at the surface and basal cells being more distinguishable from each other. The PBS treated samples also appeared very similar to the controls, which correlate with other similar studies (Ojewole et al., 2012). Both the placebo film (Fig. 4b) and drug treated film (Fig. 4c) mucosal treatments following 6 h of permeation studies displayed no noticeable histomorphological changes that were indicative of tissue damage. The basal cell layer (Fig. 4d) appeared intact and darkly stained in H&E reflecting their greater mitotic activity characteristic of these basal cells, therefore suggesting that any changes from DDI or polymers would not be permanent.

These observations were then further confirmed using TEM. TEM images allow a deeper investigation at a cellular level to assess any destruction of the cellular membranes, of individual cells, as well as to the cellular organelles that ensure proper functioning of these cells. Damage to the cellular membrane, destruction of the nuclear membrane and the nucleus, as well as cytoplasmic blebbing are all markers of necrosis (Zong and Thompson, 2006). TEM images can also allow for the evaluation of tight-junctions or similar interconnections between adjacent cells, as well as the evaluation of intercellular spaces that can be used as a route of paracellular transport. The control (Fig. 5a) displayed cells that are characteristic of normal healthy cells, with no signs of either apoptosis or necrosis. These images also displayed very small intercellular

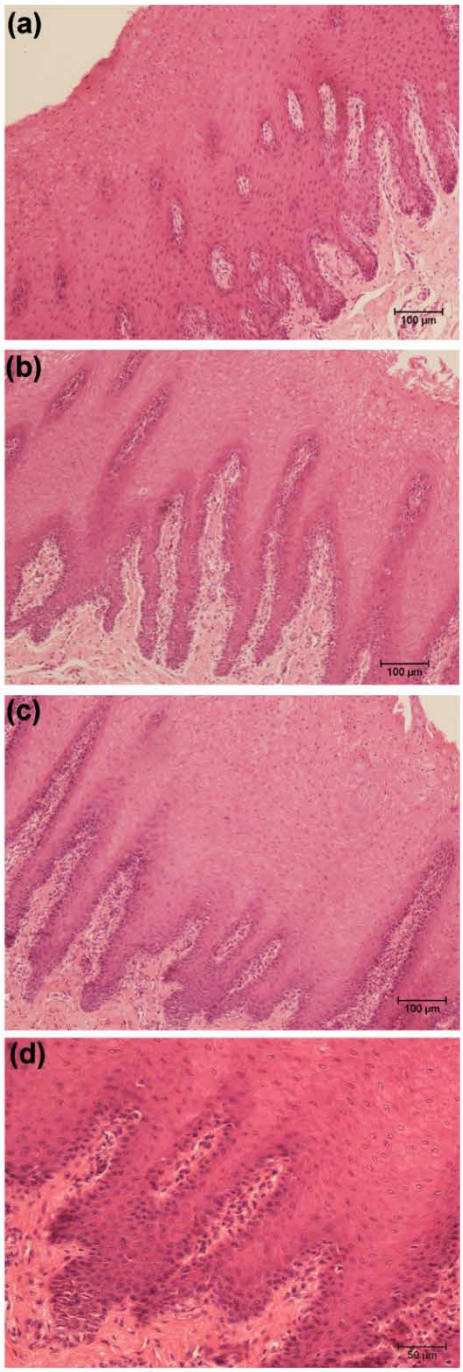

Fig. 4. Microphotographs of the control and treated ultra-thin buccal mucosal sections for light microscopy (LM): (a) untreated control, (b) placebo film, (c) drug film and (d) basal cells of drug film.

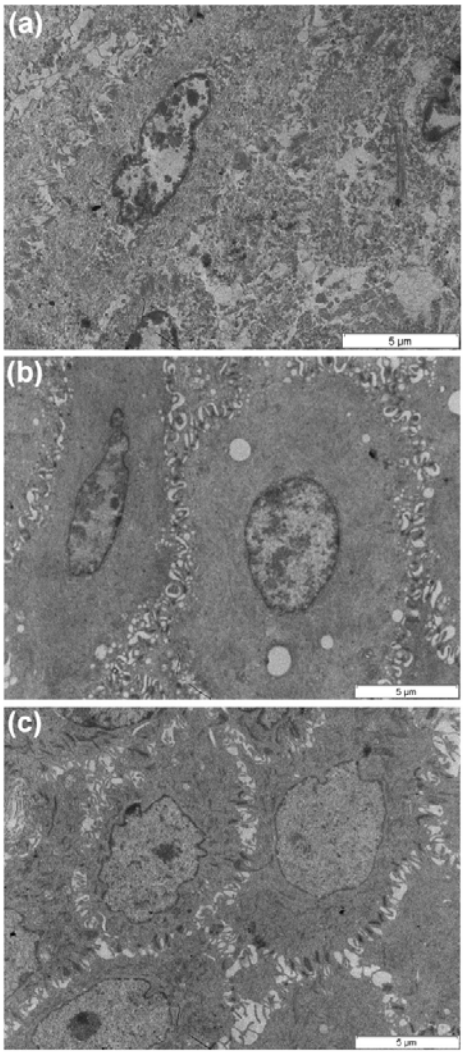

Fig. 5. Microphotographs of the control and treated ultra-thin buccal mucosal sections for transmission electron (TEM): (a) untreated control, (b) placebo film and (c) drug Film.

spaces and relatively closely packed cells. The placebo film treatment (Fig. 5b) displayed cellular morphology similar to those of the controls, with slightly enlarged intercellular spaces as compared to the control samples. This increase in intercellular spaces could be attributed to the polymers that were incorporated into the design of the film. The drug loaded film treatment showed a further increase in these intercellular spaces, which possibly aided in the transport of the drug via the paracellular route through the buccal mucosa. Although there was an increase in the size of the intercellular spaces in the drug loaded film, the tight junctions were still intact which is indicative that the changes caused by the drug treatment are not permanent. Apart from the observed changes mentioned above, no other detrimental changes to the buccal mucosa due to the placebo/drug treatment were observed.

Both the LM and TEM studies confirmed that there was no tissue damage or distress due to either placebo or drug loaded film treatment. These studies further identified a possible route of transport for the DDI loaded film across the buccal mucosa and also showed evidence that the changes observed were temporary.

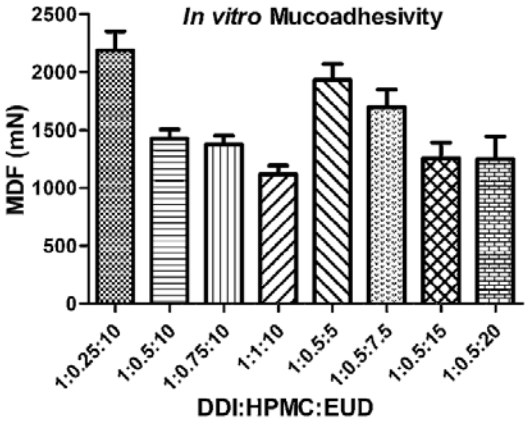

Fig. 6. Effect of HPMC and EUD on in vitro mucoadhesivity.

3.5. Mucoadhesivity of films

The majority of mucoadhesive polymers investigated for buccal films are hydrophilic (Morales and McConville, 2011). Conversely hydrophobic Eudragit® can also demonstrate mucoadhesiveness when used separately or together with other hydrophilic polymers such as chitosan (Pendekal and Tegginamat, 2012; Perumal et al., 2008b). Prepared formulations were subsequently tested for their mucoadhesivity, since a prerequisite for buccal controlled drug delivery systems is adhesion on the oral mucosa (Eouani et al., 2001). A maximum detachment force (MDF) of 2184 mN per film (Fig. 6) seen with 1:0.25:10 is comparable to optimized buccal films (Ayensu et al., 2012b; Eouani et al., 2001) and buccal tablets (Boyapally et al., 2010; Cappello et al., 2006) published previously. This indicates that the films prepared in this study show potential for retention at the site of drug absorption for prolonged time periods.

The effect of HPMC content on mucoadhesivity was investigated (Table 3). The decrease in MDF observed as HPMC content increased could be attributed to the increased intermolecular interactions possibly between the higher levels of plasticizers and polymers in the subsequent formulations. HPMC was selected as the hydrophilic polymer for the formulation based on preliminary drug–polymer interaction studies. Molecular mechanistic simulations as discussed later under Section 3.8.4 indicated that HPMC showed few electrostatic interactions with –COOH and $-NH_2$ groups of the mucin potentially explaining the decrease in MDF observed as the HPMC content increased from 0.25% to 1% (Table 3).

The effect of EUD content on mucoadhesivity was also investigated (Fig. 6). A decrease in MDF was also noted as EUD content increased. EUD, a cationic polymer, is positively charged and could interact to some extent with the negatively charged mucin glycoproteins. Molecular simulations supported the level of mucoadhesivity observed. The quaternary ammonium groups of EUD seemed to form the much needed electrostatic interaction to impart mucoadhesivity to the buccal films, but the hydrophobic nature of EUD may have caused destabilization of H-bonding. The mucoadhesive interaction was reduced (1930.2 ± 137.9 to 1245.7 ± 196.1 mN) as the EUD content increased from 5% to 20% respectively. EUD being a hydrophobic polymer restricted the free entry of water causing less efficient chain mobility and physical entanglement with mucus (Perumal et al., 2008b). Work of adhesion is the area under the force/distance curve generated by the TA-XT2*i*. High coefficient of variance (CV) percentages for work of adhesion reflects the difficulty in accurately measuring the area under the curve (AUC) of the narrow peaks generated on the stress-strain curves. Work of adhesion and MDF

Table 3
Effect of polymer concentration on mucoadhesivity of films.

Formulation (DDI:HPMC:EUD)	Maximum detachment force (mN) $n=9$		Work of adhesion (mJ) $n=9$	
	Mean ± SD	CV%	Mean ± SD	CV%
Effect of HPMC				
1:0.25:10	$2184.56 \pm 164.28^{***}$	7.52	1.42 ± 0.42	29.63
1:0.5:10	$1425.00 \pm 77.15^{N/A}$	5.41	1.21 ± 0.32	26.18
1:0.75:10	1371.33 ± 79.62^{NS}	5.81	1.02 ± 0.13	12.47
1:1:10	$1116.44 \pm 75.08^{***}$	6.72	0.90 ± 0.18	20.48
Effect of EUD				
1:0.5:5	$1930.22 \pm 137.87^{***}$	7.14	1.31 ± 0.23	17.17
1:0.5:7.5	$1695.78 \pm 151.96^{***}$	8.96	1.25 ± 0.15	12.00
1:0.5:10	$1425.00 \pm 77.15^{N/A}$	5.41	1.21 ± 0.32	26.18
1:0.5:15	$1253.67 \pm 134.62^{*}$	10.74	0.99 ± 0.17	16.82
1:0.5:20	$1245.67 \pm 196.05^{*}$	15.74	1.04 ± 0.33	31.88

Statistical significance compared to 1:0.5:10—$^{***}p<0.001$; $^{**}p=0.001$–0.01; $^{*}p=0.01$–0.05; $^{NS}p>0.05$; N/A—non-applicable.

values followed similar trends throughout. Statistical significant ($p<0.05$) differences between mucoadhesion (MDF) values were observed between the identified 1:0.5:10 formulation and other ratios prepared (Table 3). Films had acceptable mucoadhesivity but increased polymeric content affected mucoadhesion negatively in the multipolymeric films. A balance needs to be achieved between acceptable mucoadhesivity and desired drug release.

3.6. Mechanical testing

The mechanical strength of films reflects their ability to withstand mechanical damage during production, handling and application (Yoo et al., 2006). It was therefore necessary to assess the mechanical properties of the DDI monolayered multipolymeric films (Table 4). The investigated mechanical properties together represent film abrasion resistance, ductility and stiffness or elasticity. Increases in HPMC concentration resulted in increased tensile strength, Young's modulus and force required to rupture the films (Table 4). This would lead to improved abrasion resistance making films less prone to breakage and more durable to handle. The effect of EUD on mechanical properties was investigated. Overall improved mechanical properties were noted for films as the EUD content increased from 5% to 20%. In this series film elongation increased up until 1:0.5:10 (69.54 ± 7.77%) then decreased as polymer content further increased in 1:0.5:15 and 1:0.5:20 (54.68 ± 0.49% and 45.36 ± 6.47%). This decrease could be attributed to the increased stiffness and thickness resulting in tougher films. These films were rigid and could cause discomfort when being administered into the buccal cavity. Interestingly, the tensile strength increased for films with increasing amounts of HPMC, whereas the opposite was noted for films containing increasing amounts of EUD. A tensile strength of 0.698 N/mm^2 compares favourably to previously produced buccal formulations (El-Kamel et al., 2007; Shidhaye et al., 2008; Sievens-Figueroa et al., 2012). Polymers selected and the concentration in the buccal formulations affect all mechanical properties. It is clear that films should have sufficient tensile strength to withstand necessary handling yet be flexible enough to ensure patient comfort. It is for this reason that optimal polymeric blends need to be identified.

3.7. Surface pH

The surface pH of buccal polymeric films is an important characteristic to evaluate. *In vivo* studies by Bottenberg et al. (1991) demonstrated that fluctuations in pH beyond the normal range of saliva (pH 5.8–7) may cause local irritation to the buccal mucosa. A minimal decrease of 0.17 in pH, from pH 6.8–6.63 was measured *in vitro* over 24 h for 1:1:10 films. The slight decreases in pH for films over time can be attributed to the availability of polymer that can ionise at PBS pH. As the film swells more polymer from the inner areas of the film become available to ionise. *In vivo* this does not pose a problem since the buccal environment is an open system, with a continuous production and flow of saliva (Cavallari et al., 2013). The pH values for all the formulations remained within a suitable range indicating that buccal mucosal irritation is unlikely to occur. These results indicate that the multipolymeric buccal films were suitable for buccal application owing to the acceptable pH measurements.

3.8. Establishment of the polymeric complexation profile and potential impact on mucoadhesion and drug release via SLAS

3.8.1. Molecular mechanics assisted model building and energy refinements

Molecular mechanics energy relationship (MMER), a method for analytico-mathematical representation of potential energy surfaces, was used to provide information about the contributions of valence terms, non-covalent Coulombic terms, and non-covalent van der Waals interactions for polymer/plasticizer/mucin interactions. The MMER model for potential energy factor in various molecular complexes can be written as:

$$E_{\text{molecule/complex}} = V_{\sum} = V_b + V_\vartheta + V_\varphi + V_{ij} + V_{hb} + V_{el} \quad (1)$$

where $V_{\sum}$ is related to total steric energy for an optimized structure, V_b corresponds to bond stretching contributions (reference values were assigned to all of a structure's bond lengths), V_ϑ denotes bond angle contributions (reference values were assigned to all of a structure's bond angles), V_φ represents torsional contribution arising from deviations from optimum dihedral angles, V_{ij} incorporates van der Waals interactions due to non-bonded interatomic distances, V_{hb} symbolizes hydrogen-bond energy function and V_{el} stands for electrostatic energy.

In addition, the total potential energy deviation, ΔE_{Total}, was calculated as the difference between the total potential energy of the complex system and the sum of the potential energies of isolated individual molecules, as follows:

$$\Delta E_{\text{Total(A/B)}} = \text{Total}_{(A/B)} - [E_{\text{Total(A)}} + E_{\text{Total(B)}}] \quad (2)$$

The molecular stability can then be estimated by comparing the total potential energies of the isolated and complexed systems. If the total potential energy of complex is smaller than the sum of the potential energies of isolated individual molecules in the same

Table 4
Effect of polymer concentration on mechanical properties of films.

Formulation (DDI:HPMC:EUD)	Rupture force (N) $n=3$	Tensile strength (N/mm^2) $n=5$	Young's modulus (N/mm) $n=5$	Elongation (%) $n=5$	Toughness (N mm) $n=5$
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Effect of HPMC					
1:0.25:10	2.62 ± 0.60	0.2733 ± 0.051***	0.10 ± 0.04	53.75 ± 15.61	41.27 ± 3.89
1:0.5:10	6.69 ± 0.39	0.6976 ± 0.064$^{N/A}$	0.23 ± 0.05	69.54 ± 7.77	111.70 ± 12.98
1:0.75:10	11.90 ± 0.40	0.7288 ± 0.057^{NS}	0.36 ± 0.03	57.41 ± 2.18	99.46 ± 17.26
1:1:10	19.47 ± 0.48	1.1301 ± 0.054***	0.59 ± 0.08	56.00 ± 6.37	102.91 ± 10.64
Effect of EUD					
1:0.5:5	5.46 ± 0.68	2.5545 ± 0.144***	1.13 ± 0.17	25.20 ± 2.90	42.71 ± 7.74
1:0.5:7.5	5.42 ± 0.86	1.1387 ± 0.097***	0.53 ± 0.04	38.02 ± 4.75	57.66 ± 13.04
1:0.5:10	6.69 ± 0.39	0.6976 ± 0.064$^{N/A}$	0.23 ± 0.05	69.54 ± 7.77	111.70 ± 12.98
1:0.5:15	25.43 ± 0.66	0.6315 ± 0.029^{NS}	0.43 ± 0.04	54.68 ± 0.49	187.58 ± 15.54
1:0.5:20	27.75 ± 1.25	0.4358 ± 0.007***	0.50 ± 0.09	45.36 ± 6.47	279.89 ± 33.32

Statistical significance compared to 1:0.5:10—$^{***}p<0.001$; $^{**}p=0.001$–0.01; $^{*}p<0.01$–0.05; $^{NS}p>0.05$; N/A—non-applicable.

conformation, the complexed form is more stable and its formation is favoured (Yu et al., 2008).

3.8.2. Effect of the incorporation of plasticizer on the individual polymer's performance

$$E_{HPMC} = 49.713\,V_{\sum} = 2.089\,V_b + 18.821\,V_\theta + 22.360\,V_\varphi + 6.776\,V_{ij} - 0.335\,V_{hb} \quad (3)$$

$$E_{GLY} = 2.863\,V_{\sum} = 0.046\,V_b + 0.269\,V_\theta + 2.123\,V_\varphi + 0.437\,V_{ij} - 0.013\,V_{hb} \quad (4)$$

$$E_{HPMC-GLY} = 39.317\,V_{\sum} = 2.337\,V_b + 20.420\,V_\theta + 30.335\,V_\varphi - 11.012\,V_{ij} - 2.762\,V_{hb} \quad (5)$$

$$E_{EUD} = 43.885\,V_{\sum} = 5.002\,V_b + 21.451\,V_\theta + 8.255\,V_\varphi + 2.398\,V_{ij} + 6.777\,V_{el} \quad (6)$$

$$E_{TEC} = 3.913\,V_{\sum} = 0.497\,V_b + 2.524\,V_\theta + 1.206\,V_\varphi - 0.105\,V_{ij} - 0.209\,V_{hb} \quad (7)$$

$$E_{EUD-TEC} = 25.912\,V_{\sum} = 5.766\,V_b + 27.741\,V_\theta + 9.680\,V_\varphi - 23.556\,V_{ij} - 0.477\,V_{hb} + 6.758\,V_{el} \quad (8)$$

The plasticization and filler effects of GLY and TEC w.r.t. HPMC and EUD are depicted in Eqs. (3)–(5) and Eqs. (6)–(8), respectively. Both the plasticized complexes, HPMC-GLY and EUD-TEC, were energetically stable with negative energy of formation (ΔE) values of ≈13 kcal/mol and ≈22 kcal/mol, respectively, justifying the selection of plasticizers for the respective polymers for the film formation and performance.

In case of HPMC-GLY, glycerine formed a well-connected H-bonded molecular complex with HPMC as shown in Fig. 7a with a space-filling ability to accommodate within the van der Waals space of HPMC molecule which is strengthened by the stabilization of V_{ij} and

V_{hb} (Table 5). With a closer look at the HPMC-GLY complex, one can assume that one glycerine molecule can additionally form an "intermolecular-bridge" between two adjacent HPMC molecules inducing an "adjacent chain-sliding phenomenon" resulting in an increase in elasticity. Interestingly, introduction of glycerine to the HPMC led to an increase (destabilization) in V_φ due to the torsional constraints experienced by the polymer which in turn was due to the filling of the intramolecular-space providing the much needed alignment and distribution of polysaccharide side-chains. On another note, the H-bonding may however decrease the accessibility of HPMC functional groups as discussed later in this paper.

Unlike HPMC-GLY, EUD-TEC was characterized by the absence of H-bonding at all modelling poses tested (data not shown) which may be due to different polarities of the complexing molecules. Additionally, the space-filling appeared more intermolecular because of the size of the TEC molecule w.r.t. EUD modelled which is evident from more stabilized V_{ij}. Furthermore, as the filling was intermolecular; the torsional strain was much reduced as compared to HPMC-GLY (V_φ values in Table 5). These two reasons made EUD-TEC more stable than HPMC-GLY. As evident from Fig. 7b, the EUD–EUD interpolymeric interaction appeared less likely because of the presence of plasticizer molecules between the adjacent polymeric chains which may further lead to increased elasticity or decreased rigidity of the EUD-component of the films (Gutiérrez-Rocca and McGinity, 1994).

3.8.3. HPMC-EUD co-blending and co-plasticization

$$E_{HPMC-EUD} = 84.573\,V_{\sum} = 6.628\,V_b + 39.552\,V_\theta + 43.674\,V_\varphi - 10.033\,V_{ij} - 0.606\,V_{hb} + 5.357\,V_{el} \quad (9)$$

$$E_{EUD\text{-}TEC/HPMC\text{-}E} = 47.431\,V_{\sum} = 7.928\,V_b + 48.246\,V_\theta + 41.782\,V_\varphi - 52.791\,V_{ij} - 4.061\,V_{hb} + 6.327\,V_{el} \quad (10)$$

The films were fabricated using a unique blend of two polymers, with their respective plasticizers, in a binary-solvent system. To simulate the fabrication conditions; the polymers were modelled together along with their respective plasticizers forming a quadra-molecular system with HPMC-GLY/EUD-TEC: co-blended-co-plasticized polymeric architecture (BPPA). A binary blend system sans plasticizers was also modelled to elucidate the compatibility of the two polymers.

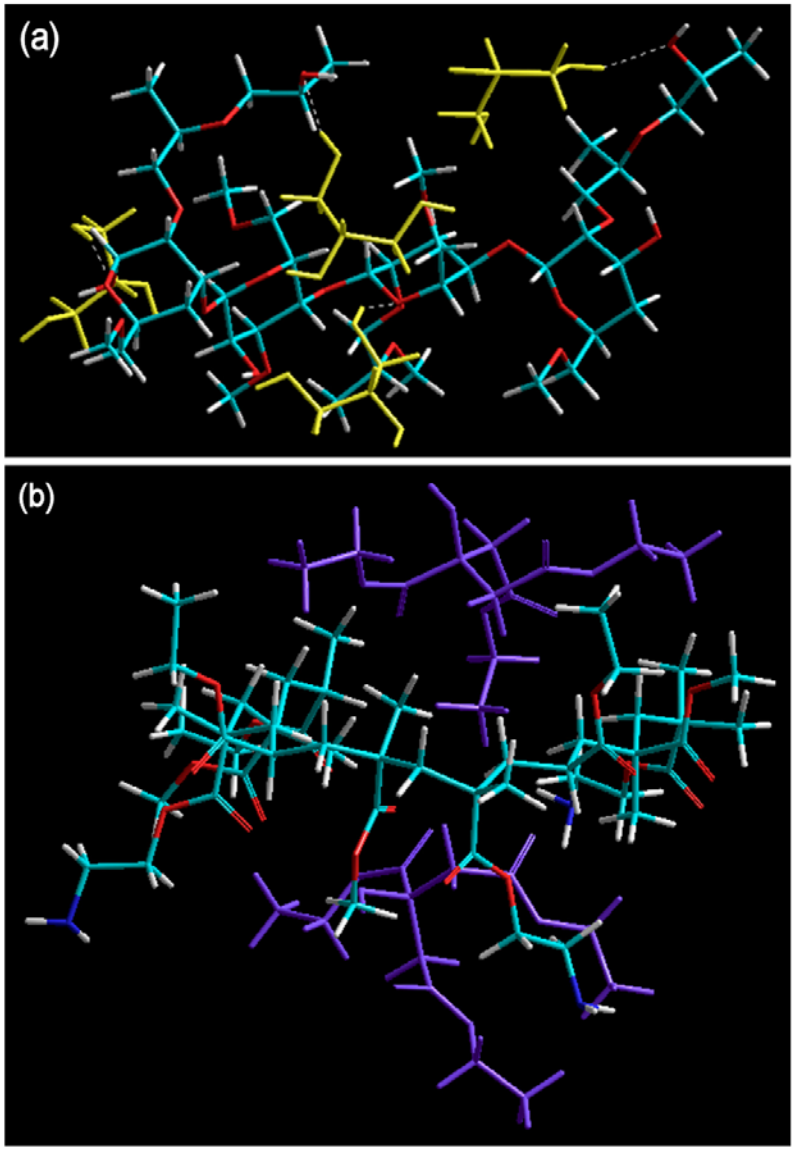

Fig. 7. Visualization of geometrical preferences of (a) HPMC–GLY: HPMC (standard colours) in molecular complexation with GLY (yellow rendering); and (b) EUD–TEC: EUD (standard colours) in molecular complexation with TEC (violet rendering), after molecular simulations in vacuum. Colour codes for HPMC and EUD tube rendering: C (cyan), O (red), H (white), and P (yellow) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.).

From Eq. (9) and Table 5; it can be deduced that the polymers formed a stabilized geometrically and energetically stable system with all the bonding (V_b and V_θ) and non-bonding (V_{ij}, V_{hb}, and V_{el}) interaction energies except V_φ. The HPMC-EUD molecular complex failed to demonstrate an H-bonded system with all the conformation poses tried (Fig. 8a and b). Furthermore, the total energy of stabilization, $\Delta E(V_e)$ recorded as ≈−9 kcal/mol, was too low to justify an efficient blending. However, an intramolecular EUD H-bonding was reported in the binary mixture which may be due to the torsional constraints caused by the presence of HPMC which was further proven by the instability caused by V_φ (Fig. 8b).

The HPMC-GLY/EUD-TEC quadra-molecular system is depicted in Fig. 8c where a well-connected intermolecular architecture is evident by the presence of H-bonding involving HPMC-GLY-EUD linked structure. Additionally, the TEC molecule was well-fitted into the intramolecular space of EUD against the intermolecular space in case of EUD-TEC discussed in the previous section. Now this well-fitted and well-connected structure presented the "standard pattern of energy stabilization" wherein all the bonding interactions (V_b, V_φ, and V_θ) were destabilized and the non-bonding (V_{ij}, V_{hb}, and V_{el}) ones were stabilized. Numerically, the total energy of stabilization, $\Delta E(V_e) = -52.943$, for HPMC-GLY/EUD-TEC was ≈6 times that of HPMC-EUD justifying the "novel co-blending-co-plasticizing strategy" of preparing buccal films.

The drug release profile of the developed buccal films in this study can be explained molecular mechanistically taking the geometrical stabilization of the components in consideration. As explained previously under Section 3.2; an increase and decrease in drug release was observed with an increase in concentration of HPMC and EUD, respectively. We hereby hypothesize that the

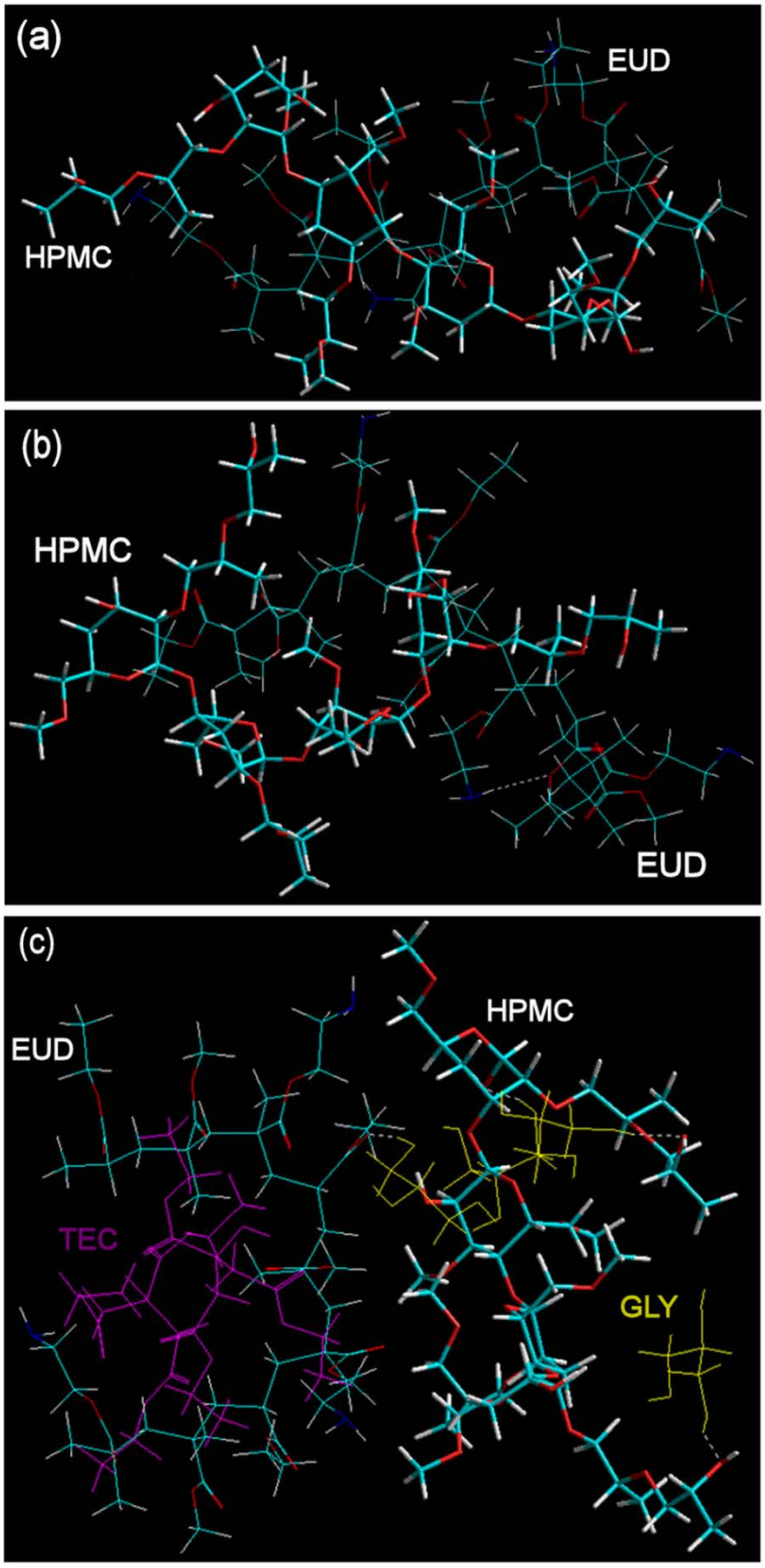

Fig. 8. Visualization of geometrical preferences of (a) HPMC-EUD: HPMC (tube rendering) in molecular complexation with EUD (stick rendering) with no H-bonding; and (b) HPMC-EUD: HPMC (tube rendering) in molecular complexation with EUD (stick rendering) with intramolecular H-bonding; and (c) HPMC-GLY/EUD-TEC: HPMC-GLY in molecular complexation with EUD-TEC, after molecular simulations in vacuum. Colour codes for HPMC and EUD: C (cyan), O (red), H (white), and P (yellow). GLY and TEC molecules are shown in yellow and purple colour coding, respectively (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.).

Table 5
Inherent energy attributes representing the molecular assemblies modelled using static lattice atomistic simulations in vacuum.

Molecular complex	ΔE^{a} $(V_{\Sigma})^{b}$	ΔE $(V_b)^{c}$	ΔE $(V_\theta)^{d}$	ΔE $(V_\phi)^{e}$	ΔE $(V_{ij})^{f}$	ΔE $(V_{hb})^{g}$	ΔE $(V_{el})^{h}$
HPMC-GLY	-13.259 [i]	0.202 [j]	1.330	5.852	-18.225	-2.414	0.000
EUD-TEC	-21.886	0.267	3.766	0.219	-25.849	-0.268	-0.019
HPMC-EUD	-9.025	-0.463	-0.720	13.059	-19.207	-0.271	-1.420
EUD-TEC/HPMC-GLY	-52.943	0.294	5.181	7.838	-62.297	-3.504	-0.45
HPMC-MUC	-31.073	0.075	0.534	6.798	-35.727	-0.502	-2.249
EUD-MUC	-80.275	-0.177	3.011	0.842	-25.097	0.180	-59.033
HPMC-MUC-EUD	-65.011	-1.054	-6.788	24.282	-45.795	-0.658	-34.994

[a] $\Delta E_{(A/B)} - E_{(A/B)} - [E_{(A)} + E_{(B)}]$.
[b] Total steric energy for an optimized structure.
[c] Bond stretching contributions.
[d] Bond angle contributions.
[e] Torsional contribution arising from deviations from optimum dihedral angles.
[f] van der Waals interactions.
[g] Hydrogen-bond energy function.
[h] Electrostatic energy.
[i] Values inked green depicts the structure stabilizing contribution.
[j] Values inked red depicts the structure destabilizing contribution.

component stabilizing a vacuum system (non-aqueous system) and an aqueous system would lead to a respective increase in hydrophobicity and hydrophilicity of the matrix. Convincingly, the presence of EUD-TEC increased the stability of the films (Eqs. (5) and (8); Table 5) leading to slower release of the drug as there would be less tendency of the stabilized system to undergo a change in terms of chain relaxation (release via diffusion) or degradation (release via erosion). Correspondingly, the comparatively higher concentration of HPMC-GLY will make the quadra-molecular architecture less stable (Eqs. (5) and (8); Table 5) in vacuum leading to an increase in hydrophilicity and chain relaxation or degradation–causing an increase in drug release.

3.8.4. *Effect of component polymers on the mucoadhesivity of buccal films*

$$E_{MUC} = -166.812\,V_{\sum} = 5.474\,V_b + 70.351\,V_\theta + 55.173\,V_\varphi - 29.066\,V_{ij} - 7.096\,V_{hb} - 261.649\,V_{el} \quad (11)$$

$$E_{HPMC-MUC} = -148.172\,V_{\sum} = 7.638\,V_b + 89.706\,V_\theta + 84.331\,V_\varphi - 58.017\,V_{ij} - 7.933\,V_{hb} - 263.898\,V_{el} \quad (12)$$

$$E_{EUD-MUC} = -203.202\,V_{\sum} = 10.299\,V_b + 94.813\,V_\theta + 64.270\,V_\varphi - 51.765\,V_{ij} - 6.916\,V_{hb} - 313.905\,V_{el} \quad (13)$$

$$E_{HPMC-MUC-EUD} = -138.225\,V_{\sum} = 11.511\,V_b + 103.835\,V_\theta + 110.07\,V_\varphi - 65.687\,V_{ij} - 8.089\,V_{hb} - 289.866\,V_{el} \quad (14)$$

In the present molecular mechanics simulations; a standard muco-platform was employed wherein glycosylated mucopeptide was modelled with EUD and HPMC individually and in combination (Murphy et al., 2012; Ndesendo et al., 2011). HPMC, though hydrophilic, is a non-ionic polymer with hydroxyl- and carboxymethyl-functionalities in the structure whereas EUD is a cationic polymer with quaternary ammonium groups capable of forming electrostatic interactions with the mucin. Referring to ΔE values in Table 5; HPMC-MUC and EUD-MUC were stabilized by ≈−31 and ≈−80 kcal/mol, respectively, with non-bonding interaction playing the major part. The neutral nature of HPMC showed few electrostatic interactions with close proximity of –OH groups of HPMC to the –COOH and $-NH_2$ groups of MUC. However, the HPMC fitted well in the steric environment created by the van der Waals radii of MUC resulting in a geometrical stabilization [$\Delta E(V_{ij}) \approx -25$ kcal/mol] (Fig. 9a). The quaternary ammonium groups of EUD seemed to form the much needed electrostatic interactions to impart mucoadhesivity to the buccal films. This electrostatic stabilization [$\Delta E(V_{el}) \approx -59$ kcal/mol] was affected by the interaction of $-NH_3^+$ functionality of EUD with the –COOH functionality of MUC (Fig. 9b). The hydrophobic nature of EUD may have caused the destabilization of H-bonding energy by 0.18 kcal/mol. In case of HPMC-MUC-EUD; the value of ΔE of stabilization for HPMC-MUC-EUD [$\Delta E(V_{\sum}) \approx -65$ kcal/mol] lied between that of HPMC-MUC and EUD-MUC validating the molecular modelling approach employed in this study (Table 5). Interestingly, the stabilized and destabilized energy terms were alike in case of HPMC-EUD and HPMC-MUC-EUD with all the bonding and non-bonding interaction terms except V_φ were lowered (and stabilized) during the formation of bimolecular and trimolecular assemblies, further validating the accuracy and appropriateness of the computational method applied. A high numerical decrease (and hence stabilization) in the van der Waals forces represented by V_{ij} [$\Delta E(V_{ij}) \approx -45$ kcal/mol] confirms the better fit of HPMC-EUD combination with MUC as compared to the individual polymers—hence justifying the use of HPMC-EUD as a blend for the formulation of the buccal films. Predictably; an increase in HPMC concentration may lead to a comparative increase in HPMC:EUD ratio which may further lead to a decrease in mucoadhesion as mentioned in the experimental finding under Section 3.5.

The above molecular mechanistic studies therefore provide useful quantitative information to simultaneously predict the stability of polymeric and plasticizer blends film formulation and to also identify the potential mechanisms for the observed drug release and mucoadhesion shown previously in Sections 3.2 and 3.5.

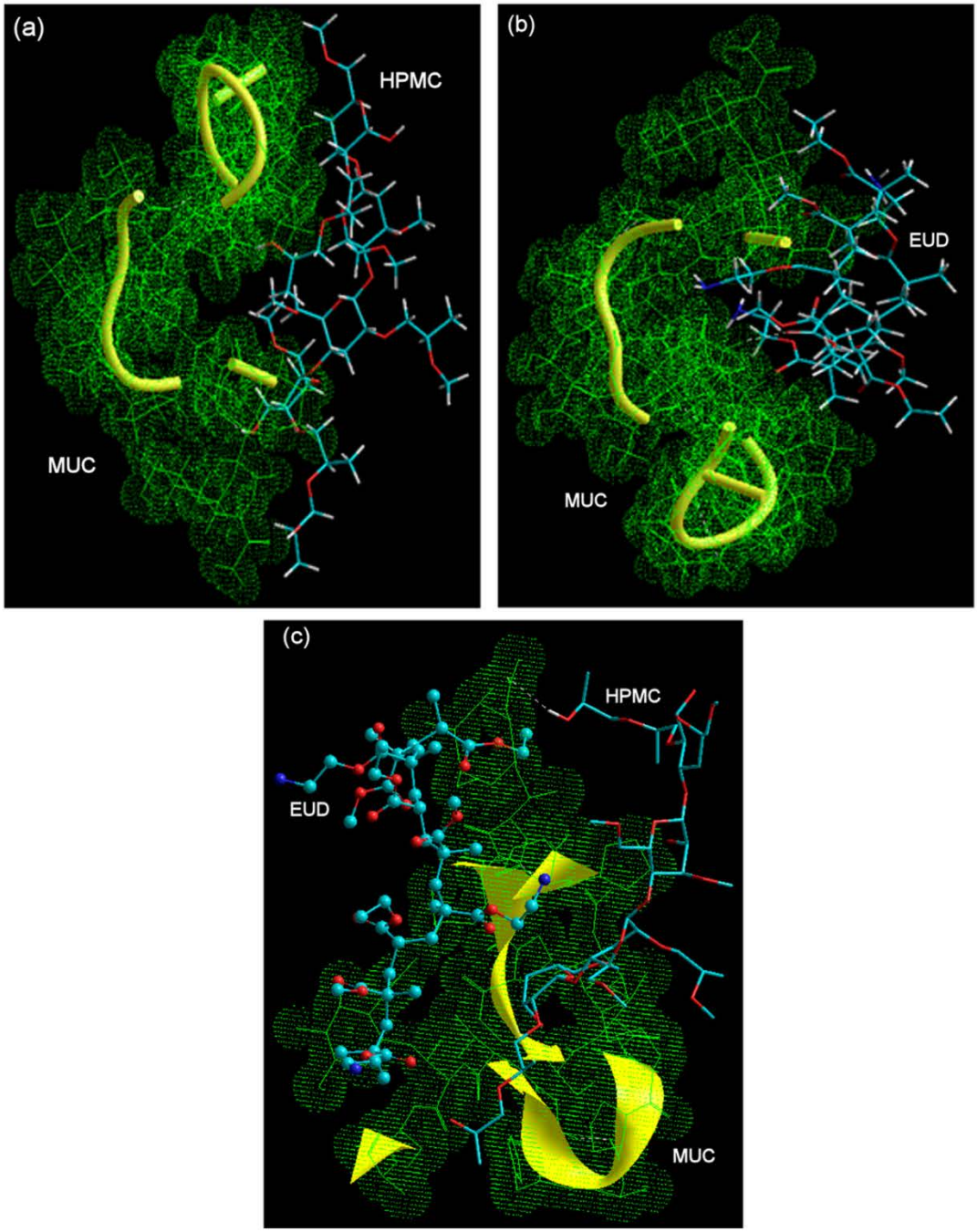

Fig. 9. Visualization of geometrical preferences of (a) HPMC-MUC: HPMC (tube rendering) in molecular complexation with MUC (dot rendering); (b) EUD-MUC: EUD (tube rendering) in molecular complexation with MUC (dot rendering); and (c) HPMC-MUC-EUD: MUC (dot rendering) in molecular complexation with HPMC (tube rendering) and EUD (ball-and-tube rendering), after molecular simulations in vacuum. Colour codes for HPMC and EUD: C (cyan), O (red), H (white), and P (yellow). Secondary structure of MUC is shown in yellow tube or ribbon rendering (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.).

4. Conclusions

The aim of this study was to formulate and characterize monolayered mucoadhesive multipolymeric films comprising of various ratios of co-blended polymers for buccal delivery of DDI. Films containing DDI were successfully prepared with HPMC and EUD by a simplified solvent casting/evaporation technique that eliminated the need for homogenization and cooling, carcinogenic solvents and additional emulsifiers. Drug content was uniform and within required specifications. Controlled release of DDI from MMFs could be obtained by modifying the ratios of HPMC and EUD. Formulations exhibiting desired controlled drug release and drug content uniformity had acceptable mechanical strength and mucoadhesivity. The buccal permeability potential of DDI from polymeric films was successfully demonstrated for the first time and histomorphological studies confirmed no buccal tissue damage or distress due to drug loaded MMFs. Static lattice atomistic simulations (SLAS) provided a mechanistic understanding of the molecular interactions involved in film formation and confirmed the corroboration of the *in silico* and *in vitro* mucoadhesive and drug release experimental

data. SLAS further justified the "novel co-blending-co-plasticizing strategy" of preparing buccal films. The data obtained in the study demonstrated for the first time the potential of buccal polymeric films to serve as platforms for delivery of DDI. These extensive physico-mechanical and molecular atomistic studies have confirmed the use of MMFs containing DDI, HPMC and EUD as a potential buccal drug delivery system to enhance patient therapy. They further serve as a platform for future studies to statistically optimize the formulations for simultaneous enhancement of drug release, permeation, mucoadhesion and mechanical strength.

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Manuscript Submission Cover Page

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In vitro comparative evaluation of monolayered multipolymeric films embedded with didanosine-loaded solid lipid nanoparticles: A potential buccal drug delivery system for ARV therapy

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International Conference Poster Presentations

Formulation and Characterization of Hydroxypropyl methylcellulose and Eudragit RS 100® co-blended Films for Transbuccal Delivery of Didanosine

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BACKGROUND & AIM

- The selection of optimal polymers in developing buccal drug delivery systems remains fundamental in achieving controlled drug release and enhanced mucoadhesivity. (1)
- The aim of the study was to formulate and evaluate mucoadhesive buccal films comprising of both hydrophobic and hydrophilic polymers to achieve controlled drug release which would have suitable physico-mechanicca properties. (1)
- Didanosine (ddI) was selected as a model drug due to its short half-life and extensive first pass metabolism making it an ideal candidate for transbuccal delivery.

METHOD

- **Formulation of Co-blended Films**
Hydroxypropyl methylcellulose (HPMC) and Eudragit RS 100® (EUD) together with required plastisizers (at 30% w/w of the polymers) were co-blended in various ratios in 50% v/v methanol as solvent before incorporating a set amount of ddI via sonification. Films were prepared via solvent casting/evaporation in silicone moulded trays.
- **Characterization of Co-blended Films**
The formulations were characterized in terms of drug content uniformity (assay) and drug release (BP2009 – modified rotating paddle method) and samples quantified via UV spectrophotometry at 250 nm. In vitro mucoadhesivity and tensile properties of film were measured using a TA-XT2i texture analyser (StableMicroSytems, UK).(2)
- **Data Analysis**
Data was captured using Microsoft Excel® 2007 (Microsoft Office, USA) and analysed with GraphPad Prism® Version 5 (GraphPad Software Inc., USA).

RESULTS & DISCUSSION

FORMULATION (% w/v)		ASSAY (%)		TENSILE STRENGTH (N/m^2)	YOUNG'S MODULUS (N/mm)	ELONGATION (%)	MAXIMUM DETATCHMENT FORCE (mN)	WORK OF ADHESION (mJ)
ddI:HPMC:EUD		MEAN ± SD	CV %	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD
EFFECT OF HPMC	1:0.25:10	91.17 ± 0.85	0.93	2861.50 ± 532.75	0.10 ± 0.04	53.75 ± 15.61	2184.56 ± 164.28	1.416 ± 0.42
	1:0.5:10	95.62 ± 5.41	5.66	10450.50 ± 956.94	0.23 ± 0.05	69.54 ± 7.77	1425.00 ± 77.15	1.212 ± 0.32
	1:0.75:10	91.69 ± 2.11	2.30	11226.00 ± 878.64	0.36 ± 0.03	57.41 ± 2.18	1371.33 ± 79.62	1.020 ± 0.13
	1:1:10	91.64 ± 2.48	2.70	18066.50 ± 860.83	0.59 ± 0.08	56.00 ± 6.37	1116.44 ± 75.08	0.896 ± 0.18
EFFECT OF EUD	1:0.5:5	96.79 ± 0.36	0.37	16750.50 ± 1710.12	1.29 ± 0.19	22.08 ± 6.03	1930.22 ± 137.87	1.313 ± 0.23
	1:0.5:10	95.62 ± 5.41	5.66	10450.50 ± 956.94	0.24 ± 0.05	69.54 ± 7.77	1425.00 ± 77.15	1.212 ± 0.32
	1:0.5:15	102.83 ± 3.06	2.98	13714.00 ± 618.10	0.43 ± 0.04	54.68 ± 0.49	1253.67 ± 134.62	0.994 ± 0.17
	1:0.5:20	105.24 ± 1.69	1.60	14522.50 ± 221.07	0.50 ± 0.09	45.36 ± 6.47	1245.67 ± 196.05	1.040 ± 0.33

Table 1: Effect of HPMC and EUD on physico-mechanical film properties.

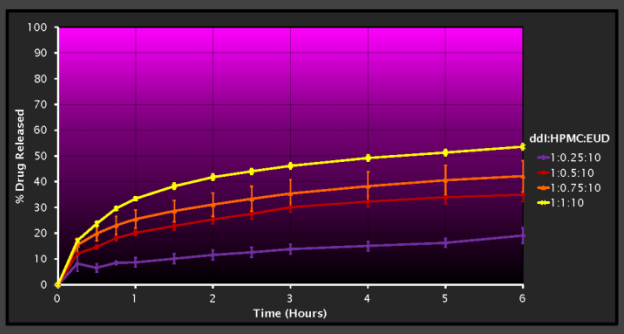

Figure 1: Effect of HPMC on drug released from co-blended films. (n=3)

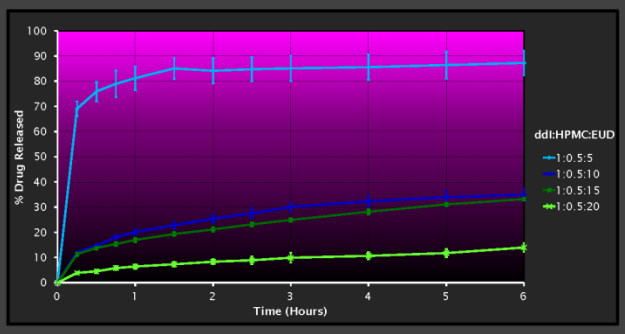

Figure 2: Effect of EUD on drug released from co-blended films. (n=3)

- Assay values for all formulations indicate uniform drug content and were within the required compendial specifications (90-110%) for current ddI oral formulations.(3)
- As the amount of EUD increased from 5% to 20% w/v, the drug release was significantly retarded. This could be attributed to the hydrophobic nature of EUD, which prevented free and deep water penetration into the film, thus only the ddI that was near the external surface of the film was initially released into the dissolution medium. Increasing HPMC from 0.25% to 1% led to increased drug release (33% after 1st hour with 1% HPMC:10% EUD) which was expected due to the hydrophilic nature of HPMC. (2,4)
- Mucoadhesion serves as prerequisite for buccal controlled release formulations. The highest mucoahesivity (2185mN) was noted for films co-blended with relatively high amounts of EUD (10%) and low HPMC (0.25%) content. A reduction of adhesion with increasing amounts of HPMC could be attributed to the intermolecular attraction between the co-blended polymers, making less interpenetration of the polymer chain with the mucosal tissue possible. (2,4)
- Films should be flexible and elastic, yet adequately strong to withstand required handling forces. Mechanical testing revealed that increasing polymeric contents (HPMC or EUD), positively influenced all mechanical properties investigated (Table 1).
- Tensile strength (TS) and maximum detachment force (MDF) results showed statistically significant differences exist between all polymeric ratios ($p < 0.05$), except for 1:0.5:10 vs 1:0.75:10 in terms of TS ($p = 0.2187$) and MDF ($p = 0.1658$). The difference in MDF for 1:0.5:15 vs 1:0.5:20 were also not significant ($p = 0.9209$).

CONCLUSION

- The drug release, mucoadhesion and physicochemical/mechanical data obtained in this study, confirmed the potential of these films as promising candidates for buccal controlled release drug delivery. These films could serve as a basis for other novel drug delivery platforms for anti-retrovirals. Further investigation on the transbuccal permeability of ddI from these polymeric films is ongoing.

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Static lattice atomistic simulations for co-blended-co-plasticized multipolymeric films for buccal drug delivery

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OBJECTIVE OF STUDY

- Molecular mechanics energy relationship, a method for analytico mathematical representation of potential energy surfaces, can be used to provide information about the intermolecular attractive forces between polymer/plasticizer/mucin molecules.
- The aim of this study was to identify the suitability of co-blended polymers comprising of Eudragit® RS100 (EUD) and hydroxypropyl methylcellulose (HPMC), along with their respective plasticizers for use in buccal mucoadhesive films.

METHODS

- The 3D structures of EUD, HPMC, glycerine (GLY), triethycitrate (TEC) and mucin (MUC) were generated using HyperChem™ 8.0.8 Molecular Modeling Software (Hypercube Inc., USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, UK). The glycosylation of the mucopeptide analogue (MUC) mucin was performed at the threonine amino acid residues.
- Energy minimizations were performed on the polymer-polymer, polymer-plasticizer and polymer-mucin complexes. The molecular stability was estimated by comparing the total potential energies of the isolated and complexed systems. If a complex had a lower total potential energy than the sum of the potential energies of isolated individual molecules, the complexed form was regarded as more stable [1].

RESULTS & DISCUSSION

Table 1: Inherent energy attributes representing the molecular assemblies modelled using static lattice atomistic simulations.

MOLECULAR COMPLEX	ΔE^a (V_Σ)^b	ΔE (V_b)^c	ΔE (V_θ)^d	ΔE (V_φ)^e	ΔE (V_{ij})^f	ΔE (V_{hb})^g	ΔE (V_{el})^h
HPMC-GLY	-13.259	0.202	1.330	5.852	-18.225	-2.414	0.000
EUD-TEC	-21.886	0.267	3.766	0.219	-25.849	-0.268	-0.019
HPMC-EUD	-9.025	-0.463	-0.720	13.059	-19.207	-0.271	-1.420
EUD-TEC/HPMC-GLY	-52.943	0.294	5.181	7.838	-62.297	-3.504	-0.45
HPMC-MUC	-31.073	0.075	0.534	6.798	-35.727	-0.502	-2.249
EUD-MUC	-80.275	-0.177	3.011	0.842	-25.097	0.180	-59.033
HPMC-MUC-EUD	-65.011	-1.054	-6.788	24.282	-45.795	-0.658	-34.994

Key for Table 1:
a - $\Delta E(A/B) = E(A/B) - [E(A) + E(B)]$
b - total steric energy for an optimized structure
c - bond stretching contributions
d - bond angle contributions
e - torsional contribution arising from deviations from optimum dihedral angles
f - van der Waals interactions
g - hydrogen-bond energy function
h - electrostatic energy

Green values depicts the structure **stabilizing** contribution.
Red values depicts the structure **destabilizing** contribution.

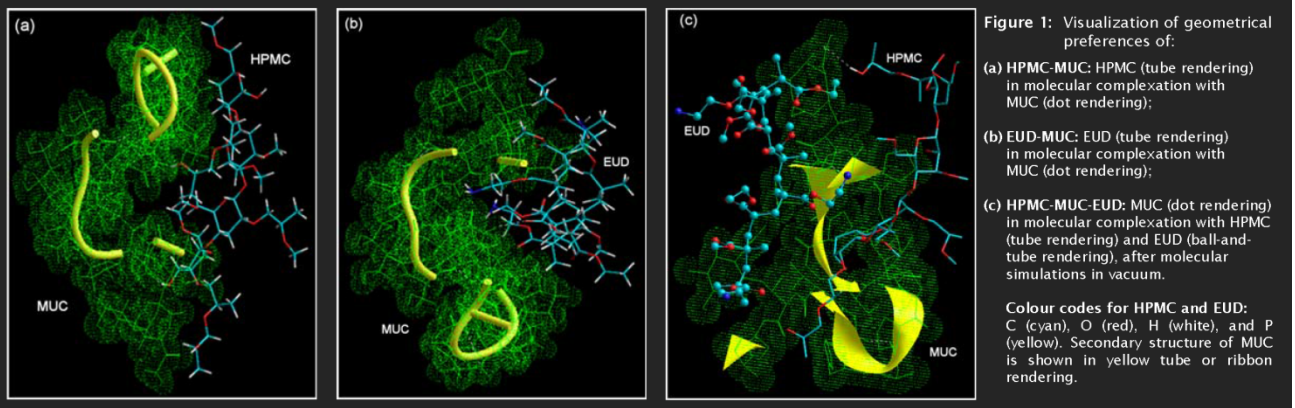

Figure 1: Visualization of geometrical preferences of:

(a) HPMC-MUC: HPMC (tube rendering) in molecular complexation with MUC (dot rendering);

(b) EUD-MUC: EUD (tube rendering) in molecular complexation with MUC (dot rendering);

(c) HPMC-MUC-EUD: MUC (dot rendering) in molecular complexation with HPMC (tube rendering) and EUD (ball-and-tube rendering), after molecular simulations in vacuum.

Colour codes for HPMC and EUD: C (cyan), O (red), H (white), and P (yellow). Secondary structure of MUC is shown in yellow tube or ribbon rendering.

- Both the plasticized complexes, HPMC-GLY and EUD-TEC, were energetically stable with negative energy of formation (ΔE) values (≈13 kcal/mol and ≈22 kcal/mol respectively), justifying the selection of plasticizers for the film formulation. The HPMC-GLY/EUD-TEC quadra-molecular system had a well-connected intermolecular architecture with a total energy of stabilization, of -52.943 kcal/mol, which was ≈6 times that of HPMC-EUD justifying the "novel co-blending-co-plasticizing strategy" of preparing buccal films.
- Drug release can also be explained molecular mechanistically taking the geometrical stabilization of the polymeric film components in consideration. We hereby hypothesize that the component stabilizing a vacuum system (non-aqueous system) and an aqueous system would lead to a respective increase in hydrophobicity and hydrophilicity of the matrix. Convincingly, the presence of EUD-TEC increased the stability of the films (Table 1) leading to slower release of the drug as there would be less tendency of the stabilized system to undergo a change in terms of chain relaxation (release via diffusion) or degradation (release via erosion). Correspondingly, the comparatively higher concentration of HPMC-GLY will make the quadra-molecular architecture less stable in vacuum leading to an increase in hydrophilicity and chain relaxation or degradation – causing an increase in drug release.
- The high numerical decrease (and hence stabilization) in the van der Waals forces represented by Vij [ΔE(Vij) ≈-45kcal/mol] confirmed the better fit of HPMC-EUD combination with MUC as compared to the individual polymers – hence justifying the use of HPMC-EUD as a blend for the formulation of the buccal films. In addition to the hydrophilic nature of HPMC, enhanced mucoadhesivity may be due to electrostatic interactions of the quaternary ammonium groups of EUD with MUC [2].

CONCLUSION

- Molecular mechanics energy relationships can assist in the identification of suitable polymeric blends for the design of optimal mucoadhesive delivery systems for the buccal route.
- Static lattice atomistic simulations (SLAS) provided a mechanistic understanding of the molecular interactions involved in film formation and confirmed the corroboration of the *in silico* and *in vitro* mucoadhesive and drug release experimental data (data not shown). SLAS further justified the "novel co-blending-co-plasticizing strategy" of preparing buccal films.

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Mucoadhesivity and mechanical evaluation of Hydroxypropyl methylcellulose and Eudragit® RS100 copolymeric films for buccal delivery of Didanosine

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OBJECTIIVE OF STUDY

Introduction
Selection of optimal polymers in developing buccal drug delivery systems remains fundamental in achieving the required mechanical strength and enhanced mucoadhesivity [1].

Single polymers used in buccal films very often fail to demonstrate all the ideal characteristics. To overcome this problem researchers have been shifting their attention to the blending of polymers and drugs of opposing solubilities.

While multipolymeric multilayered films and wafers have been prepared with drugs and polymers of opposing solubilities [2,3], monolayered multipolymeric films (MMFs) have been reported to offer more advantages in terms of lower production costs, improved drug release, mucoadhesivity and size [3].

The *in vitro* mucoadhesivity and mechanical properties of hydroxypropyl methylcellulose (HPMC) and Eudragit RS100® (EUD) in combination with the antiretroviral drug, didanosine (ddI), have not been previously studied for buccal films.

Aim
The aim of this study was to prepare MMFs comprising of both hydrophobic and hydrophilic polymers for buccal delivery of ddI and to evaluate the in vitro mucoadhesivity and mechanical properties of the films.

METHOD

Formulation of Monolayered Multipolymeric Films (MMFs)
MMFS were prepared in various polymeric ratios using the solvent casting/evaporation technique as follow: specified quantities of EUD and Triethyl citrate (TEC) as its plasticizer together with HPMC and Glycerol (GLY) as its plasticizer were dissolved in 50:50 water and methanol as solvent before adding the required amount of ddI. Thereafter 2 ml of each polymeric solution was syringed into each 6 cm^2 well of the silicone moulded trays (SMT) containing Teflon coated Perspex inserts. The drug-polymeric mixture was allowed to dry in an oven at 43 ºC for approximately 24 h, until constant film weight was achieved.

Characterization of Mucoadhesivity
The effects of polymeric ratios on the *in vitro* mucoadhesivity of films were measured using a TA.XT2*i* Texture Analyser (Stable Micro Systems, UK) equipped with a 5 kg load cell in tension mode and removable aluminium probes. Film samples (*n=3*), were individually attached to probes using double sided adhesive tape. Solidified 10% w/v gelatine gel covered in 30% w/v mucin served as buccal mucosa substrate. The film were brought into contact with the mucin covered gelatin with a force of 100 g for 60 before the mobile arm was raised. This generated a plot from which the Maximum Detachment Force (MDF) (*mN*) and Work of Adhesion (*mJ*) could be determined.

Characterization of Mechanical Properties
In vitro mechanical properties of films were measured using a TA-XT2i texture analyser. Individual film samples (*n=5*), were clamped between Teflon coated grips (TA-96) prior to being pulled by the top grip at a rate of 1 mm/s until the film ruptured completely. The tensile strength, percent elongation, and Young's modulus were determined from the stress-strain plots generated with the Texture Expert™ software. The rupture force was also determined using a film support rig with an exposed area of 0.786 cm^2 and a 5 mm stainless steel ball probe.

Data Analysis
Data was captured using Microsoft Excel® 2007 (Microsoft Office, USA) and analysed with GraphPad Prism® Version 5 (GraphPad Software Inc., USA).

RESULTS & DISCUSSION

Table 1: Effect of HPMC and EUD on physico-mechanical film properties.

FORMULATION (% w/v)		MAXIMUM DETATCHMENT FORCE (*mN*)	WORK OF ADHESION (*mJ*)	TENSILE STRENGTH (N/mm^2)	YOUNG'S MODULUS (*N/mm*)	ELONGATION (*%*)	RUPTURE FORCE (*N*)
ddI:HPMC:EUD		MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD
EFFECT OF HPMC	1:0.25:10	2184.56 ± 164.28***	1.42 ± 0.42	0.2733 ± 0.051***	0.10 ± 0.04	53.75 ± 15.61	2.62 ± 0.60
	1:0.5:10	1425.00 ± 77.15$^{N/A}$	1.21 ± 0.32	0.6976 ± 0.064$^{N/A}$	0.23 ± 0.05	69.54 ± 7.77	6.69 ± 0.39
	1:0.75:10	1371.33 ± 79.62^{NS}	1.02 ± 0.13	0.7288 ± 0.057^{NS}	0.36 ± 0.03	57.41 ± 2.18	11.90 ± 0.40
	1:1:10	1116.44 ± 75.08***	0.90 ± 0.18	1.1301 ± 0.054***	0.59 ± 0.08	56.00 ± 6.37	19.47 ± 0.48
EFFECT OF EUD	1:0.5:5	1930.22 ± 137.87***	1.31 ± 0.23	2.5545 ± 0.144***	1.13 ± 0.17	25.20 ± 2.90	5.46 ± 0.68
	1:0.5:7.5	1695.78 ± 151.96***	1.25 ± 0.15	1.1387 ± 0.097***	0.53 ± 0.04	38.02 ±4.75	5.42 ± 0.86
	1:0.5:10	1425.00 ± 77.15$^{N/A}$	1.21 ± 0.32	0.6976 ± 0.064$^{N/A}$	0.23 ± 0.05	69.54 ± 7.77	6.69 ± 0.39
	1:0.5:15	1253.67 ± 134.62*	0.99 ± 0.17	0.6315 ± 0.029^{NS}	0.43 ± 0.04	54.68 ± 0.49	25.43 ± 0.66
	1:0.5:20	1245.67 ± 196.05*	1.04 ± 0.33	0.4358 ± 0.007***	0.50 ± 0.09	45.36 ± 6.47	27.75 ± 1.25

Key for Table 1: Statistical significance compared to 1:0.5:10 :*** $p < 0.001$; ** p 0.001 to 0.01; * p 0.01 to 0.05; NS $p > 0.05$; N/A Non-applicable

Maximum mucoadhesivity of 2185 mN was measured for formulation 1:0.25:10 (ddI:HPMC:EUD). The decrease in mucoadhesivity (from 2185 mN to 1116 mN) as HPMC content increased could be attributed to increased intermolecular interactions possible between the higher levels of plasticizers and polymers in the subsequent formulations. Increases in HPMC concentration resulted in increased tensile strength (18067 N/m2), Young's modulus (0.59 N/mm) and force required to rupture the films (19.47 N) for formulation 1:1:10. This would lead to improved abrasion resistance making films less prone to breakage and more durable to handle. Improved mechanical properties were also noted for films as the EUD content increased from 5 % to 20 %, the latter requiring a force of 27.75 N to rupture the film.

Mucoadhesion serves as a prerequisite for buccal controlled release formulations. The highest mucoahesivity (2185mN) was noted for films co-blended with relatively high amounts of EUD (10%) and low HPMC (0.25%) content. A reduction of adhesion with increasing amounts of HPMC could be attributed to the intermolecular attraction between the co-blended polymers, making less interpenetration of the polymer chain with the mucosal tissue possible.

Films should be flexible and elastic, yet adequately strong to withstand required handling forces. Mechanical testing revealed that increasing polymeric contents (HPMC or EUD), positively influenced all mechanical properties investigated (Table 1).

Tensile strength (TS) and maximum detachment force (MDF) results showed statistically significant differences exist between all polymeric ratios ($p < 0.05$), except for 1:0.5:10 vs 1:0.75:10 in terms of TS ($p = 0.2187$) and MDF ($p = 0.1658$). The difference in MDF for 1:0.5:15 vs 1:0.5:20 were also not significant ($p = 0.9209$).

CONCLUSION

Monolayered copolymeric films using polymers and drug of opposing solubilities were successfully prepared. Mucoadhesion and mechanical data demonstrated the potential of buccal polymeric films to serve as a platform for delivery of ddI.

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Local Conference Poster Presentation

Development of an Assay Method for Didanosine Quantification in Buccal Polymeric Films

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BACKGROUND & AIM

- Didanosine (ddI) is only available for the oral route of administration. Its controlled transbuccal administration may improve bioavailability by avoiding hepatic first-pass metabolism and gastrointestinal degradation.
- Poor drug content for controlled release buccal polymeric films has been reported previously. [1-3] In order to comply with compendial specifications, oral dosage forms of ddI should contain between 90% and 110% of the stated amount of ddI. [4]
- This study aimed to develop a suitable assay method for quantification of ddI in buccal polymeric films.

METHOD

- **Preparation of Films**

Multipolymeric films containing ddI were prepared using Eudragit® RS100 (EUD) and Hydroxypropyl methylcellulose (HPMC) in a ratio of 1 : 0.5 : 10 (ddI : HPMC : EUD) by the homogenization / solvent casting/evaporation method.[2,3] The films were prepared in a silicone-moulded tray and dried at 37°C.

- **Assay of Buccal Polymeric Films**

Films were assayed for ddI content using an initial method consisting of a 95 % ethanol in distilled water (5 %) mixture.[5] Individual films were cut into pieces, crushed in the solvent and then agitated in a heated shaking water-bath (40 °C) for 24 hours before drug quantification.

This method was then optimized by altering the ratio between the organic and aqueous phase of the solvent system and by changing the individual solvent components in a stepwise manner.

The final optimized assay method entailed soaking the polymeric films in the required amount of methanol with manual agitation for 3 hours prior to making up to volume with phosphate buffered saline (PBS) pH 7.4.

- **Drug Quantification**

All samples were filtered (0.45µm syringe filter) prior to appropriate dilutions (1 in 10) being made, and then quantified via UV spectroscopy (Shimadzu, 1650-PC, Japan) at 250 nm. All assays were preformed in 5 replicates.

- **Data Analysis**

Data was captured with the aid of Microsoft Excel 2007 (Microsoft Office 2007, USA) and analysed with a statistical package (Graph Pad Instat Version 3.05 - GraphPad software Inc., USA).

RESULTS & DISCUSSION

- The initial assay method (95 % ethanol in distilled water) resulted in 82.9±1.7 % ddI content. By alteration of the method to consist of 20 % ethanol in distilled water, we were able to produce assay values of 82.4±1.6 %. Further modification of the method to 20 % methanol in distilled water yielded 87.0±1.2 % ddI. As a final modification, the distilled water was changed to PBS pH 7.4. This resulted in 100.1±2.8 % drug content, which is well within compendial specifications.[4]
- The final optimized assay method allowed for complete dissolution of the films in the solvent system thereby eliminating the need for cutting and crushing of individual films. Complete drug extraction was achieved from the polymeric films using an assay solvent consisting of 20 % methanol in PBS 7.4.
- The newly developed assay method holds additional benefits. The need for a heated shaking water-bath was eliminated, the duration of the process was reduced from 24 hours to 3 hours and less organic solvent is required, making this optimized assay method also cost-effective with a reduced environmental impact.

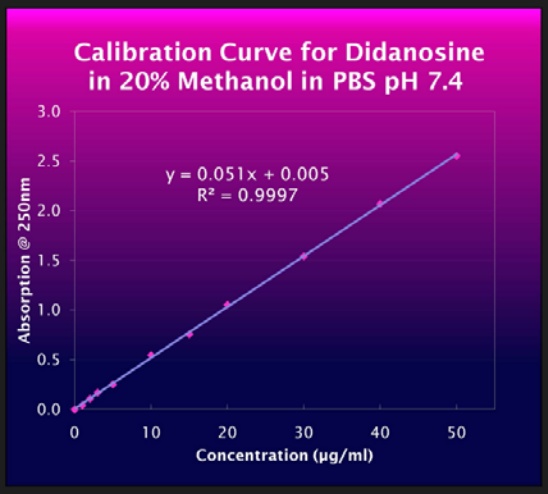

Figure 1: Calibration curve used for drug quantification

	Assay Solvent	Assay (%) (n=5)	P values (vs. A)
A	95 % Ethanol in Distilled Water	82.9±1.7	-
B	20 % Ethanol in Distilled Water	82.4±1.6	> 0.05
C	20 % Methanol in Distilled Water	87.0±1.2	< 0.05
D	20 % Methanol in PBS 7.4	100.1±2.8	< 0.001

Table 1: Comparison between different assay solvents

CONCLUSION

An assay method with the potential for effective quantification of active pharmaceutical ingredients, such as ddI, in polymeric buccal films was developed. A solvent system comprising of 20% methanol in PBS pH 7.4 was found to be optimal for this purpose.

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