EXPLORING THE BUCCAL DELIVERY POTENTIAL OF AN ANTIRETROVIRAL DRUG

By

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This thesis is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Pharmaceutics) in the Discipline of Pharmaceutical Sciences School and College of Health Sciences at the University of KwaZulu-Natal

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DEDICATION

This thesis is dedicated to the Almighty God for giving me both health and strength to complete this study. You are the pillar that holds my life! Lord, I owe everything to you.

I also dedicate the thesis to my late Mom, Madam Florence Adunola Jacobs, for her selfless love and commitment towards all her children's educational career. Mom, you were my inspiration to being the best that I have been, even in your eternal sleep, the memory of you lingers on. Thank you Mom, for believing in me and for supporting me throughout my life endeavors. Sweetest Mom of all, continue to rest in perfect and eternal peace.

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To God Be the Glory!

QUOTES

"After climbing a great hill, one only finds that there are many more hills to climb." *- Nelson Mandela*

"Everything has its time"
- Ecclesiastes 3:14-15 and 9: 10- 11" In: The Woman's Study Bible, Nelson Thomas Inc. USA, 2006

DECLARATION ON PLAGIARISM

I, the undersigned candidate, hereby declare that:-

i. The work contained in this thesis is my original work except where otherwise specified in the relevant sections of this thesis.

ii. I have not submitted the thesis previously in part or in its entirety for any degree in this University or at any other University.

iii. This thesis does not contain any text, graphics, pictures, tables or any other data copied and pasted from the internet, unless specifically acknowledged, and the sources where obtained detailed in the thesis and in the references sections.

iv. Permission was obtained for all the figures and tables or graphics that were included in the publications in this thesis.

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Name of candidate: Elizabeth B. Ojewole

Signature: _____ Date: _____

Consent obtained for submission of thesis from Supervisor.

Name of Supervisor: Prof Thirumala Govender

Signature: _____ Date: _____

DECLARATION ON PUBLICATIONS

Details of my contributions for the three publications that form part of and / or include research presented in this thesis are highlighted as follows:-

1. **E. Ojewole**, R. Kalhapure, K. Akamanchi, T. Govender Novel Oleic acid derivatives enhance buccal permeation of didanosine. ***Drug Development and Industrial Pharmacy 2014;*** 40(5): 657-668.

   E. Ojewole contributed to the design of the project, and was responsible for the excision and preparation of the buccal mucosae for the permeation and histomorphological studies. She performed all *in vitro* permeation experiments and was responsible for the interpretation of the permeability data. She undertook the histological studies and prepared samples for light microscopy (LM) and transmission electron microscopy (TEM) evaluations. She interpreted both the photo-micrographs and eletro-micrographs of the buccal mucosae. Furthermore, she was responsible for the writing of the manuscript, from the first draft and up until the final manuscript submission. R. Kalhapure served as a postdoctoral mentor and was responsible for the synthesis and characterization of the novel OA derivatives. K. Akamanchi served as an international collaborator and T. Govender served as the supervisor.

2. **E. Ojewole,** I. Mackraj, K. Akhundov, J. Hamman, A. Viljoen, E. Olivier, J. Wesley-Smith and T. Govender Investigating the effect of *Aloe vera* gel on the buccal permeability of didanosine. ***Planta Medica 2012*****;** 78(4): 354-361.

E. Ojewole contributed to the design of the project, was responsible for the excision and preparation of the buccal mucosae for the permeation and histomorphological studies. She performed the *in vitro* permeation experiments, the final viscosity determinations and the interpretation of the data. She undertook histological studies and prepared samples for LM and TEM evaluations. Furthermore, she contributed to the overall interpretation of the results as well as the writing of the manuscript. J. Wesley-Smith assisted with the LM and TEM evaluations and the interpretations of both the photo-micrographs and eletro-micrographs, as well as assisted with the writing of the LM and TEM sections of the manuscript. I. Mackraj assisted with the harvesting of the buccal mucosa from the cheeks of the pigs, and K. Akhundov demonstrated and assisted with the surgical removal of the excessive connective tissue from the buccal mucosa. J. Hamman and A. Viljoen donated Aloe vera gel for the permeability enhancement studies and assisted in writing the manuscript. E. Olivier assisted with the initial viscosity experiments.

3. **E. Ojewole**, I. Mackraj, P. Naidoo and T. Govender Exploring the use of novel drug delivery systems for antiretroviral drugs ***European Journal of Pharmaceutics and Biopharmaceutics 2008;*** 70: 697–710.

E. Ojewole contributed to the overall design of the contents for this review paper. She performed the literature search and identified the relevant articles used in the writing of the manuscript. She wrote the initial outline of the topics and the sections covered in the review paper. She was responsible for writing the sections on buccal, transdermal and rectal delivery. She also contributed to the writing of all the sections in collaboration with I. Mackraj, P. Naidoo and the supervisor (T. Govender). Furthermore, she contributed to the final draft of the manuscript and revised all the sections together with the supervisor before submission for journal publication.

DECLARATION ON ANIMAL ETHICS APPLICATION AND APPROVAL

Ethical application was made and approval was obtained annually from the University of KwaZulu-Natal Animal Ethics Committee, for the porcine buccal mucosae which were harvested, excised, prepared and used for all experimental studies undertaken and reported in this thesis. The reference numbers for the Ethics approval letters obtained for this study are 043/14/Animal, 039/13/Animal, 07/12/Animal, 025/11/Animal, 029/10/Animal, 028/09/Animal and 001/08/Animal. The approval letters and their respective reference numbers can be found in appendix II.

RESEARCH OUTPUT FROM THE THESIS

1. PUBLICATIONS IN ISI JOURNALS

The following first / prime authored papers were published from the literature (review paper) and data (original research papers), generated during this study. The years of publications were 2008, 2012 and 2014 which are reflected in their respective journals (i.e. Institute of Scientific Information (ISI) – journals).

1.1 **Elizabeth Ojewole**, Irene Mackraj, Panjasaram Naidoo and Thirumala Govender. Exploring the use of novel drug delivery systems for antiretroviral drugs. ***European Journal of Pharmaceutics and Biopharmaceutics 2008;*** 70: 697–710. (Impact factor = 4.245)

****The published paper can be found in Appendix III.*

1.2 **Elizabeth Ojewole,** Irene Mackraj, Kamil Akhundov Josias Hamman, Alvaro Viljoen, Eugene Olivier James Wesley-Smith and Thirumala Govender. Investigating the effect of *Aloe vera* gel on the buccal permeability of didanosine. ***Planta Medica 2012*****;** 78(4): 354-361. (Impact factor = 2.339)

****The published paper can be found in Appendix IV.*

1.3 **Elizabeth Ojewole**, R. Kalhapure, K Akamanchi, T Govender. Novel Oleic acid derivatives enhance buccal permeation of didanosine. ***Drug Development and Industrial Pharmacy 2014;*** 40(5): 657-668. (Impact factor = 2.006)

****The published paper can be found in Appendix V.*

2. PUBLISHED ABSTRACTS IN ISI JOURNALS

The following first / prime authored research abstracts were published from data generated during this study. The year of publications were 2009, which are reflected in their respective ISI journals.

2.1 **E Ojewole**, I Mackraj, K Akhundov, J Hamman, A Viljoen, T Govender. Exploring the effect of Aloe vera gel on the buccal permeability of didanosine: Permeability and Histomorphological studies. **Journal of Pharmacy and Pharmacology 2009;** 61: A34-A35. (BPC Science Proceedings in accredited ISI journals ISSN 0022-3573). (Impact factor = 2.161)

****The published abstract can be found in Appendix VI.*

2.2 **E Ojewole**, I Mackraj, E Jones, Z Madida, Z Mohajane, M Nkukhu, M Somtsewu, T Govender. Preparation and Evaluation of mucoadhesive polymeric films for buccal delivery of anti-HIV-AIDS drug (didanosine). **Journal of Pharmacy and Pharmacology 2009;** 61: A35-A35. (Published BPC Science Proceedings in accredited ISI journals ISSN 0022-3573). (Impact factor = 2.161)

*****The published abstract can be found in Appendix VI.*

3.0 CONFERENCE PRESENTATIONS

The following international and local / national conference presentations were produced from data generated during this study:

3.1 International Conference Presentations

3.1.1 **E Ojewole,** I Mackraj, K Akhundov, J Hamman, A Viljoen, J Wesley-Smith, T Govender. Exploring the effect of Aloe vera gel on the buccal permeability of didanosine: Permeability and Histomorphological studies. Presented at the British Pharmaceutical Conference (***BPC2009)*** 6 – 9 September, Manchester Central, **UK**.

3.1.2 **E Ojewole**, I Mackraj, E Jones, Z Madida, Z Mohajane, M Nkukhu, M Somtsewu, TGovender. Preparation and Evaluation of mucoadhesive polymeric films for buccal delivery of an anti-HIV-AIDS drug (didanosine). Presented at the British Pharmaceutical Conference (***BPC2009)*** 6 – 9 September, Manchester Central, **UK**.

3.1.3 **Elizabeth Ojewole** Irene Mackraj, Josias Hamman, Alvaro Viljoen, James Wesley-Smith, Eugene Olivier, Thirumala Govender. "Permeability and Mucosal Ultrastructural Analyses for Transbuccal Delivery of Didanosine". Presented at the 2nd Conference on Innovation in Drug Delivery: From Preformulation to Development through Innovative Evaluation Process, 2010, Aix-en-Provence, **France**.

3.2 Local / National Conference Presentations

3.2.1 **Elizabeth Ojewole**, Irene Mackraj, Kamil Akhundov and Thirumala Govender. Comparing the buccal delivery potential of two antiretroviral drugs: permeability & histological studies on didanosine & zalcitabine. Presented at the 29th Annual Academy of Pharmaceutical Sciences Conference, 22-26 September (APSSA **2008**) Magaliesberg, Gauteng **South Africa** - ***Awarded the Best Research Poster prize in the Pharmaceutics category.***

3.2.2 **E Ojewole**, I Mackraj, K Akhundov J Hamman, A Viljoen, J Wesley-Smith, T Govender. Buccal Permeability Enhancement of Didanosine using Aloe Vera Gel: Histological and Microscopical Evaluations. Presented at the 5th International Conference on Pharmaceutical and Pharmacological Sciences (5th ICPPS **2009**) 23rd to 26th September, North-West University, Portchefstroom, **South Africa.**

3.2.3 **E Ojewole**, I Mackraj, K Akhundov, T Govender. In Vitro Transbuccal Delivery of An Antiretroviral Drug: Effect of Donor Concentrations on Didanosine Permeation. Presented at the 5th International Conference on Pharmaceutical and Pharmacological Sciences (5th ICPPS **2009**) 23rd to 26th September, North-West University, Portchefstroom, **South Africa**.

3.2.4 **E Ojewole**, I Mackraj, K Akhundov, T Govender. Comparing the buccal delivery potential of two antiretroviral drugs: permeability and histological studies on didanosine and zalcitabine. Presented at the 16th scientific meeting of The South African Association for Laboratory Animal Science, (SAALAS **2009**) 16 -18 September, 1on1 - Gateway, **South Africa.**

3.2.5 **E Ojewole,** I Mackraj, K Akhundov J Hamman, A Viljoen, J Wesley-Smith, T Govender. Buccal Permeability Enhancement of Didanosine using Aloe Vera Gel: Histological and Microscopical Evaluations. Presented at the 47th Annual Microscopy Society of Southern Africa Conference, (MSSA 2009) 8-11 December, University of KwaZulu-Natal Durban **South Africa**. Publisehd as proceedings (Published Proceedings ISSN 0250-0418 and ISBN 0-620-350563), Microscopy Society of Southern Africa **2009**; 39: 33.

3.2.6 **Elizabeth Ojewole**, Irene Mackraj, Josias Hamman, Alvaro Viljoen, James Wesley-Smith, Eugene Olivier, Thirumala Govender Permeability and Mucosal Ultrastructural Analyses for Transbuccal Delivery of Didanosine. Presented at the 31st Annual Conference of the Academy of Pharmaceutical Sciences of South Africa (APSSA **2010**), 22-26 September, Turfloop Campus University of Limpopo, **South Africa.**

3.2.7 **E Ojewole**, E Jones, Z Madida, Z Mohajane, M Nkukhu, M Somtsewu I Mackraj, and T Govender. "Preparation and Evaluation of mucoadhesive polymeric films for buccal delivery of an antiretroviral drug (didanosine)" College of Health Sciences Research Symposium September **2010**, Nelson R Mandela

Medical School, University of KwaZulu-Natal, Durban **South Africa.**

3.2.8 **E Ojewole**, J Hamman, A Viljoen, and T Govender. Effect of Aloe Vera Gel on the Buccal Polymeric Films of Didanosine. Presented at the 6th International Conference on Pharmaceutical and Pharmacological Sciences, 25th to 27th September **2011** University of KwaZulu-Natal, **South Africa**

ABSTRACT

Whilst antiretroviral drugs (ARVs) have significantly improved treatment of Human Immunodeficiency Virus infection and Acquired Immune Deficiency Syndrome (HIV and AIDS), several limitations exist with their oral route of administration. Several orally administered ARVs such as, didanosine, saquinavir, tenofovir and zidovudine are associated with low and erratic bioavailability due to extensive first pass effect (FPE) as well as gastrointestinal (GI) acids and enzymatic degradation. Moreover, the half-life for several ARV drugs is short, which requires frequent administration of doses leading to systemic side effects and decreased patient compliance. Alternative routes of administration, such as buccal, rectal and vaginal, are widely investigated in the literature. Buccal delivery of drugs may therefore overcome the above limitations by bypassing FPE and GI degradation, thus improving bioavailability. Furthermore, drug absorption following buccal administration is not influenced by the potential variations in the gastric emptying rate or the presence of food. However, drug absorption can be limited by low buccal permeability due to the epithelial lining the mucosa. Identifying optimal novel enhancers is paramount to designing and developing drugs as buccal delivery systems. In an attempt to explore the potential of the buccal mucosal route for the delivery of an ARV drug using didanosine (ddI) as a model drug, the aims of this study were to: 1] investigate the permeability properties of ddI across the buccal mucosa route in order to determine its suitability for development as a buccal delivery system, 2] determine the effects of novel permeation enhancers, i.e. aloe vera gel (AVgel), oleic acid (OA) and its novel synthesized oleodendrimer derivatives, on the buccal permeability of ddI, and 3] to find out (through histo-morphological evaluation) whether ddI and the novel enhancers, i.e. AV gel and novel OA derivatives have any toxic effects on the buccal mucosa.

The buccal mucosa was harvested from pigs, and all the excess connective tissue was surgically removed. *In vitro* buccal permeation experiments were undertaken using modified vertical Franz diffusion cells, with phosphate buffered saline pH 7.4 (PBS) at 37 °C. ddI was quantified at 250 nm using a validated UV spectrophotometric method. The histomorphological evaluations were undertaken using light microscopy (LM) and transmission electron microscopy (TEM).

ddI permeated through the buccal mucosa and its permeability was concentration-dependent. A linear relationship (R^2 = 0.9557) between the concentrations and flux indicated passive diffusion as the mechanism of drug transport. AVgel, at concentrations of 0.25 to 2 %w/v, significantly enhanced ddI flux ($p<0.05$), with permeability enhancement ratios from 5.09 (0.25 %w/v) to 11.78 (2 %w/v), but decreased permeability at 4 and 6 %w/v. OA and its derivatives,

i.e. ester (OA1E), the dicarboxylic acid (OA1A), the bicephalous dianionic surfactant (OA1ANa) and their parent compound, OA, all enhanced the buccal permeability of ddI. OA, OA1E, OA1A and OA1ANa at 1 %w/w all showed potential, with enhancement ratios (ER) of 1.29, 1.33, 1.01 and 1.72 respectively. OA1ANa at 1 %w/w demonstrated the highest flux (80.30 ± 10.37 µg cm^{-2}.hr), permeability coefficient (4.01 ± 0.57 x 10^{-3} cm hr^{-1}) and enhancement ratio (1.72). The highest flux for ddI (144.00 ± 53.54 µg cm^{-2}.hr) was reported with OA1ANa at 2 %w/w, which displayed an ER of 3.09 more than that with ddI alone ($p=0.0014$). At equivalent concentrations, OA1ANa (ER=3.09) had a significantly higher permeation enhancing effect than its parent OA (ER=1.54).

Histomorphological studies showed that ddI did not have any adverse effects on the buccal mucosae. Ultrastructural analysis of the buccal mucosae treated with phosphate buffer saline pH 7.4 (PBS), ddI/PBS and ddI/PBS/AVgel 0.5 %w/v showed cells with normal plasmalemma, well-developed cristae and nuclei with regular nuclear envelopes. However, cells from 1, 2 and 6 %w/v AVgel-treated mucosae showed irregular nuclear outlines, increased intercellular spacing and plasmalemma crenulations. AVgel enhanced the buccal permeation of ddI and 0.05 %w/v was identified as a potentially safe and effective concentration for developing and optimizing buccal delivery systems. OA1ANa at all concentrations, except 6.0 %w/w had no adverse effects on the mucosae. OA1ANa at 2 %w/w was identified as a potentially safe concentration, and the optimal novel OA derivative that can widen the pool of fatty acid derivatives as chemical permeation enhancers for buccal drug delivery. The cellular changes, such as vacuoles formation and increased intercellular spaces, were attributed to the buccal permeation enhancing effects of AVgel and OA1ANa.

The results in this study confirmed the potential of buccal delivery of ddI, identified permeability parameters of ddI across the buccal mucosa and its permeability enhancement by both AVgel and OA derivatives as novel permeation enhancers. The study showed that both OA1ANa at 2 %w/w and AVgel at 0.5 %w/v, or lower concentrations, can be used as buccal permeation enhancers to develop and optimize novel buccal delivery systems for ddI to improve ARV therapy. The novel enhancers are recommended for selection as buccal permeation enhancers, to design and optimize ddI buccal delivery systems, and application to other ARV drugs for improved therapy.

Keywords: HIV and AIDS, Antiretroviral drugs, Buccal, Didanosine, Permeation enhancer, *Aloe vera* (L.) Burm. F. (*Aloe barbadensis* Miller), Oleic acid derivatives, Histomorphology, Transmission electron microscopy.

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LIST OF ABBREVIATIONS

3TC	Lamivudine
AcCl	Acetyl Chloride
AIDS	Acquired Immunodeficiency Syndrome
AZT	Zidovudine
ANOVA	Analysis of Variance
ARV	Antiretroviral
AVgel	Aloe Vera Gel
BCS	Biopharmaceutics Classification System
BIOSIS	Biosciences Information Service
BPC	British Pharmaceutical Conference
BRU	Biomedical Resource Unit
CCR5	Chemokine Receptor Type 5
CXCR4	Chemokine Receptor Type 4
CPE	Chemical Permeation Enhancer
CNS	Central Nervous System
CV	Coefficient of Variation
DCM	Dichloromethane
d4T	Stavudine
ddC	Zalcitabine
ddI	Didanosine
DLV	Delaviridine
DMAP	Dimethylaminopyridine
DS	Diclofenac Sodium
EC	Enteric Coated
EDAC.HCL	Ethyldimethylaminopropylcarbodiimide Hydrochloride

EFV	Efavirenz
EMU	Electron Microscope Unit
ER	Enhancement Ratio
EtOAc	Ethyl Acetate
EUD	Eudragit
FA	Fatty Acids
FI	Fusion Inhibitor
FPE	First Pass Effect
FTIR	Fourier Transform Infra-Red
GIT	Gastrointestinal Tract
H & E	Hematoxylin and Eosin
HAART	Highly Active Antiretroviral Therapy
HCl	Hydrochloric Acid
HIV	Human Immunodeficiency Virus
HLB	Hydrophilic-Lipophilic Balance
HPMC	Hydroxypropyl methylcellulose
IASC	International Aloe Science Council
IF	Impact Factor
II	Integrase Inhibitor
INV	Indinavir
IS	Intercellular Spaces
ISI	Institute of Scientific Information
KH_2P0_4	Potassium Dihydrogen Phosphate
LM	Light Microscopy
Log P	Logarithm of Partition Coefficient
MeOH	Methanol
MMF	Monolayered Multipolymeric Film
NaCl	Sodium Chloride
Na_2P0_4	Disodium Phosphate
Na_2S0_4	Disodium Sulphate

$NaHC0_3$	Sodium Bicarbonate
NaOH	Sodium Hydroxide
NDDS	Novel Drug Delivery Systems
NMR	Nuclear Magnetic Resonance
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NtRTI	Nucleotide Reverse Transcriptase Inhibitor
OA	Oleic Acid
OA1E	Ester Derivative of Oleic Acid
OA1A	Dicarboxylic Acid Derivative of Oleic Acid
OA1ANa	Sodium Salt of Dicarboxylic Acid Derivative of Oleic Acid
PBS	Phosphate Buffered Saline
PEG	Polyethylene Glycol
PI	Protease Inhibitor
SD	Standard Deviation
SEM	Scanning Electron Microscopy
SMT	Silicone Molded Tray
SPSS	Statistical Package for Social Sciences
SQV	Saquinavir
TEER	Transepithelial electrical resistance
TEM	Transmission Electron Microscopy
TFV	Tenofovir
UKZN	University of Kwazulu-Natal
UNAIDS	Joint United Nations Programme On HIV/AIDS
USA	United States of America
UV	Ultraviolet
WoS	Web of Science
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

The background to the studies presented in the thesis is described in this chapter. The potential of the buccal mucosa route to address the limitations of other routes of administration are highlighted. It outlines the epithelium lining of mucosa as the barrier to drug permeation and proposes strategies to address this. It also describes aloe vera gel (AVgel) as a permeation enhancer from natural product origin and derivatives of fatty acids as chemical permeation enhancers (CPE). The aim and the objectives of the study, its significance and novelty as well as a general overview of the thesis are further presented.

1.2 BACKGROUND

This section presents a quick overview of buccal delivery and applicability to various drugs and disease conditions. The strategies to enhance permeation of drugs across the buccal mucosa including the use of permeation enhancers was highlighted. Further, the status of HIV and AIDS, the challenges of current ARV therapy as well as motivation for buccal delivery of ARV drugs were highlighted.

1.2.1 BUCCAL DELIVERY

While the oral route is convenient and remains the most popular and preferred route of drug administration by both patients and healthcare practitioners, it has several disadvantages (Morales and McConville, 2014; Sattar et al., 2014; Sohi et al., 2010). These include degradation of drugs due to acid and enzymatic attack in the gastrointestinal (GI) tract, as well as susceptibility to first pass hepatic metabolism, thereby reducing drug bioavailability and necessitating higher doses for administration (Desai et al., 2012). A major area of research is therefore seeking alternative routes of drug delivery, such as transdermal (Chen et al., 2013; Lam and Gambari, 2014; Patel et al., 2012a), buccal (Morales et al., 2013; Senel et al., 2012; Zeng et al., 2014) and vaginal (Berginc et al., 2014; D'Cruz and Uckun, 2014; Ghosal et al., 2014; Ndesendo et al., 2008).

The buccal route is receiving increasing interest, as it is an attractive alternate and non-invasive site for delivering both locally and systemically active drugs (Dhiman et al., 2009; Teubl et al., 2013). Drug in a dosage form, such as films, patches and gels, is applied and adheres to the mucosa lining, the inner cheeks of the mouth, where drug is absorbed (Hearnden et al., 2012; Patel et al., 2011). The buccal mucosal route has several advantages. It avoids the degradation of drugs by both the GI acids and enzymes and bypasses hepatic first pass metabolism, thereby improving the systemic bioavailability of various

drugs (Madhav et al., 2012; Singh, 2013). Furthermore, absorption following administration via the buccal route is not influenced by potential variations in the gastric emptying rate or the presence of food (Li and Chan, 1999; MacBrayne et al., 2014). The permeability of the buccal mucosa is also higher than that of the skin (Patel et al., 2011), and a lower loading dose in a transbuccal device could provide the same therapeutic effect as a transdermal patch.

Moreover, the buccal mucosa has a larger area for drug application, and has good accessibility compared to other mucosae, such as the nasal, rectal and vaginal (das Neves et al., 2011; Mallipeddi and Rohan, 2010; Ndesendo et al., 2008). In addition there is increased ease of dosage form application and easy removal if therapy is required to be discontinued. Various classes of drugs, including antiretroviral (zalcitabine (ddC), tenofovir (TFV), and saquinavir (SQV)) (Rambharose et al., 2014b; Shojaei et al., 1999; Xiang et al., 2002), non-steroidal anti-inflammatory) (piroxicam) (Attia et al., 2004), opiate (morphine) (Senel et al., 1997), proton-pump inhibitor (omeprazole) (Figueiras et al., 2010), anti-diabetic hormone and blood glucose lowering agent(insulin)(Morales et al., 2014; Xue et al., 2012) and (beta blocker) (e.g. metoprolol) (Patel et al., 2012b), have been studied for delivery via the buccal mucosa to exploit its above advantages. The buccal route therefore has wide applicability for diverse drugs and disease conditions.

Some disadvantages include:

1) the buccal epithelium lining the mucosa is a barrier to drug permeation which reduces the bioavailability of drugs applied via the buccal route (Hearnden et al., 2012).
2) the bucal mucosa is largely hindered by the thickness of mucus viscoelastic layer and a continuous secretion of the saliva which may potentially lead to drug dilution, lowered drug concentration, and reduced bioavailability (Madhav et al., 2009).
3) the chewing and swallowing of food can potentially lead to drug loss due to involuntary removal and swallowing of the dosage form and drugs from the application site as well as may cause possible hazard of choking (Heemstra et al., 2010).

However, these disadvantages are superseded by the advantages, and the delivery of drugs via the oral mucosal route and modification of dosage forms are currently a major focus of international pharmaceutical science research for enhancing drug therapy. Studies on buccal delivery will contribute to developing cost-effective dosage forms, leading to an improved disease management, improved quality of life, and ultimately a reduction in health care costs both in South Africa and internationally. An improved economy at a global level is expected with improved drug therapy, as better drug treatment will lead to a reduction in work absenteeism, promote better work output and

general health state of the global community. Therefore, strategies to enhance the formulation and properties of buccal delivery systems are essential.

1.2.2 PERMEATION ENHANCEMENT STRATEGIES FOR BUCCAL DRUG DELIVERY

One of the main challenges with buccal mucosal therapy is its limited mucosal permeability due to the epithelial lining of the membrane which acts as a barrier to drug permeation (Giannola et al., 2007; Sattar et al., 2014; Şenel and Hıncal, 2001; Teubl et al., 2013). The outermost layer of the stratified squamous epithelium is non-keratinized, covered by a thin layer of mucus and is comparatively thicker than the rest of the oral mucosal lining. The basement membrane lies directly underneath the epithelium, followed by the lamina propria and the submucosa. The mucosa is made up of about 40-50 cell layers and a thickness of 500–800 μm has been reported (Dodla and Velmurugan, 2013; Pather et al., 2008). The mucosal structure thus contributes to the challenges and factors that are responsible for the limited buccal permeability of drugs. Enhancing permeation of drugs across the buccal mucosa is therefore critical for optimizing bioavailability of various drugs (Hearnden et al., 2012; Madhav et al., 2012).

Maximizing the bioavailability of several drugs after buccal administration for absorption through the mucosal lining will be beneficial to reducing intra and inter subject variability as well as side effects of the drugs (Kapil et al., 2013; Patel et al., 2012b). Moreover, the cost of manufacture will be reduced by

decreasing drug wastage owing to its low systemic bioavailability (Aungst, 2012), especially where drugs have limited permeability and subsequently low bioavailability. Hence, the use of permeation enhancing strategies in many cases is essential to overcome the limited permeability of the buccal mucosae for improved buccal drug delivery (Hassan et al., 2010; Senel et al., 2012; Sohi et al., 2010).

Approaches to promote buccal permeation of drugs thus include the use of chemical permeation enhancers and physical methods, such as particle size reduction by ball milling (Hu et al., 2011a; Hu et al., 2011b; Rambharose et al., 2014a; Rao et al., 2011). Other approaches include ultrasound and electrical assisted methods (iontophoresis and electroporation), as well as thermal enhancement (Morales and McConville, 2014; Senel et al., 2012; Wei et al., 2012). A buccal delivery system that employs a permeation enhancer to deliver insulin is commercially available. The system uses bile salts encapsulated as an enhancer in mixed micelles for improved hypoglycaemic therapy (Palermo et al., 2011). There is therefore a scope to develop buccal drug delivery systems to utilize permeation enhancers for improved buccal drug delivery.

1.2.3 PERMEATION ENHANCERS FOR BUCCAL DELIVERY

Permeation enhancers are substances or techniques that are widely used in promoting the permeability of drugs across mucosal membranes including buccal mucosa. Chemical permeation enhancers (CPEs) have been employed and proved promising for enhancing permeability of various drugs (Hassan et

al., 2010; Sohi et al., 2010). Examples include bile salts, fatty acids and surfactants, i.e. sodium deoxycholate for 5-FU (Pendekal and Tegginamat, 2012), oleic acid for clonazepam (Sakata et al., 2011) and sodium dodecyl sulfate for insulin, caffeine and estradiol (Bernstein, 2008; Nicolazzo et al., 2004). The discovery of new permeation enhancers and the careful selection for buccal permeability enhancement of drugs are essential to optimize drug delivery via the buccal route.

1.2.3.1 ALOE VERA GEL

Currently, there is an increasing interest in drug products that either are of natural origin or contain components of natural products. Permeation enhancers from natural origin have become popular as they offer numerous benefits over their synthetic counterparts. These benefits include sustainable mass production from renewable resources as well as lower cost of production depending on the extraction method used (Fox et al., 2011; Maurya et al., 2006; Rodríguez-González et al., 2012).

Aloe vera (*Aloe barbadensis* Miller) is a succulent plant with strap-shaped green leaves (Kiran and Rao, 2014; Lad and Murthy, 2013). The aloe latex (or exudate), the aloe gel and the whole leaf (or whole leaf extract) are the main parts used for medicinal applications (Chen et al., 2009). The inner pulp of the fresh leaves is used for gel extrusion (Hamman, 2008; Reynolds and Dweck, 1999). The gel obtained from aloe vera, i.e. aloe vera gel (AVgel), is composed mainly of water (>99 %), and the remaining 0.5 – 1 % of solid material

comprises several polysaccharides, vitamins, enzymes, lipids, inorganic and small organic compounds (Boudreau and Beland, 2006). Additional properties identified for Aloe vera are anti-inflammatory and antifungal, as well as soothing effect on the mucosal lining and wound healing properties (Pugh et al., 2001). While AVgel has been shown to be an effective transdermal (Cole and Heard, 2007) and intestinal (Chen et al., 2009) permeation enhancer for various drugs, its applicability for buccal permeation enhancement has not been previously investigated. In addition, those studies with AVgel as an enhancer for the intestinal and transdermal routes did not report its histomorphological effects (Chen et al., 2009; Cole and Heard, 2007), which is important for assessing its preliminary suitability.

In the *in vitro* permeation studies reported by Chen et al 2009, it was reported that the polysaccharides from Aloe vera gel is capable of reducing the TEER of excised rat intestinal tissue, thus enhancing the transport of atenolol across this tissue to a significant extent. Moreover, Aloe vera gel materials could significantly decrease the TEER of Caco-2 cell monolayers and this reduction in TEER was associated with the opening of tight junctions between adjacent epithelial cells and this effect was completely reversible after removal of the Aloe vera leaf materials from the cell monolayers.

Cole and Heard (2007) also stated that the skin penetration enhancement effect of AVgel was due to a probable pull effect of complexes formed between the compound and the enhancing agent within the aloe gel, however, the proposed mechanism of action has to be further investigated and confirmed as stated by the authors.

Furthermore, the histomorphological studies could be useful for identifying the potential mechanisms of permeation enhancers and drug permeation pathways.

Aloe vera gel has also been shown to have the potential to modify drug release profiles in pharmaceutical dosage forms (Jani et al., 2007). Moreover, polysaccharides form a major component of AVgel, therefore unlike several other existing permeation enhancers, it has the potential to also provide multifunctional properties in buccal drug delivery systems. Furthermore, aloe vera has been reported as useful to treat bacterial and fungal infections, as well as sexually transmitted diseases, including HIV and AIDS (Kamatenesi-Mugisha et al., 2008; Pugh et al., 2001). It was therefore the focus of this study to explore the potential of AVgel in enhancing the buccal permeation of drugs, using a model ARV drug.

1.2.3.2 FATTY ACIDS DERIVATIVES

Fatty Acids (FAs) are widely used chemical permeation enhancers for various drugs (Dodla and Velmurugan, 2013). For example, oleic acid, sodium caprate, caprylic acid, sucrose esters and lauric acid have been reported for enhancing the permeation of drugs such as propranolol, lidocaine, ergotamine, insulin and sumatriptan across the buccal mucosa (Artusi et al., 2004; Bhati, 2012; Bigucci et al., 2014; Senel and Hincal, 2001; Sohi et al., 2010; Tsutsumi et al., 1998). It has been reported that FAs can disrupt the lipid bilayer of the mucosal lining

thereby increasing drug transport and bioavailability (Dodla and Velmurugan, 2013; Hassan et al., 2010; Sohi et al., 2010). Oleic acid (OA) in particular has been reported as an effective chemical permeation enhancer for drugs, such as levothyroxine sodium via the intestinal route (Pabla et al., 2010), caffeine and diclofenac sodium (DS) via the transdermal route (Ochalek et al., 2012), and propranolol as well as 5-fluorouracil via the buccal route (Bigucci et al., 2014; Dhiman et al., 2009).

Recent reports are emerging on the use of derivatives of common chemical enhancers for further maximising mucosal drug permeation (Caon et al., 2014; Hassan et al., 2010). For example, newly synthesised propanoyloxy derivatives of 5b-cholan-24-oic acid were more effective in enhancing permeation of theophylline as compared to its parent compound, cholic acid, thereby potentiating the efficacy of bile acids as a class of chemical permeation enhancers (Coufalová et al., 2013; Mrózek et al., 2013). There is therefore a need to explore and identify new derivatives of chemical enhancers to widen the pool of available superior enhancers for buccal drug delivery. Novel derivatives of OA will therefore be useful to further improve the permeation enhancing potential of fatty acids, and to contribute to the pool of enhancers for enhancing drug permeability.

Synthesizing novel OA derivatives i.e. A1E, A2E, E1E and E2E of G1 and G2 was reported for transdermal delivery of diclofenac sodium (DS), a non-steroidal anti-inflammatory drug (Kalhapure and Akamanchi, 2013).

Oleodendrimers A1E and A2E have an amide linkage whereas, E1E and E2E have an ester linkage between the dendron and OA moiety. The derivatives of OA were shown to be more effective in enhancing the transdermal permeation of DS compared to the parent OA. The potential of these novel derivatives i.e. oleodendrimers as permeation enhancer for buccal delivery, have not been studied for any drug to date. The buccal permeation enhancement of these OA derivatives may expand its applicability in drug delivery systems. Investigating aloe vera gel and novel derivatives of fatty acids as buccal permeation enhancers remains to be explored.

1.2.4 HIV AND AIDS AND CHALLENGES OF CURENT ARV THERAPY

Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS), remain a major health challenge in Africa and globally. It constitutes one of the most serious infectious disease challenges to public health globally, and has had a crippling effect in certain parts of the world, especially sub-Saharan Africa (Mazzeo et al., 2012). There are currently 35.3 million people living with HIV and AIDS globally, of whom 25 million are in sub-Saharan Africa, and represent 71% of the global number. HIV and AIDS is therefore still the leading fatal disease worldwide (UNAIDS, 2013; WHO, 2013).

While ARV drug therapy has contributed significantly to improved management of patients with HIV and AIDS, its current use is associated with several challenges and inconveniences (Boffito et al., 2014; Ramana et al., 2014).

Many ARV drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. The half-lives of several ARV drugs are short, which then requires frequent administration of doses, leading to dose-dependent side effects and decreased patient compliance (Li and Chan, 1999; MacBrayne et al., 2014; Rakhmanina et al., 2012).

Despite the availability of ARV therapy, the above statistics indicate that much remains to be accomplished, as the number of newly reported HIV infections is still unacceptably high (UNAIDS, 2013). In addition to acid degradation and first pass hepatic metabolism, several ARV drugs suffer from physicochemical problems, such as poor solubility that may lead to formulation difficulties (Li and Chan, 1999; Xiang et al., 2002). Strategies currently being investigated to overcome these limitations include: identifying new and chemical modification of existing chemical entities (Ghosh et al., 2008; Schroeder et al., 2014; Vivet-Boudou et al., 2011), examining various dosing regimens (de Kock et al., 2014; Larson et al., 2014), designing and developing novel drug delivery systems (das Neves et al., 2010; Ramana et al., 2014; Sosnik et al., 2009) that can improve the efficacy of both existing and new ARV drugs. Moreover, different routes of drug administration, including buccal (Jones et al., 2014; Rambharose, 2013; Xiang et al., 2002), rectal (das Neves et al., 2013; Dezzutti et al., 2014), and transdermal (van Heerden et al., 2010; Zidan and Habib, 2014) are being explored.

1.2.5 ANTIRETROVIRAL DRUGS FOR BUCCAL DELIVERY

Drugs administered via the buccal route will avoid degradation by both the GI acids and enzymes, and will also bypass the hepatic first pass metabolism, thereby improving their bioavailability (Morales and McConville, 2011; Singh, 2013). Buccal delivery will benefit most ARV drugs as it can address several disadvantages and challenges that exist with current ARV therapy, such as:

- Most ARVs have low bioavailability due to hepatic first pass metabolism and their degradation in the acidic and enzymatic conditions of the gastrointestinal tract. Their absorption may also be influenced by variations in the gastric emptying rate as well as presence of food. The buccal route as an alternative to oral will bypass the hepatic first pass effect and the harsh conditions of the GI, thus improving the bioavailability of the ARVs (Desai et al., 2012).
- Most ARVs have short half-lives that necessitate that higher doses be incorporated into the dosage forms, and that they be given frequently to treat HIV and AIDS. As a result of these higher and more frequent doses, patients experience severe dose-dependent systemic side and adverse effects, and may therefore not comply with ARV dosage regimens. Buccal route will require lesser amount of such drugs as compared to oral route, since the buccal mucosal site for the dosage application is highly vascularised, and can promote higher absorption of drugs. The dose-dependent toxicities and adverse effects experienced with ARV therapy will be significantly reduced and patient compliance

improved by using the buccal route (Chandwani et al., 2012; Hien et al., 2013).

- Most ARVs, due to their low bioavailability, are formulated as high-dose oral dosage forms which translate into larger oral delivery systems such as tablet. This presents with swallowing challenges in paediatrics as well as in adults with dysphagia. The amount of drug incorporated into a buccal delivery system is smaller compared to the amount of drug incorporated into an oral delivery system. The formulation of ARVs for buccal delivery may also exhibit controlled-release kinetics of drugs, which requires that drug release is prolonged at the site of application over a predetermined period of time. The frequency of dosing as well as the systemic side effects are reduced, and patient compliance with their ARV therapy is improved (Lam et al., 2014; Madhav et al., 2009).
- Most ARVs that are formulated for oral administration are influenced by food content in the GIT and the timing of food ingestion. This poses certain challenges in children especially in paediatrics that require frequent feeding. The buccal route of delivery will not be affected by food timing as compared to oral. Hence buccal delivery of drugs will be applicable in most patient populations, particularly the paediatrics and geriatrics.

Didanosine (ddI) is a nucleoside reverse transcriptase inhibitor used as a second line ARV regimen in both adult and paediatric population (Schulenburg and Le Roux, 2008). The pharmacological and physicochemical properties of

ddI makes it a suitable candidate for buccal delivery. The oral bioavailability of ddI is as low as 30 % to 40 % which may further be reduced in the presence of a meal, and its half-life is 1.3 to 1.6 hours (Bettini et al., 2010; Li and Chan, 1999). Additionally, the molecular weight is 236.3 Dalton, its pKa in water is 9.12 and Log P has been reported as being -1.24 (Moffat et al., 2004). The challenges with the current oral use of ddI, in addition to the above properties, therefore necessitates that alternative route be explored, and that ddI can be considered as a model ARV drug for buccal delivery. The small molecular weight will ensure that the drug can diffuse across the cell membranes with less difficulty. Furthermore, at the physiological pH of the buccal mucosa of 6 to 7, ddI will remain mostly in its unionized form which will ensure its interaction with the lipoidal cell membrane and improved absorption.

Didanosine has a logP value of -1.24 (Moffat et al., 2004), hence classified as a hydrophilic drug and this property may limit its permeability across the lipoidal cell membrane. Also, ddI is classified as BCS Class III drug, exhibiting poor/low permeability. Moreover, the buccal permeability of drugs is limited by the epithelium lining the buccal mucosa. This suggests that the inclusion of a safe and effective buccal permeation enhancer will therefore promote the delivery of ARVs such as ddI for improved therapy.

At the time of this study, only one other ARV, namely ddC, was investigated and reported for buccal delivery (Shojaei et al., 1999; Xiang et al., 2002). The buccal delivery potential of ddC was investigated by these two researchers,

and they employed bile salts (Xiang et al., 2002), and menthol (Shojaei et al., 1999) as permeation enhancers. To the best of our knowledge, these two reports on ddC were the only ones found on buccal delivery of ARVs in the literature until the studies on ddI, and AVgel as permeation enhancer were reported in this study. Therefore, the potential of the buccal mucosa route for delivering ARVs, such as ddI, and carefully selecting permeation enhancers needed to be explored.

Recently, studies have reported on permeation potential of other ARVs, i.e. tenofovir (TFV) and saquinavir (SQV) (Rambharose et al., 2014a; Rambharose et al., 2014b).The incorporation of zidovudine (AZT) into buccal mucoadhesive patches (Reddy et al., 2012) and ddI into buccal polymeric films (Jones et al., 2013) as well as nano-enabled films (Jones et al., 2014; Jones et al., 2013) have also been recently reported.

Studies on the buccal mucosa route are ongoing in anticipation of contributing to an improved ARV therapy. Buccal delivery of drugs are still attractive to scientists across the pharmaceutical industries and in academia. The identification of safe and effective permeation enhancers that will contribute to the development of buccal delivery systems also continues to attract researchers across the globe, until such a time that dosage forms for buccal delivery of ARVs are commercially available.

No studies were reported on the buccal delivery potential of ddI, and none on the potential of AV gel and OA synthetic derivatives as buccal permeation enhancers, particularly using ddI as a model ARV drug. Therefore, the aim of the studies reported in this thesis was to explore the potential of the buccal mucosa route to deliver an ARV drug, and to identify novel buccal permeation enhancers in order to contribute to developing and optimizing novel buccal delivery systems that will be useful to improve ARV therapy.

1.3 AIM AND OBJECTIVES

In order to explore the potential of the buccal mucosal route for the delivery of an ARV drug, the sub-Aims and their respective objectives of the study were:

Aim 1.

To investigate the potential of the buccal mucosal route for delivery of an ARV drug using ddI as a model drug.

Objectives:

1. Determine the permeability parameters of ddI across the buccal mucosa
2. Investigate the effects of ddI concentrations on its permeability parameters.

Aim 2.

To determine the effects of different novel enhancers such as AV gel, oleic acid and the chemically synthesized oleodendrimer derivatives on the buccal permeability of ddI.

Objectives:

3. Investigate the buccal enhancement potential of novel enhancer from natural origin, AV Gel by identifying the permeability parameters of ddIin the absence and presence of AV gel.
4. Determine the concentration effects of AV Gel on the buccal permeability of ddI.
5. Investigate the buccal enhancement potential of OA and its chemically synthesized oleodendrimer derivatives by identifying the permeability parameters of ddI in the absence and presence of chemically synthesized novel OA derivatives.
6. Identify the OA derivative with the best buccal permeation enhancement and determine its concentration effects on the permeability properties of ddI.

Aim 3.

To assess the histo-morphological effects of both ddI and the novel enhancers, AV gel and OA derivatives on the buccal mucosa.

Objectives:

7. To assess the morphological effects of ddI, and the concentrations of AV gel and the selected OA derivative as novel permeation enhancers on the buccal mucosa.
8. To evaluate the ultrastructure of the buccal mucosa in order to determine the safe concentrations of the novel buccal permeation enhancers.

It should be noted that the original protocol for this study included an objective for formulation studies, i.e. to incorporate a selected ARV into a novel buccal drug delivery system such as monolayered multipolymeric films (MMFs) for the buccal route. This objective was indeed addressed in this study and preliminary film formulation experiments of MMFs were performed. The preliminary experimental findings of this objective have been published as research abstracts in ISI journals, i.e. Journal of Pharmacy and Pharmacology, Impact factor = 2.006. This published abstract is listed on Pages x – xi, and has been included in Appendix VI on page 274. The experimental findings have also been presented at both local and international conferences, and the presentations have been included in Appendix VII, pages 279 – 285. These preliminary experimental findings were not explored further and are not presented as a chapter in this thesis, as the thesis met the current UKZN criteria of three (3) publications in the same topic area for an award of a PhD degree and the criteria can be found in Appendix I, pages 221 – 224.

1.4 IMPORTANCE OF THE STUDY

The experimental findings of this study will be of significant benefits in that an alternate buccal route for the delivery of ARV drugs, such as ddI, could be identified. The identification of alternative route, such as the buccal mucosal route, could offer a superior approach over the current oral route of drug administration for HIV and AIDS therapy. This could be particularly useful for patients for improving compliance and providing better therapeutic outcomes of ARV drugs.

The findings could also be significant in widening the pool of buccal permeation enhancers for the delivery of ARVs. The buccal permeability of ddI was enhanced successfully using products from both natural origin (AVgel) as well as chemically synthesized OA derivatives. This means that more sources of buccal permeation enhancers could be available, which will broaden the categories of permeation enhancers that are suitable in the formulation and optimization of buccal drug delivery systems containing ddI. The novel AVgel and OA derivatives as buccal permeation enhancers could be applicable to a wide range of ARVs as well as other classes of drugs.

The experimental findings of this study could significantly create new knowledge in terms of identifying the buccal delivery potential and mechanism of buccal permeation of an ARV drug. The mechanism of action of the novel buccal permeation enhancers, i.e. AV gel and OA derivatives will create new

knowledge in the field of pharmaceutical sciences, and particularly useful for the formulation scientists, and for research and development in buccal delivery of ARV drugs. The new knowledge created in this study will be a great manufacturing resource for the pharmaceutical industries.

Moreover, the enhancers used in this study are synthetic derivatives and natural products. They would be of benefit to formulation scientists in that different classes of permeation enhancers will be available for selection in the formulation, optimization and eventual manufacture and commercialization of buccal delivery systems containing ddI, or any other ARVs, and could be applicable to other various classes of drugs.

1.5 NOVELTY OF THE STUDY

At the time of this study, and to the best of our knowledge, only one other ARV, in particular, zalcitabine (ddC), was reported for buccal delivery. For the first time in the area of buccal delivery research investigating antiretroviral drugs, this study identified the buccal delivery potential of ddI, and a possible mechanism of ddI permeation across the buccal mucosa was predicted.

AVgel was previously identified as a permeation enhancer for both intestinal and skin permeation. This is the first time that AVgel was identified as a buccal permeation enhancer particularly for an antiretroviral drug, ddI and for any other drug for that matter. The effective and safe concentration of AVgel as buccal permeation enhancer for ddI was also identified in this study. The

histomorphological evaluations reported in the previous studies on the buccal permeability of ddC were limited to the use of light microscopy (LM).This is the first time we show that histomorphological evaluations, using both LM and transmission electron microscopy (TEM), can be used to access the effects of ddI and enhancers on the buccal mucosa. Particularly, the analysis of the buccal mucosa using TEM was reported for the first time in buccal delivery of ARV. This study shows that the buccal permeation enhancing properties of AVgel can be correlated with its effects on the buccal mucosa.

While fatty acids have been reported as chemical permeation enhancers for other drugs, the derivatives of fatty acids OA have not been indicated for buccal delivery. This is the first time that fatty acids and derivatives, i.e. OA and its oleodendrimer derivatives, were used as buccal permeation enhancers for an antiretroviral drug, ddI. Identifying the best derivative, as well as its effective and safe concentration is reported for the first time. These novel enhancers can widen the pool of buccal enhancers for the formulation scientists to explore in the development of buccal drug delivery systems, especially for ARVs. This new knowledge is particularly desirable, as to date, no specific ARV drug has been developed as buccal drug delivery system for commercialization.

This study also identified the most suitable derivative of OA (OANa1A), and reported its safe and effective concentration as revealed by TEM analysis and proposed its use as buccal enhancer for formulation and optimization of novel buccal delivery systems for improved ARV therapy.

1.6 THESIS OVERVIEW

This thesis is divided into the following five chapters:

Chapter 1. Introduction - This chapter outlines the background to the study, which included buccal delivery of drugs, permeation enhancement strategies and the challenges of current ARV therapy. The buccal mucosa route as alternate routes to others such as oral and parenteral as well as the advantages and disadvantages of buccal delivery are described. This chapter also described AV gel as permeation enhancer from natural products origin as well as fatty acids derivatives as synthetic chemical permeation enhancers. The potential of the buccal mucosa for delivery of ARV drugs was highlighted and the aims and objectives are presented in the chapter.

Chapter 2. Publication one - Literature Review – This chapter presents the literature which was reviewed and then published as a review article in an international ISI journal. The publication's authors are **Elizabeth Ojewole**, Irene Mackraj, Panjasaram Naidoo and Thirumala Govender Other details are: Exploring the use of novel drug delivery systems for antiretroviral drugs. European Journal of Pharmaceutics and Biopharmaceutics 2008; 70: 697–710 (Impact Factor = 4.245). The Citation counts are reported as follows: Google Scholar = 76, Web of Science = 48, and BIOSIS = 31.This publication is a first-authored paper

published from the literature reviewed during this study. The review article describes several drug delivery systems that were developed for ARVs, including buccal, rectal and transdermal drug delivery systems. It highlights the significant potential that novel drug delivery systems have for the future of ARV drug therapy. This chapter presents the literature review in the final revised and accepted version and the format required for publication by the journal.

Chapter 3. Publication two - This chapter presents an original research from the data generated in this study which was published in an international ISI journal. The publication's authors are **Elizabeth Ojewole,** Irene Mackraj, Kamil Akhundov Josias Hamman, Alvaro Viljoen, Eugene Olivier James Wesley-Smith and Thirumala Govender. Other details are: Investigating the effect of Aloe vera gel on the buccal permeability of didanosine. Planta Medica **2012**; 78(4): 354-361 (Impact Factor = 2.339). The Citation counts are reported as follows: Google Scholar = 8, Web of Science = 6, and BIOSIS = 2). This publication is a first-authored paper that reported the experimental findings on the potential of the buccal mucosal route for delivery of didanosine. It also highlights the significant findings that no adverse effects were observed with ddI and AVgel at their selected concentrations. This chapter presents the research paper in the final revised and accepted version and the format required for publication by the journal.

Chapter 4. Publication three - This chapter presents an original research from the data generated in this study which was published in an international ISI journal. The publication's authors are **Elizabeth Ojewole**, R. Kalhapure, K Akamanchi, and T. Govender. Other details are: Novel Oleic acid derivatives enhance buccal permeation of didanosine. Drug Development and Industrial Pharmacy **2014**; 40(5): 657-668 (Impact Factor = 2.006). The citation counts are reported as follows: Google Scholar = 1, Web of Science = 0, and BIOSIS = 0. This publication is a first-authored paper that reported the potential of oleic acid and its oleodendrimer derivatives as buccal permeation enhancers for ddI. This chapter presents the research paper in the final revised and accepted version, and in the format required for publication by the journal.

Chapter 5. Conclusion. This chapter describes the general conclusions drawn from the experimental findings in this study, identifies possible study limitations and highlights recommendations for future work.

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CHAPTER TWO

LITERATURE REVIEW - PUBLISHED PAPER

2.1 INTRODUCTION

The following paper was published in an international peer reviewed ISI journal and reports the literature review generated during this study.

Elizabeth Ojewole, Irene Mackraj, Panjasaram Naidoo and Thirumala Govender. Exploring the use of novel drug delivery systems for antiretroviral drugs. **European Journal of Pharmaceutics and Biopharmaceutics** 2008; 70 697–710.

E. Ojewole contributed to the overall content design of this review paper. She performed the literature search and identified the relevant articles used in the writing of the manuscript. She wrote the initial outline of the topics for the review paper. She was responsible for writing the sections on buccal, transdermal and rectal delivery. She contributed to writing all the sections in collaboration with I. Mackraj, P. Naidoo and the supervisor (T. Govender). Furthermore, she contributed to the final draft of the manuscript and revised all the sections together with the supervisor before submission for ISI journal publication.

This chapter is presented in the required format by the ISI journal and is in the final revised and accepted version, published in the European Journal of Pharmaceutics and Biopharmaceutics.

The review article has been cited 48 times according to the cited counts by the Web of Science core collection, and 31 times cited counts according to the BIOSIS citation Index, accessed 02/11/2014.

In 2012, the review article was listed among the “Top 20 Articles, in the Domain of Article 18720133, since its Publication (2008)”.The ISI journal article can be found in Appendix III.

2.2 PUBLISHED PAPER

Exploring the use of novel drug delivery systems for antiretroviral drugs.

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Abstract

Novel drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral (ARV) drug therapy, thereby improving the management of patients with HIV/AIDS. This paper provides a comprehensive review of the various ARV delivery systems that have been developed for achieving sustained drug release kinetics, specifically targeting drugs to the macrophages, brain and gastric mucosa, and for addressing formulation difficulties such as poor solubility, stability and drug entrapment. Studies on the potential of systems for alternative routes of ARV drug administration, i.e., transdermal, buccal and rectal are also highlighted. The physico-chemical properties and the in vitro/in vivo performances of various systems such as sustained release tablets, ceramic implants, nanoparticles, nanocontainers, liposomes, emulsomes, aspasomes, microemulsions, nanopowders and Pheroid™ are summarised. Further studies that remain to be undertaken for formulation optimisation are also identified. This review highlights the significant potential that novel drug delivery systems have for the future effective treatment of HIV/AIDS patients on ARV drug therapy.

KEYWORDS:

HIV/AIDS, Antiretroviral drugs, Novel drug delivery systems, Sustained release, Targeting.

1. Introduction to HIV/AIDS

Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS), commonly referred to as HIV/AIDS, constitute one of the most serious infectious disease challenges to public health globally, and has had a crippling effect in certain parts of the world especially Sub-Saharan Africa [1-3]. There are currently 33.2 million people living with HIV/AIDS globally. Of this total number, an overwhelming 22.5 million people are HIV positive in Sub-Saharan Africa specifically, representing 67.8% of the global number [3]. Interventions such as AIDS counselling, educational tools and antiretroviral drug therapy have contributed to transforming HIV infection from a fatal to a manageable chronic infectious disease [4]. Despite the availability of these measures, the above statistics indicate that much remains to be accomplished as the number of newly reported HIV infections still remains unacceptably high.

There are currently two known species of HIV, viz., HIV-1 and HIV-2, with their respective subspecies. HIV-1 is the globally common infection while HIV-2 is more prevalent in West Africa, and takes a longer time to develop into immunodeficiency from infection than HIV-1 [5, 6]. HIV infection in the human body results mainly from integration of the viral genome into the host cell for the purpose of cell replication, and AIDS is the advanced stage of the disease caused by HIV infection. The virus infects the host cell by binding of the viral gp120 protein to two transmembrane receptors, i.e., CD4+ and either of the

two chemokine receptors, CCR5 and CXCR4 [7]. HIV infects macrophages and T-helper lymphocytes (CD4+); but the defining feature of AIDS is the depletion of CD4+ cells. T-tropic viruses prefer to replicate in T cells while M-tropic viruses prefer the macrophage. Of the HIV-1 viruses, M tropic types predominate in the brain [8].

The viral genome contains 3 structural genes – gag, pol and env and six regulatory genes –tat, rev, nef, vif, vpr, and vpu [5]. The virus utilizes some of these genes to maximize its production using host cell resources. DNA microarray studies have implicated HIV encoded Nef protein in this process [9], and humans infected with the nef-deleted form of HIV have remained disease free for several years [10]. Interestingly, HIV has been referred to as a "master regulator" of cellular gene expression [9] as a means to augment expression of its own genome. An understanding of these processes is critical to developing novel therapeutic strategies for the suppression or elimination of the virus.

The immunopathogenesis of HIV/AIDS has been previously amply documented; from the time of infection to the end stage of the disease [5]. The end stage of the disease may be characterised by a spectrum of diseases [11] including opportunistic infections (such as Pnuemocystis carinii and Mycobacteruim tuberculosis), dementia and cancer [6, 11]. In addition to macrophages, lymph nodes, bone marrow, spleen and lungs, the CNS represents one of the most important anatomical sites of the virus after infection. This causes significant neuronal damage and loss that often leads to

HIV associated dementia [12]. Without treatment, HIV 1 infection is nearly uniformly fatal within 5-10 years [11].

2. HIV/AIDS Drug Therapy and its Current Limitations

Although the development of drugs for HIV infection has undergone substantial progress, numerous uncertainties persist about the best way to manage this disease. Reports addressing this aspect have appeared in the literature [13]. At present, the different ARVs are classified under categories such as nucleoside reverse transcriptase inhibitors (NRTI), nucleotide reverse transcriptase inhibitors (NtRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), and more recently fusion and integrase inhibitors [14]. Table 1 [15-19] lists the various drugs under the different classes, the available dosage forms as well as their half-lives and bioavailabilities. These drugs are administered as combined therapy as in the case of Highly Active Antiretroviral Therapy (HAART) [20]. Among the newer classes of drugs under investigation are the assembly and budding inhibitors [21], as well as the zinc finger inhibitors [22]. Virus assembly and disassembly are particularly attractive candidate processes for antiviral intervention. HIV-1 capsid (CA) protein and human cyclophilin A (CypA) play important roles in these processes, which consequently make them attractive targets of high priority [23].

Table 1: Examples of antiretroviral drugs, their commercially available dosage forms, bioavailabilities and half-lives.

Name and Class of Drug	Dosage form [15-19]	F (%)* [15-19]	Half-Life (hrs) [15-19]
Zidovudine(NRTI)	Capsule, Liquid	60	1.1
Lamivudine(NRTI)	Tablet, Liquid	86	3-6
Didanosine (NRTI)	Tablet, Capsule (EC), Liquid	30-40	1.3-1.6
Zalcitabine(NRTI)	Tablet	85	1-3
Stavudine(NRTI)	Capsule, Powder for reconstitution	80	1-1.6
Abacavir(NRTI)	Tablet, Liquid	83-100	1-2
Emtricitabine(NRTI)	Capsule	93	10
Tenofovir NtRTI	Tablet	25-39	17
Nevirapine (NNRTI)	Tablet, Syrup	>90	25-30
Efavirenz(NNRTI)	Tablet, Capsule, Solution	42-80	40-50
Delavirdine(NNRTI)	Tablet	85	5.8
Etravirine (NNRTI)	Tablet	unknown	30-40
Amprenavir(PI)	Capsule, Solution	No data.	7-10
Indinivir(PI)	Capsule	65	1.2-2
Saquinavir(PI)	Tablet, Capsule	Erratic, 4	1.5-2
Nelfinavir(PI)	Tablet, Powder	20-80	3.5-5
Ritonavir (PI)	Tablet, Capsule, Liquid	65	3-5
Atazanavir(PI)	Capsule	No data	7
Darunavir(PI)	Tablet	37	15
Enfuvirtide (Entry and FI)	Powder for subcutaneous injection	84.3	3.8
Maraviroc (Entry and FI)	Tablet	23-33	14-18
Raltegravir (II)	Tablet	No data	9

NRTI=Nucleoside Reverse Transcriptase Inhibitors

NtRTI=Nucleotide Reverse Transcriptase Inhibitors

NNRTI= Non-nucleoside Reverse Transcriptase Inhibitors

PI=Protease Inhibitors

FI=Fusion Inhibitors

II=Integrase Inhibitors

F=Bioavailibility

EC = Enteric Coated

Although ARV drug therapy has contributed significantly to improved patient/disease management, its current use is associated with several disadvantages and inconveniences to the HIV/AIDS patient. Many ARV drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. The half-life for several ARV drugs is short, which then requires frequent administration of doses leading to decreased patient compliance [24]. A major limitation is that HIV is localised in certain inaccessible compartments of the body such as the CNS, the lymphatic system and within the macrophages. These sites cannot be accessed by the majority of drugs in the therapeutic concentrations required; and the drugs also cannot be maintained for the necessary duration at the site of HIV localization [25]. These sub therapeutic drug concentrations and short residence time at the required sites of action contribute significantly to both the failure of eliminating HIV from these reservoirs, as well as the development of multidrug-resistance against the ARVs [26]. The severe side effects associated with ARV therapy can therefore be attributed to the subsequent large doses essential for achieving a therapeutic effect, due to the inadequate drug concentrations at the site of action, and/or the poor bioavailability of several ARV drugs. These drugs also suffer from physico-chemical problems such as poor solubility that may lead to formulation difficulties [27, 28]. Strategies currently being investigated to overcome these limitations include; the identification of new and chemical modification of existing chemical entities, the examination of various dosing regimens, as well as the design and

development of novel drug delivery systems (NDDS) that can improve the efficacy of both existing and new ARV drugs. More specifically, in the past decade there has been an explosion of interest in the development of NDDS for the incorporation of ARV drugs as a way of circumventing the problems described above and optimising the treatment of HIV/AIDS patients. To the best of our knowledge, the last review paper on NDDS for ARV drugs appeared in 1993 [28]. There have since been significant advancements of the systems described in that paper and further new NDDS for ARV drugs have since emerged in the literature. The purpose of this paper is therefore to present a comprehensive review of the various NDDS, including studies on alternative routes of administration that have emerged for ARV drugs. This will identify the progress that has been achieved both for the technological development of these delivery systems, as well as their clinical potential for overcoming the limitations associated with current ARV therapy. This review will also enable the identification of future studies that remain to be undertaken for its optimisation and ultimately its commercialisation.

3. Novel Drug Delivery Systems for ARV Drugs

3.1 Sustained Release/Bioadhesive/Enteric Coated Matrix Tablets

Sustained drug delivery systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include: minimisation of drug related side effects due to controlled therapeutic blood levels instead of oscillating blood levels, improved patient compliance due to

reduced frequency of dosing and a reduction of the total dose of drug administered [29, 30]. Bioadhesive drug delivery systems are designed for prolonged retention on the mucosa to facilitate drug absorption over a prolonged period of time by interacting with mucin [31]. Hence, the combination of both sustained release and bioadhesive properties in a delivery system would further enhance therapeutic efficacy. ARVs such as didanosine (ddI) would be an ideal candidate for sustained drug release due to its short half life of 1.3-1.6 hours, necessitating frequent administration of doses, as well as its severe dose dependent side effects [24]. In an attempt to improve the oral absorption of ddI by delivering it over a prolonged period of time as well as prolonging retention on the mucosae, Betageri et al. [32] prepared a sustained release bioadhesive tablet formulation of ddI, containing Polyox WSRN-303, Carbopol 974P-NF and Methocel K4M as polymeric matrix materials. Hydrogel forming tablet formulations with 10% and 30% Polyox WSRN-303 were able to extend the release of ddI (Figure 1) while 30% Methocel K4M was required for extending the drug release in other formulations. Preparations with Carbopol 934P prevented complete release of ddI from the tablet during the test period and the authors attributed this to drug-polymer interactions. The bioadhesivity also increased with an increase in polymer concentration. These researchers concluded that a single polymer could be used for the preparation of hydrogel matrix ddI tablets designed to provide both sustained release and bioadhesivity. However, while a single polymer may provide both bioadhesivity and sustained drug release, it has since become well recognised in the literature, via various in vitro drug release and bioadhesivity tests during

formulation studies, that simultaneous optimisation of both these properties may require the blending of various polymers [33-35] for both single and multiple unit systems. These systems remain to be investigated for their clinical applicability.

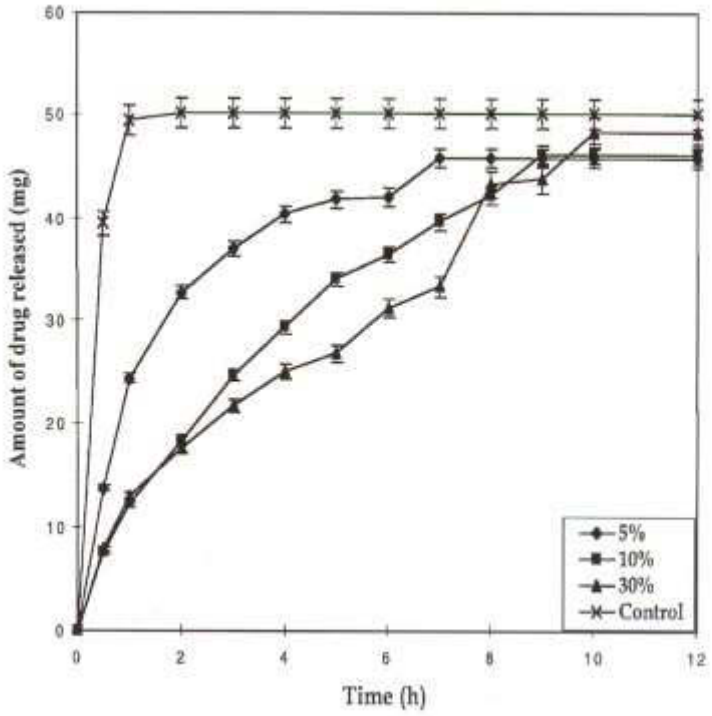

Figure 1: Effect of Polyox WSRN-303 on the release of ddI from tablets (Reproduced from Betageri et al., 2001).

ddI controlled release matrix tablets containing methacrylic (Eudragit RSPM) and ethylcellulose (Ethocel 100) polymers have also been prepared by Sanchez-Lafuente et al. [36]. The ddI 500 mg tablets (5, 10 or 15 %w/w) were prepared by direct compression and comprised of Eudragit® RSPM and Ethocel® 100 in varying ratios (75/25, 50/50 and 25/75 w/w). The physical

characteristics in terms of weight, thickness and diameter confirmed the excellent compactibility properties of these polymers with ddI, which allowed for direct compression in the absence of other excipients. The drug release studies showed that varying polymer ratios could modulate the release of ddI as a result of the swelling properties of Eudragit® RSPM and plastic properties of the hydrophobic Ethocel® 100. Since these two polymers showed potential for modulating drug release, the subsequent study by this group focused on the use of a statistical experimental design for formulation optimisation as well as for identifying and quantifying the effects of formulation variables on drug release. Therefore, a Doehlert design was applied to evaluate the influence of variables and possible interactions among such variables on ddI release from the directly compressed matrix tablets based on the blends of the two insoluble polymers, Eudragit® RSPM and Ethocel® 100 [37]. The drug content and the polymers had the most significant effect on drug release while the compression force had no significant effect. The optimum formulation conditions identified in the studied experimental design for a formulation with optimum drug release was Eudragit-Ethocel ratio of 83/17 (w/w) and a drug content of 13 %w/w. The experimental values obtained from the optimised formulation highly agreed with the predicted values, thereby validating the mathematical model used in the preparation of ddI tablets.

ddI also undergoes acid degradation in the gastric medium [38]. An enteric coated matrix tablet formulation that combines sustained drug release, bioadhesivity and an enteric coating to resist acid degradation to maximise

therapeutic efficacy has also been reported. Deshmukh et al. [39] reported the preparation of enteric coated, sustained release bioadhesive matrix tablets of ddI comprising of Polyox, WSRN-303 and Methocel K4M with hydroxypropylmethylcellulose phthalate (HPMCP 5.5). The formulation was shown to be resistant to dissolution in 0.1N HCl but dissolved within 10 minutes in PBS pH 7.4. Furthermore, the stability of the formulation for 6 months at varying storage conditions was confirmed. Permeation studies on the matrix tablets showed that Polyox WSRN-303 containing tablets demonstrated higher ddI permeability across live intestinal tissue compared with conventional tablets.

While the above tablets sought to provide sustained drug release, bioadhesion and resistance to gastric acid degradation, a possible limitation could be the fact that it would still undergo extensive first pass degradation since it is meant for oral administration.

3.2 Ceramic Implants

Attempts have been made in the literature to explore the use of ceramic implants to modulate the release of antiretroviral drugs. Due to the adverse effects of AZT associated with oral and intravenous administration, Benghuzzi et al. [40] in early in vivo studies investigated the release of deoxynucleoside thymidine, the normal counterpart of azidothymidine (AZT), by means of alumino-calcium-phosphorous oxide (ALCAP) ceramic implantable capsules in rats. The results showed that thymidine could be released from the ALCAP

ceramic capsules in a sustained manner for a minimum duration of 120 days. Based on the results with thymidine, they subsequently concluded that these implantable capsules could be considered for the delivery of AZT. Consequently, in a follow up study [41], AZT was loaded into tricalcium phosphate (TCP) and ALCAP ceramic capsules. They showed that the rate of release of AZT from TCP capsules were lower than from ALCAP capsules. Figure 2 confirms the sustained release of AZT from TCP ceramic capsules over 26 days when loaded with 20, 40 and 60 mg AZT.

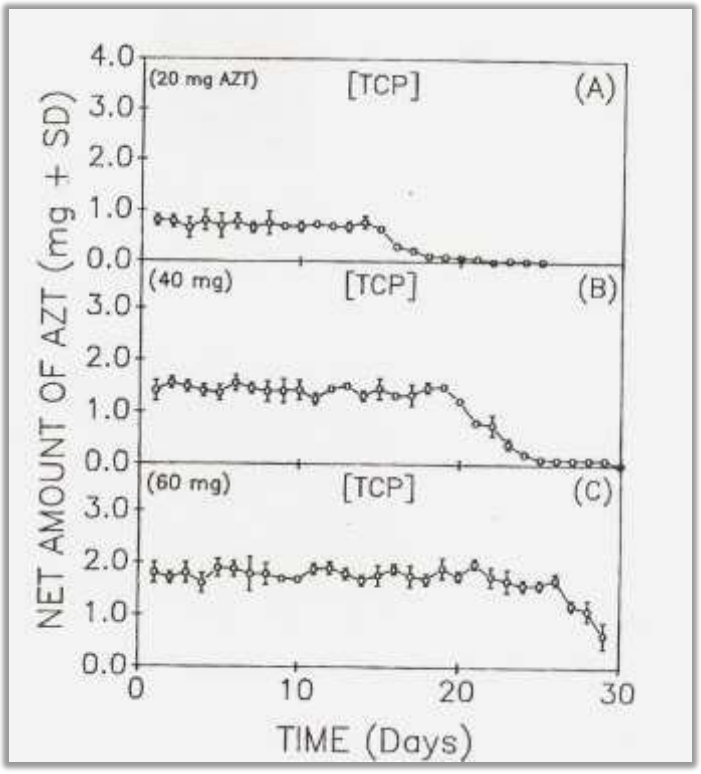

Figure 2: Release of AZT from TCP ceramic capsules (Reproduced from Benghuzzi et al., 1990)

To further control release, Nagy and Bajpai [42] extended this in vitro study by preparing a TCP ceramic delivery system containing thymidine and AZT and determining the effect of sesame seed oil or wheat germ oil on their release. Ceramic capsules were prepared by pressing 1 gram of <38 microns beta-TCP particles with or without the stipulated quantity of thymidine or AZT in a 10 mm die at a load of 4000 lbs in an electric hydraulic press. They found that sesame seed oil and wheat germ oil (Vitamin E) could delay the release of thymdine and AZT from TCP drug loaded capsules. Further, incorporation of thymidine or AZT in the form of a compressed pellet also retarded its release from the TCP ceramic capsules prepared with oil treated ceramic particles. The above studies were extended to an in vivo study later [43]. Three ceramic devices were implanted subcutaneously in Sprague Dawley rats for two weeks. The in vivo studies showed that oil saturated TCP and AZT device as well as the AZT pellet inserted in an oil saturated TCP shell device were able to retard AZT release at a significantly lower rate than the AZT and TCP untreated devices. These authors concluded that the treatment of ceramic devices with oil decreased the release rate and prolonged the delivery of AZT. The inclusion of wheat germ into another ceramic device, hydroxyapatite (HA) composite, was also able to deliver AZT for prolonged periods in vitro [44].

A subsequent in vivo study by Benghuzzi [45] compared the release of AZT from two commonly studied ceramic implants, i.e., TCP and HA. Sterilised drug loaded ceramics containing AZT in three dosages (40, 60 and 90 mg) were inserted under the skin of rats using standard surgical techniques. The data

from this study showed that AZT release rates from TCP ceramic implants (30 mg=2.38±0.23 ng/mL, 60 mg=4.64±1.03 ng/mL and 90 mg=11.92±2.35 ng/mL serum AZT) were significantly higher than from HA ceramic implants (30 mg=0.84±0.05 ng/mL, 60 mg=2.40±0.83 ng/mL and 90 mg=6.41±1.24 ng/mL serum AZT). The authors concluded that TCP and HA ceramic implants could be considered effective for delivering AZT in quantities required for providing physiological responses in vivo. The sustained drug release profiles obtained indicated that large fluctuations of AZT concentrations in the blood stream and tissues, as with conventional routes of administration, could be eliminated using ceramic drug delivery systems.

While ceramic implants were actively studied between 1990 and 2000, there appears to be no further work since reported for ARV containing ceramic implants.

3.3 Liposomes

Liposomes, ranging in size between 25 nm and several microns, are microscopic vesicles that comprise one or more phospholipid bilayers which surround an aqueous core. They are prepared from natural or synthetic phospholipids and cholesterol and may also additionally include other lipids and proteins. The aqueous core facilitates the entrapment of hydrophilic drugs, while hydrophobic drugs are bound to or incorporated in the lipid bilayer. When administered, liposomes are recognised as being foreign and are immediately taken up by cells of the mononuclear phagocytic system (MPS). Since the HIV

virus localises in these cells, liposomes therefore represent a suitable drug delivery system for targeting ARVs into infected cells; and thus have the potential of improving the efficacy of drugs and reducing side effects [46, 47, 48].

The effect of liposomal encapsulation of AZT in mice was determined in early studies [49-50]. Unlike injections of free AZT, liposomal encapsulated AZT showed no bone marrow toxicity with normal erythrocyte and leukocyte profiles. Also, enhanced localisation in the liver, spleen and lung was found with the AZT liposomes. Liposomal encapsulated AZT further reduced haematopoietic toxicity and resulted in enhanced antiretroviral activity in mice. Liposomal formulations have also been prepared for administration of AZT by the transdermal route [51]. The optimised liposomal formulation showed a transdermal flux of 98.8±5.8 μg/cm^2 across rat skin as compared to 5.72±0.3 μg /cm^2 for the free drug and this should contribute to an improved bioavailability. These liposomes for the transdermal route were also able to target the RES organs more effectively.

Liposomes containing ddI was initially studied by Harvie et al. [52]. They found that the elimination plasma half life of 112 nm and 83 nm liposomal ddI was 46 and 14 times higher than that of the free drug, respectively. They also reported efficient targeting of lymph nodes and macrophage–rich tissue with these conventional liposomes. In a subsequent study, they were able to extend further the ddI half life in plasma from 3.9 hours for conventional liposomes to

14.5 hours by incorporating it into sterically stabilized liposomes. Following intravenous injection, the majority of the sterically stabilised liposomes also concentrated in the spleen with a peak level at 24 hours (Figure 3) [53].

Apart from AZT and ddI, zalcitabine (ddC) has also been investigated for encapsulation into liposomes by Makabi-Panzu et al. [54-55]. The ddC loaded liposomes were more rapidly taken up by the mouse macrophage cell line than the free ddC. They also reported that a high intracellular uptake of ddC was facilitated by the anionic nature of liposomes. To be pharmacologically active, dideoxynucleosides such as ddC must be phosphorylated into 5'-triphosphates by cellular kinases. Since some cell types have a low ability to phosphorylate these compounds, administration of the phosphorylated form of the drug would be most suitable. However, this would not be feasible as cell membranes are impermeable to the phosphorylated form, and phosphatases present in body fluids hydrolyse nucleotides into the corresponding nucleosides [56]. To overcome this limitation and to obtain site specific delivery, the antiviral effects of ddC and ddC-triphosphate(ddC-TP) and liposome encapsulated ddCTP (L(ddCTP)) were established and compared in cultured, human monocyte macrophages infected with HIV-1 [57]. ddCTP was dephosphorylated before entering the cells while L(ddCTP) remained stable over days. These preparations were also able to inhibit replication at nanomolar drug levels. Data obtained from liposome encapsulated ddCTP in a murine acquired immunodeficiency syndrome (MAIDS) model has also showed reduced proviral DNA in cells of the MPS in both spleen and bone marrow [58].

Liposomes have also been explored for the encapsulation and delivery of newly synthesised prodrugs. Lalanne et al. [59] synthesized two novel glycerolipidic ddI conjugates as prodrugs to avoid hepatic first pass metabolism. Liposomal formulations (1160±nm) of the prodrugs displayed antiviral activity and showed promise as formulations for enhancing drug bioavailability. Due to the low entrapment efficiency and high leakage of AZT from liposomes [48], AZT-myristate (AZT-M) has been synthesized as a prodrug and investigated for its potential for liposomal encapsulation. A high entrapment efficiency of 98% was achieved with higher plasma AZT being achieved with the AZT-M liposomes as compared to free AZT solution. Higher concentrations of AZT in organs of the RES and brain were also found with the liposomal preparation. This study could have been enhanced if AZT-M liposome preparations were compared not only with free AZT, but also with AZT entrapped liposomes. Prodrug liposomal preparations therefore offer the opportunity of not only more efficient targeting but also improved drug action and formulation processing.

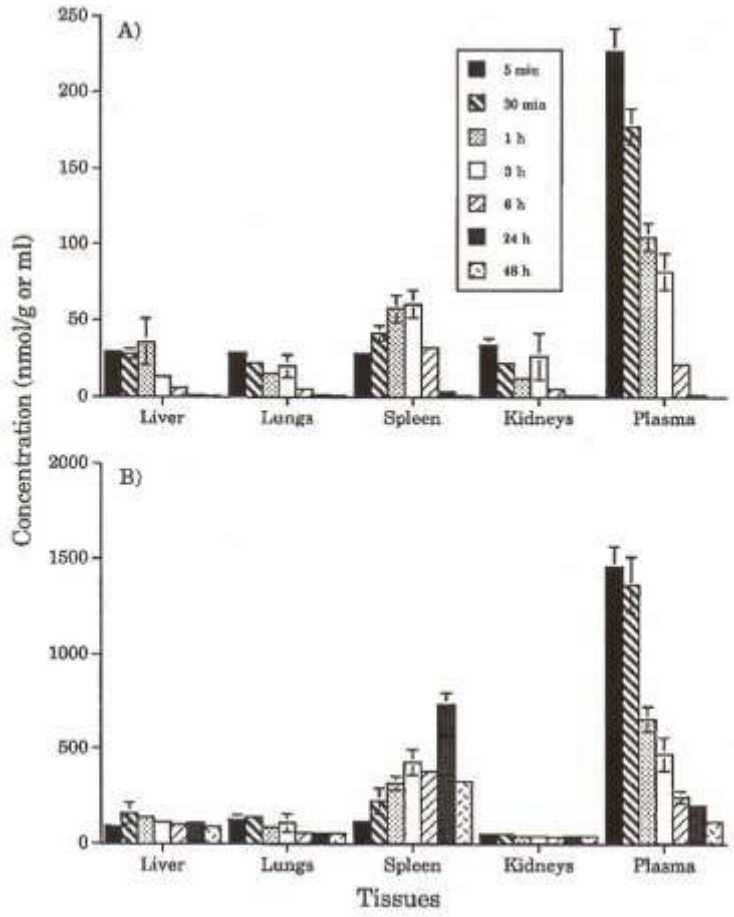

Figure 3: Plasma and tissue distribution of ddI ([^{3}H]ddI) (A) and liposomal lipids ([^{14}C]DPPC) (B) from sterically stabilized liposome-encapsulated ddI after the administration of a single intravenous dose (3 mg of ddI per kg) to rats. Values are the means obtained for four to six animals per group per time point (Reproduced from Harvie et al., 1996)

In addition to liposomes having PEG chains attached to its surface, for increasing circulation time in vivo [60, 61], active targeting of HIV infected cells can also be obtained by using liposomes that have surface attached ligands that specifically promote receptor interaction at the site of targeting [47] as well. Using the antibody, H-2-K(k), for Fc-mediated targeting; Betageri and

Burrell [62] showed that the lipid composition of ddI-triphosphate liposomes influenced conjugation of antibodies and also retention of the encapsulated drug. Sterically stabilised immunoliposomes containing grafted anti-HLA-DR antibodies were effective in enhancing the concentrations of indinavir (INV) in all tissues leading to a 21-126 fold increased accumulation as compared to the unencapsulated drug (Table 2) [63]. Also, immunoliposomal INV was as efficient as the free agent to inhibit HIV-1 replication in cultured cells. Lectin receptors, which act as molecular targets for sugar molecules, are found on the surface of cells of the mononuclear macrophage system (MPS) and have also been included in the strategy to improve site specific drug delivery. Using a mannose binding protein, concanavalin A, maximum cellular drug uptake occurred when mannosylated liposomes containing stavudine (D4T) were used [64]. Other sugar molecules used for liposomal formulations to target cells of the MPS include galactosylated D4T and AZT liposomes [65-66]. Together, these studies confirmed enhanced targeting to tissues rich in galactose specific receptors and confirmed their potential of providing sustained drug release characteristics. Slepushkin et al. [67] has also reported that synthetic peptides can bind specifically to HIV infected cells. The potential of various ligands for active targeting of ARV loaded liposomes have therefore been confirmed and show potential for formulation optimisation.

Table 2: Area under the curve for free and immunoliposomal indinavir in tissues after a single subcutaneous administration in mice (Reproduced from Gagne et al., 2002).

Tissue	Immunoliposomal indinavir	Free indinavir	Ratio immuno-liposomal/ free indinavir
Cervical lymph nodes	523.2	7.6	68.8
Brachial lymph nodes	617	4.9	126.0
Mesenteric lymph nodes	192.8	6.4	30.1
Iguinal lymph nodes	144.5	4.1	35.2
Popliteal lymph nodes	134.2	4.5	29.8
Liver	733.3	35.0	21.0
Spleen	211.3	5.3	39.9
Plasma	77.8	2.3	33.8

In addition to targeting liposomes to the phagocytic system, other areas in the body have also been of interest. Kompella et al. [68] evaluated the effect of neutral liposomes on corneal and conjuctival permeability of ddI. While the liposomal formulations were able to encapsulate ddI and permeate through the rabbit conjuctivial mucosa, the permeability coefficient, initial flux and tissue levels of ddI at the end of the transport study were actually lower in the presence of liposomal formulations. These neutral liposomes failed to enhance the corneal or conjuctival transport or uptake of ddI.

One of the disadvantages of liposomes is poor stability in terms of drug retention and poor encapsulation. When assessing the stability of ARVs incorporated into liposomes, Betageri [69] found that lipid composition influenced encapsulation and retention of ddI-triphosphate (ddITP); and that its retention in the DMPC:CHOL liposomes was maximum when stored at 4 °C.

A novel liposomal formulation, i.e., “emulsomes” for sustained and targeted delivery of AZT to the liver has recently been described by Vyas et al. [25]. Emulsomes are a novel lipoidal vesicular system with an internal solid fat core surrounded by a phospholipid bilayer. In addition to demonstrating a retarded drug release profile (12-15% after 24 hours), studies in rats showed better uptake of the emulsomal formulations by the liver cells. We agree with the researchers that this proposed cationic emulsome-based system shows excellent potential for intracellular hepatic targeting.

Liposomes have clearly been more extensively investigated for their in vitro and in vivo properties than other NDDS for ARV delivery. A greater number of drugs and prodrugs have been encapsulated and additional formulation optimisation techniques and in vivo evaluations have been undertaken. These studies highlight and underscore the potential benefits of liposomes for improving ARV drug therapy.

3.4 Nanoparticles

Drug encapsulated nanoparticles are solid colloidal particles that range from 10-1000 nm in size [70]. Based on their size and polymeric composition, they are able to target drug to specified sites in the body and have also shown potential for sustained drug delivery [71]. Nanoparticles have also been explored for improving the formulation and efficacy of drugs with physicochemical problems such as poor solubility and stability [72]. They are being increasingly investigated for targeted delivery of ARVs to HIV infected cells and to achieve sustained drug release kinetics. Their encapsulation into such systems may provide improved efficacy, decreased drug resistance, a reduction in dosage, a decrease in systemic toxicity and side effects, and an improvement in patient compliance.

Cells of the mononuclear phagocytic system (MPS), such as the monocytes/macrophages (Mo/Mac), act as a reservoir for the HIV virus [73]. Therefore, drug treatment of HIV infection should involve targeting drugs to these cells in addition to the lymphocytes. Several studies involving ARV loaded nanoparticles for targeting to the macrophages have consequently emerged. In an early preliminary study, Schafer et al. [74] prepared AZT loaded polyalkycyanoacrylate (PACA), polymethylmethacrylate (PMMA) and human serum albumin (HSA) nanoparticles. This study confirmed uptake of the nanoparticles into macrophages isolated from HIV infected patients. The same group also later prepared and confirmed the potential of human serum albumin

and poly(hexylcyanoacrylate) nanoparticles loaded with the nucleoside analogues, AZT and ddC for the targeting of macrophages. These in vitro studies were also undertaken using macrophages isolated from the peripheral blood of healthy blood donors and transmission electron microscopy [75]. Saquinavir (SQN) and ddC, have also been loaded into poly(hexylcyanoacrylate) nanoparticles [76] by emulsion polymerization. While ddC showed no superiority to an aqueous solution of the drug in terms of reducing the HIV-1 antigen production, a significantly higher efficacy was observed for SQN loaded nanoparticles as compared to its aqueous solution. An in vivo study in rats to investigate the oral delivery of AZT bound to hexylcyanoacrylate nanoparticles for delivery to the reticuloendothelial cells was undertaken by Löbenberg, Araujo, and Kreuter [77]. The area under the curve (AUC) of [^{14}C] AZT in the liver was 30% higher when the drug was bound to nanoparticles than after administration of the solution. Higher AZT levels were also found in the blood and brain when nanoparticles were used as compared to the control solution. In an in vivo study a year later using the intravenous route instead, they showed that AZT concentrations were up to 18 times higher in organs of the RES if the drug was bound to nanoparticles as compared to unbound AZT [78]. Surface modification of nanoparticulate systems with hydrophilic groups such as polyethylene glycol has been shown to influence the biodistribution of nanoparticles [79]. Using THP-1 human monocyte /macrophage (Mo/Mac) cell line, Shah and Amiji [80] showed that a significantly higher percentage of the administered dose of nanoparticles was internalized within the cells when SQN was incorporated into poly(ethylene

oxide)-modified poly (epsilon-caprolactone) nanoparticles (200 nm). Also, intracellular SQN concentrations were significantly higher when administered in the surface modified nanoparticles as compared to its aqueous solution. A possible limitation of this study is that while aqueous solutions of SQN were compared to SQN PEG modified nanoparticles, a comparative study with surface unmodified SQN nanoparticles was not performed. This would have provided greater insight to the contribution of PEG specifically for ARV delivery. Most recently, the uptake of AZT loaded poly(lactic acid)-poly(ethylene glycol) nanoparticles by polymorphonuclear leucocytes in vitro was shown to be dependant on PEG and its ratio in the polymer [81].

Since the HIV virus can migrate to, multiply and localise in the CNS causing several neurological disorders, targeting of ARV drugs to the brain has become a significant goal for drug therapy. The blood brain barrier (BBB) prevents access of ARVs to the brain due to the tight endothelial cell junctions of the brain capillaries and the presence of efflux transporters on the cell surface [81]. Nanoparticulate systems promote drug delivery in the brain, since they may gain entry by means of endocytosis/phagocytosis and are also moved away from the vicinity of efflux pumps [82, 83]. Kuo [84] therefore loaded D4T into polybutylcyanoacrylate (PBCA) and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) nanoparticles for brain targeting. Drug loading of the nanoparticles (59.5-149.2 nm) was inversely proportional to particle size and was also affected by freeze-drying and preservation as it influenced particle size. Similar to other studies [85], they also found pH to be

critical, since variation in pH value of the loading medium from pH 7.2 led to a reduction in the loading efficiency of D4T. Kuo and Chen [86] then evaluated the effects of size of PBCA and MMA-SPM nanoparticles and alcohol on the permeability of AZT and lamivudine (3TC) across the BBB using blood brain-microvascular endothelial cells model (BMEC). Both loading efficiency and permeability of AZT and 3TC decreased with an increase in the particle size of the two polymeric carriers. While PBCA nanoparticles increased the BBB permeability of AZT and 3TC 8-20 and 10-18 fold respectively, the MMA-SPM nanoparticles led to a significant 100% increase in the BBB permeability of both drugs. A 4-12% enhancement in the BBB permeability of the two drugs with 0.5% ethanol was attributed to temporary unfolding of tight junctions among BMECs upon treatment with alcohol. In a subsequent paper, these authors compared the transport of D4T, delaviridine (DLV) and SQV across the in vitro BBB using (PBCA), (MMA-SPM) and also solid lipid nanoparticles (SLNs) [83]. These various polymeric systems investigated enhanced permeability of the drugs with higher permeabilities being reported with smaller particle sizes. In their most recent paper, Kuo and Kuo [87] showed that exposure to an electromagnetic field (EMF) could further enhance drug permeability across the BBB. The potential of SLNs for targeted brain delivery of another ARV, atazanavir, has also recently been confirmed [88].

More recently, a novel approach was proposed by Dou et al. [89, 90]. They postulated that the mononuclear phagocytes, as the principal reservoir for viral dissemination, could also serve as a transporter of antiretroviral drugs

themselves, since they are responsible for dissemination of HIV, i.e., macrophages can enter into tissues that limit entry of many ARV drugs. In these two papers, they describe a macrophage based nanoparticulate system as a carrier itself for indinavir (INV). A nanoparticle indinavir (NP-INV) formulation was prepared and packaged into bone marrow-derived macrophages (BMMs). The effects of this drug carrier on drug distribution and disease outcomes were assessed in immune competent and human immunodeficiency virus type 1 (HIV-1) infected humanised immune-deficient mice [89]. Significant lung, liver and spleen BMMs and drug distribution were observed. This initial study also reported reduced numbers of virus infected cells in plasma, lymph nodes, spleen, liver and lung as well as CD4(+) T-cell protection when the NP-IDV BMMs were administered to HIV-1 challenged humanised mice. Later, a similar NP-INV formulation was prepared with Lipoid E80 [90]. They reported sustained drug release from the macrophages. The administration of NP-INV, when compared to equal drug levels of free soluble INV, also significantly blocked induction of multinucleated giant cells, production of reverse transcriptase activity in culture fluids and cell associated HIV-lp24 antigens after HIV-1 infection. This study proved that use of a macrophage based NP delivery system has potential for the treatment of HIV-1 infections.

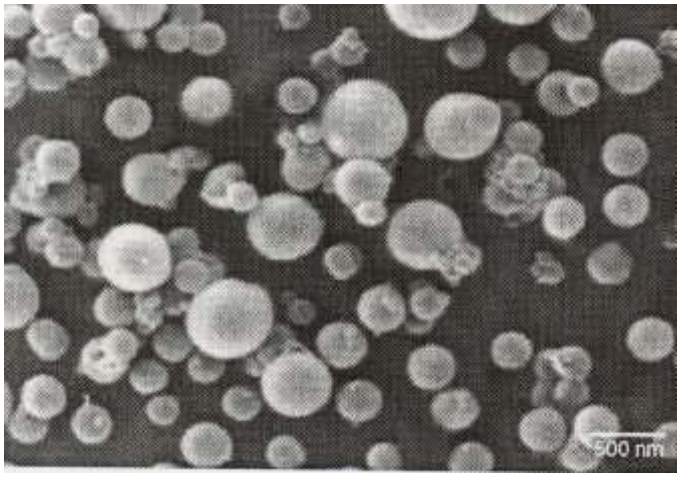

Figure 4: Scanning electron micrograph of ddI loaded mannose coupled gelatin nanoparticles (x30000) (Reproduced from Jain et al., 2008).

The use of ligands on nanoparticles for receptor mediated targeting has just been reported in the literature [91-92]. Since macrophages contain various receptors such as mannosyl, galactosyl and others, Jain et al [91] prepared mannosylated gelatine nanoparticles (MN-G-NP) (248-325 nm) (Figure 4) with a drug encapsulation of 40.2-48.5%. Via fluorescence and ex vivo studies using alveolar macrophages from rats, they showed a 18.0 and 2.7 times higher uptake by the macrophages from MN-G-NPs as compared to the free drug and uncoated G-NPs (Figure 5).

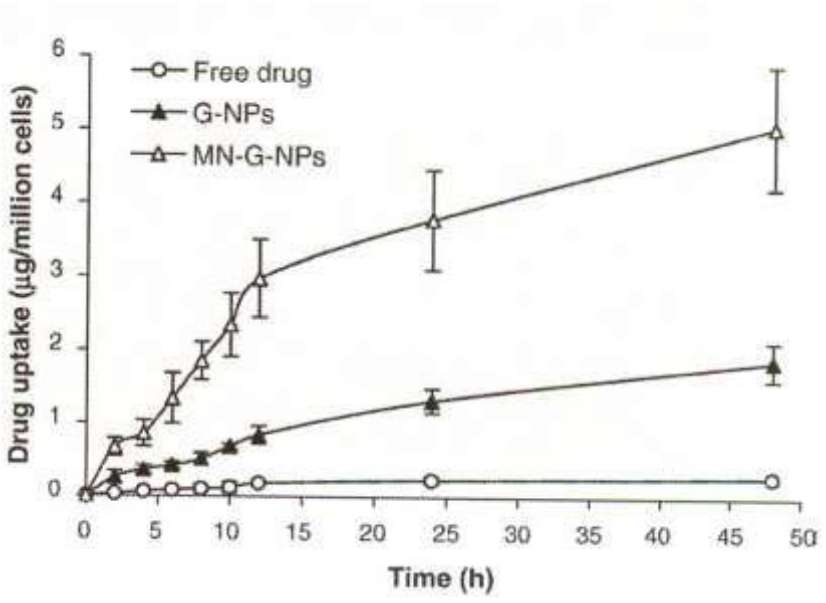

Figure 5: Drug uptake from ddI containing mannosylated gelatin nanoparticles by alveolar macrophages at different time points at 37±2°C (Reproduced from Jain et al., 2008).

The use of nanoparticles for targeting other areas, such as the gastrointestinal mucosa and associated lymphoid tissues has also been reported by Dembri et al. [93]. As compared to the drug solution, AZT loaded isohexylcyanoacrylate nanoparticles were able to efficiently concentrate AZT in the intestinal mucosa. They also found that the nanoparticles were also able to control the release of free AZT.

Solid lipid nanoparticles (SLNs) are prepared from lipids that remain in a solid state at room and body temperature. Heiati et al. [94] initially prepared SLNs consisting of AZT-palmitate (AZT-P) and trilaurin (TL) as the solid core with dipalmitoylphosphatidylcholine (DPPC), and a mixture of DPPC and dimyristoylphosphatidylglycerol (DMPG). Their study concluded that the

loading of AZT-P was proportional to the concentration of phospholipids content and was independent of the amount of trilaurin used. Phospholipids with transition temperatures below 37°C increased drug release. In a subsequent study, coating the SLNs with a PEG layer on its surface further increased the levels of AZT in the blood, since PEG creates a steric barrier that reduces particle uptake, thereby prolonging circulation [95]. They also found that the SLN-PEG nanoparticles were able to decrease the drug release rate in plasma as compared to SLN particles without PEG. The studies by this research group confirmed that surface modification with PEG could be used for controlling drug release and the pharmacokinetic behaviour of SLNs.

While the majority of studies have focused on targeted delivery of ARVs with nanoparticles, some studies have also focused on modifications to its preparation to enhance drug loading and decrease toxicity; and also to increase its absorption by facilitating pH-sensitive drug release. Boudad et al. [96] prepared SQN loaded poly(alkylcyanoacrylate) nanoparticles and showed that incorporation of cyclodextrins enhanced the entrapment of SQN. Studies on the Caco-2 cell line showed that incorporation of cyclodextrins with nanoparticles decreased cytotoxicity when compared to blank and SQN loaded nanoparticles. The ability of cyclodextrins to mask to some extent the cytotoxic effects of the aliphatic alcohols originating from the hydrolytic degradation of the polymers was proposed as a possible reason for this effect. The oral bioavailability of a poorly water soluble HIV-1 protease inhibitor (CGP 70726-Novartis) was also enhanced when incorporated into pH sensitive

nanoparticles prepared from poly(methacrylic acid-co-ethacrylate) copolymer Eudragit L100-55 [72].

The surge of interest in nanoparticulate systems for ARV therapy has led to several drugs being studied for its incorporation. These in vitro/in vivo studies clearly confirm the ability of nanoparticles to enhance the therapeutic efficacy of ARVs, as well as, addressing formulation problems.

3.5 Nanocontainers

Dendrimer based systems have also been explored for the concept of ARV targeting. Dendrimers are characterized as being synthetic, highly branched, spherical monodispersed macromolecules. Due to their unique architecture and macromolecular characteristics, they have emerged as an important class of drug carrier for targeted delivery [97-98]. Hence, not surprisingly, they have just been reported for targeting of ARV drugs. Recently, Dutta et al. [99] prepared poly(propyleneimine) (PPI) dendrimer based nanocontainers for targeting of efavirenz (EFV) to Mo/Mac. Fifth generation PPI dendrimer, t-Boc-lycine conjugated PPI dendrimer (TPPI) and mannose conjugated dendrimers (MPPI) were synthesized and used to prepare “nanocontainers”. Like a dendritic box, these molecules act as closed containers of nanoscopic size containing the entrapped drug and are therefore called nanocontainers. The drug entrapment efficiency of the nanocontainers varied, with the mannose conjugated dendrimer being 47.4%, followed by that of the PPI dendrimer (32.15%) and t-Boc-glycine conjugated dendrimer (23.1%). While the PPI

dendrimer released the drug by 24 hours, the dendrimer based nanocontainers of t-Boc glycine and mannose conjugated dendrimers prolonged the release rate up to 144 hours. The authors found significant increase in cellular uptake of EFV by Mo/Mac with nanocontainers of the mannose conjugated dendrimer being 12 times higher than that of free drug and 5.5 times higher than those of t-Boc-glycine conjugated dendrimer. Further, PPI showed a very high toxicity on HEPG2 cells while TPPI and MPPI had negligible toxicity (Figure 6). These differences were attributed to the free terminal amino groups in PPI which is masked in MPPI and TPPI. This study therefore showed that mannosylated PPI dendrimers could be an effective carrier system for targeted delivery of EFV and possibly other ARVs.

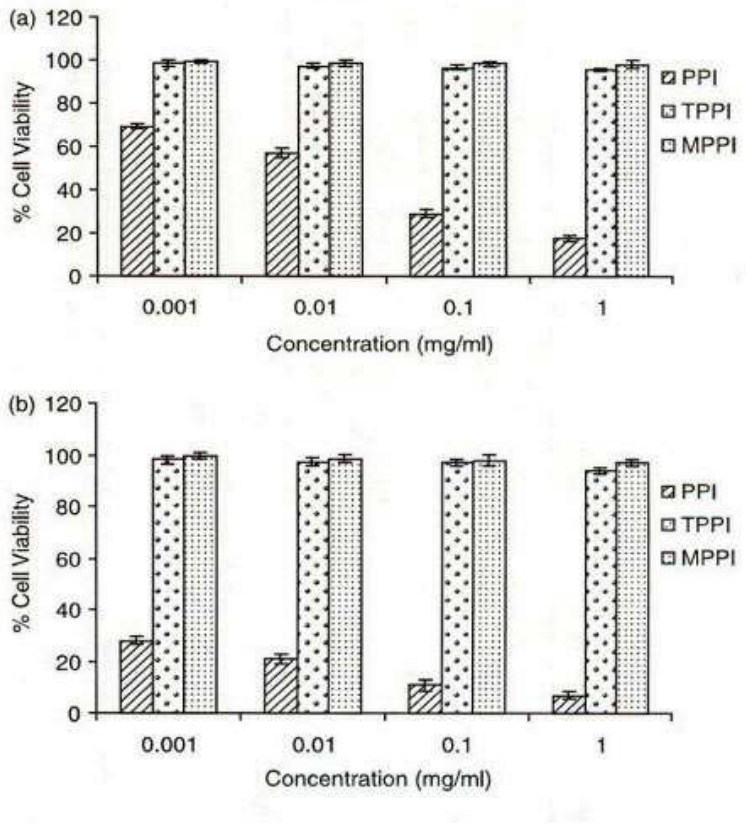

Figure 6: Cytotoxicity of poly(propyleneimine) (PPI) dendrimer and its nanocontainers, t-Boc-lycine conjugated PPI dendrimer (TPPI) and mannose conjugated dendrimers (MPPI) (a) after 24 h and (b) after 48 h of incubation for targeting of efavirenz (EFV) to Mo/Mac. (values = mean ±SD, n=3) (Reproduced from Dutta et al., 2007).

3.6 Micelles and Microemulsions

Microemulsions have been studied for ARV drug delivery as an approach to redirect the absorption of ARV from the portal blood to the HIV-rich intestinal lymphatics, thus enhancing the bioavailability of drugs that undergo extensive first-pass metabolism and have poor oral bioavailability. Three formulations of SQN containing oleic acid have been studied [100] for targeted intestinal lymphatic transport using rats as the in vivo model: cremophor-oleic acid mixed

micelles, D-Alpha tocopheryl polyethylene glycol 1000 succinate (TPGS)-oleic acid mixed micelles and an oleic acid microemulsion. The extent of lymphatic transport from the lipid vehicles was 0.025-0.5% of the dose administered. The microemulsion generated higher and more prolonged mesenteric lymph concentrations than the micellar formulations (Figure 7). The systemic bioavailability was estimated to be 8.5% and 4.8% for the cremophor mixed micelle and the microemulsion, respectively. Since the cremophor mixed micelles produced higher bioavailability than TPGS mixed micelles, the researchers concluded that the nature of the surfactant can influence biodistribution of the drug between lymph and plasma.

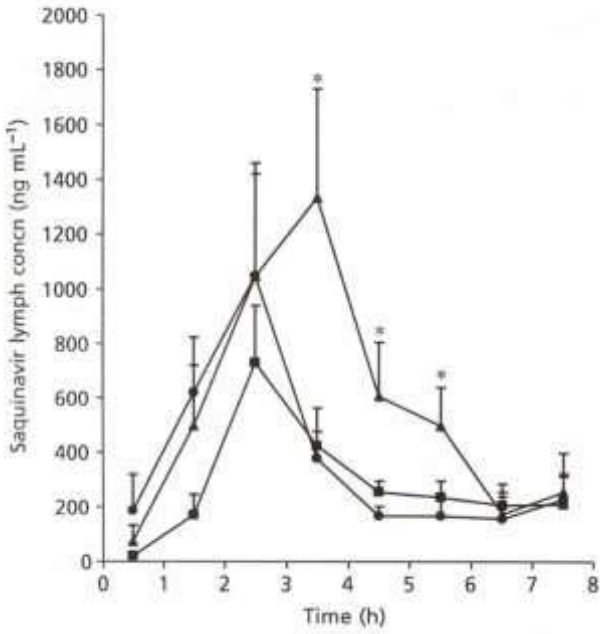

Figure 7: Concentration of SQN in intestinal lymph versus time (Mean ±S.E., n≥5). SQN (5 mg) was administered intraduodenally to anaesthetized rats in a cremophor-oleic acid mixed micellar formation (closed circle), a TPGS-oleic acid mixed micellar formulation (closed circle) or as an oleic acid microemulsion (closed triangle) (Reproduced from Griffin and O'Driscoll, 2006).

3.7 Nanopowders

Most recently, nanopowders have been used as a delivery system for oral administration to enhance the dissolution rates of poorly soluble drugs. Tween 80/poloxamer 188 stabilised nanosuspensions of the hydrophobic ARV, loviride, were prepared by media milling, and sucrose co-freeze dried to obtain solid nanopowders [101]. Morphological characterisation showed plate like structures in the nanopowder which was different from the morphology of untreated loviride crystals (Figure 8) Loviride showed higher dissolution rates in nanosized products than in their respective physical mixtures, i.e., the amount of drug released after 15 minutes was 104.2% for the nanopowder prepared from freeze drying with sucrose, 58% for the freeze dried nanosuspension without sucrose, 54.8% for the physical mixture containing sucrose, 14.5 % for the physical mixture without sucrose and 64.7% for the pure untreated loviride (Figure 9). The addition of sucrose also further enhanced the dissolution rates. Caco-2 experiments revealed a significantly higher transport of loviride from the nanopowder formulation as compared to the physical mixture and the untreated loviride. Nanopowders were able to increase the dissolution rate due to its high surface area while sucrose had an additional enhancing effect due to its disintegrant properties.

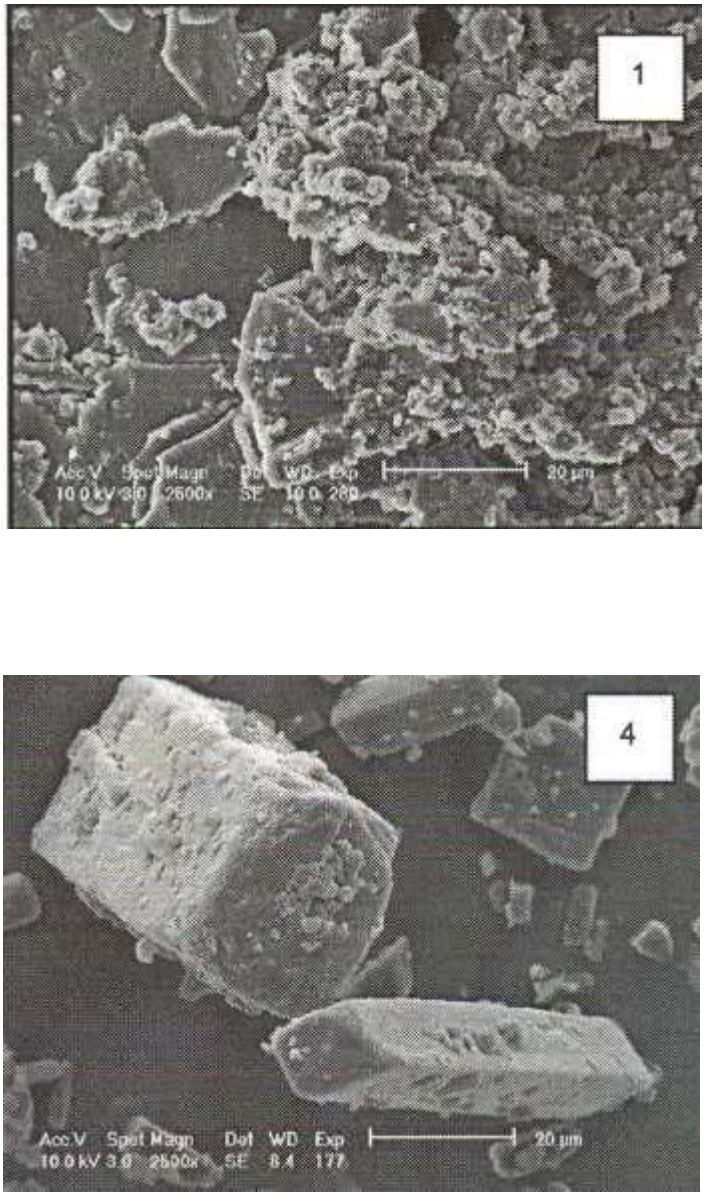

Figure 8: Scanning electron micrographs of 1) nanopowder and 4) untreated loviride crystals (Reproduced from Van Eerdenbrugh et al., 2007).

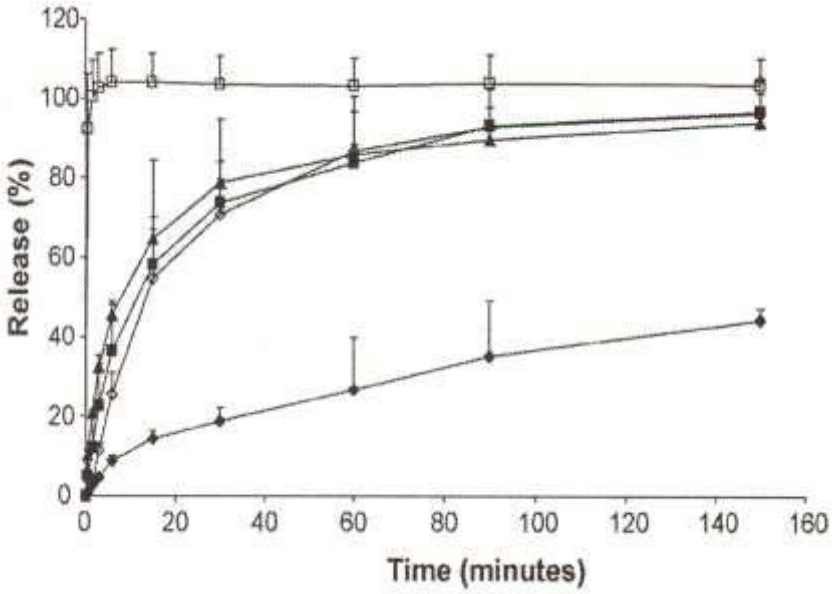

Figure 9: Dissolution profiles: freeze-dried nanosuspension without sucrose (open diamond), physical mixture without sucrose (closed diamond), nanopowder (Open Square), physical mixture with sucrose (closed square), and untreated loviride (closed triangle). (Reproduced from Van Eerdenbrugh et al., 2007).

3.8 Suspensions

Since studies with INV in HIV positive patients have indicated that drug concentrations in lymph node mononuclear cells were about 25-35% of mononuclear cells in blood, in a proof of concept study, Kinman et al. [102] showed that association of INV with lipids could enhance localisation in lymphoid tissues and also reduce the viral load. This was accomplished by preparing lipid associated complexes in suspension for subcutaneous injection to HIV-2287-infected macaques. They showed that INV concentrations in both

peripheral and visceral lymph nodes were 250-2270% higher than plasma as compared with <35% with soluble lipid-free drug administration in humans. Also, administration of the INV-lipid complexes reduced significantly the viral RNA load and increased CD4 T cell number concentrations (Figure 10).

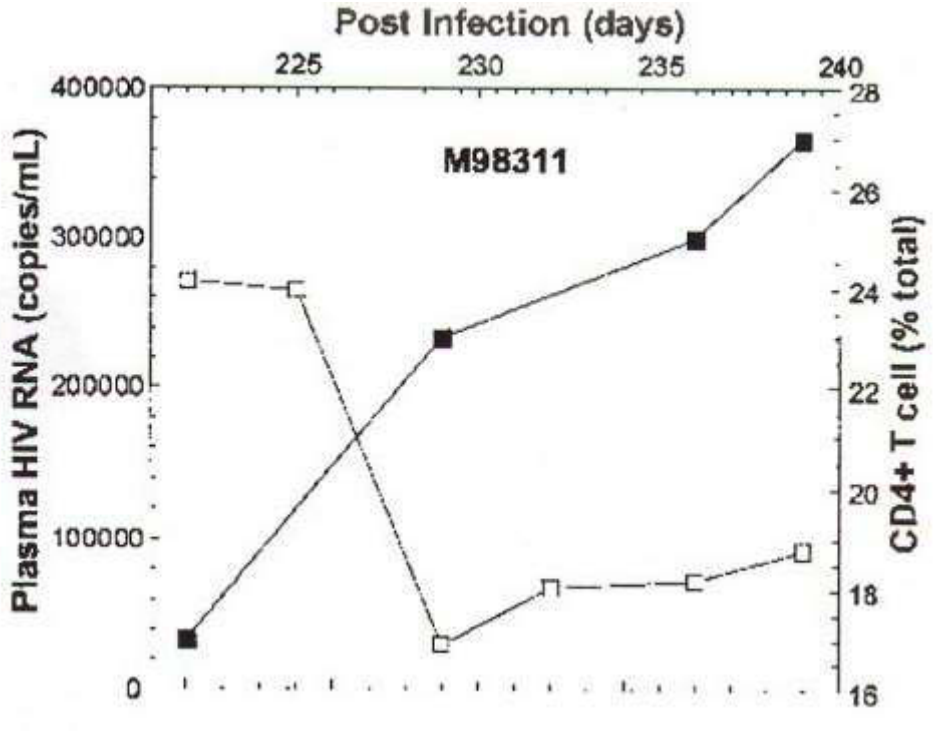

Figure 10: Changes in plasma viral load of one HIV-2_{287}-infected macaques at 25 weeks postinfection and treated with 10 daily 20-mg/kg (SC) doses of lipid-associated indinavir over 14 days. (Reproduced from Kinman et al., 2003).

3.9 Transdermal delivery

The advantages offered by drug administration via the transdermal route include; avoidance of first pass effect and/or GI degradation, reduced fluctuations in plasma drug concentrations, excellent targeting of the drug for

local effect as well as improved patient compliance [103, 104]. The potential of ARVs for transdermal administration has therefore been extensively reported. The various transdermal permeation studies with ARV drugs specifically in terms of the focus/foci of the particular investigation and main outcomes of the study are summarised in Table 3. The most commonly investigated drug thus far for transdermal delivery has been AZT, although there are some studies that have also investigated ddC and ddI for transdermal delivery. One of the limitations of transdermal delivery of drugs is poor skin/percutaneous penetration/absorption of drugs. Hence, the majority of ARV transdermal studies have focused on permeation enhancement investigating, inter alia, various chemical enhancers, type of vehicles (solvents/cosolvents), as well as iontophoresis and anodal current application. Table 3 identifies specifically the various penetration enhancers and vehicles that have been specifically investigated thus far. These various permeation enhancement variables either alone or in combination have been found to be beneficial in promoting ARV drug permeation through the skin.

In addition to comparative permeation enhancement studies with drug solutions, some studies have developed and evaluated transdermal delivery systems of an ARV drug. Gels containing AZT [105, 106] and AZT patches using a gum matrix [107, 108] have been developed. Both were found to be capable of facilitating ARV permeation and the gel formulations were also found to be more stable than drug solutions. One of the first vesicular carriers to be studied for transdermal delivery of AZT was aspasomes [109]. These are

vesicles formed from ascorbyl palmitate (ASP) in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). Figure 11 shows that aspasomal AZT (ASP-AZT) was able to significantly enhance transdermal permeation of drug as compared to the AZT solution. Although lower than ASP-AZT, the higher drug permeation of ASP-AZT dispersion as compared to AZT free drug solution showed that ascorbyl palmitate had skin permeation enhancing properties. An elastic liposomal formulation of AZT has also enhanced transdermal flux, provided sustained drug release and improved site specificity of the drug [51]. Pheroid™ is a patented submicron emulsion which has been shown to entrap, transport and deliver several pharmacological compounds for enhanced therapeutic action [110, 111]. Pheroid™ comprises essential and plant fatty acids, i.e., ethyl esters of the essential fatty acids, oleic, linolenic and linoleic acids, which are emulsified in water and saturated with nitrous oxide. As shown in Table 2, oleic acid is an effective permeation enhancer due to its kinked structure that briefly disrupts the packed formation of the intercellular lipids [112]. Recently, the use of Pheroid ™ was investigated for its potential to enhance the transdermal permeation of ddC, 3TC and several N-acyl lamivudine esters [113]. However, while the drugs were shown to be entrapped in the Pheroid™, the transdermal flux of the drugs in Pheroid™ was lower than in PBS. Hence, the Pheroid™ delivery system showed no practical advantage in terms of its transdermal application.

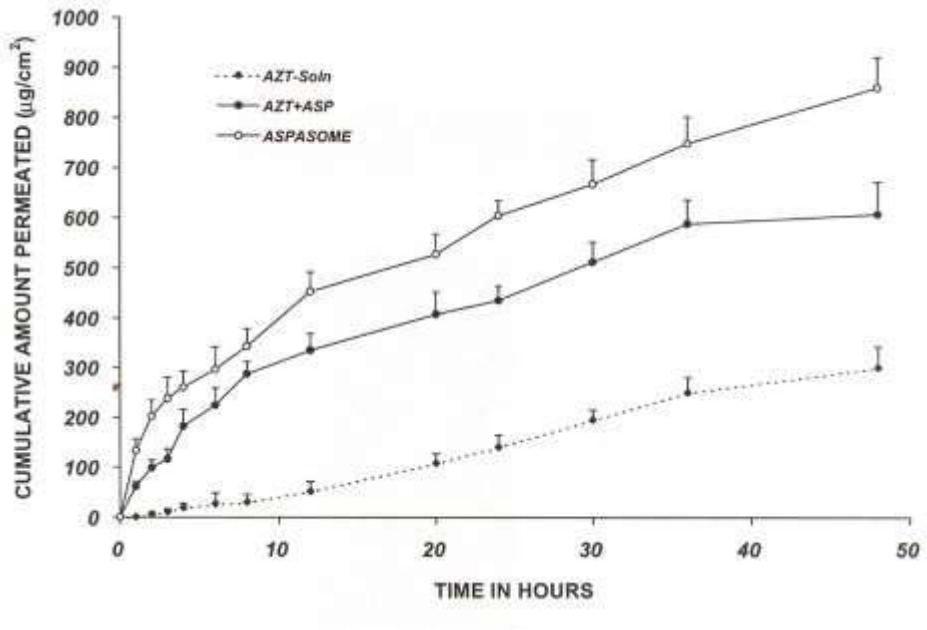

Figure 11: In vitro permeation profiles of AZT across excised rat skin following treatment with various systems i.e. aspasomal AZT (ASPASOME); AZT-ASP dispersion (AZT+ASP), free AZT solution (AZT-Soln) (Reproduced from Gopinath et al., 2004).

The various transdermal delivery studies with drugs such as ddI, ddC and AZT using various animal models such as the skin of rat, mouse, pig and human cadaver have confirmed the potential of ARV drugs for transdermal delivery.

3.10 Buccal delivery

Delivery of drugs via the buccal mucosa has received increased attention in the literature as an attractive alternative to the traditional oral and other conventional routes of drug administration. Use of the buccal mucosal route presents several advantages, such as the bypass of first pass hepatic

metabolism and avoidance of gastrointestinal enzymatic degradation, thereby increasing the bioavailability of drugs [114]; higher permeability than that of the other routes such as the skin [115]; larger surface area for drug application, and good accessibility compared to other mucosal surfaces such as nasal, rectal and vaginal mucosa [116]. ARV drugs may therefore benefit from buccal mucosal administration instead of traditional oral administration.

Studies investigating the feasibility of the systemic buccal delivery of anti-HIV drugs have emerged. Shojaei et al. [117] initially investigated the use of a safe and effective permeation enhancer, i.e., menthol, on the buccal permeation of ddC. This study showed that the in vitro transbuccal permeation of ddC increased significantly in the presence of 1-menthol with an enhancement factor of 2.02 and a t_{lag} of 6 hours. The permeation enhancement was not concentration dependent as no significant difference was observed between the permeation enhancement of ddC in the presence of 0.1, 0.2, and 0.3 mg/mL of 1-menthol [117]. Later, Xiang et al. [27] also studied the feasibility of transbuccal delivery of ddC using McIlvaine buffer solution (IMB). Their study focused on identifying the major permeation barrier within the epithelium of the buccal mucosa, the influence of sodium glycodeoxycholate (GDC) as a permeation enhancer as well as the histological effects of ddC on the buccal mucosa. These researchers reported that the basal lamina layer within the epithelium of buccal mucosa acted as an important barrier to the permeation of ddC. They also found that the permeability of ddC was significantly enhanced by GDC up to 32 times (Figure 12). Histological studies revealed

that the basal lamina remained intact and no nucleated cell leakage was found within 24 hours. These studies also showed that the thickness of epithelium was greatly reduced after buccal tissues were immersed in IMB solution for 12 and 24 hours, and no difference was observed between the tissue samples incubated in the IMB and ddC IMP solution. These two research groups concluded that transbuccal delivery is a potential route of administration of ddC, and hence for enhancing antiretroviral drug therapy.

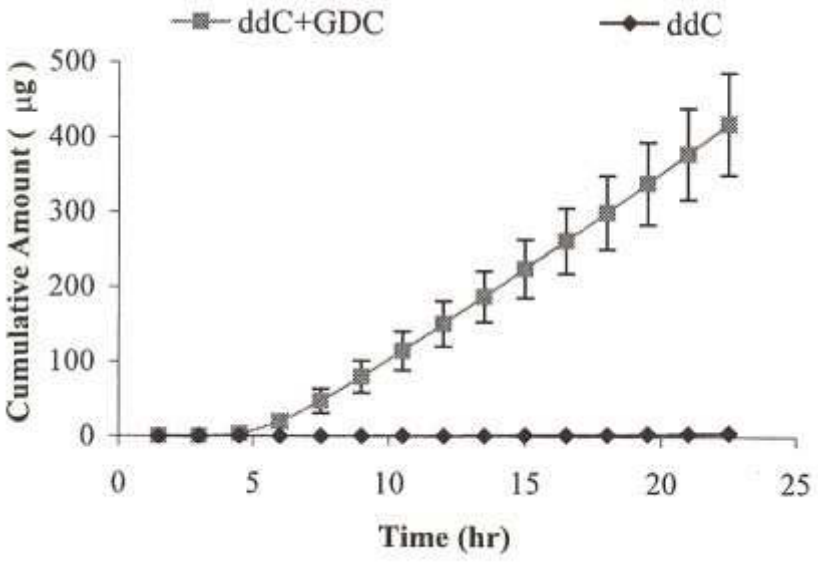

Figure 12: Cumulative amount of ddC permeating through the porcine buccal mucosa without GDC (closed triangle) and with co-administration of GDC (closed square). Data are presented as means±S.D. (n=3) (Reproduced from Xiang et al., 2002).

Unlike the transdermal route, the buccal route for ARV permeation potential has not been comprehensively investigated. The reported studies to date have focused only on 2 different permeation enhancers and no studies on the formulation and assessment of buccal delivery systems of ARVs could be found.

3.11 Rectal delivery

The rectal route has also been considered for effective delivery of ARV drugs that undergo first pass hepatic metabolism and/or extensive GI degradation. Two studies were found to have been reported in the literature. Sustained-release AZT suppositories were prepared [118] using hydroxypropyl cellulose (HPC) and assessed in rats. It was found that AZT suppositories at 10 mg/kg maintained constant plasma levels above 1 μM for more than 6 hours and they subsequently proposed suppositories as an alternative drug delivery system for AZT (Figure 13).

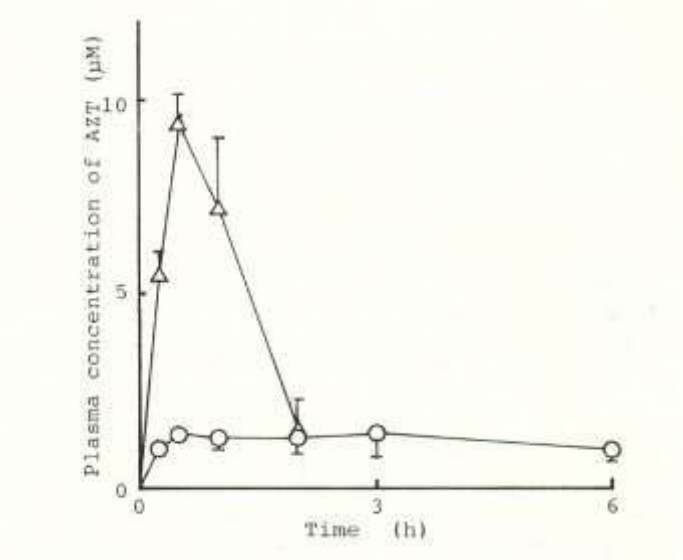

Figure 13: Plasma concentration-time profiles following the administration of AZT suppositories: conventional (open triangle) and sustained release (open circle) (Reproduced from Kawaguchi et al., 1991).

A further study of rectal administration of AZT [119] showed that the drug was considerably absorbed after rectal administration, with a pharmacokinetic profile that resembled that of a sustained-release delivery device No further studies on this approach have since been identified in the literature. The work in this area appears to be limited, most probably due to patient inconvenience, as well as the fact that HIV/AIDS patients often suffer from diarrhoea.

Table 3: Summary of transdermal delivery studies on ARVs.

ARV Drugs	Focus/Foci of study	Summary of main findings	Reference
AZT	Investigated effect of N-methyl-2-pyrrolidone (MP) as a penetration enhancer and ethylene-vinyl acetate copolymer membrane for controlled-release.	Permeation of AZT was significantly enhanced and plasma concentration of AZT maintained for 10 hours after the application of MP controlled - release transdermal system.	[120]
ddI	Explored transfollicular absorption route for ddI and investigated effect of penetration enhancers, i.e. azone and propylene glycol. Determined the pharmacokinetics of ddI after topical application.	Systemic bioavailability in high and low follicular density rats was similar indicating unimportant role of the transfollicular route for ddI. Transdermal delivery of ddI exceeded the oral bioavailability and was further increased by pre-treatment with absorption enhancers.	[121]
AZT	Investigated the effect of t-anethole, carvacrol, thymol, linalool and L-menthol. Determined the *in vivo* performance of AZT gel formulation.	Transport of AZT was optimum with 5% enhancer concentrations. *In vitro* studies produced higher amount and rate of AZT transport than *in vivo* studies.	[106]
ddC ddI AZT	Determined stability profiles of drugs in solution when in contact with hairless rat skin and identified the degradation mechanisms of ddC and ddI.	AZT was found to be stable for 30 hours at 37^{o}C. ddC and ddI degraded by bacterial and ddI by cutaneous enzyme- degradation mechanisms. ddC was stabilized with thimerosal or gentamicin, while ddI was stabilized with para-chloromercuricbenzoic acid	[122]

ddC	Investigated the effects of ethanol/water and ethanol/tricaprylin cosolvents and other permeation enhancers such as oleic acid and N-methyl-2-pyrrolidone.	Permeation rate across human cadaver skin was significantly lower than across hairless rat skin. Enhancement of ddC permeation using 1 %v/v of oleic acid in ethanol/water (60:40) cosolvent was 4-5 times higher than target rate of 0.14mg/cm^2/h to maintain the therapeutic blood level.	[123]
AZT	Determined drug release from AZT patches made from Karaya gum through excised hairless mouse skin and also investigated the effect of enhancers.	Thickness of gum matrix and enhancers such as propylene glycol, oleic acid, and sodium dodecyl sulphate influenced drug release from patches. Permeation was best enhanced with propylene glycol/oleic acid/sodium dodecyl sulfate ternary system.	[108]
ddC ddI AZT	Investigated effects of ethanol/water and ethanol/tricaprylin as cosolvent systems and oleic acid as permeation enhancer on permeation rate of each of the drugs alone.	Permeation rates of AZT, ddC and ddI increased with ethanol/water and ethanol/tricaprylin cosolvent systems. Addition of oleic acid to the ethanol/water system enhanced permeation but did not with the ethanol/tricaprylin system. Permeation rates reached the target for required therapeutic levels with ethanol/water (60:40) containing oleic acid at 1.0 %v/v	[124]
DdC DdI AZT	Investigated effects of ethanol/water and ethanol/tricaprylin as cosolvent systems and oleic acid as permeation enhancer on the simultaneous skin permeation of the 3 drugs together using hairless rat skin.	Permeation rates of AZT, ddC and ddI increased with ethanol/water and ethanol/tricaprylin. Addition of oleic acid in ethanol/water (80:20) significantly increased permeation but not in the ethanol/tricaprylin (50:50) solvent.	[125]

ddC ddI AZT	Compared the skin permeation rates of ddC, ddI and AZT, alone or in combination with various compositions of ethanol/water and ethanol/tricaprylin cosolvent systems, across human cadaver and rat skins.	Human cadaver skin permeation rates of the drugs alone, or in combination were lower than the rat skin. The addition of oleic acid at 0.3 – 1% v/v increased permeation rate of all three drugs. 5 % v/v oleic acid increased permeation rate of ddC and ddI in combination and saturated in ethanol/water (80:20).	[126]
ddC ddI AZT	Compared permeation rates of drugs. Permeation enhancing effects of ethanol/water systems and oleic acid were investigated.	Permeation increased as volume fraction of ethanol increased. For ddC, ddI and AZT, addition of oleic acid (>2.0%w/v) in ethanol/water (70:30) further enhanced skin permeation rate. Enhancement for hydrophilic drugs was greater than for lipophilic drugs.	[127]
AZT	Investigated transdermal flux of AZT using iontophoresis and propylene glycol/oleic acid. Effect of flux enhancement by iontophoresis was also investigated using a karaya gum matrix formulation of AZT and compared with AZT solution.	Enhancement of transdermal flux by iontophoresis was smaller with the karaya gum matrix containing AZT. The iontophoretic flux from AZT solution increased about 4-5 fold. Penetration enhancers increased the passive flux 2-50 fold and worked synergistically with iontophoresis.	[107]

AZT	Investigated permeation of AZT using penetration enhancers such as: menthol, cineole, linolenic acid, oleic acid, in combinations of cineole or menthol with either oleic acid or linolenic acid or anodal current application.	Permeability enhancing properties of the penetration enhancers were in the order of linolenic acid > menthol > oleic acid > cineole > vehicle. Combination of cineole and oleic acid enhanced permeation. Simultaneous application of current with menthol and cineole significantly increased AZT permeation.	[128]
AZT	Compared permeation of a AZT gel formulation including penetration enhancers (menthol and oleic acid) with solutions.	Gel formulation was found to be more stable than solutions. There was no retardation in permeability of AZT in the gel formulation across the rat skin compared to the AZT solution. Combination of penetration enhancers at 2.5% w/w enhanced permeation.	[105]
AZT	Investigated effects of binary vehicles [ethanol/water; isopropyl alcohol/water; polyethylene glycol/water; and ethanol/isopropyl myristate (IPM)], penetration enhancers [N-methyl-2-pyrrolidone (NMP); oleic acid; and lauric acid] and polymer [microporous polyethylene (PE) membrane] on permeation.	Ethanol/IPM (50/50, v/v) demonstrated highest transdermal flux. Use of vehicle and enhancer combinations (ethanol/IPM 20/80 plus 10% NMP and ethanol/IPM 30/70 plus 10% NMP) resulted in increased AZT solubility as well as high AZT flux values, when compared to vehicles without enhancers.	[129]
AZT	Investigated permeation of AZT across human cadaver skin and the effect of terpenes [L-menthol and 1, 8-cineole] on phase behavior and molecular organization of a model Stratum Corneum (SC) lipid system.	Terpenes enhanced permeation of AZT by transforming SC lipids from a highly ordered orthorhombic perpendicular subcellular packing to a less ordered hexagonal subcell packing. Terpenes caused disruption / alteration in the barrier property of SC and enhanced permeation of AZT more than ethanol and water.	[130]

AZT	Evaluated the formation and transdermal permeation properties of aspasomes containing AZT.	Proportion of cholesterol affected drug release rate with maximum retardation achieved with 45 mol% of cholesterol. Aspasomes had better antioxidant activity than ascorbic acid. Asposomal AZT enhanced transdermal permeation of the drug.	[115]
AZT	Evaluated use of elastic liposomes for transdermal delivery of AZT	Elastic liposomes enhanced transdermal flux, provided sustained drug release and improved site specificity of AZT.	[51]
3TC ddC N-acyl-3TC esters	Determined the *in vitro* transdermal permeation of ddC, 3TC and synthesized 3TC esters through human epidermis with or without Pheroid™ as drug delivery system	Drugs with higher aqueous solubilities displayed greater transdermal flux values both in PBS and Pheroid™ .Transdermal flux values of drugs in Pheroid™ were lower than in PBS.	[113]

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4. Conclusions and Future Studies

Despite significant advances that have been made in understanding the mechanism of HIV infection and identifying effective treatment approaches, the search for optimum treatment strategies for AIDS still remains a major challenge. Results presented in this review indicate that novel drug delivery systems clearly present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral drug therapy. The use of such systems began in the early 1990's but it is only within the past 5 years that there appears to be a sudden surge of interest and publications in the use of novel drug delivery systems for ARV drugs. While several novel drug delivery systems have been investigated for ARV delivery, recently there appears to be

greater interest and advancement in the use of liposomes and nanoparticles as compared to other systems. While the clinical potential for several NDDS have been reported from in vitro and animal studies, there is a lack of data on formulation optimisation and detailed physico-chemical/mechanical characterisation of these NDDS. Since HIV/AIDS treatment involves combination drug therapy, the potential of these novel drug delivery systems for simultaneous loading of various drug combinations needs to be investigated. While the potential of alternate routes of ARV drug administration such as transdermal and buccal has been confirmed, the design and development of drug delivery systems for these routes specifically are currently lacking. Correlations between the performances of these systems with their permeation potential need to be established. Although various papers report efficacy studies under in vitro conditions including experimental animal studies, there is a significant lack of data on the clinical applicability (human in vivo studies) and toxicity of these preparations. These therefore need to be extensively explored. Based on the complexity of the disease and the formulation optimisation and evaluation studies required, multidisciplinary research would be essential for eventual commercialisation of NDDS containing ARV drugs.

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CHAPTER THREE

PUBLISHED PAPER

3.1 INTRODUCTION

The following paper was published in an international peer reviewed ISI journal and reports the original research on data generated during this study.

E. Ojewole, I. Mackraj, K. Akhundov, J. Hamman, A. Viljoen, E. Olivier, J. Wesley-Smith and T. Govender Investigating the effect of Aloe vera gel on the buccal permeability of didanosine. **Planta Medica** 2012; 78(4): 354-361.

E. Ojewole contributed to the design of the project, was responsible for the excision and preparation of the buccal mucosae for the permeation and histomorphological studies. She performed the in vitro permeation experiments, the final viscosity determinations and the interpretation of the data. She undertook histological studies and prepared samples for LM and TEM evaluations. Furthermore, she contributed to the overall interpretation of the results as well as the writing of the manuscript. J. Wesley-Smith assisted with the LM and TEM work, the evaluations and interpretations of both the photo-micrographs and eletro-micrographs as well as assisted with the writing of the manuscript. I. Mackraj assisted with the identification and harvesting of the buccal mucosa from the pigs, and K. Akhundov demonstrated and assisted with the surgical removal of the excessive connective tissue from the mucosae.

J. Hamman and A. Viljoen donated Aloe vera gel for the permeability enhancement studies and assisted in writing the manuscript. E. Olivier assisted with the initial viscosity experiments.

This chapter is presented in the required format by the journal and is in the final revised and accepted version, published in the Planta Medica.

This research article has been cited 6 times according to cited counts reported by the Web of Science core collection, and 2 times cited counts according to the BIOSIS citation Index, accessed 02/11/2014.

3.2. PUBLISHED PAPER

Investigating the effect of *Aloe vera* gel on the buccal permeability of didanosine

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ABSTRACT

The buccal mucosal route offers several advantages but the delivery of certain drugs can be limited by low membrane permeability. This study investigated the buccal permeability properties of didanosine (ddI) and assessed the potential of *Aloe vera gel* (AVgel) as a novel buccal permeation enhancer. Permeation studies were performed using Franz diffusion cells and drug was quantified by UV spectroscopy. Histomorphological evaluations were undertaken using light and transmission electron microscopy. The permeability of ddI was concentration-dependent and it did not have any adverse effects on the buccal mucosae. A linear relationship (R^2 = 0.9557) between the concentrations and flux indicated passive diffusion as the mechanism of drug transport. AVgel at concentrations of 0.25 to 2 %w/v enhanced ddI permeability with enhancement ratios from 5.09 (0.25 %w/v) to 11.78 (2 %w/v), but decreased permeability at 4 and 6 %w/v. Ultrastructural analysis of the buccal mucosae treated with PBS, ddI/PBS and ddI/PBS/AVgel 0.5 %w/v showed cells with normal plasmalemma, well-developed cristae and nuclei with regular nuclear envelopes. However cells from 1, 2 and 6 %w/v AVgel-treated mucosae showed irregular nuclear outlines, increased intercellular spacing and plasmalemma crenulations. This study demonstrates the potential of AVgel as a buccal permeation enhancer for ddI to improve anti-HIV and AIDS therapy.

KEYWORDS:

Buccal, Didanosine, Permeation enhancer, Histomorphology, *Aloe vera* (L.) Burn. F., *Aloe barbadensis* Miller, Asphodelaceae

Introduction

Antiretroviral (ARV) drugs have revolutionized the treatment of HIV (Human Immunodeficiency Virus) infection and AIDS (Acquired Immune Deficiency Syndrome) [1], widely acknowledged as being among the most serious public health problems [2]. However, several limitations exist with current ARV drug therapy via the oral route [3, 4]. These drugs suffer from low bioavailability due to extensive first pass effects and gastrointestinal degradation. Also, short half-lives necessitate frequent administration of doses and severe dose dependent side-effects may occur.

Buccal drug delivery, which is administration of drug from a delivery system (e.g. films, patches and gels) through the mucosae lining the cheeks of the mouth, has received increased interest as an alternative to the oral route. Drugs administered via the buccal route can bypass enzymatic degradation and hepatic first pass metabolism thereby improving bioavailability [5,6]. It has a high patient acceptability compared to other non-oral routes [7]. Buccal delivery systems offer an attractive approach for pediatrics and for patients with swallowing problems. Buccal delivery of ARV drugs can therefore contribute to overcoming some of their current disadvantages. While the potential of ARV drugs for administration via another non-oral route namely the transdermal route, has been explored [8, 9]; their buccal delivery potential remains to be investigated.

The epithelium lining the oral cavity is a barrier to drug permeation. The use of permeation enhancers in many cases is essential for efficient buccal drug delivery [10, 11]. The discovery of new permeation enhancers is essential for optimizing drug delivery via the buccal route. Currently, there is an increasing interest for drug products that either are of natural origin or contain such components [12]. *Aloe vera* (*Aloe barbadensis* Miller) is a succulent plant with strap-shaped green leaves [12]. For medicinal applications, the aloe latex (or exudate), the aloe gel and the whole leaf (or whole leaf extract) are the main parts used [13]. The inner pulp of the fresh leaves is used for gel extrusion [14]. The gel is composed mainly of water (>99%) and the remaining 0.5-1% of solid material comprises several polysaccharides, vitamins, enzymes, lipids and inorganic and small organic compounds [15]. It is recognized as an important medicinal plant that has effective anti-inflammatory, antifungal, soothing effect on the mucosal lining and wound healing properties (16). While it has recently been shown to be an effective transdermal [17] and intestinal [13] penetration enhancer for various drugs, its applicability for buccal permeation enhancement has not been investigated before. In these *in vitro* permeation studies by Chen et al 2009, it was reported that the polysaccharides from Aloe vera gel is capable of reducing the TEER of excised rat intestinal tissue, thus enhancing the transport of atenolol across this tissue to a significant extent. Moreover, Aloe vera gel materials could significantly decrease the TEER of Caco-2 cell monolayers and this reduction in TEER was associated with the opening of tight junctions between adjacent epithelial cells and this effect was

completely reversible after removal of the Aloe vera leaf materials from the cell monolayers. (Chen et al 2009).

Cole and Heard (2007) also stated that the skin penetration enhancement effect of AVgel was due to a probable pull effect of complexes formed between the compound and the enhancing agent within the aloe gel, however, the proposed mechanism of action has to be further investigated and confirmed as stated by the authors. Those studies with AVgel as an enhancer for the intestinal and transdermal routes did not report its histomorphological effects [13, 17], which is important for assessing its preliminary suitability. Recently, it has been shown to have the potential to modify drug release profiles in dosage forms [18]. It appears that *Aloe vera* gel, with polysaccharides as a significant component, has the potential unlike several existing penetration enhancers, to also provide multifunctional properties in buccal drug delivery systems. These multifunctional properties include mucoadhesion, absorption-enhancing, sustaining drug release and modified drug release properties. A buccal controlled release product based on *Aloe vera gel* (AVgel) will therefore be an attractive system for the administration of ARV drugs.

The aim of this study was therefore to identify the buccal permeability potential of a model ARV drug i.e. didanosine (ddI) in the absence and presence of a potential novel buccal permeation enhancer, namely AVgel. In addition the study also aimed at evaluating the histomorphological effects of ddI and AVgel on the buccal mucosa.

Materials and Methods

Ethical Clearance

Ethical approval was obtained from University of KwaZulu-Natal Animal Ethics Committee in 2008 (001/08/Animal) and renewed annually in 2009 (028/09/Animal), 2010 (029/10/Animal) and 2011 (25/11/Animal).

Materials

Didanosine (ddI) (Chromatographic purity (HPLC) = 99.4 %) was donated by Aspen Pharmacare (South Africa). AVgel, in dry powder form, was received from the International Aloe Science Council (IASC, 051309, Texas, USA) and was the same sample used in our previously reported study in Planta Medica [15]. The ^{1}H-NMR spectrum of the AVgel and the quantities of chemical markers as determined by NMR spectroscopy are available as supporting information (Figure 1S and Table 1S) and are discussed under the Results section. Disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium chloride were purchased from Sigma-Aldrich (Germany). All other reagents used were of analytical grade.

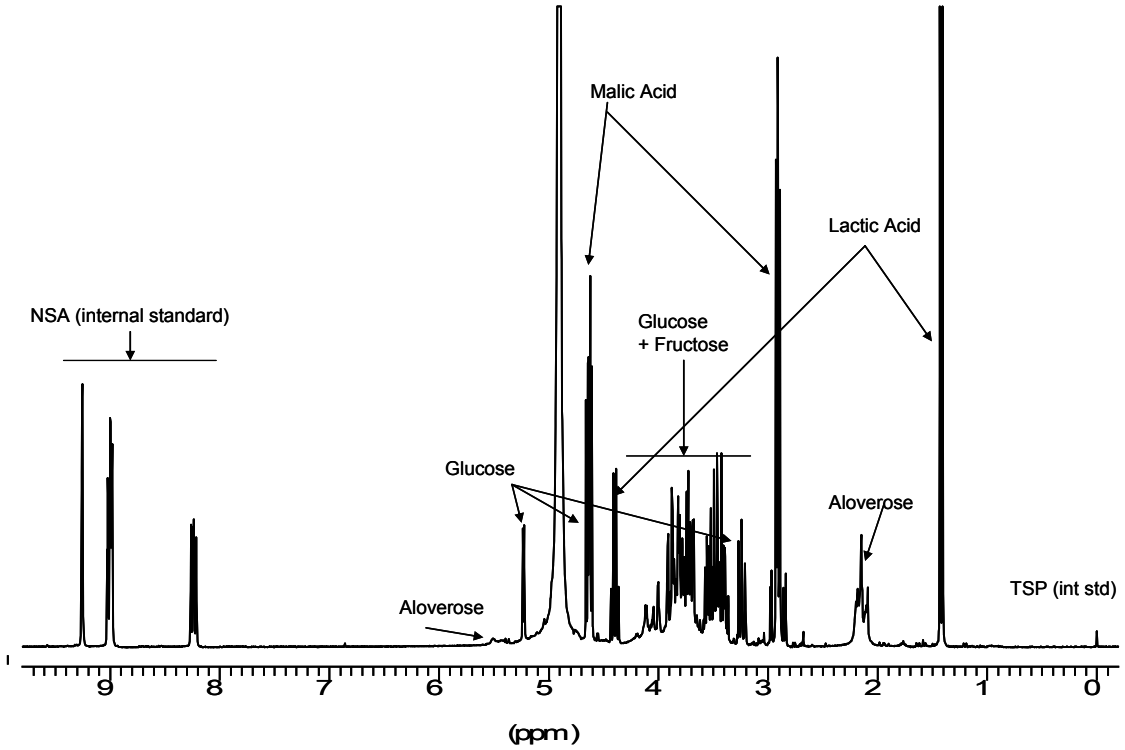

Fig. 1S: ^{1}H-NMR spectrum of AVgel labeled with the main chemical constituents and markers [15]

Table 1S: Chemical composition of AVgel as determined by ^{1}H-NMR [15]

Chemical	AVgel	
	Content (%)	Content (mg/L)
Aloverose	12.7	892.1
Glucose	16.7	1171.2
Malic acid	20.0	1403.4
Lactic acid	5.1	359.2
Citric acid	not detected	
WLM	detected	
Maltodextrin	not detected	
Acetic acid	not detected	
Succinic acid	trace	
Fumaric acid	not detected	
Formic acid	not detected	
Sodium benzoate	not detected	
Potassium sorbate	not detected	

Methods

Preparation of Porcine Buccal Mucosae

Buccal mucosae harvested from pigs (30–40 kg) (Biomedical Resource Unit, UKZN) and sacrificed by LECO euthanasia were appropriately excised. The thickness of the buccal mucosa was 665±72 µm (CV=8.3%). Fresh buccal mucosae were used for histological evaluations. For buccal permeability studies, the buccal mucosae were snap frozen in liquid nitrogen, stored in a biofreezer (-85 °C) and used within three months [12].

In Vitro Permeation

Frozen buccal mucosae were allowed to thaw and equilibrated in phosphate buffer saline pH 7.4 (PBS). Franz diffusion cells (PermeGear, Inc., Bethlehem, USA) with a diffusional area of 0.786 cm^2 were used for permeation experiments. The buccal mucosa was mounted to the diffusional area between the donor and receptor cells and was equilibrated with PBS at 37 °C. The donor compartment contained either varying concentrations of ddI in PBS alone (5, 10, 15 and 20 mg mL^{-1}) or ddI (20 mg mL^{-1}) in the presence of AVgel (0.25, 0.5, 1.0, 2.0, 4.0 and 6.0 %w/v). The receptor compartments were filled with PBS. Samples were removed from the receptor compartments at predetermined time intervals and replaced with the same volume of ddI–free PBS. Each experiment was undertaken using a minimum of three replicates. Similar to permeation studies with other drugs [19, 20], ddI was quantified by a validated UV Spectrophotometry method at a λ_{max} of 250 nm (UV Spectrophotometer 1650, Shimadzu, Japan).

Permeability Data Analysis

The cumulative amount of ddI permeated per unit surface area was plotted against time. The steady state flux (J_{ss}) was determined from the linear part of the permeability curve by linear regression analysis (Microsoft Excel 2007, USA). The permeability coefficient (P) was calculated as follows [21]:

$$P = (dQ/dt)/A \times Cd = Jss/Cd \quad (1)$$

Where dQ/dt is the cumulative amount permeated per unit time, A is the diffusion area and C_d is the drug concentration in the donor compartment. The permeability of ddI was evaluated in the presence of various concentrations of AVgel. The enhancement ratio (ER) was calculated as follows [21]:

$$ER = \frac{\text{Permeability coefficient of drug in the presence of enhancer}}{\text{Permeability coefficient of drug in the absence of enhancer}} \quad (2)$$

Viscosity Determination

The viscosities of ddI (20 mg mL^{-1}) only and ddI (20 mg mL^{-1}) in the presence of AVgel (0.25, 0.50, 1.0, 2.0, 4.0 and 6.0 %w/v) were determined with a Modular Advanced Rheometer (ThermoHaake MARS, Thermo Fischer Scientific, Germany), equipped with a titanium cone (C35 / 1° Ti) set at a sample gap of 0.051 mm and a Thermocontroller (UTC-MARS II). The relationships between the viscosity and shear stress as a function of shear rate were analyzed using HaakeRheoWin, 3.50.0012 software.

Light Microscopy and Transmission Electron Microscopy

Fresh buccal mucosa was cut into 1 x 1 x 0.1 cm cross sections. Mucosae were incubated in bottles containing either PBS only, or ddI/PBS (20 mg mL^{-1}) or ddI/PBS (20 mg mL^{-1}) / AVgel in varying concentrations. The bottles were kept in a water bath at 37 °C over six hours. Untreated buccal mucosa was transferred from normal saline into 10% buffered formalin without incubation in PBS and served as the control. Both the control and treated buccal mucosae were fixed in formalin for seven days. Buccal mucosa was dehydrated using an ethanol gradient and embedded in paraffin wax. The sections were collected on slides, dried and stained with Hematoxylin and Eosin (H&E). Semi-thin sections (1 µm) of the epoxy-embedded samples were also obtained and stained with Toluidine Blue. Sections were examined using a light microscope (Nikon 80i, Japan) and bright field images were captured using NIS Elements D software and a camera (Nikon U2, Japan).

Samples for transmission electron microscopy (TEM) were incubated as described above. Samples were cut into pieces not exceeding 0.5 mm^3, and fixed for 24 hours (4°C) using Karnovsky's fixative [22] buffered to pH 7.2. Samples were processed and embedded in epoxy resin using standard protocols. Ultrathin sections (90 nm) were cut and contrasted with uranyl acetate and lead citrate and viewed with a transmission electron microscope (JEOL 1010, Japan).

All experiments were performed using a minimum of three replicates.

Statistical Analysis

The results, expressed as Mean ± standard deviation (SD), were analyzed using One-way ANOVA followed by Mann Whitney test using GraphPad Prism® (Graph Pad Software Inc., Version 3). Differences were considered significant at $p < 0.05$.

Results and Discussion

The permeability potential of ddI in the absence of an enhancer was initially investigated. Fig. 1 shows the cumulative amount of ddI permeated at different donor concentrations. The flux values increased with an increase in ddI concentration and ranged from 25.94±1.35 µg cm^{-2} hr^{-1} to 71.57±3.12 µg cm^{-2} hr^{1} (Table 1). There was a significant difference ($p=0.001$) between all concentrations except between the flux values of 15 mg $mL^{-1}$and 20 mg mL^{-1} ddI, which were not significant ($p=0.302$).

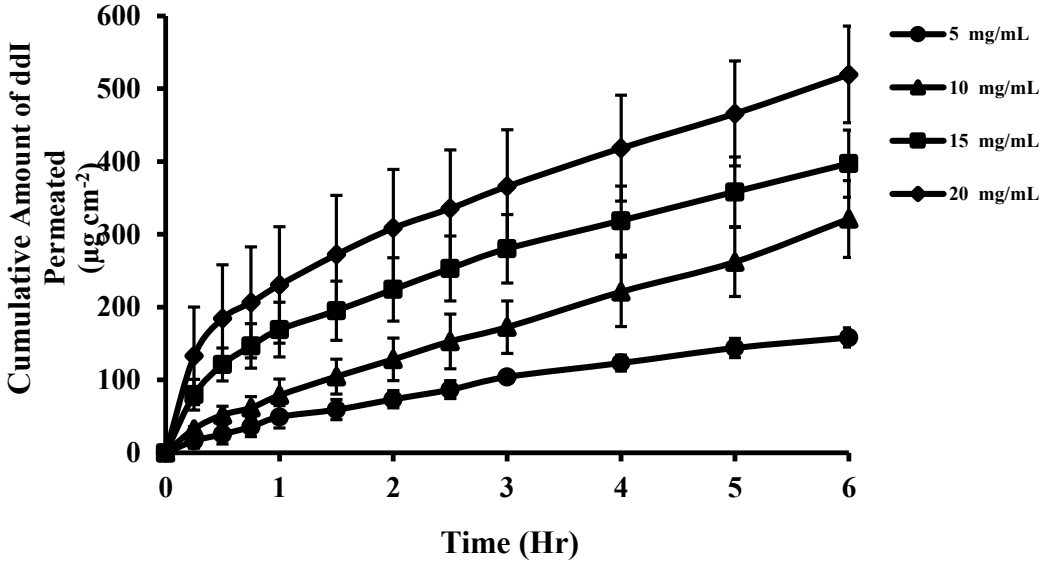

Fig. 1: Cumulative amount of ddI permeated per unit surface area vs. time profiles observed for ddI donor concentrations (Mean values ± SD; N ≥ 3).

The faster and non-linear drug release observed at earlier times as compared to slower and linear drug release thereafter may be due to lack of equilibration between the mucosal absorption site and the permeating drug molecules. Once an equilibrium exists between the drug molecules and the mucosa, the non-linearity disappears due to reservoir of permeating molecules created from the partitioning of the drug into the deeper mucosal layers, which slows the diffusion rate, hence the slower kinetics observed in this study (Niccolazzo et al 2003; Mashru et al 2005; Birudaraj et al 2005).

A linear relationship (R^2=0.9557) between the flux and ddI concentrations was obtained (Fig. 2), indicating passive diffusion as the main mechanism of ddI

transport across the buccal mucosa [23, 24]. Didanosine is hydrophilic and its passive diffusion should favour the paracellular pathway [25, 26].

Table 1: Effect of ddI donor concentration on its permeability parameters

Donor Concentration of ddI (mg mL^{-1})	Cumulative Amount of ddI permeated (µg cm^{-2})	Linear Equation ($y = mx + c$)	Correlation coefficient (R^2)	Flux (Jss) (µg $cm^{-2}hr^{-1}$)	Permeability coefficient (P) $x10^{-2}$ (cm hr^{-1})
5	158.15 ± 13.17	$Q = 25.94_t + 15.65$	0.97	25.94 ± 1.35	0.52 ± 0.03
10	321.08 ± 52.82	$Q = 49.85_t + 22.23$	0.99	49.85 ± 8.99	0.49 ± 0.09
15	397.03 ± 46.01	$Q = 57.35_t + 85.14$	0.92	57.35 ± 5.88	0.38 ± 0.04
20	456.89 ± 57.11	$Q = 71.57_t + 128.70$	0.89	71.57 ± 3.12	0.36 ± 0.02

Xiang et al [3] highlighted the promising potential of zalcitabine (ddC), the only other ARV reported to date for buccal delivery. They reported a flux of 13.42±6.35 µg cm^{-2} $hr^{-1}$for ddC at 20 mg mL^{-1} which is lower than the flux of ddI (71.57±3.12 µg cm^{-2} hr^{-1}). Several drugs with similar and lower flux values have been reported as having the potential for improving drug therapy via the buccal route [23, 24, 27]. ddI may therefore be regarded as having the potential for improving HIV and AIDS drug therapy when administered by the buccal route.

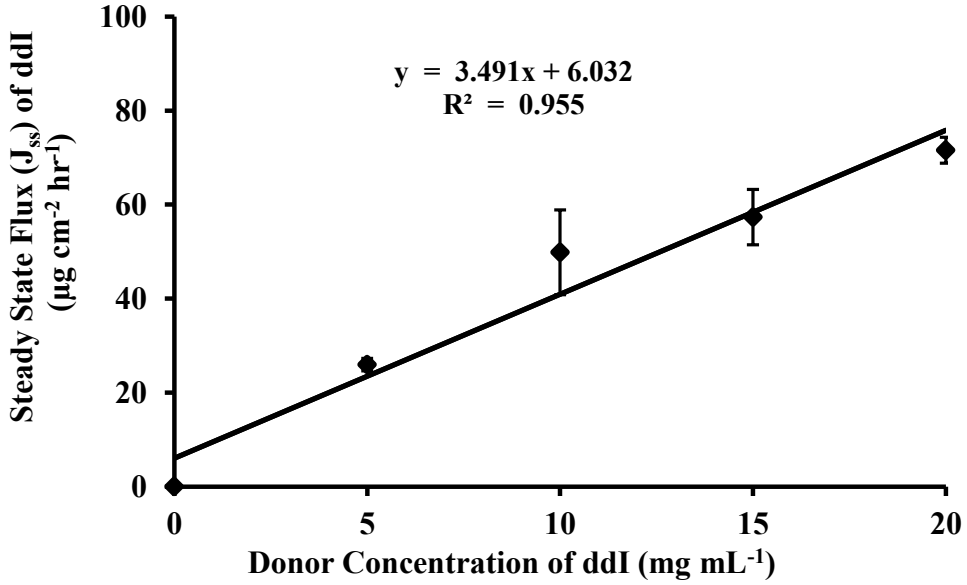

Fig. 2: Effect of donor concentration on the steady state flux of ddI at pH 7.4 (Mean values ± SD; N ≥ 3)

The AVgel employed in this study to investigate its effect on ddI permeation was the same as used by Chen et al. [13] to study its effects on intestinal drug permeability. The ^{1}H-NMR spectrum of the AVgel is shown in Fig. 1S and the quantities of chemical markers as determined by NMR spectroscopy in Table 1S. The results indicate that the AVgel material contained all the essential markers especially aloverose.

The buccal permeability of ddI in the presence of AVgel (Fig. 3) was investigated. The flux of ddI in the absence of AVgel was 71.57±3.12 µg cm^{-2} hr^{-1}. It increased significantly (*p<0.001*) with an increase in AVgel concentration

up to 2 %w/v (Table 2), which demonstrated the highest permeability coefficient of 3.3 x 10^{-2} cm hr^{-1} and an enhancement ratio (ER) of 11.78, thereby confirming for the first time the buccal permeation enhancement property of AVgel.

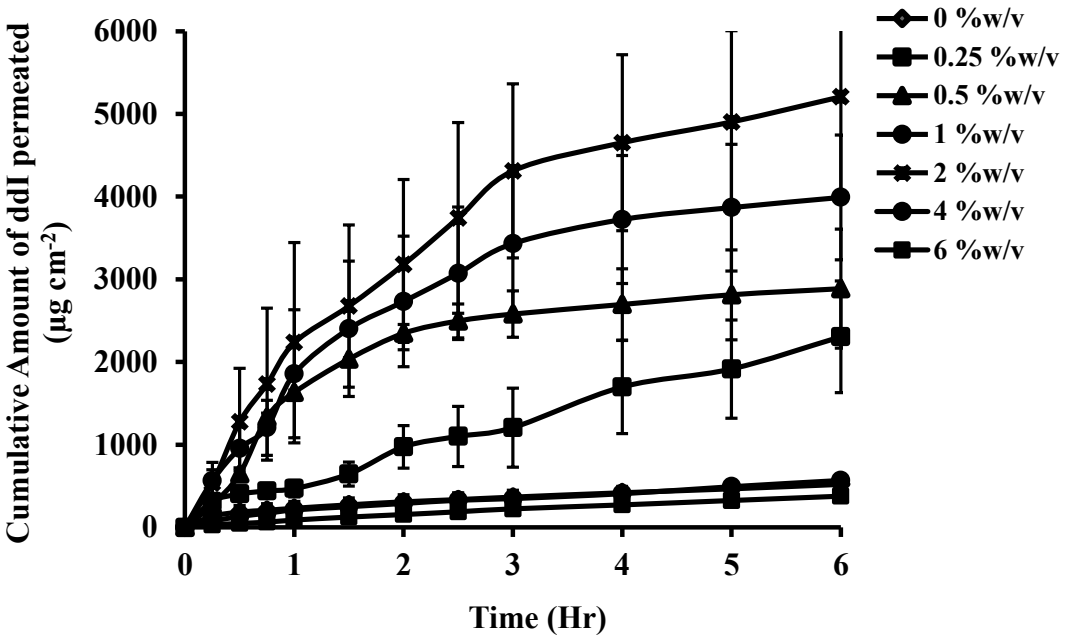

Fig. 3 Cumulative amount of ddI permeated per unit surface area vs. time profiles observed for AVgel concentrations (Mean values ± SD; N ≥ 3).

The permeation enhancing potential of AVgel from 0.25 to 2.0 %w/v may be similar to proposed mechanisms for other polysaccharides reported as permeation enhancers [28]. Polysaccharides such as chitosan are known to demonstrate mucoadhesivity, which causes prolonged drug retention on mucosae. It has been proposed that chitosan enhances buccal permeability by

interactions with the epithelial barrier that may weaken it, partially dismantling the extracellular matrix structure and intercellular joint. Since the major component of AVgel is polysaccharides [18], a similar mechanism may apply. Furthermore, AVgel is cationic and its possible ionic interaction with sialic acid residues on the buccal mucosae could alter membrane permeability [25, 28].

Table 2: Effect of AVgel concentration on the permeability parameters of ddI

Concentration of AVgel (%w/v)	Correlation Coefficient (R^2)	* Flux (Jss) (μg cm^{-2} hr^{-1})	Permeability Coefficient (P) x 10^{-2} (cm hr^{-1})	Enhancement Ratio (ER)
0.0	0.89	71.57 ± 3.12[a]	0.36 ± 0.02	1
0.25	0.99	364.69 ± 92.59[b]	1.82 ± 0.46	5.09
0.5	0.89	613.69 ± 292.49[b]	3.07 ± 1.46	8.58
1.0	0.85	650.07 ± 164.41[b]	3.25 ± 0.82	9.08
2.0	0.88	842.73 ± 129.24[b]	4.21 ± 0.65	11.78
4.0	0.95	83.95 ± 11.71[c]	0.42 ± 0.06	1.17
6.0	0.99	62.02 ± 5.41[c]	0.31 ± 0.03	0.87

***[(a vs b; $p < 0.05$), (a vs c; $p > 0.05$)]; [a]flux of the control; [b]statistically significant higher than control (ANOVA); [c]statistically non-suignificant compared to control**

Further increases in AVgel to 4.0 and 6.0 %w/v led to a decrease in flux to 83.95±9.24 and 62.06±5.58 μg cm^{-2} hr^{-1} respectively. Although there is a 10 fold reduction in the flux between 2 and 4 %w/v AVgel, the flux at 4 and 6 %w/v is reduced to a value which is statistically similar to the flux in the absence of

AVgel (Table 3). The decrease may be attributed to a higher viscosity of AVgel at higher concentrations that can increase resistance to drug diffusion and hinder drug movement [18, 29]. Increasing the concentration of AVgel in the ddI/PBS/AVgel formulations led to an increased viscosity of the formulations (Fig. 4) and displayed a linear correlation (R^2=0.972). The viscosity of AVgel at 6.0 %w/v (2.84 mPa) was almost three times (up to 240%) higher than that at 0.25 %w/v (0.94 mPa) (Table 3). The viscosities of AVgel at 4.0 and 6.0 %w/v may have been high enough to impede the buccal permeability enhancing potential of AVgel. Similar trends, with an initial increase in flux with increase in enhancer concentrations (propylene glycol) but resultant flux decreases with further increases have been reported in another study [30], although possible reasons were not investigated.

Table 3: Effect of AVgel concentration on the viscosity of ddI/PBS/AVgel formulations

Concentration of AVgel (% w/v)	Viscosity (η) (mPa)	Percentage increase in viscosity (%)
0	0.84 ±0.00	0
0.25	0.94 ±0.05	12.19
0.5	1.05 ±0.01	25.14
1	1.22 ±0.04	45.51
2	1.86 ±0.52	121.89
4	2.21 ±0.14	163.38
6	2.84 ±0.11	238.72

The ER of ddI increased approximately 12-fold with AVgel 2.0 %w/v, but decreased to 0.87-fold with AVgel 6.0 %w/v (Table 2). The ER values in this study are within the range of previous studies using AVgel at similar concentrations for other routes. The ER for colchicine through porcine skin was 11.2 (AVgel 3.0 %w/v) [17] while that of insulin through the intestinal epithelial monolayer was 2.31 (2.5 %w/v AVgel) [13]. A higher ER at a slightly lower concentration of 2 %w/v is reported for the buccal mucosa in this study. One explanation is that the buccal mucosa is more permeable than skin. Also, insulin in the previous study is a larger molecule, and may not permeate to a similar extent as ddI. The ER of other buccal enhancers were found comparable to those observed with AVgel in this study. Other chemical enhancers such as sodium glycodeoxycholate (ER=32), menthol (ER=2.02) and sodium glycolate (ER=9) have been reported as effective enhancers for buccal delivery [3, 21].

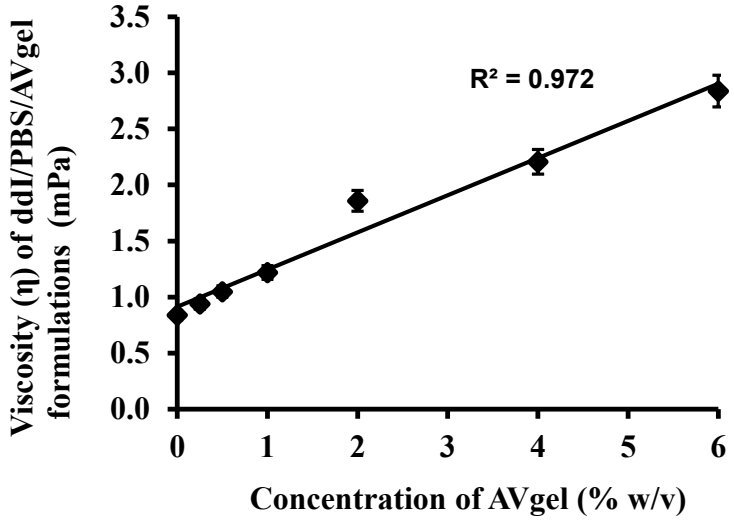

Fig. 4: Effect of AVgel concentration on the viscosity of ddI/PBS/AVgel formulations (Mean values ± SD; N = 3).

While permeation enhancing effects of substances are extensively reported, their effects on buccal mucosa morphology are limited [3, 31]. Since buccal delivery involves retention of a delivery system on the mucosae, an assessment of histological effects of a drug and or enhancer/s to evaluate their suitability is essential.

Histomorphological effects of the control/untreated and the treated porcine buccal mucosae (PBS alone and ddI/PBS in the absence and presence of AVgel) were assessed. The morphology of pig buccal mucosa has been described previously and it closely resembles human buccal epithelium [32, 33]. In the control group the buccal epithelium resembled that of a normal non-keratinized stratified squamous layer (Fig. 5a). Basal cells appeared oval and darkly stained in H&E (Fig. 5b) and toluidine blue (Fig. 5c) sections, reflecting their greater mitotic activity. The middle region showed large polygonal cells and superficial cells showed desquamation (Fig. 5d). Basal cells were nucleated while some of the superficial cells were anucleate. The basal cell layer represents the germinal tissue from which new cells are produced and should form the focus of such studies. Damage to superficial layers can be rectified by renewed growth from the germinal layer, but chronic or severe damage to the basal cell layer is probably irreversible [34]. The appearance of the control, PBS and ddI/PBS samples in H&E (Figs 5a-b) and toluidine blue (Figs 5c-e) respectively, were similar suggesting no influence of PBS or ddI (either alone or in combination) on tissue morphology. Therefore, ddI at the highest concentration had no adverse effects on the buccal mucosae.

The buccal mucosa upon treatment with ddI/PBS in combination with AVgel was examined. The addition of 0.5 %w/v AVgel led to an increase in intercellular spaces and darker staining of the cytoplasm, resembling the structure of control samples (Fig. 5f). However, increased AVgel concentration to 1%w/v showed a marked increase in intercellular spaces and distortion of cellular outlines (Fig. 5g). Cells appeared irregular and crenulated compared to controls. This was accentuated in 6 %w/v AVgel samples where extreme compaction of cells in the basal region was observed (Fig. 5h). Although not shown, cells from the middle and superficial layers also appeared severely damaged. Furthermore, the epithelial surface and basal lamina of the mucosa in the H&E sections of the control, PBS alone, ddI/PBS and ddI/PBS/AVgel 0.5 %w/v still appeared intact after six hours, but extensive disordering of this cell layer was observed in toluidine blue sections of the ddI/PBS/AVgel 6 %w/v. This disorder increased towards the epithelial surface, and may be due to the higher concentration effect of AVgel on the buccal mucosa.

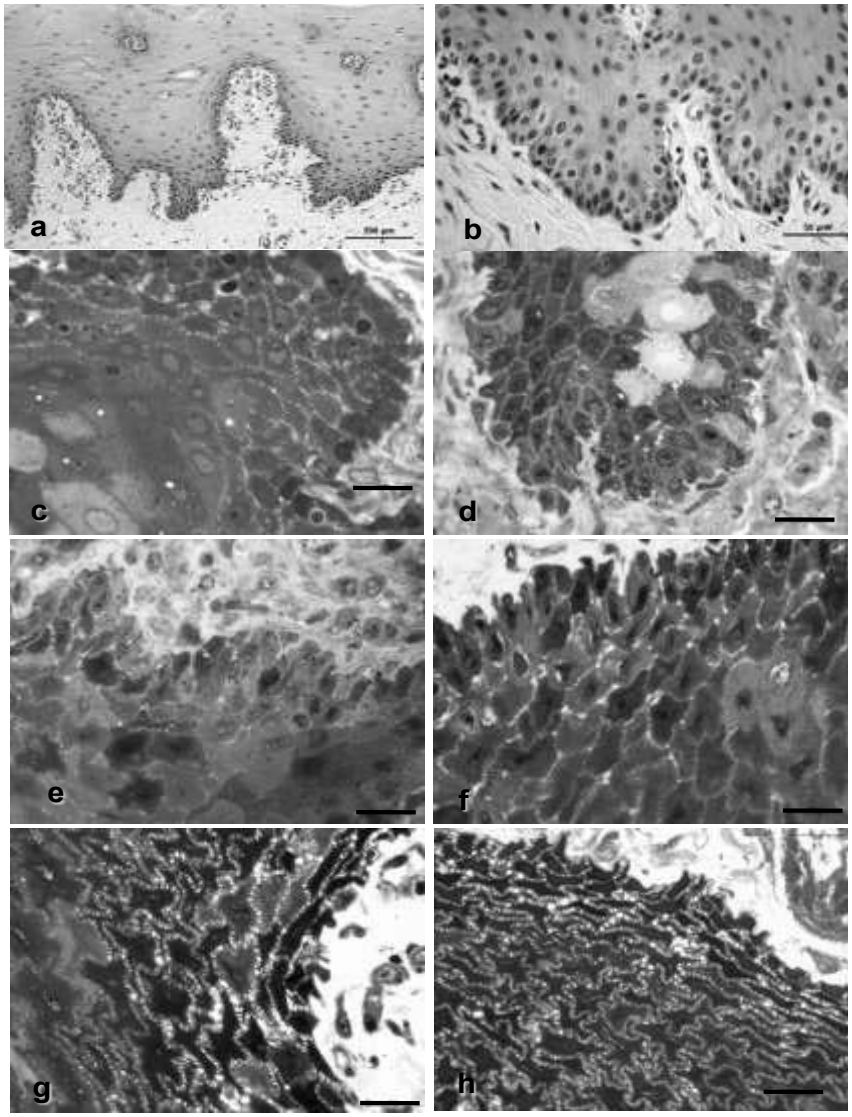

Fig. 5: Microphotographs of the control and treated buccal mucosal sections for Light Micrsoscopy (LM); stained with *H&E:* (a) control / untreated, (b)ddl/PBS, and with *toluidine blue*: (c) control/untreated, (d) PBS, (e) ddl/PBS, (f) ddl/PBS/AVgel 0.5 %w/v, (g) ddl/PBS/AVgel 1.0 %w/v, (h) ddl/PBS/AVgel 6.0 %w/v

The ultrastructure of buccal mucosae was evaluated. The control buccal mucosae showed short profiles of endoplasmic reticulum, an abundance of ribosomes and regular nuclei with evenly-dispersed chromatin (Fig. 6a).

Mitochondria appeared dense with well-developed cristae suggesting normal cellular activity (Fig. 6b). Intercellular spaces were small and clearly defined desmosomes between attachment plaques in neighbouring cells were observed (Fig. 6c). PBS and ddI/PBS treated mucosae showed a similar ultrastructure to the saline control, confirming trends observed at light microscope level. Cells from 0.5 %w/v AVgel samples also showed signs of active cellular metabolism and regular nuclear outlines (Fig. 6d). While electron translucent clearings within the mitochondria were occasionally observed, cellular damage was not evident (Fig. 6e). However, increasing AVgel concentration to 1 %w/v led to cellular damage evident by irregular nuclear outlines, peripheral distribution of visibly-compacted chromatin, electron-lucent mitochondria containing little internal detail and distended endoplasmic reticulum profiles (Fig. 6f). Increased intercellular spacing and crenulation of the plasmalemma also became evident (Fig. 6g). Further increases in AVgel concentrations to 2 and 6 %w/v led to disruption of basal cell layers, severe cellular compaction and larger intercellular spaces (Figs. 6h and 6i).

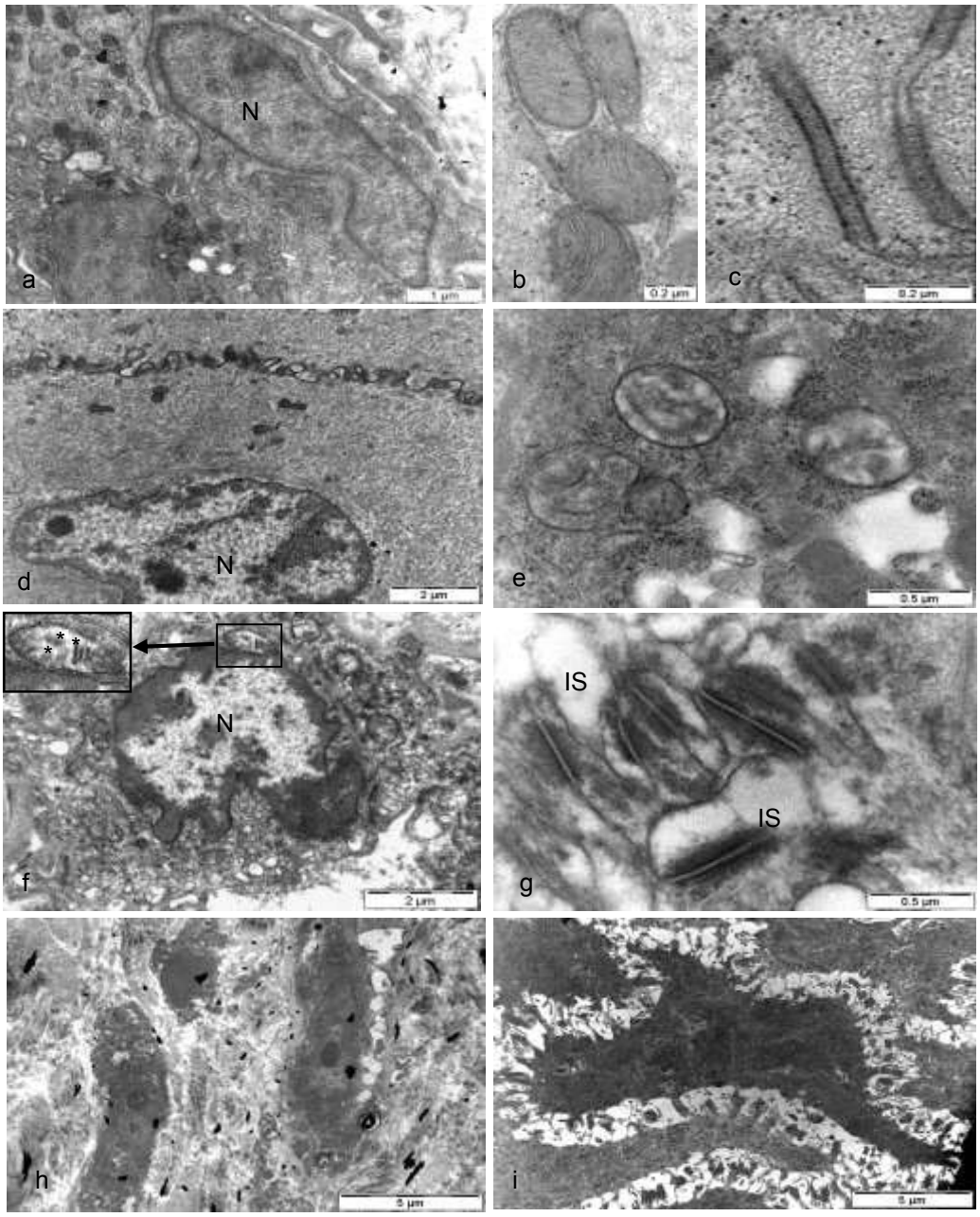

Fig. 6: Microphotographs of the control and treated ultra-thin buccal mucosa sections for transmission electron microscopy (TEM): (a) control / untreated, (b) PBS, (c) ddI/PBS, (d-e) ddI/PBS/AVgel 0.5 %w/v (f-g) ddI/PBS/AVgel 1.0 %w/v (h) ddI/PBS/AVgel 2.0 %w/v, (i) ddI/PBS/AVgel 6.0 %w/v

Histomorphological evaluations showed that AVgel caused adverse effects on the mucosa at higher concentrations of 1, 2 and 6 %w/v. Since the buccal mucosa was not adversely affected at lower concentration of 0.5 %w/v, AVgel may therefore be considered as a safe permeation enhancer up to this concentration. At 0.5 %w/v, AVgel showed an ER of 5.09 which is still higher than several other reported enhancers [3, 23, 27].

The study has shown that ddI can permeate the buccal mucosa without adversely affecting its morphology. AVgel at concentrations up to 2 %w/v was identified as an effective buccal permeation enhancer for ddI. Based on the findings; it is proposed that AVgel be used in concentrations at or lower than 0.5 %w/v due to adverse mucosal effects at higher concentrations. Histomorphological evaluations therefore proved useful in correlating the permeation enhancing properties of AVgel with its effects on the buccal mucosa. The results confirm the potential of developing a buccal drug delivery system containing ddI and AVgel as an enhancer for improving drug therapy.

Acknowledgements

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Declaration of interest

The authors declare that there are no conflicts of interest for this study. The authors alone are responsible for the design, content and writing of this paper.

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Supporting Information

Investigating the effect of *Aloe vera* gel on the buccal permeability of didanosine

Elizabeth Ojewole[1], Irene Mackraj[2], Kamil Akhundov[3], Josias Hamman[4], Alvaro Viljoen[4], Eugene Olivier[4], James Wesley-Smith[5] and Thirumala Govender[1]

Table 1S: Chemical composition of AVgel as determined by ^{1}H-NMR [15]

	AVgel	
Chemical	**Content (%)**	**Content (mg/L)**
Aloverose	12.7	892.1
Glucose	16.7	1171.2
Malic acid	20.0	1403.4
Lactic acid	5.1	359.2
Citric acid	not detected	
WLM	detected	
Maltodextrin	not detected	
Acetic acid	not detected	
Succinic acid	trace	
Fumaric acid	not detected	
Formic acid	not detected	
Sodium benzoate	not detected	
Potassium sorbate	not detected	

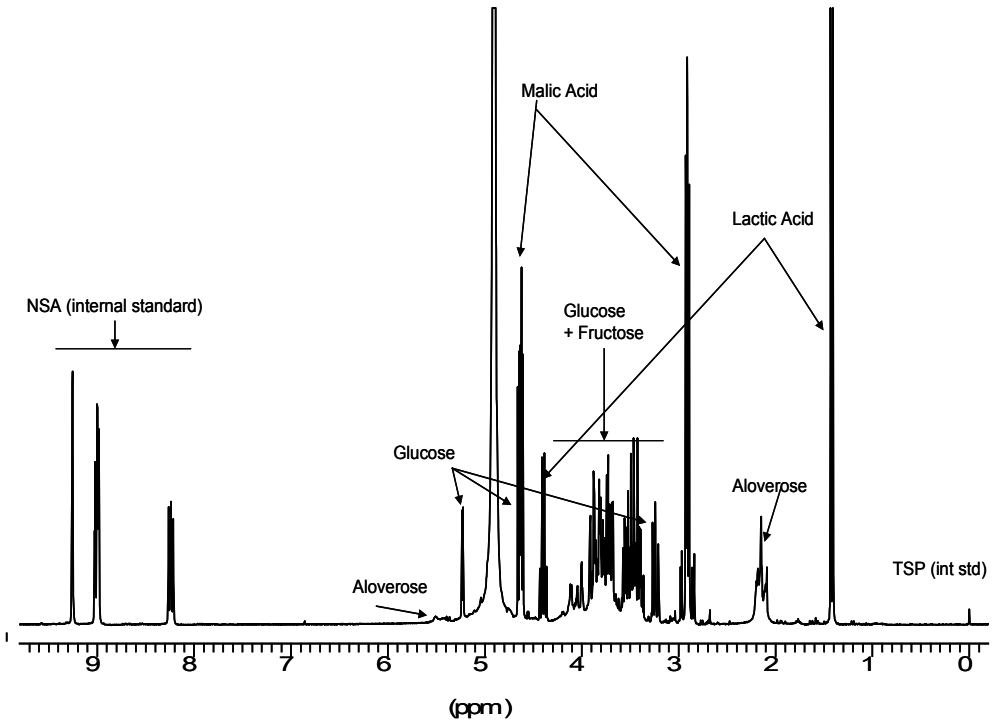

Fig. 1S: ^{1}H-NMR spectrum of AVgel labeled with the main chemical constituents and markers [15].

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CHAPTER FOUR

PUBLISHED PAPER

4.1. INTRODUCTION

This paper was published in an international peer reviewed ISI journal and reports the original published research on data generated in this study.

E. Ojewole, R. Kalhapure, K. Akamanchi, T. Govender Novel Oleic acid derivatives enhance buccal permeation of didanosine. ***Drug Development and Industrial Pharmacy 2014;*** 40(5): 657-668.

E. Ojewole contributed to the design of the project, and was responsible for the excision and preparation of the buccal mucosae for the permeation and histomorphological studies. She performed all *in vitro* permeation experiments and was responsible for the interpretation of the permeability data. She undertook the histological studies and prepared samples for light microscopy (LM) and transmission electron microscopy (TEM) evaluations. She interpreted both the photo-micrographs and eletro-micrographs of the buccal mucosae. Furthermore, she was responsible for the writing of the manuscript, from the first and up until the final draft. R. Kalhapure served as a postdoctoral mentor and was responsible for the synthesis and

characterization of the novel OA derivatives. K. Akamanchi served as an international collaborator and T. Govender served as the supervisor.

This chapter is presented in the required format by the journal and is in the final revised and accepted version, published in Drug Development and Industrial Pharmacy.

4.2 PUBLISHED PAPER

Novel Oleic acid derivatives enhance buccal permeability of didanosine

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ABSTRACT

The aim of this study was to explore the potential of novel oleic acid (OA) derivatives as buccal permeation enhancers for the delivery of didanosine (ddI). The OA derivatives, i.e. ester derivative (OA1E), the dicarboxylic acid derivative (OA1A) and the bicephalous dianionic surfactant (OA1ANa) were synthesised and their permeation effects were compared to the parent OA. OA, OA1E, OA1A and OA1ANa at 1 %w/w all showed potential for enhancing the buccal permeability of ddI with enhancement ratio (ER) of 1.29, 1.33, 1.01 and 1.72 respectively. OA1ANa at 1 %w/w, demonstrated the highest flux (80.30 ± 10.37 μg cm^{-2}.hr), permeability coefficient (4.01 ± 0.57 x 10^{-3} cm hr^{-1}) and enhancement ratio (1.72). The highest flux for ddI (144.00 ± 53.54 μg cm^{-2}.hr) was reported with OA1ANa 2 %w/w, which displayed an ER of 3.09 more than that with ddI alone. At equivalent concentrations, OA1ANa (ER=3.09) had a significantly higher permeation enhancing effect than its parent OA (ER=1.54). Histomorphological studies confirmed that OA1ANa at all concentrations except 6.0 %w/w had no adverse effects on the mucosae. Morphological changes such as vacuoles formation and increased intercellular spaces were attributed to the buccal permeation enhancing effect of OA1ANa. This study demonstrated the potential of novel OA derivatives as buccal permeation enhancers. OA1ANa at 2 %w/w was also identified as the optimal novel OA derivative to widen the pool of fatty acid derivatives as chemical permeation enhancers for buccal drug delivery.

Keywords: Buccal, Didanosine, Oleic acid derivatives, Permeation enhancers, Antiretroviral.

1. Introduction

The buccal mucosa remains an attractive alternate and non-invasive site for the delivery of both locally and systemically active drugs [1,2]. It avoids the degradation of drugs by both the GI acids and enzymes and also bypasses hepatic first pass metabolism, thereby improving the systemic bioavailability of various drugs[3-5]. Furthermore, absorption following administration via the buccal route is not influenced by potential variations in the gastric emptying rate or the presence of food[6]. The permeability of the buccal mucosa is also higher than that of skin [7]. Hence, a lower loading dose in a transbuccal device could provide the same therapeutic effect as a transdermal patch. The buccal mucosa also has a larger area for drug application and has good accessibility compared to other mucosae such as the nasal, rectal and vaginal mucosae[7]. Various classes of drugs including zalcitabine (ddC) (antiretroviral)[8,9], piroxicam (non-steroidal anti-inflammatory)[10], morphine (opiate)[11], omeprazole (proton-pump inhibitor)[12], insulin (anti-diabetic hormone and blood gloucose lowering agent)[13] and metoprolol (beta blocker)[14], have been studied for delivery via the buccal mucosa to exploit its above advantages. The buccal route therefore has wide applicability for diverse drugs and disease conditions.

One of the main challenges with buccal mucosal therapy is its limited mucosal permeability due to the epithelial lining of the membrane which acts as a barrier to drug permeation [15]. The outermost layer of the stratified squamous

epithelium is keratinized, covered by a thin layer of mucus and is comparatively thicker than the rest of the oral mucosal lining. The basement membrane lies directly underneath the epithelium, followed by the lamina propria and the submucosa. The mucosa is made up of about 40-50 cell layers and a thickness of 500–800 μm has been reported[16,17]. The mucosal structure thus contributes to the challenges and factors that are responsible for the limited buccal permeability of drugs. Enhancing permeation of drugs across the buccal mucosa is therefore critical for optimising bioavailability of various drugs [5]. Maximising the bioavailability of several drugs after buccal administration for absorption through the mucosal lining will be beneficial to reducing intra and inter subject variability as well as side effects of the drugs [14,18]. Moreover, the cost of manufacture will be reduced by decreasing drug wastage owing to its low systemic bioavailability [19], especially where drugs have limited permeability and subsequently low bioavailability. Hence, the use of permeation enhancing strategies in many cases is essential to overcome the limited permeability of the buccal mucosae for improved buccal drug delivery[20,21]. Recent advancements in the use of permeation enhancing strategies have identified various approaches, including physical and chemical methods, to enhance the buccal permeability of drugs. It has been reported that chemical permeation enhancers (CPEs), for example bile salts, surfactants and fatty acids (FAs), have proved promising for enhancing buccal permeability of drugs[22,23]. Other approaches include drug particle size reduction[24], ultrasound and electrical assisted approaches (iontophoresis and electroporation) as well as thermal enhancement[25-27]. More specifically, recent reports are emerging on the use of

derivatives of common chemical enhancers for further maximising mucosal drug permeation. For example, newly synthesised propanoyloxy derivatives of 5b-cholan-24-oic acid were more effective in enhancing permeation of theophylline as compared to its parent compound, cholic acid, thereby potentiating the efficacy of bile acids as a class of chemical permeation enhancers [28,29]. There is therefore a need to explore and identify new derivatives of chemical enhancers to widen the pool of available superior enhancers for buccal drug delivery.

Fatty Acids (FAs) are widely used chemical permeation enhancers for various drugs [17]. Sodium caprate, caprylic acid, sucrose esters and lauric acid have been reported for enhancing the permeation of drugs such as lidocaine, ergotamine, insulin and sumatriptan across the buccal mucosa [23,30-33]. It has been reported that FAs can disrupt the lipid bilayer of the mucosal lining thereby increasing drug transport and bioavailability. Oleic acid (OA) in particular has been reported as an effective chemical permeation enhancer for drugs such as levothyroxine sodium via the intestinal route [34], caffeine and diclofenac sodium (DS) via the transdermal route [35] and 5-fluorouracil via the buccal route [1]. Novel derivatives of OA will therefore be useful for further improving their permeation enhancing potential and will contribute to the pool of permeation enhancers for enhancing drug permeability.

In a previous study by our group, the synthesis of novel OA derivatives, known as oleodendrimers A1E, A2E, E1E and E2E were reported. Additionally, the potential of these derivatives as transdermal permeation enhancers for the delivery of a non-steroidal anti-inflammatory drug, diclofenac sodium were identified [36]. These derivatives, A1E, A2E, E1E and E2E are G1 and G2 Janus type dendrimers in which the dendron moiety is linked with OA through ester and amide bonds. Oleodendrimers A1E and A2E have an amide linkage whereas E1E and E2E have an ester linkage between the dendron and OA moiety. This study showed the OA derivatives, oleodendrimers, as being far more effective in enhancing the transdermal permeation of diclofenac sodium as compared to the parent OA. The specific mechanism of permeation enhancement was not cited in the previous study. However, as fatty acids, it could be proposed as the perturbation /disruption of the lipid bilayer of the stratum corneum which could have led to increased drug permeation across the epidermis of the skin [23]

The potential of these novel oleodendrimers as permeation enhancers for buccal drug delivery has not been studied for any drug to date. The buccal permeation enhancement probability of the OA derivatives can expand its applicability in drug delivery systems. Therefore, in this study it was decided to explore the potential of the oleodendrimer E1E (OA1E), its dicarboxylic acid derivative (OA1A) and a sodium salt of dicarboxylic acid (bicephalous dianionic surfactant, OA1ANa) as buccal permeation enhancers. OA1E has been

studied previously as a transdermal enhancer only and derivatives OA1A and OA1ANa have not been studied as permeation enhancers for any drug by any route.

In the previously reported study, OA1E was synthesised by methods that involved the use of hazardous reagents such as thionyl chloride, harsh reaction conditions involving high reaction temperatures and it was furthermore a detailed multistep process [37]. In keeping with the current trends of applying green chemistry approaches [38], the synthesis of the OA1E derivative, from which OA1A and OA1ANa are obtained, by a modified method to eliminate the above drawbacks will be beneficial for its commercial application as enhancers for various routes.

In this study, didanosine (ddI) was selected as a model drug for *in vitro* buccal permeation investigations. Antiretroviral (ARV) drugs have improved the treatment of Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS), diseases that significantly affect the global population [39,40]. Most ARVs, including ddI, have low bioavailability due to the first pass effect and gastrointestinal acidic and enzymatic degradation. Additionally, they exhibit dose-dependent toxicities and adverse effects [41,42]. ARV drugs such as ddI may therefore benefit from buccal delivery. The permeability potential of ARV drugs via the buccal route has been investigated for ddC, ddI, TFV, AZT and SQV [7,9,43-45] and these ARVs have all shown potential for permeability via the buccal mucosa. There are limited studies on

the identification of chemical permeation enhancers for ARV drugs. The latter have included polymeric excipients [44], aloe vera [43], bile salts [9] and menthol [8].

The aim of this study was therefore to synthesise novel OA derivatives by a greener chemistry approach and explore the potential of OA and its oleodendrimer derivatives as novel permeation enhancers for buccal permeability using ddI as a model ARV drug.

2. Materials and Methods

2.1. Ethical Clearance

Ethical approval (Reference 039/13/Animal) was obtained from the University of KwaZulu-Natal Animal Research and Ethics Committee.

2.2. Materials

Oleic acid (OA) (technical grade, 90 %), *N*-Ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC.HCl), *p*-dimethylaminopyridine (DMAP) were obtained from Sigma, USA. 3-Amino-1-propanol and *tert*-butyl acrylate were purchased from Alfa-Aesar (USA).

Acetyl chloride (AcCl) and dichloromethane (DCM) were from Merck Chemicals (Germany). All other solvents used were of analytical grade and were procured from Merck Chemicals (Germany). Merck precoated Silica-gel $60F_{254}$ plates were used for thin layer chromatography. Didanosine (ddI) [Chromatographic

purity (HPLC) = 99.4 %] was purchased from Ruland Chemistry Co., Ltd (Nanjing, China). Disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl) and hydroxypropyl methyl cellulose (HPMC) were purchased from Sigma-Aldrich (Germany). Sodium Hydroxide (NaOH) and Hydrochloric Acid (HCl) were of analytical grade. Milli-Q Purified water was obtained from the purification system (Millipore Corp., USA) in our laboratories. Pigs (40 - 60 kg) were supplied by the Biomedical Resource Unit, UKZN (South Africa).

2.3 Methods

2.3.1 Synthesis and characterisation of OA Derivatives

2.3.1.1 **Synthesis**

The synthetic scheme in Figure 1 shows the reaction sequences involved in the synthesis of the different, three OA derivatives used in this study [37,46] i.e. ester derivative, oleodendrimer E1E (OA1E), the dicarboxylic acid derivative (OA1A) and the bicephalous dianionic surfactant (OA1ANa). The derivatives, i.e. OA1E, OA1A and OA1ANa as well as their molecular mass, formulae and chemical structures are presented in Table 1.

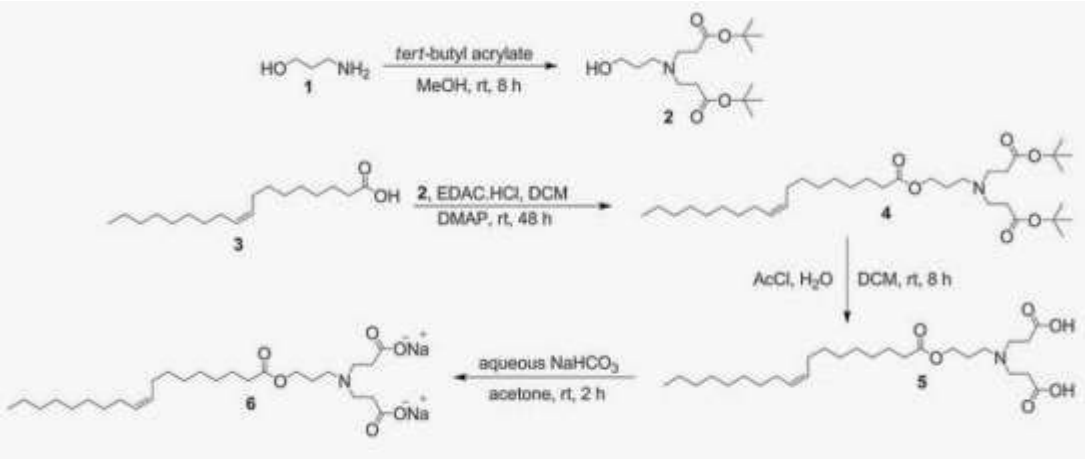
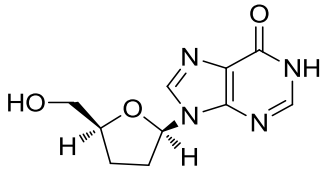
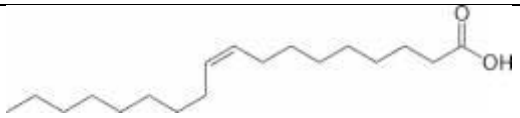
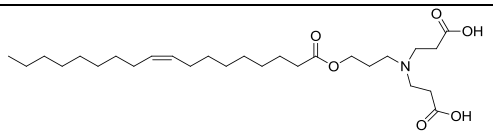
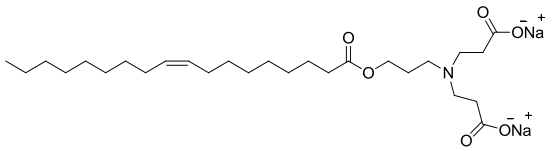
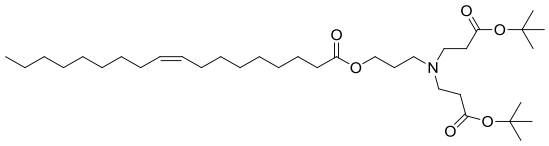


Figure 1: Scheme showing the reaction sequences involved in the synthesis of different OA derivatives.

2.3.1.1.1 Synthesis of 3-N, N-di- (tert-butyloxycarbonylethyl) aminopropanol ***2***

Compound **2** was synthesised following the literature procedure. Briefly, to a solution of *tert*-butyl acrylate (19.2 g, 1.5 mol) in MeOH (200 ml), 3-amino-1-propanol **1** (3.75 g, 0.5 mol) in MeOH (100 ml) was added drop wise maintaining the reaction temperature below 30 ^{o}C. The reaction mixture was allowed to stand overnight after stirring at room temperature for 8 h. The solvent (MeOH) and excess *tert*-butyl acrylate were removed under vacuum to afford compound **2** as a colorless liquid (16.4 g, 99 %).

Table 1: Molecular Mass, Formula and chemical structure of ddI and OA, OA1A, OA1E and OA1ANa

	Molecular Mass g mol^{-1}.	Molecular formula	Chemical Structure
ddI	**236.23**	**$C_{10}H_{12}N_4O_3$**	
OA	**282.46**	**$C_{18}H_{34}O_2$**	
OA1A	**483.36**	**$C_{27}H_{49}NO_6$**	
OA1ANa	**527.32**	**$C_{27}H_{47}NNa_2O_6$**	
OA1E	**595.48**	**$C_{35}H_{65}NO_6$**	

2.3.1.1.2 Synthesis of Oleodendrimer E1E (OA1E) ***4***

A mixture of compound **2** (2.84 g; 0.90 mol) and DMAP (0.68 g, 0.56 mol) in DCM (25 ml) was stirred at room temperature for 10 minutes. To this mixture EDAC.HCl (2 g, 1.12 mol) was added followed by a mixture of OA (2 g, 0.75 mol) in DCM (25 ml). The formed clear solution was stirred at room temperature

for 48 h. Solvent was evaporated under vacuo and crude product obtained was purified by column chromatography using silica gel # 60-100 mesh with hexane/EtOAc, 9:1 as an eluent to obtain compound **4** as a colorless liquid (3.84 g, 91 %).

*2.3.1.1.3 Dicarboxylic acid derivative of OA (OA1A) **5** and its sodium salt (OA1ANa) **6***

Literature reported procedures [37] with slight modifications were followed for the synthesis of compound **5** and **6**. In short, to a mixture of compound **4** (5.95 g, 0.01 mol), H_2O (0.72 g, 0.04 mol) in DCM (100 ml), acetyl chloride (3.14 g, 0.04 mol) was added drop wise, over a period of 10 min and stirred for 8 h. The reaction mixture was washed with brine water and concentrated in vacuo after drying over anhydrous Na_2SO_4 to afford compound **5** as a viscous liquid (3.98 g, 82 %). This compound **5** was utilised, without further purification, for the preparation of compound **6**. In short, a solution of **5** (5 g, 0.01 mol) in acetone (200 ml) was neutralised with 20 % hot aqueous $NaHCO_3$ solution (8.35 ml, 0.019 mol) under vigorous stirring for 2 h. The precipitated white solid was further dried by removal of solvent under vacuum to afford compound **6** as an off white solid (5.30 g, 97 %).

2.3.1.2 Characterisation of Derivatives

The synthesised derivatives were characterised by standard analytical techniques for structural confirmation. FT-IR spectra were recorded using a

Bruker Alpha spectrophotometer (Germany). ^{1}H NMR and ^{13}C NMR were recorded using a Bruker NMR instrument (Germany) operating at 400 and 100 MHz respectively. HLB and log $P_{octanol/water}$ values of the OA derivatives were calculated using ChemSW® software (ChemSW Inc., Version 6.33, California, USA). The chemical structures, molecular formulae and mass of the derivatives are as presented in Table 1.

2.3.2 Buccal Permeation studies

2.3.2.1 Formulation and preparation of Gels for Permeation Studies

To determine the effect of OA and its derivatives, simple gels containing ddI (2 %w/w), HPMC (4 %w/w), OA and its derivatives (OA1A, OA1E and OA1ANa) were prepared using 1 %w/w of either OA or each of the derivatives. The compositions of the prepared gel formulations, in the absence of the enhancers and in the presence of either OA or its derivatives are described. Briefly, HPMC (4 %w/w) was weighed and mixed in a beaker with sufficient quantity of purified water. Also, ddI (2 %w/w), weighed was separately mixed with a small quantity of purified water and added to the HPMC. The gel formulation was then made up to weight with further purified water, and then stirred on a magnetic stirrer until all dissolved and was coded as formulation F1. Each enhancer, i.e. OA, OA1A, OA1E and OA1ANa was separately weighed and added to formulation F1 at a concentration of 1 %w/w to form the drug / enhancer gels, and were coded as formulations F2, F3, F4 and F5 respectively. For the concentration effects experiments, the compositions of the prepared formulations are described. Briefly, ddI gel in the absence of the enhancers (i.e. OA and its

derivatives) was prepared as per formulation F1. OA at varying concentrations of 0.5, 1.0, 2.0, 4.0, 6.0 %w/w were weighed, and separately incorporated with a sufficient quantity of formulation F1 to make the required formulations F6, F7, F8, F9 and F10 respectively. Similarly, OA1ANa in varying concentrations of 0.5, 1.0, 2.0, 4.0, 6.0 %w/w, was each added to a sufficient quantity of formulation F1 to make formulations F11, F12, F13, F14 and F15 respectively. Each prepared formulation was stored in an airtight amber container.

2.3.2.2 Preparation of Porcine Buccal Mucosae

Porcine buccal mucosa has many similarities to the human buccal mucosa and was chosen as the biological membrane for the permeation experiments [47-49]. Pigs were sacrificed using the standard operating procedure at Biomedical Resource Unit, University of KwaZulu-Natal (BRU, UKZN). Briefly, the pig was weighted and appropriate drugs were mixed to form a cocktail for intramuscular injection, by the registered, licensed veterinary doctor at BRU, UKZN. The cocktail contained the following drugs, i.e. Domitor ® (Zoetis, Australia), which contains medetomidine 1mg/ml and dosed at 0.06 mg/kg; Zoletil ® 100 (Virbac, Mexico), which contains a mixture of tiletamine 50 mg/ml; zolazepam 50 mg/ml, and dosed at 1.5 mg/kg and lastly, Butorphanol 10 mg/ml, (V-Tech Dispensing Pharmacy, South Africa) dosed at 0.17 mg/kg. The appropriate, measured doses per kilogram body weight of these drugs were each placed in a sterile vial and subsequently mixed together and then drawn up into a single 5 ml syringe. The pig was restrained by placing it in a squeeze cage and the cocktail was injected into the quadriceps muscle. The ear vein became easily

accessible within 5-7 minutes such that the pig was at a stage of sedation and free of pain. The pig was then euthanized with Eutha-Naze® (Bayer, South Africa), containing sodium pentobarbitone 200 mg/ml, dosed at 1 ml/kg and administered intravenously at a rapid rate. Generally a state of death was achieved with about 10-15 ml of Eutha-Naze. Buccal mucosae harvested from euthanized pigs, were appropriately excised and prepared for the permeation experiments. The thickness of the buccal mucosa was 665±72 µm (CV=8.3%). For buccal permeability studies, the buccal mucosae were wrapped in foil, snap-frozen in liquid nitrogen and then stored in a bio freezer (-85 °C) until further use within three months according to previous reports [20,50].

2.3.2.3 In Vitro Permeation

Frozen buccal mucosae were allowed to thaw and equilibrated in phosphate buffer saline pH 7.4 (PBS). Franz diffusion cells (PermeGear, Inc., Bethlehem, USA) with a diffusional area of 0.786 cm^2 were used for permeation experiments. The buccal mucosa was mounted to the diffusional area between the donor and receptor cells and was equilibrated with PBS at 37 °C. Initially, just the ddI gel, in the absence of any enhancer (formulation F1) and presence of OA and the oleodendrimer derivatives, i.e. OA1A, OA1E and OA1ANa; formulated at only one gel concentration as formulations F2, F3, F4 and F5 respectively were employed in the permeation experiments. Briefly, the donor compartment contained either ddI (2 %w/w) / HPMC (4 %w/w) gel alone, or in the presence of 1 %w/w of either OA or its oleodendrimer derivatives (OA1A,

OA1E and OA1ANa). In the subsequent experiments, i.e. the concentration effect studies, ddI gel in the presence of either OA or OA1ANa, at varying concentrations, 0.5, 1.0, 2.0, 4.0 and 6.0 %w/w; was placed in the donor compartment. The receptor compartments were filled with PBS. Samples were removed from the receptor compartments at predetermined time intervals and replaced with the same volume of PBS (drug–free). Each experiment was undertaken using a minimum of three replicates. ddI was quantified by a validated UV Spectrophotometry method at a λ_{max} of 250 nm (UV Spectrophotometer 1650, Shimadzu, Japan) as employed by previous buccal permeation studies with antiretrovirals such as ddI [43] and ddC [8,9]; as well as other drugs including ondasetron [51], galantamine [52] and carbamazepine [53].

2.3.2.4 Permeability Data Analysis

The cumulative amount of ddI permeated per unit surface area versus time was plotted. The steady state flux (J_{ss}) across the mucosal membrane was determined from the linear part of the permeation graph by linear regression analysis (Microsoft Excel® 2010, USA). The permeability coefficient (P) was calculated using the following equation [8]:

$$P = (dQ/dt\ )/A\ x\ Cd = Jss\ /\ Cd \qquad (1)$$

Where dQ/dt is the cumulative amount (Q) of ddI permeated per unit time (t), A is the active, cross-sectional diffusion area and C_d is the drug concentration in the donor compartment. The effects of OA, OA1A, OA1E and OA1ANa and

the various concentrations of OA and OA1ANa on the permeability of ddI were evaluated. The enhancement ratio (ER) was calculated using the following equation [8]:

$$ER = P\ (Enhancer) / P\ (No\ Enhancer) \quad (2)$$

2.3.2.5 Morphological Evaluations using Light Microscopy and Transmission Electron Microscopy

Histological evaluations were performed on freshly harvested, excised buccal mucosa. Untreated buccal mucosa was transferred directly after excision from normal saline into 10% buffered formalin without any equilibration in PBS and served as the control. Treated samples of the buccal mucosae comprised of those that were exposed to PBS only, or ddI gel formulation with and without OA1ANa at varying concentrations of 0.5, 2.0 and 6.0 %w/w. Permeation experiments were performed as described in previous studies, without drug quantification [25,43,51]. At the end of the permeation experiments, the buccal mucosa was cut into cross sections. For light microscopy (LM), the samples were fixed in 10% buffered formalin for seven days, washed in water, dehydrated using an ethanol gradient and embedded in paraffin wax using previously described standard procedures [20,43,50]. The sections were collected on slides, dried and stained with Hematoxylin and Eosin (H&E). Sections (in 1 µm thick slices) were examined using a light microscope (Nikon 80i, Japan) and bright field images were captured using NIS Elements D software and a camera (Nikon U2, Japan).

The samples for Transmission Electron Microscopy (TEM) were obtained after the permeation experiments described above. They were cut into pieces not exceeding 0.5 mm^3, and fixed for 24 hours (4°C) using 4 % glutaraldehyde fixative, buffered to pH 7.2 [54]. Samples were processed and embedded in epoxy resin using standard protocols. Ultrathin sections (90 nm) were cut and contrasted with uranyl acetate and lead citrate and viewed with a transmission electron microscope (JEOL 1010, Japan). All experiments were performed using a minimum of three replicates.

2.3.2.6 Statistical Analysis

The results, expressed as mean ± standard deviation (SD) in values, were analysed using one-way analysis of variance (ANOVA), and followed by a Tukey's Multiple Comparison Test using GraphPad Prism® (Graph Pad Software Inc., Version 5). Differences were considered significant at $p < 0.05$.

3 Results and Discussion

3.1 Synthesis and Characterisation of Novel Oleodendrimer Derivatives

The OA derivatives, i.e. ester derivative (OA1E), the dicarboxylic acid derivative (OA1A) and the bicephalous dianionic surfactant (OA1ANa) were successfully synthesised by a modified method that excluded harsh reaction conditions in keeping with the current trends of greener approaches to synthetic chemistry. The literature reported procedure for the synthesis of OA1E from

which OA1A and OA1ANa were subsequently derived in this study [46] required a high reaction temperature and the use of thionyl chloride, a hazardous chemical. In the present work, the synthetic methodology was successfully modified by the use of coupling agents, viz., DMAP and EDAC.HCl. This synthetic modification enabled the avoidance of thionyl chloride to transform OA into oleoyl chloride, synthesis at room temperature instead of 110 ^{o}C as well as a reduction in the number of steps involved in the synthesis of OA1E and the other OA derivatives.

The structures of the newly synthesised compounds by the modified method were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopic analysis and compared to the spectroscopic data for the OA derivatives synthesised by the original method from the literature [46]. The data below show that the compounds were identical to the compounds prepared by the previously reported method [37,46]. Therefore the modified method in line with green chemistry approaches could be used to successfully prepare the OA derivatives.

*a) 3-N,N'-di-(tert-butyloxycarbonylethyl)aminopropanol (compound **2**)*

^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 18 H), 1.72 (q, 2H), 2.42 (t, 4H), 2.64 (t, 2H), 2.77 (t, 4H), 3.75 (t, 2H).

*b) OA1E (compound **4**)*

^{1}H NMR ($CDCl_3$) δ: 0.89 (t, 3H), 1.30 (m, 20H), 1.44 (s, 18H), 1.63 (q, 2H), 1.77 (m, 2H), 2.04 (m, 4H), 2.34 (m, 6H), 2.49 (t, 2H), 2.73 (m, 4H), 4.09 (t, 2H), 5.35 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.67, 24.98, 26.65, 27.20, 27.89,

28.09, 29.18, 29.31, 29.51, 29.70, 29.75, 31.89, 33.76, 34.34, 49.38, 50.13, 62.43, 80.31, 129.74, 129.97, 171.96, 173.85.

*c) OA1A (compound **5**)*

^{1}H NMR ($CDCl_3$) δ: 0.89 (t, 3H), 1.30 (m, 20H), 1.45 (s, 18H), 1.64 (m, 4H), 1.77 (m, 2H), 2.01 (m, 6H), 2.35 (m, 6H), 2.95 (m, 4H), 3.32 (m, 4H), 4.16 (t, 2H), 5.34 (m, 2H), 6.25 (br s, 2H). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.67, 24.82, 27.15, 27.21, 29.06, 29.14, 29.31, 29.51, 29.58, 29.67, 29.76, 31.89, 33.99, 48.53, 50.68, 55.40, 60.93, 129.72, 130.02, 173.56, 179.50.

3.2 *In-vitro* Buccal Permeation

3.2.1 Effects of OA, OA1E, OA1A and OA1ANa as permeation enhancers on the buccal permeability of ddI

In this study, the permeability enhancement potential of OA and its oleodendrimer derivatives, i.e. OA1A, OA1E and OA1ANa as novel buccal permeation enhancers to enhance the buccal permeability of ddI is reported. The cumulative amounts of ddI permeated over six hours, its *in vitro* permeation parameters in the absence of any enhancer and in the presence of either OA or the novel oleodendrimer derivatives, i.e. OA1A, OA1E and OA1ANa are shown in Figure 2 and Table 2 respectively. The flux of ddI in the absence of any of the enhancers was 46.57 ± 10.15 μg cm $^{-2}$.hr. This flux was marginally increased to 60.08 ± 10.34 μg cm $^{-2}$.hr in the presence of OA, the parent compound with ER of 1.29 although the increase was not statistically

significantly different from that of the control (p = 0.0674). The flux values for ddI in the presence of OA derivatives) i.e. OA1A, OA1E and OA1ANa were 46.57 ± 4.93 µg cm $^{-2}$.hr, 61.88 ± 9.75 µg cm $^{-2}$.hr and 80.30 ± 10.37 µg cm $^{-2}$.hr respectively with ER values of 1.01, 1.33 and 1.72 respectively. Therefore, these OA derivatives were able to enhance permeation of ddI, with OA1E and OAIANa specifically having higher ER values than the parent OA itself. OA1ANa with an enhancement ratio (ER) of 1.72 had the highest permeation enhancing effect as compared to OA, OA1A and OA1E. Statistical analysis showed a highly significant difference (p = 0.0004) between the flux with OA1ANa when compared to the control (ddI without any enhancer). A highly significant difference was also observed between OA1ANa when compared to OA1A (p<0.0001) as well as to OA (p<0.0001). Moreover, there was an overall significant difference (p = 0.0116) between the permeability enhancing efficacy of OA1A and OA1E when compared with OA1ANa (Table 2). In comparisons to other studies, the permeability enhancements obtained in this study were consistent with data from the previously published literature sources with oleic acid enhancers. The buccal mucosal permeability of Fluorouracil in a gel formulation incorporated with oleic acid as an enhancer, was increased by 1-6 fold as compared to gels without enhancer [1,55,56]. However, lower permeability values have been reported where oleic acid was used for drug permeability enhancement across the transdermal route [57,58]. In a recent study reported on OA's enhancing effect on diclofenac sodium (DS), OA enhanced the transdermal permeation of DS (ER = 1.87) [36]. This is comparable to the ER obtained for OA's enhancing effect on ddI in this study (ER = 1.29).

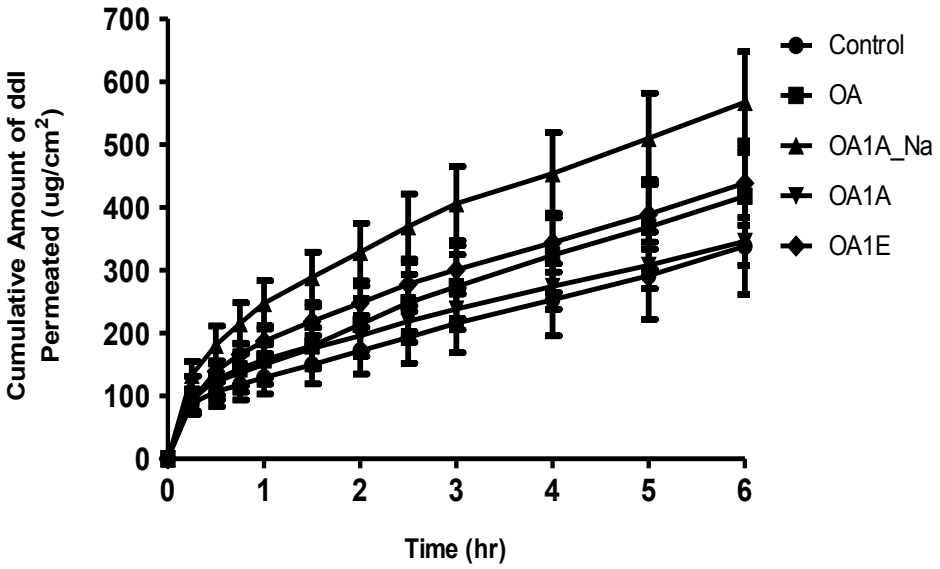


Figure 2: The effect of OA, OA1E, OA1A and OA1ANa on the cumulative amount of ddI permeated across the buccal mucosae.

Drugs generally can use either the paracellular or transcellular pathways or both for permeating the lipoidal membrane, and enhancement across the buccal mucosa [59,60]. Hydrophilic drugs in particular can permeate the lipoidal membrane via the paracellular route. FAs as chemical permeation enhancers can improve the permeability of drugs via the paracellular pathway [60]. It has been postulated that OA enhances permeability of drugs across the membrane by disrupting the lipid structure of the membrane, causing solubilisation by formation of micelles to create aqueous channels. One other probable mechanism is that FAs cause extraction of the inter- and intra-cellular lipids and proteins of the membrane [31], thereby causing an increased fluidity in the membrane. Various reasons could be attributed to the observed permeation enhancement differences between the OA1ANa and the other two derivatives. With available literature findings on percutaneous and oro-mucosal permeation

and the experimental observations in this study, a discussion to correlate the structural effects of OA derivatives on the permeation of ddI through porcine buccal mucosa is presented. The chemical structures, molecular mass, HLB, Log $P_{o/w}$ and ER_{flux} for the novel derivatives, are shown in Tables 1 and 3. The basic structural difference in the three OA derivatives is that OA1E has a branched diester function, OA1A is a dicarboxylic acid and OA1ANa is a bicephalous dianionic surfactant. These structural differences of OA derivatives along with their physicochemical properties are taken into consideration to correlate structural effects on buccal permeability. Based on this, OA1E showed higher ER as compared to OA1A which can be attributed to the higher lipophilicity of OA1E than OA1A [37]. The results correlate with previous findings where it has been observed that an increase in lipophilicity results in an increase in transdermal permeation of diclofenac sodium [36]. Also OA1E has a branched *tert*-butyl ester function at its periphery which may have contributed to enhanced ddI permeation since the branched diesters can provide better permeation enhancement [61]. OA1ANa showed the highest ER amongst all the three derivatives although less lipophilic. Its higher ER as compared to the other 2 derivatives may be due to a combination of effects. It may be due to its amphiphilic nature and surfactant characteristics which O1AE and OA1A lack. Like other surfactants such as sodium lauryl sulphate, OA1ANa might have showed effects like, disorganisation of the entire membrane architecture due to the extraction of the inter- and intra- cellular lipids and proteins of the membrane; the expansion of intercellular spaces, and the insertion of OA1ANa molecules into the lipid structure [62] which facilitated

better permeation of the drug through the lipid bilayer. These results are in good agreement with previous studies where oral mucosal absorption of lidocaine significantly increased in the presence of a surfactant derived from fatty acid [63]. In addition to the disruption of the lipid bilayer by the FAs, the sodium derivative could have an added advantage of ionic interactions between the free Na^+ ions and the negatively charged sialic acid residues of mucin on the mucosae that may have further altered the membrane permeability [64]. This is proposed as an additional mechanism since in contrast, OA1A and OA1E are acidic and ester compounds respectively and lacked the Na^+ ions that may have caused the ionic interaction between sialic acid and the enhancer as in OA1ANa. Furthermore, similar interactions have been reported in previous studies where interaction of the enhancer with the sialic acid residues has been proposed as a mechanism of permeation enhancement, i.e. a cationic charged molecule with the mucosal layer [7, 64].

Table 2: Permeability parameters of the OA, OA1A, OA1E and OA1ANa as novel buccal permeation enhancers for ddI (Mean ± SD; n ≥3)

	Treatment	Amount permeated ($\mu g.cm^{-2}$)	Flux, J_{ss} ($\mu g\ cm^{-2}\ .hr$)	Permeability, P x 10^{-3} cm hr^{-1}	Enhancement Ratio (ER)	pValue for Flux, J_{ss}
Control	Didanosine	338.07 ± 76.65	46.57 [a] ± 10.15	2.33 ± 0.56	---	---
Enhancer 1	OA	418.86 ± 77.31	60.08 [b] ± 10.34	3.00 ± 0.57	1.29	0.0674
Enhancer 2	OA1A	345.98 ± 37.96	46.57 [b] ± 4.93	2.34 ± 0.27	1.01	0.9747
Enhancer 3	OA1ANa	567.14 ± 80.46	80.30 [c] ± 10.37	4.01 ± 0.57	1.72	0.0004***
Enhancer 4	OA1E	438.56 ± 66.99	61.88 [c] ± 9.75	3.09 ± 0.53	1.33	0.0361*

{"a versus b" demonstrates statistically non-significant difference ($p > 0.05$), in the flux values of the enhancers compared to the control}.

{"a versus c" demonstrates statistically significant difference ($p < 0.05$), in the flux values of the enhancers compared to the control}.

The enhanced transbuccal permeation of ddI in the presence of OA1ANa is therefore presumably attributed to the disruption of the lipid bilayer as well as the ionic interaction between the sodium ions and sialic acid of the mucosal lining.

It has been reported that the log *P* values and HLB number can play an important role in differentiating enhancement ratios, flux and permeability coefficient values [28]. Moreover, it has been shown that the ester derivatives have better enhancement potential than the acid derivatives [36]. HLB and

hydrophilicity order of OA derivatives was OA1ANa > OA1A > OA1E (Table 3). Significant increase (p < 0.05) in ER_{flux} with OA1ANa (ER_{flux} =1.72) was observed than with OA1E (ER_{flux} = 1.33). From this observation it can be concluded that an increase in HLB value and hydrophilicity of OA derivative due to conversion into a surfactant molecule, resulted in increased ER_{flux} of ddI with OA1ANa. However, ER_{flux} value with OA1A was lower than with OA1E though the HLB and hydrophilicity of OA1A was more than OA1E. These contrasting results may be due to the fact that OA1E and OA1A as lipidic structures act by the same mechanism of action like other FA derivatives [61] and OA1ANa as a surfactant also acts by a mechanism of action similar to other surfactant like molecules [63]. It should be noted that the difference in ER_{flux} of OA1A and OA1E was not significant (p >0.05).

Table 3: Log $P_{oil/water}$, HLB and ER_{flux} of the OA derivatives, i.e. OA1E, OA1A and OA1ANa

Derivative	**HLB**	**Log $P_{octanol/water}$**	**ER_{flux}**
OA1E	4.79	9.40	1.33
OA1A	7.41	6.00	1.01
OA1ANa	36.2	-2.96	1.72

The faster and non-linear drug release observed at earlier times as compared to slower and linear drug release thereafter may be due to lack of equilibration between the mucosal absorption site and the permeating drug molecules. Once an equilibrium exists between the drug molecules and the mucosa, the non-linearity disappears due to reservoir of permeating molecules created from the partitioning of the drug into the deeper mucosal layers, which slows the diffusion rate, hence the slower kinetics observed in this study (Niccolazzo et al 2003; Mashru et al 2005; Birudaraj et al 2005).

Another observation is that there was no lag time in the permeation profiles obtained for the enhancing effect of OA and its derivatives on ddI permeability (Figure 2). By nature of the permeation enhancing effect of the enhancers, the cell layers would have been disrupted, hence reducing and/or removing any lag time that the drug might experience. Furthermore, as ddI is hydrophilic, permeation may be via the paracellular pathway thereby further reducing the lag time despite the thickness of the mucosae. Similar lack of lag times during buccal permeation studies on various drugs including didanosine, lidocaine and tenofovir, using porcine buccal mucosa has been reported previously [24,43,44,63]

In this study, it has therefore been shown that OA and its novel derivatives, i.e. OA1E, OA1A and OA1ANa, to a different extent enhanced the buccal permeability of ddI. The oleodendrimer derivative OA1ANa further had the highest enhancement factor for ddI as compared to the parent OA and other derivatives.

3.2.2 Effects of varying concentrations of OA and OA1ANa on the buccal permeability of ddI.

The above study identified OA1ANa as having the highest permeation enhancing effect. The effects of varying concentrations of the OA1ANa derivative was then studied and compared to OA (Figure 3 and Table 4). This study also identified the concentration range at which it will be effective and potentially safe for buccal permeation enhancement of ddI. The permeation enhancing potential of OA1ANa, as shown by the permeability properties of ddI, increased significantly (p = 0.0014) from 0.5 %w/w (ER = 1.14) to 2 %w/w (ER = 3.09), but decreased at 4 %w/w (ER = 1.55) and at 6 %w/w (ER = 1.50). Similar trends in the permeability properties of ddI in the presence of OA1ANa were also observed for OA at varying concentrations (Figure 3 and Table 4). Increase in OA1ANa concentrations from 0.5 to 2 %w/w led to increasing ER values but further increases at 4 and 6 %w/w led to subsequent decreases in ER values and they did not show any further enhancement in the ddI flux; and this was similarly observed for OA. Interestingly, the highest flux of ddI was obtained for both OA and OA1ANa at the same concentration of 2 %w/w.

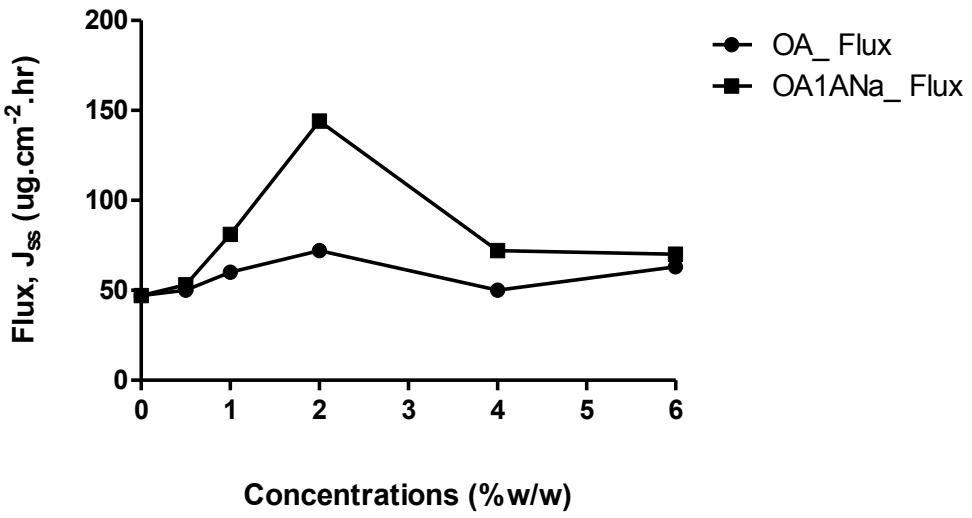


Figure 3: Concentration Effects of OA and OA1ANa on the Flux of ddI.

The results obtained in this study are similar to previous studies where increasing the concentration of the enhancers increased the buccal permeability enhancement initially, but further increases in concentration led to decreased permeability [31,43,57]. Reasons for the trend observed could be due to disruption of the lipid bilayer of the buccal mucosa occurring at the 2 %w/w concentration to allow for optimum permeation, whilst increased concentrations to 4 and 6 %w/w could have increased the viscosity at the mucosal layer, resulting in low drug movement [65]. Higher concentrations may have also decreased partitioning of ddI from the gel. Previous studies that used OA as a permeation enhancer have reported that the flux of piroxicam in the presence of OA increased from 0.3 %w/w to 1 %w/w but decreased at 5 %w/w [57]. The reason attributed to this was that at a 5 %w/w OA concentration, the presence

of a large amount of the FA could have slowed down the partitioning of the drug out of the gel base, thereby reducing the permeability rate of piroxicam [57]. The maximum ER value for OA1ANa obtained (ER=3.09) is similar to and in some cases higher than other reported CPEs stated as being promising enhancers for buccal permeation e.g. 3.2 for propylene glycol and dodecyl-2-dimethylamino propionate (DDAIP) HCl with ondasetron [25] and 1.8 for sodium dodecyl sulphate with caffeine [66]. It has been previously shown that the novel derivative, OA1E, used in this study could enhance the transdermal permeation of drugs [36]. The ER of the OA1ANa derivative compares with the oleodendrimers reported in that study. The ER ranged from 2.02 to 2.58 for oleodendrimers with the amide linkage. Interestingly, these ER values for transdermal permeation (2.02 to 2.58) are lower than the ER values obtained for OA1ANa (ER = 3.09). This can be due to the higher permeability of the buccal mucosa than that of the skin; since the skin unlike the buccal mucosa is keratinized and the application site in the skin layers could be thicker and greater thus posing more challenges for drug permeability[7]. Additionally, possible interaction of the Na^+ ions with negatively charged sialic acid residues and the mucin molecules may have further led to the higher ER value for buccal permeation.

Table 4: Effects of concentrations of OA and OA1ANa on the Permeability parameters of ddI (Mean ± SD; n ≥3)

	Cum Amt Permeated, Q_{6Hr} (µg.cm $^{-2}$)		**Flux, J_{ss} (µg cm $^{-2}$·hr)**		**Enhancement Ratio (ER)**	
Control	**338.07 ± 76.66**		**46.57 ± 10.16 ± 10.15**		**--**	
Concentrations [%w/w]	**OA**	**OA1ANa**	**OA**	**OA1ANa**	**OA**	**OA1ANa**
[0.5]	**381 ±63**	**392 ±55**	**49.19 ± 5.90**	**52.78 ± 6.86**	**1.05**	**1.14**
[1]	**419 ± 77**	**567 ± 80**	**60.08 ± 11.51**	**80.50 ± 11.33**	**1.29**	**1.72**
[2]	**496 ± 93**	**970 ± 289**	**71.80 ± 12.03**	**144.00 ± 53.54**	**1.54**	**3.09**
[4]	**400 ± 105**	**508 ± 126**	**49.85 ± 11.50**	**72.25 ±16.34**	**1.07**	**1.55**
[6]	**404 ± 36**	**440 ± 106**	**62.57 ± 6.38**	**69.91 18.26**	**1.35**	**1.50**

This study has identified for the first time a novel derivative of OA, OA1ANa, as an effective buccal permeation enhancer, thereby adding to the pool of chemical permeation enhancers for buccal delivery of ddI and other drugs.

3.3 Light Microscopy (LM) and Transmission Electron Microscopy (TEM) for the Histomorphological Evaluations of the mucosa

Permeation enhancers can play a role in the improvement of the permeability of drugs; however their suitability needs to be established as they may have membrane damaging effects [8,67]. Buccal delivery involves retention of the drug delivery system on the buccal mucosal site for diffusion to occur across the mucosa. Hence, the basal membrane of the mucosa must remain intact to ensure an effective diffusion of the drug. LM and TEM have been used in the literature to assess integrity of and histomorphological changes on the buccal mucosae after drug permeation studies [43,44,68].

In this study the effects of OA1ANa, being the buccal permeation enhancer amongst the three OA derivatives studied with the highest permeability coefficient and flux values, were investigated using LM and TEM. It must be noted that the barrier function of the stratified epithelium lends itself to a 'rebound' effect after prolonged exposure to a drug, which was not assessed in this study. However, adequate information can be obtained from LM and TEM images to determine whether the tissue suffered permanent/irreversible damage after exposure to the drug and enhancer treatment [64,68].

The morphology of the porcine buccal mucosa has been described previously and its characteristics resemble that of the human buccal epithelium[49,69]. The LM and TEM treated sections were compared to the controls. In LM investigations, the untreated mucosae/controls resembled that of a normal

non-keratinized, stratified squamous layer and the basal cells appeared oval and darkly stained in H&E (Figure 4A). Hence, the cells observed in the control sections could be regarded as healthy cells. The cells observed in the ddI gel treated mucosae (Figure 4B) resembles those seen in the control, therefore confirming that ddI gel and the excipients in the formulation did not adversely affect the mucosa.

The cells of the mucosa treated with ddI+ OA1ANa 0.5 %w/w (Figure 4C) closely resemble that of the control (Figure 4A) and the ddI gel treated mucosae (Figure 4B). This shows that no adverse effect was displayed in the cells of the mucosa treated with OA1ANa 0.5 %w/w. Moreover, the appearance of the control, ddI and ddI+ OA1ANa 0.5 %w/w mucosal sections were all similar suggesting that no adverse influence of ddI or OA1ANa at 0.5%w/w in the tissue morphology was evident. The outermost layer of the stratified squamous epithelium, basement membrane, lamina propria and submucosa were all intact in both the treated and untreated mucosae (Figures 4A-4E). Therefore OA1ANa 0.5 %w/w had no adverse effect on the buccal mucosae. There were no morphological changes observed in the mucosae treated with ddI alone (Figure 4B) and those treated with ddI+OA1ANa 0.5%w/w (Figures 4C). The superficial, prickle and basal cells all remained intact and no loss of the superficial cell layers and no formation of vacuoles in both the prickle and basal cells were observed.

However, the neatly aligned cells observed in Figures 4A and 4B which are the control and ddI gel treated mucosae respectively appeared to be interspaced in Figures 4C-4E which are ddI+OA1ANa 0.5 %w/w, ddI+OA1ANa 2 %w/w and ddI+OA1ANa 6%w/w treated mucosae respectively. The increased intercellular spaces (Figures 4C-4E)) could be attributed to the permeation enhancing effect of OA1ANa. Furthermore, the intercellular spaces between the epithelial cells increased with an increase in the concentration of OA1ANa from 0.5 %w/w to 2 %w/w, and further increased with ddI+OA1ANa 6%w/w (Figures 4C-4E). Moreover, swellings and vacuoles formation were observed in the prickle layers of the enhancer-treated mucosae compared to the control and drug-treated mucosae. When compared to the ddI+OA1ANa 0.5%w/w treated mucosae, increased formation of vacuoles, swelling and increased intercellular spaces in both the basal and prickle cell layers were observed in the ddI+OA1ANa 2 and 6 %w/w treated mucosae (Figures 4D-4E). The progressive morphological changes observed in treated cells could therefore explain the increased permeation of ddI observed in this study with OA1ANa at these concentrations (Figure 3). Increase in swelling and vacuole formation may also be attributable to accumulation of both drug and enhancer in the mucosa cells without the loss of the superficial layer. Increasing permeation was observed from ddI+OA1ANa 0.5 %w/w to ddI+OA1ANa 2 %w/w which correlates with the increased intercellular spaces, formation of vacuoles and swelling observed at these two concentrations.

There were clear distinctions between the cells treated with ddI+OA1ANa 2 %w/w (Figure 4D) and with ddI+OA1ANa 6 %w/w (Figure 4F). The cells treated with 6 %w/w were not as darkly stained as in the control, 0.5 %w/w and 2 %w/w treated cells. They showed probable reduced mitotic activity in both the prickle and basal layers, which may prevent diffusion of the drug across the basal cuboidal cell layer. The partitioning of drug across the biological membrane is possibly due to the mitotic activity in the mucosal cells. Thus, if a disruption is shown in the cells, such that cell organelles are disorganized, there could be a reduced mitotic activity and hence reduced partitioning of drugs [70]. Evidence of disruption to the cell organelles could indicate lack of partitioning of drugs into the membrane, hence lack of drug transport by diffusion across the membrane. This slight reduction in the mitotic activity observed in the basal cell layers could have led to the reduced permeability observed at the higher concentration (ddI+OA1ANa 6 %w/w). With ddI+OA1ANa 6 %w/w treated mucosae, the basal cells were additionally not totally oval in shape, showing some distortions in the membrane. However, any damage to the mucosa is considered non-permanent as the mucosa can regenerate from its damaged status by renewed growth of the mucosa from the germinal layer [71]. The results of this study correlated with trends observed in the concentration effect of aloe vera gel on the enhancing properties of ddI that was reported previously [43]

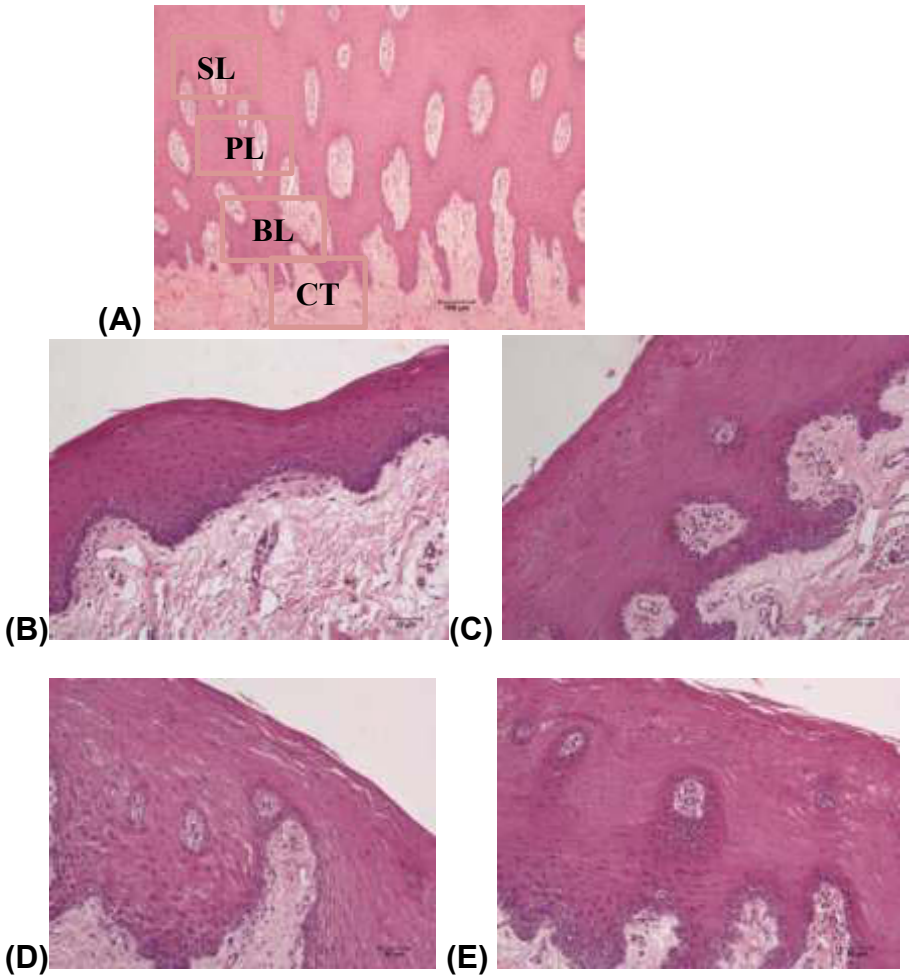


Figure 4: Photomicrographs of the control (untreated) and treated buccal mucosal sections stained with H&E (LM): (**A)** control /untreated (SL: superficial layer; PL: prickle layer; BL: basal layer; CT: connective tissue); (**B)** ddI gel (in the absence of any enhancers); (**C)** ddI + 0.5 %w/w OA1ANa; (**D)** ddI + 2 %w/w OA1ANa and **(E)** ddI + 6 %w/w OA1ANa.

In TEM investigations, an in-depth ultrastructural analysis of the cellular organelles of the buccal mucosa sections is made possible. TEM was therefore undertaken to confirm the effect of the novel derivative OA1ANa on the cellular organelles of the buccal mucosa that was observed at the LM level and reported above. The ultrastructure of the untreated buccal mucosa (control) showed regular nuclear profiles with closely packed cellular walls and well-arranged desmosomes at the gap junctions. The nuclear outlines appeared regular in the control buccal mucosal section (Figure 5A).

Both the ddI and OA1ANa 0.5 %w/w treated mucosae resembled the control section by way of their regular nuclear outlines and absence of cellular distortions. The mitochondria appeared dense in both the ddI gel treated mucosa (Figures Bi-ii) and the OA1ANa 0.5 %w/w treated mucosae (Figure Cii), although they showed slight electron translucent clearings within the mitochondria. An increase in the intercellular spaces was noted with ddI gel only (Figures 5B) as well as ddI+OA1ANa 0.5 and 2 %w/w treated mucosae (Figures 5Ci and 5Di). The increased intercellular spaces observed could be attributed to the partitioning of the permeant (ddI) as well as the enhancing effect of OA1ANa.

A permeant can use either the paracellular, transcellular or both pathways mechanisms for permeability enhancement [7,69]. The increase in the intercellular spaces did not damage the gap junctions as evident by the presence of the desmosomes. Interestingly, the desmosomes appeared intact in the ddI+OA1ANa 2 %w/w treated mucosa (Figure 5D (ii)). This may indicate that the highest permeability observed at this concentration level could be via

the paracellular transport pathway. Surfactants can disrupt the lipid bilayer / the entire membrane architecture of the buccal mucosa, cause an expansion in intercellular spaces and can lead to an insertion of the OA1ANa molecules into the lipid structure [72] which can encourage the enhanced permeability of drugs. Though appearing slightly swollen, no evidence of damage to the desmosomes at the gap junction could be seen except for increased intercellular spaces. This can also be due to the enhancing effect of OA1ANa on the buccal permeability of ddI [17]. While the nuclear envelope appeared slightly irregular as observed in the ddI+OA1ANa 2 %w/w treated mucosa, cellular damage was not evident. The nuclear membrane showed slight distortion in the outlines which do not differ much from that of the ddI+OA1ANa 0.5 %w/w treated mucosae. However, crenulation of the nuclear envelope was observed in the ddI+OA1ANa 6 %w/w (Figure 5E).

In this study, LM confirmed that there was no adverse effect and no tissue damage as a result of the ddI gel when used alone and when in combination with OA1ANa 0.5 and 2 %w/w except for the OA1ANa 6 %w/w where adverse effects were observed. TEM revealed similar effects of OA1ANa on the mucosa as observed with the LM. Since the mucosa was not adversely affected at these concentrations, i.e. 0.5 and 2 %w/w, OA1ANa could be used at these concentrations to enhance the permeability of ddI. In permeation studies, OA1ANa was identified as the enhancer with highest ER for ddI. Based on these results, OA1ANa at 2 %w/w can be proposed as an enhancer in a delivery system since the exposure of OA1ANa concentrations up to 2 %w/w

did not show any adverse effects on the buccal mucosa and simultaneously displayed the highest permeability enhancement.

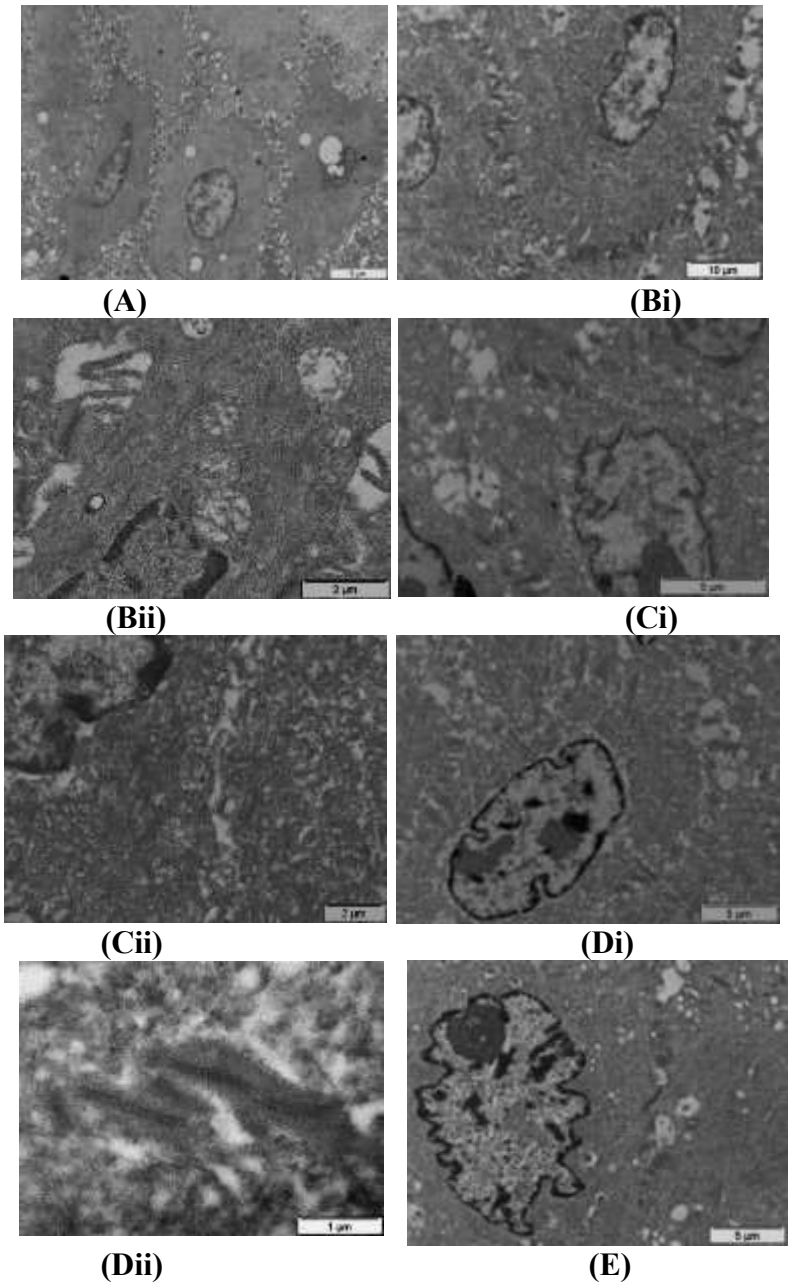


Figure 5: Electro-micrographs of the control (untreated) and treated ultra-thin buccal mucosal sections (TEM): **(A)** control /untreated; (**Bi and ii) ddI** gel only (in the absence of any enhancers); (**Ci and ii**) ddI + 0.5 %w/w OA1ANa; (**Di and ii)** ddI + 2 %w/w OA1ANa; (**E)** ddI + 6 %w/w OA1ANa

4 Conclusions

The derivatives of OA were successfully synthesised incorporating the use of a coupling agent i.e DMAP and EDAC.HCl that eliminated the use of thionyl chloride and high reaction temperatures as well as reduced the number of synthetic steps. This study clearly demonstrated that OA and its novel derivatives, i.e. OA1E, OA1A, and OA1ANa could enhance the buccal permeability of ddI. All the novel derivatives of OA that were explored and reported in this study increased the buccal permeability of ddI, with the OA1ANa derivative having the best enhancing potential than other derivatives and its parent compound, OA. OA1ANa 2 %w/w displayed the highest flux and permeability for ddI across the buccal mucosa with an enhancement ratio of 3.09 more than that of the ddI alone. The permeability enhancing effects OA1ANa as a novel buccal permeation enhancer for ddI was shown to be concentration-dependent. Interestingly, both OA and OA1ANa also showed a similar trend with flux values as their concentrations were increased. Maximum flux for both OA and OA1ANa were observed at 2 %w/w with ER values of 1.54 and 3.09 respectively. The morphological changes reported in this study, i.e. vacuoles formation, increased intercellular spaces and swelling were attributed to, and correlated with the permeation enhancing effect of the novel OA1ANa derivative. No adverse effects were observed in all treated and untreated mucosae in this study. The novel OA derivatives show potential for the enhancement of the buccal permeability of ddI and can widen the pool of

chemical permeation enhancers for buccal delivery of various drugs for drug therapy optimisation.

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Declaration of interest

The authors report no declaration of interest

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CHAPTER FIVE

CONCLUSION

5.1 INTRODUCTION

The conclusions drawn from the main experimental findings in this study are presented in this chapter. It also identifies possible study limitations, and highlights the significance of the study as well as recommendations for future work.

The potential of the buccal mucosa route to deliver different classes of drugs, including antiretroviral, specifically ddI, may overcome certain challenges suffered by the current use of other routes, such as oral, parenteral and rectal, to increase therapeutic outcomes and patient compliance. A major focus in the area of buccal delivery of drugs is identifying approaches to overcome the challenges of limited buccal permeability due to the epithelium layer that acts as a barrier to drug permeation. Thus, the selection of a safe and effective permeation enhancer is essential for the design of buccal drug delivery systems to promote the permeation of drugs across the buccal mucosa and improve bioavailability. The potential of the buccal mucosa route for delivery of ARVs have not been comprehensively investigated. The ultimate aim of this study therefore, was to explore the potential of the buccal mucosal route for the delivery of an antiretroviral drug.

The permeation experiments using vertical Franz diffusion cells were undertaken, and permeability of ddI across the buccal mucosa was confirmed. Histomorphological studies using light and transmission electron microscopy to evaluate the effect of ddI and the novel permeation enhancers on the buccal mucosa were performed. Furthermore, the preliminary formulation experiments were undertaken and reported as published research abstracts. Novel MMFs containing ddI for buccal delivery were prepared using homogenization, casting and solvent evaporation methods. *In vitro* dissolution studies of the MMFs containing ddI were performed using a shaking water bath. Scanning electron microscopy was employed to evaluate the surface morphology of the films before and after *in vitro* dissolution testing. The ddI MMFs prepared in this study were confirmed as potential candidates for optimization into buccal delivery systems.

5.2 CONCLUSIONS FROM THE STUDY FINDINGS

This study purposed to explore the potential of the buccal mucosa route to deliver a model ARV drug, i.e. ddI. The study had three sub-aims as presented below, and each had a number of objectives. The conclusions drawn from each aim, based on the main experimental findings in this study will be outlined respectively:

1. Investigate the permeability properties of ARV drugs across the buccal mucosa using ddI as a model ARV drug.

   - The potential for delivering an antiretroviral drug such as ddI using the buccal mucosa as an alternate route was confirmed. The buccal permeability parameters of ddI were identified, and the effects of ddI concentration study showed that its permeation across the buccal mucosa was concentration-dependent. A linear relationship (R^2=0.9557) between the flux and ddI concentrations indicated that passive diffusion was the mechanism of its transport across the buccal mucosa.

2. Determine the effects of different novel enhancers such as, AVgel, OA and the synthesized oleodendrimer derivatives of OA, on the buccal permeability of ddI.

   - The effect of AVgel as a buccal permeation enhancer obtained from a natural plant product was investigated. The study showed that ddI can permeate the buccal mucosa in the absence of AVgel, and without adversely affecting the mucosal morphology. AVgel, at concentrations 0.25 to 2 %w/v, was identified as an effective buccal permeation enhancer for ddI. The permeability enhancement potential of AVgel for ddI increased significantly from 0.25 to 2 %w/v, with enhancement ratio (ER) of 5.09 (AVgel 0.25 %w/v) to 11.78 (AVgel 2 %w/v). The

enhancement of ddI increased approximately 12-fold with AVgel 2.0 %w/v, but decreased to 0.87-fold with AVgel 6.0 %w/v. Therefore, AVgel can increase the permeation of ddI across the buccal mucosa with the enhancement being concentration-dependent.

- The effects of OA and its novel derivatives were investigated i.e. ester derivative (OA1E), dicarboxylic acid derivative (OA1A) and bicephalous dianionic surfactant (OA1ANa) as chemical permeation enhancers for buccal delivery of ddI. This study clearly demonstrated that OA and its derivatives, i.e. OA1E, OA1A, and OA1ANa, all at 1 %w/w, could enhance the buccal permeability of ddI. OA and all the novel derivatives that were explored and reported in this study increased the buccal permeability of ddI, with the OA1ANa derivative having the best enhancing potential than other derivatives and its parent compound, OA.

- The concentration effect of the best identified oleodendrimer derivative, OA1ANa, was also investigated at varying concentrations of 0.5 to 6 %w/w, and compared with its parent compound, OA. The permeability enhancing effects of OA1ANa as a novel buccal permeation enhancer for ddI was shown to be higher than its parent compound, OA, and was concentration-dependent. OA1ANa at 2 %w/w displayed the highest flux of 144.00 ± 53.54 μgcm^{-2}.hr, and enhanced the permeability of ddI

across the buccal mucosa with an enhancement ratio of 3.09, more than that with the ddI alone. At equivalent concentrations, OA1ANa (ER = 3.09) showed higher enhancement of ddI than the parent compound, OA (ER = 1.54). This novel OA derivative displayed the best potential for enhancing the buccal permeability of ddI, and it can widen the pool of chemical permeation enhancers for the buccal delivery of ddI and other drugs for therapy optimization.

3. Assess the histo-morphological effects of both ddI and the novel enhancers, AVgel and OA oleodendrimer derivatives on the buccal mucosa, using light and transmission electron microscopical studies.

- Histomorphological investigations of the buccal mucosae in the absence or presence of AVgel were performed. This study therefore demonstrated that there were adverse effects on the cell organelles of the 1, 2 and 6 %w/v AVgel-treated mucosae. However, the cell organelles of the buccal mucosae treated with PBS, ddI/PBS and ddI/PBS/AVgel 0.5 %w/v were still intact, and showed that there were no adverse mucosal effects. Based on the findings in this study, it is proposed that AVgel can be used in concentrations at or lower than 0.5 %w/v due to adverse mucosal effects at the higher concentrations. The histomorphological evaluations in this study also proved useful in correlating the permeation enhancing properties of AVgel with its effects

on the buccal mucosa. AVgel effectively enhanced the buccal permeability of ddI at all concentrations studied. A safe and effective concentration of 0.5 %w/v w with ER of 5.09 was identified. Therefore AVgel at 0.5 %w/v showed the most buccal permeation enhancing potential for delivering an antiretroviral, ddI.

- Histomorphological investigations of the buccal mucosae in the absence or presence of the selected OA derivatives were also performed. Light and transmission electron microscopical analysis confirmed that OA1ANa at all concentrations, except the 6 %w/v, had no adverse effects on the mucosae. Morphological changes, such as vacuoles formation and increased intercellular spaces, noted that 2 %w/w were attributed to the buccal permeation enhancing effect of OA1ANa.This study also showed that OA1ANa is safe and effective to use as buccal permeation enhancer for ddI at the identified concentration of 2 %w/v.

- The potential of using AVgel at a concentration of 0.5 %w/v or lower for developing a buccal drug delivery system containing antiretroviral ddI and buccal permeation enhancer of natural origin. Furthermore, the potential of a novel chemical permeation enhancer, i.e. OA1ANa at a concentration of 2 %w/v or lower, was identified. The presence of intercellular spaces in the mucosae treated with both ddI and the

enhancers suggested that they permeated the buccal mucosa using both the paracelular and transcellular routes for transport.

The findings of this study therefore contributed significantly to knowledge about the buccal delivery potential of an ARV, ddI and the permeation enhancers of both natural plant and synthetic products origin. These novel enhancers can be selected to develop buccal delivery systems containing ARVs, for improving HIV and AIDS therapy. The various ddI MMFs prepared in this study are potential candidates for optimization as novel buccal delivery systems.

5.3 STUDY LIMITATIONS

The aim of this study was to explore the buccal delivery potential of an antiretroviral drug using ddI as a model drug. The methods employed to generate the experimental findings in this study were in line with those employed in other studies reported in the literature. However, certain limitations as described below should be noted:

- The buccal mucosa, is a biological membrane, and when used for permeation studies can produce data with high variability, particularly if the membrane is not harvested and excised according to an appropriate standard procedures. The initial stage of the preparation of buccal mucosae from pigs for permeation

experiments therefore took extensive time, as very limited expertise in the mucosa excision was available in the country. Therefore, much time was taken to achieve the correct procedures to obtain the buccal mucosa, and to reduce variability in the various permeation experiments undertaken at the initial stage of this study. In addition, extensive time was taken to ensure that the reported data were reproducible. An early exposure to the standard operating procedures regarding the preparation of the buccal mucosae, including harvesting the mucosae from the pigs, as well as the excision for permeation experiments could have saved time, which could have been used to add value to the experimental studies.

- Some studies in the literature support permeation studies with transepithelial electrical resistance (TEER) which is an indicator for epithelial viability and, to demonstrate that mucosal integrity remains irreversibly affected. The publication of the findings from experimental studies in chapters 3 and 4 could have been improved if relevant TEER equipment were available for the study at the initial stage. However, the morphological characteristics of the buccal mucosae evaluated by LM and TEM as well as the reproducible data, did show that the buccal mucosa used in the study remained intact throughout the 6 hour experimental period. In addition, the permeation data in this study were obtained with acceptable standard deviations, and the histomorphological evaluations

correlated well with the permeation data. The experimental studies on buccal permeability did not include TEER measurements due to lack of equipment, which could have further strengthened the published paper at the time.

- ddI was incorporated into MMFs, and the preliminary experimental findings were published as an abstract in an international ISI journal. The characterization of the ddI buccal films (i.e. MMFs) in terms of drug uniformity content, in vitro drug release, film thickness and morphology did confirm the potential for optimization. However, an in-depth characterization of the ddI MMFs was not done. Therefore, further characterization, such as mucoadhesive and physicomechanical properties of the MMFs that were prepared in this study, could have strengthened the thesis. This was not performed, as this thesis met the criteria for PhD by publication route.

5.4 RECOMMENDATIONS FOR FUTURE WORK

The findings of this study can contribute significantly to the knowledge required by formulation scientists to optimally design and formulate ARV buccal delivery systems that will incorporate permeation enhancers for improving antiretroviral therapy. Further work will be necessary for buccal delivery systems containing an ARV such as, ddI, to formulate with permeation enhancers and manufacture

for commercialization. Therefore, future research could address the following areas:

- The mechanism of ddI permeation in the presence of the enhancers should be investigated, as ddI (pKa 9.2) has the tendency to ionize in an acidic medium, and any variation in the buccal physiological pH may influence the functionality of the dosage form as well as ddI absorption mechanism. Therefore, investigating the effect of pH on ddI permeation in the presence of the enhancers as well as pH-partitioning studies are recommended. Mechanistic studies to characterize the buccal permeation properties of both drug (ddI) and enhancers, i.e. AVgel and OA1ANa, which would be useful in determining the exact permeation pathways are proposed. In addition, molecular dynamics studies on both drug and enhancers are proposed in order to predict mechanism of permeation, as well as how the molecules of drug and enhancers interact with the components of the buccal mucosa.

- The use of conventional light and transmission electron microscopy are limited by rigorous method of fixation, sectioning and staining of the buccal mucosa. Processing techniques may interfere with the examination and interpretation of the structures of the mucosa. Hence, confocal scanning laser microscopy is proposed for future studies, as it may play a major role in detecting and determining various cell organelles in the mucosa. The pathways of the buccal permeation of ddI and the

enhancers may become clearer and mechanisms of permeation evidently determined.

- The enhancers identified in this study have the potential for being applicable to other ARVs, particularly the new generation of ARVs, such as integrase strand-transfer inhibitors (INSTI, i.e. elvitegravir), and a second generation NNRTI, i.e. rilpivirine. It is therefore recommended that the study approach described in this thesis be employed in developing and optimizing other newer ARVs. The enhancers identified in this study can also be investigated for delivery systems containing ddI or any other ARV, for various mucosal applications, including rectal and vaginal. Furthermore, studies on developing delivery systems that will contain more than one drugs for simultaneous delivery via transmucosal routes are emerging. In addition, in South Africa and globally, antiretroviral therapy using combination drugs, such as fixed dose combination (FDC) of tenofovir, emtricitabine and efavirenz or other multi-class combination ARV products, is currently a gold standard treatment for HIV and AIDS in an approach referred to as highly active antiretroviral therapy (HAART) for improved therapeutics' outcomes and patient compliance. Therefore, an investigation into the effects of the enhancers identified in this study for buccal delivery of FDC for other ARVs and multi-class combination drugs is highly recommended.

- Incorporating buccal permeation enhancers into an optimized drug delivery system for ddI or other ARVs remains to be investigated. The development of a carefully designed advanced buccal delivery system, such as nano-composite or polyelectrolyte films, or novel mucoadhesive nanoparticles to contain ddI and selected permeation enhancers for improved ARV therapy is proposed. Further, optimization and in-depth characterization of these delivery systems, in terms of physicochemical/mechanical properties, should be explored. Additionally, molecular modeling is recommended in order to determine how the enhancers will interact with the drug and in the presence of the selected excipients. Stability studies on the formulation of ddI MMFs in order to ascertain the exact effects of the enhancers on the design and formulation of buccal delivery systems, in terms of chemical and physical stability are recommended.

- *In vivo* studies using appropriate animal model, such as pig, to identify the effectiveness of the dose of drug and enhancers to be incorporated into the buccal delivery systems are proposed. Additionally, the acceptability of the buccal delivery systems by the intended human populace should be explored. Studies on irritation and toxicity of the drug and the enhancers on the buccal mucosa are recommended.

5.5 SIGNIFICANCE OF THE FINDINGS IN THE STUDY

- **Optimizing drug treatment of HIV and AIDS:**

This study has confirmed the buccal delivery potential of ddI as an alternate route for ARV therapy. The treatment of patients suffering from HIV and AIDS remains a health priority in South Africa and globally. The delivery of ARV drugs via the buccal mucosal route will overcome challenges with oral delivery. This study will benefit patients in many ways, including increase in bioavailability, reduction in systemic side effects of ARVs, and improved patient compliance. The delivery of ARV via buccal will benefit patients in many ways, including increase in bioavailability due to the highly vascularized buccal mucosal site that promote higher absorption of drugs. For buccal drug delivery, lower doses are administered compared to the high and frequent doses with oral drug delivery, hence the dose-dependent GI side/adverse effects would be reduced. Moreover, the buccal drug delivery systems may exhibit controlled-release kinetics thus decreasing frequency of dosing and reducing systemic side effects leading to improved patient compliance, hence optimized drug treatment. Identifying alternative route of delivery for ARVs, especially in paediatrics and patients with swallowing difficulty is paramount for improving ARV therapy. Furthermore, the availability of buccal delivery systems containing ddI can increase the variety of

dosage forms for various categories of patients, particularly for paediatric population.

- **Creation of new knowledge in buccal delivery of antiretroviral drugs:**

  This study has identified the buccal permeability parameters of ddI and has confirmed the potential of ddI for buccal delivery. It has also identified the permeation enhancing abilities and possible mechanisms of novel permeation enhancers of both natural plant and synthetic origins for buccal delivery. The study also contributed to establishing correlations between histological evaluations of buccal mucosa using the TEM with permeability parameters in the area of ARV buccal delivery.

- **Identification of new pharmaceutical materials for buccal delivery:**

  The project has contributed to the identification of new excipients for buccal delivery systems. The identification of synthetic derivatives of OA as well as AVgel from natural plant products as enhancers for buccal delivery was established. These novel permeation enhancers will now be available to formulation scientist for selection in formulating, optimizing and manufacturing ddI buccal films for

eventual commercialization. This will also be applicable to other various classes of drugs for various diseases, and particularly to other ARVs, fixed dose combinations, and multi-class ARV combinations. Further, the pharmaceutical industries will benefit from the findings of this study, in that the newly identified materials can be used to manufacture more cost-effective medicines.

- **Impact of this study on future research:**

  The identification of the permeability parameters of the ARV drug, ddI as well as drug permeation enhancement potential of AVgel and OA derivatives impact on future research as it will stimulate further research into the design of ddI buccal delivery systems with optimal properties. The findings in this study can also be applied to investigate the buccal delivery potential of other newer ARVs, and specifically the multi-class combination drugs for improving ARV therapy. This study is also significant for other routes of drug administration, such as the vaginal, rectal as well as ocular drug delivery routes, as these enhancers may be investigated for these routes as well.

The current trend for optimizing drug treatment for various diseases is searching for alternate routes for drug delivery, and developing novel drug delivery systems, instead of searching for new chemical entities. The buccal

mucosa as alternate route for drug administration clearly has potential for the delivery of an antiretroviral drug. The design, optimization and evaluation of a novel drug delivery system of an ARV for buccal would require multidisciplinary collaborative approach and efforts of the researchers in academia, formulation scientists as well as pharmaceutical industries. This multidisciplinary collaborative approach is particularly warranted for an eventual manufacturing and commercialization of the novel buccal delivery systems of ARV drugs.

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APPENDIX I

ACADEMIC RULES - COLLEGE OF HEALTH SCIENCES

(Adapted from the College of Health Sciences Handbook 2014, available at http://chs.ukzn.ac.za/Homepage.aspx)

COLLEGE OF HEALTH SCIENCES ACADEMIC RULES

Note:

- **The General Academic Rules of the University shall, where applicable, also apply to the qualifications offered in the College**
- **Students are advised that not all modules listed in this handbook will necessarily be offered and that the University reserves the right to withdraw modules at short notice if and when necessary**
- **All first entry undergraduate students from 2014 must pass a module in isiZulu in order to be degree complete; or obtain exemption from the module under rule GR8a.**

CHS 1 – Changes in Rules

The College may revise or add to its rules from time to time, and any such alteration or addition shall become binding upon the date of publication or upon such date as may be specified by the College, provided that no change in rules shall be interpreted so as to operate retrospectively to the prejudice of any currently registered student.

CHS 2 - Professional Registration

Where a Statutory body (e.g. the Health Professions Council of South Africa), requires the professional registration of students in a programme, then the continued registration of the student in the programme (and the University) shall be a condition of such registration with the Statutory Body.

CHS 3 Statutory Body Requirements

a) Statutory Bodies governing qualifications and programmes offered in the College may have stipulated learning activity requirements (e.g. a minimum number of hours of clinical, experiential, fieldwork and/or service learning) that must be achieved prior to graduation.
b) If necessary, such activities may need to be undertaken after normal working hours, over weekends, public holidays and during University vacations.

CHS 4 Compulsory Hepatitis B Vaccination

a) All students registered in the College for the first time (or in a new programme) shall provide proof of successful vaccination against Hepatitis B by the end of their first year.
b) There shall be no further registration without such proof.

CHS 6 Registration and Progression

a) Save in exceptional circumstances and with the express permission of the School, no student shall be allowed to register for modules where known timetable clashes exist. If a timetable clash is identified after registration, the student will have to deregister the "higher level" module in favour of the "lower level" module.
b) Students who repeat module(s) must attend all components of the module(s).

CHS 7 Readmission Following Suspension of Registration

A student who for two semesters or more has not undertaken clinical, experiential, fieldwork and/or service learning will be required to pass a test, or otherwise produce evidence of sustained clinical competence in order to be readmitted to the programme.

CHS 8 Unacceptable Behaviour

a) All students who are required to attend healthcare and other facilities, both internal and external to the college or University, shall comply with the codes of conduct, behaviour and dress of the University and of the College.
b) Where a student has been found guilty of professional misconduct by the relevant Statutory Body, and their registration cancelled by the Statutory Body, their University registration will be cancelled. The student will have a right to be heard.

CHS 9 Impaired Practitioner

A student who, after due consideration and assessment by an *ad hoc* committee (appointed by the College or School or Statutory/Professional Body) is deemed impaired and unable on *inter alia* psychiatric grounds or grounds of substance abuse to continue his/her studies, shall have his/her registration suspended or be refused readmission to the programme.

CHS10 Eligibility for Postgraduate Qualifications

Applicants shall be subject to selection based on the appropriateness of their academic background, the strength of their previous academic record, the availability of University resources and University obligations in terms of University or Government policies.

CHS 11 Eligibility for Postgraduate Diplomas in the College

a) A candidate is eligible to apply for selection to register for the qualification of a Postgraduate Diploma in the College provided that he or she holds
    (i) a Bachelor of Medical Science, or
    (ii) a Bachelor of Science, or
    (iii) an MBChB, or
    (iv) a Bachelors qualification in one of the health professions from the University
b) Applicants shall be subject to selection based on the appropriateness of their academic background, the strength of their previous academic record, the availability of University resources and University obligations in terms of University or Government policies.

CHS 12 Postgraduate Diploma module repeats and examinations

a) With the permission of the School, candidates who have failed a module shall be permitted to repeat such module or, if the module in question is not a core module, to select an alternative module to complete the Postgraduate Diploma.
b) A candidate who repeats a module shall repeat all parts of the module, including group work and assignments.
c) No module shall be repeated more than once.

CHS 13 Dissertation by Publication For Masters by Coursework

In addition to rule CR13:

a) A dissertation may comprise one or more papers of which the student is the prime author, published or in press or in manuscripts written in a paper format, in peer-reviewed journals on the SAPSE/ISI list of journals, or in manuscripts written in paper format accompanied by introductory and concluding integrative material, one of which reports original research.
b) Reviews and other types of papers in addition to original research paper/s may be included, provided they are on the same topic.

CHS 14 Doctoral Degree by Research

a) A thesis may comprise three or more papers of which the student is the prime author, published or in press or in manuscripts written in a paper format, in peer-reviewed journals on the SAPSE/ISI list of journals, or in manuscripts written in paper format accompanied by introductory and concluding integrative material, two of which reports original research.
b) Reviews and other types of papers in addition to original research paper/s may be included, provided they are on the same topic.

The following qualifications are offered in the College:

Qualification	School
Diploma in Oral Health (2 year qualification)(Only Pipeline students)	School of Health Sciences
Bachelor of Audiology	School of Health Sciences
Bachelor of Communication Pathology (Audiology)(Pipeline students)	School of Health Sciences
Bachelor of Speech Therapy	School of Health Sciences
Bachelor of Communication Pathology (Speech-Language Pathology)(Pipeline students)	School of Health Sciences
Bachelor of Dental Therapy	School of Health Sciences
Bachelor of Medical Science (Anatomy)	School of Lab Meds and Medical Sciences

APPENDIX II

ETHICAL APPROVAL LETTERS

UNIVERSITY OF
KWAZULU-NATAL

RESEARCH OFFICE
HOWARD COLLEGE CAMPUS
E-Mail: bawa@ukzn.ac.za
Tel.: 27-31-260 2273 Fax: 27-31-260 2384

12 October 2007

Reference: 001/08/Animal

Prof. T. Govender
School of Pharmacy and Pharmacology
University of KwaZulu-Natal
WESTVILLE CAMPUS

Dear Prof. Govender

Renewal: Ethical Approval of Research Project using Animals

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2008 on the following project:

Permeation mechanisms via the Buccal Mucosa and histological effects: The effect of model drugs and drug containing Polymeric Films.

Yours sincerely

HT Coetzer

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar
Research Office
Head of School

UNIVERSITY OF
KWAZULU-NATAL

RESEARCH OFFICE
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22 January 2009

Reference: 028/09/Animal

Mrs E Ojewole
Lecturer
School of Pharmacy & Pharmacology
University of KwaZulu-Natal
WESTVILLE

Dear Mrs Ojewole

Ethical Approval of Research Project using Animals

I have pleasure in informing you that on recommendation of the review panel, the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2009 on the following project:

"Investigating the potential of the buccal mucosal route for systemic delivery of antiretroviral drugs: Permeability and Formulation Studies".

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar
Research Office
Head of School

UNIVERSITY OF
KWAZULU-NATAL

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4 November 2009

Reference: 029/10/Animal

Mrs E Ojewole
Lecturer
Department of Pharmacy
Block E2, Floor 6, Room 606
University of KwaZulu-Natal
WESTVILLE CAMPUS

Dear Mrs Ojewole

Renewal: Ethical Approval of Research Project using Animals

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2010 on the following project:

"Investigating the potential of the buccal mucosal route for systemic delivery of antiretroviral drugs: Permeability and Formulation Studies".

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar
Research Office
Head of School

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21 December 2010

Reference: 25/11/Animal

Mrs E Ojewole
School of Pharmacy and Pharmacology
Medical Sciences
University of KwaZulu-Natal
WESTVILLE CAMPUS

Dear Mrs Ojewole

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 2011 on the following project:

"Investigating the potential of the buccal mucosal route for systematic delivery of antiretroviral drugs: Permeability and Formulation Studies"

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar
Research Office
Supervisor (Prof. T Govender)
Head of School (Prof. WMU Daniels)

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21 November 2011

Reference: 07/12/Animal

Ms.. E. Ojewole
Department of Pharmacy
Block E2 Floor 6
Room 606
Westville Campus

Dear Ms. Ojewole

Renewal: Ethical Approval of Research Project on Animals

I have pleasure in informing you that on recommendation of the review panel, the Animal Ethics Sub-committee of the University Research and Ethics Committee has granted ethical approval for 2012 on the following project:

"Investigating the potential of the buccal mucosal route for systemic delivery of antiretroviral drugs: Permeability and Formulation Studies."

Yours sincerely

Prof. Theresa HT Coetzer (Chair)
ANIMAL RESEARCH ETHICS COMMITTEE

Cc Registrar, Prof. J. Meyerowitz
Research Office, Mr Nelson Moodley
Head of School, Prof. F. Oosthuizen
Supervisor, Prof. T. Govender
BRU, Dr. SD Singh

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14 December 2012

Reference: 039/13/Animal

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

Dear Ms Ojewole

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:

"Investigating the potential of the buccal mucosal route for systemic delivery of antiretroviral drugs: Permeability and Formulation Studies."

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

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

23 December 2013

Reference: 043/14/Animal

Ms E Ojewole
Discipline of Pharmaceutical Sciences
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WESTVILLE Campus

Dear Ms Ojewole

RENEWAL: Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Research Ethics Committee has granted ethical approval for **2014** on the following project:

"Investigating the potential of the buccal mucosal route for systemic delivery of antiretroviral drugs: Permeability and Formulation Studies."

Yours sincerely

HTJoetzer

Professor Theresa HT Coetzer
Chairperson: Animal Research Ethics Committee

Cc Registrar – Prof. J Meyerowitz
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APPENDIX III

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Review article

Exploring the use of novel drug delivery systems for antiretroviral drugs

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ABSTRACT

Novel drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral (ARV) drug therapy, thereby improving the management of patients with HIV/AIDS. This paper provides a comprehensive review of the various ARV delivery systems that have been developed for achieving sustained drug release kinetics, specifically targeting drugs to the macrophages, brain and gastric mucosa, and for addressing formulation difficulties such as poor solubility, stability and drug entrapment. Studies on the potential of systems for alternative routes of ARV drug administration, i.e., transdermal, buccal and rectal, are also highlighted. The physico-chemical properties and the *in vitro*/*in vivo* performances of various systems such as sustained release tablets, ceramic implants, nanoparticles, nanocontainers, liposomes, emulsomes, aspasomes, microemulsions, nanopowders and Pheroid™ are summarised. Further studies that remain to be undertaken for formulation optimisation are also identified. This review highlights the significant potential that novel drug delivery systems have for the future effective treatment of HIV/AIDS patients on ARV drug therapy.

1. Introduction to HIV/AIDS

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS), commonly referred to as HIV/AIDS, constitute one of the most serious infectious disease challenges to public health globally, and has had a crippling effect in certain parts of the world especially in Sub-Saharan Africa [1–3]. There are currently 33.2 million people living with HIV/AIDS globally. Of this total number, an overwhelming 22.5 million people are HIV positive in Sub-Saharan Africa specifically, representing 67.8% of the global number [3]. Interventions such as AIDS counselling, educational tools and antiretroviral drug therapy have contributed to transforming HIV infection from a fatal to a manageable chronic infectious disease [4]. Despite the availability of these measures, the above statistics indicate that much remains to be accomplished as the number of newly reported HIV infections still remains unacceptably high.

There are currently two known species of HIV, viz., HIV-1 and HIV-2, with their respective subspecies. HIV-1 is the globally common infection while HIV-2 is more prevalent in West Africa, and takes a longer time to develop into immunodeficiency from infection than HIV-1 [5,6]. HIV infection in the human body results mainly from integration of the viral genome into the host cell for the purpose of cell replication, and AIDS is the advanced stage of the disease caused by HIV infection. The virus infects the host cell by binding the viral gp120 protein to two transmembrane receptors, i.e., CD4+ and either of the two chemokine receptors, CCR5 and CXCR4 [7]. HIV infects macrophages and T-helper lymphocytes (CD4+); but the defining feature of AIDS is the depletion of CD4+ cells. T-tropic viruses prefer to replicate in T cells, while M-tropic viruses prefer the macrophage. Of the HIV-1 viruses, M-tropic types predominate in the brain [8].

The viral genome contains three structural genes – *gag*, *pol* and *env* – and six regulatory genes – *tat*, *rev*, *nef*, *vif*, *vpr* and *vpu* [5]. The virus utilizes some of these genes to maximise its production using host cell resources. DNA microarray studies have implicated HIV encoded Nef protein in this process [9], and humans infected with the *nef*-deleted form of HIV have remained disease free for several years [10]. Interestingly, HIV has been referred to as a "master regulator" of cellular gene expression [9] as a means to augment expression of its own genome. An understanding of these processes is critical to developing novel therapeutic strategies for the suppression or elimination of the virus.

The immunopathogenesis of HIV/AIDS has been previously amply documented; from the time of infection to the end stage of the disease [5]. The end stage of the disease may be characterised by a spectrum of diseases [11] including opportunistic infections (such as *Pneumocystis carinii* and *Mycobacterium tuberculosis*), dementia and cancer [6,11]. In addition to macrophages, lymph nodes, bone marrow, spleen and lungs, the CNS represents one of the most important anatomical sites of the virus after infection. This causes

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significant neuronal damage and loss that often leads to HIV associated dementia [12]. Without treatment, HIV-1 infection is nearly uniformly fatal within 5–10 years [11].

2. HIV/AIDS drug therapy and its current limitations

Although the development of drugs for HIV infection has undergone substantial progress, numerous uncertainties persist about the best way to manage this disease. Reports addressing this aspect have appeared in the literature [13]. At present, the different ARVs are classified under categories such as nucleoside reverse transcriptase inhibitors (NRTI), nucleotide reverse transcriptase inhibitors (NtRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), and more recently fusion and integrase inhibitors [14]. Table 1 [15–19] lists the various drugs under the different classes, the available dosage forms as well as their half-lives and bioavailabilities. These drugs are administered as combined therapy as in the case of highly active antiretroviral therapy (HAART) [20]. Among the newer classes of drugs under investigation are the assembly and budding inhibitors [21], as well as the zinc finger inhibitors [22]. Virus assembly and disassembly are particularly attractive candidate processes for antiviral intervention. HIV-1 capsid (CA) protein and human cyclophilin A (CypA) play important roles in these processes, which consequently make them attractive targets of high priority [23].

Although ARV drug therapy has contributed significantly to improved patient/disease management, its current use is associated with several disadvantages and inconveniences to the HIV/AIDS patient. Many ARV drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. The half-life for several ARV drugs is short, which then requires frequent administration of doses leading to decreased patient compliance [24]. A major limitation is that HIV is localised in certain inaccessible compartments of the body such as the CNS, the lymphatic system and within the macrophages. These sites cannot be accessed by the majority of drugs in the therapeutic concentrations required; and the drugs also cannot be maintained for the necessary duration at the site of HIV localisation [25]. These subtherapeutic drug concentrations and short residence time at the required sites of action contribute significantly to both the failure of eliminating HIV from these reservoirs, and the development of multidrug-resistance against the ARVs [26]. The severe side effects associated with ARV therapy can therefore be attributed to the subsequent large doses essential for achieving a therapeutic effect, due to the inadequate drug concentrations at the site of action, and/or the poor bioavailability of several ARV drugs. These drugs also suffer from physico-chemical problems such as poor solubility that may lead to formulation difficulties [27,28]. Strategies currently being investigated to overcome these limitations include the identification of new and chemical modification of existing chemical entities, the examination of various dosing regimens, as well as the design and development of novel drug delivery systems (NDDS) that can improve the efficacy of both existing and new ARV drugs. More specifically, in the past decade there has been an explosion of interest in the development of NDDS for the incorporation of ARV drugs as a way of circumventing the problems described above and optimising the treatment of HIV/AIDS patients. To the best of our knowledge, the last review paper on NDDS for ARV drugs appeared in 1993 [28]. There have since been significant advancements of the systems described in that paper, and further new NDDS for ARV drugs have since emerged in the literature. The purpose of this paper is therefore to present a comprehensive review of the various NDDS, including studies on alternative routes of administration that have emerged for ARV drugs. This will identify the progress that has been achieved both for the technological development of these delivery systems, and their clinical potential for overcoming the limitations associated with current ARV therapy. This review will also enable the identification of future studies that remain to be undertaken for its optimisation and ultimately its commercialisation.

3. Novel drug delivery systems for ARV drugs

3.1. Sustained release/bioadhesive/enteric coated matrix tablets

Sustained drug delivery systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include minimisation of drug related side effects due to controlled therapeutic blood levels instead of

Table 1
Examples of antiretroviral drugs, their commercially available dosage forms, bioavailabilities and half-lives

Name and class of drug	Dosage form [15–19]	F (%) [15–19]	Half-life (h) [15–19]
Zidovudine(NRTI)	Capsule, liquid	60	1.1
Lamivudine (NRTI)	Tablet, liquid	86	3–6
Didanosine (NRTI)	Tablet, capsule (EC), liquid	30–40	1.3–1.6
Zalcitabine(NRTI)	Tablet	85	1–3
Stavudine(NRTI)	Capsule, powder for reconstitution	80	1–1.6
Abacavir(NRTI)	Tablet, liquid	83–100	1–2
Emtricitabine(NRTI)	Capsule	93	10
Tenofovir NtRTI	Tablet	25–39	17
Nevirapine (NNRTI)	Tablet, syrup	>90	25–30
Efavirenz(NNRTI)	Tablet, capsule, solution	42–80	40–50
Delavirdine(NNRTI)	Tablet	85	5.8
Etravirine (NNRTI)	Tablet	Unknown	30–40
Amprenavir(PI)	Capsule, solution	No data	7–10
Indinavir(PI)	Capsule	65	1.2–2
Saquinavir(PI)	Tablet, capsule	Erratic, 4	1.5–2
Nelfinavir(PI)	Tablet, powder	20–80	3.5–5
Ritonavir (PI)	Tablet, capsule, liquid	65	3–5
Atazanavir(PI)	Capsule	No data	7
Darunavir(PI)	Tablet	37	15
Enfuvirtide (Entry and FI)	Powder for subcutaneous injection	84.3	3.8
Maraviroc (Entry and FI)	Tablet	23–33	14–18
Raltegravir (II)	Tablet	No data	9

NRTI, nucleoside reverse transcriptase inhibitors; NtRTI, nucleotide reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; FI, fusion inhibitors; II, integrase inhibitors, F, Bioavailability.

oscillating blood levels, improved patient compliance due to reduced frequency of dosing and the reduction of the total dose of drug administered [29,30]. Bioadhesive drug delivery systems are designed for prolonged retention on the mucosa to facilitate drug absorption over a prolonged period of time by interacting with mucin [31]. Hence, the combination of both sustained release and bioadhesive properties in a delivery system would further enhance therapeutic efficacy. ARVs such as didanosine (ddI) would be an ideal candidate for sustained drug release due to its short half-life of 1.3–1.6 h, necessitating frequent administration of doses, as well as its severe dose dependent side effects [24]. In an attempt to improve the oral absorption of ddI by delivering it over a prolonged period of time as well as prolonging retention on the mucosae, Betageri et al. [32] prepared a sustained release bioadhesive tablet formulation of ddI, containing Polyox WSRN-303, Carbopol 974P-NF and Methocel K4M as polymeric matrix materials. Hydrogel forming tablet formulations with 10% and 30% Polyox WSRN-303 were able to extend the release of ddI (Fig. 1), while 30% Methocel K4M was required for extending the drug release in other formulations. Preparations with Carbopol 934P prevented complete release of ddI from the tablet during the test period, and the authors attributed this to drug–polymer interactions. The bioadhesivity also increased with an increase in polymer concentration. These researchers concluded that a single polymer could be used for the preparation of hydrogel matrix ddI tablets designed to provide both sustained release and bioadhesivity. However, while a single polymer may provide both bioadhesivity and sustained drug release, it has since become well recognised in the literature, via various *in vitro* drug release and bioadhesivity tests during formulation studies, that simultaneous optimisation of both these properties may require the blending of various polymers [33–35] for both single and multiple unit systems. These systems remain to be investigated for their clinical applicability.

ddI controlled release matrix tablets containing methacrylic (Eudragit RSPM) and ethylcellulose (Ethocel 100) polymers have also been prepared by Sanchez-Lafuente et al. [36]. The ddI 500 mg tablets (5, 10 or 15%w/w) were prepared by direct compression, and comprised Eudragit® RSPM and Ethocel™ 100 in varying ratios (75/25, 50/50 and 25/75 w/w). The physical characteristics in terms of weight, thickness and diameter confirmed the excellent compactibility properties of these polymers with ddI, which allowed for direct compression in the absence of other excipients. The drug release studies showed that varying polymer ratios could modulate the release of ddI as a result of the swelling properties of Eudragit® RSPM and plastic properties of the hydrophobic Ethocel™ 100. Since these two polymers showed potential for modulating drug release, the subsequent study by this group focused on the use of a statistical experimental design for formulation optimisation as well as for identifying and quantifying the effects of formulation variables on drug release. Therefore, a Doehlert design was applied to evaluate the influence of variables and possible interactions among such variables on ddI release from the directly compressed matrix tablets based on the blends of the two insoluble polymers, Eudragit® RSPM and Ethocel® 100 [37]. The drug content and the polymers had the most significant effect on drug release, while the compression force had no significant effect. The optimum formulation conditions identified in the studied experimental design for a formulation with optimum drug release were Eudragit–Ethocel ratio of 83/17 (w/w) and a drug content of 13%w/w. The experimental values obtained from the optimised formulation highly agreed with the predicted values, thereby validating the mathematical model used in the preparation of ddI tablets.

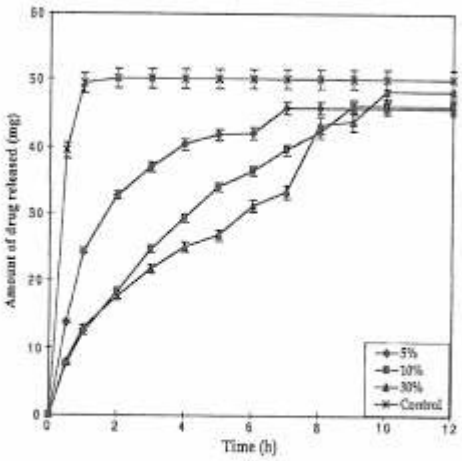

Fig. 1. Effect of Polyox WSRN-303 on the release of ddI from tablets (reproduced from Betageri et al. [32]).

ddI also undergoes acid degradation in the gastric medium [38]. An enteric coated matrix tablet formulation that combines sustained drug release, bioadhesivity and an enteric coating to resist acid degradation to maximise therapeutic efficacy has also been reported. Deshmukh et al. [39] reported the preparation of enteric coated, sustained release bioadhesive matrix tablets of ddI comprising Polyox, WSRN-303 and Methocel K4M with hydroxypropylmethylcellulose phthalate (HPMCP 5.5). The formulation was shown to be resistant to dissolution in 0.1 N HCl but dissolved within 10 min in PBS, pH 7.4. Furthermore, the stability of the formulation for 6 months at varying storage conditions was confirmed. Permeation studies on the matrix tablets showed that Polyox WSRN-303 containing tablets demonstrated higher ddI permeability across live intestinal tissue compared with conventional tablets.

While the above tablets sought to provide sustained drug release, bioadhesion and resistance to gastric acid degradation, a possible limitation could be the fact that it would still undergo extensive first pass degradation since it is meant for oral administration.

3.2. Ceramic implants

Attempts have been made in the literature to explore the use of ceramic implants to modulate the release of antiretroviral drugs. Due to the adverse effects of AZT associated with oral and intravenous administration, Benghuzzi et al. [40] in early *in vivo* studies investigated the release of deoxynucleoside thymidine, the normal counterpart of azidothymidine (AZT), by means of alumino-calcium-phosphorous oxide (ALCAP) ceramic implantable capsules in rats. The results showed that thymidine could be released from the ALCAP ceramic capsules in a sustained manner for a minimum duration of 120 days. Based on the results with thymidine, they subsequently concluded that these implantable capsules could be considered for the delivery of AZT. Consequently, in a follow-up study [41], AZT was loaded into tricalcium phosphate (TCP) and ALCAP ceramic capsules. They showed that the rate of release of AZT from TCP capsules was lower than from ALCAP capsules. Fig. 2 confirms the sustained release of AZT from TCP ceramic capsules over 26 days when loaded with 20, 40 and 60 mg AZT.

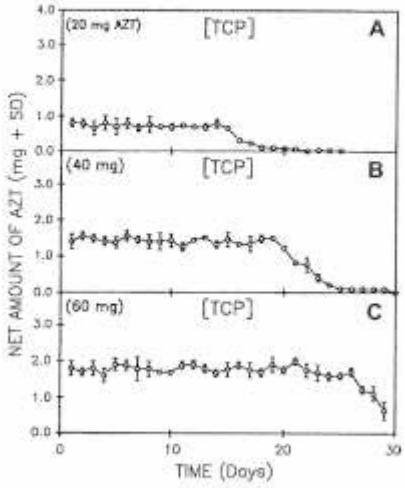

Fig. 2. Release of AZT from TCP ceramic capsules (reproduced from Benghuzzi et al. [41]).

To further control release, Nagy and Bajpai [42] extended this *in vitro* study by preparing a TCP ceramic delivery system containing thymidine and AZT by determining the effect of sesame seed oil or wheat germ oil on their release. Ceramic capsules were prepared by pressing 1 gram of <38 μm beta-TCP particles with or without the stipulated quantity of thymidine or AZT in a 10 mm die at a load of 4000 lbs in an electric hydraulic press. They found that sesame seed oil and wheat germ oil (Vitamin E) could delay the release of thymdine and AZT from TCP drug loaded capsules. Further, incorporation of thymidine or AZT in the form of a compressed pellet also retarded its release from the TCP ceramic capsules prepared with oil treated ceramic particles. The above studies were extended to an *in vivo* study later [43]. Three ceramic devices were implanted subcutaneously in Sprague–Dawley rats for 2 weeks. The *in vivo* studies showed that oil saturated TCP and AZT devices as well as the AZT pellet inserted in an oil saturated TCP shell device were able to retard AZT release at a significantly lower rate than the AZT and TCP untreated devices. These authors concluded that the treatment of ceramic devices with oil decreased the release rate and prolonged the delivery of AZT. The inclusion of wheat germ into another ceramic device, hydroxyapatite (HA) composite, was also able to deliver AZT for prolonged periods *in vitro* [44].

A subsequent *in vivo* study by Benghuzzi [45] compared the release of AZT from two commonly studied ceramic implants, i.e., TCP and HA. Sterilised drug loaded ceramics containing AZT in three dosages (40, 60 and 90 mg) were inserted under the skin of rats using standard surgical techniques. The data from this study showed that AZT release rates from TCP ceramic implants (30 mg = 2.38 ± 0.23 ng/mL, 60 mg = 4.64 ± 1.03 ng/mL and 90 mg = 11.92 ± 2.35 ng/mL serum AZT) were significantly higher than from HA ceramic implants (30 mg = 0.84 ± 0.05 ng/mL, 60 mg = 2.40 ± 0.83 ng/mL and 90 mg = 6.41 ± 1.24 ng/mL serum AZT). The authors concluded that TCP and HA ceramic implants could be considered effective for delivering AZT in quantities required for providing physiological responses *in vivo*. The sustained drug release profiles obtained indicated that large fluctuations of AZT concentrations in the blood stream and tissues, as with conventional routes of administration, could be eliminated using ceramic drug delivery systems.

While ceramic implants were actively studied between 1990 and 2000, there appears to be no further work since reported for ARV containing ceramic implants.

3.3. Liposomes

Liposomes, ranging in size between 25 nm and several microns, are microscopic vesicles that comprise one or more phospholipid bilayers which surround an aqueous core. They are prepared from natural or synthetic phospholipids and cholesterol, and may also additionally include other lipids and proteins. The aqueous core facilitates the entrapment of hydrophilic drugs, while hydrophobic drugs are bound to or incorporated in the lipid bilayer. When administered, liposomes are recognised as being foreign, and are immediately taken up by cells of the mononuclear phagocytic system (MPS). Since the HIV virus localises in these cells, liposomes therefore represent a suitable drug delivery system for targeting ARVs into infected cells; and thus have the potential of improving the efficacy of drugs and reducing side effects [46–48].

The effect of liposomal encapsulation of AZT in mice was determined in early studies [49,50]. Unlike injections of free AZT, liposomal encapsulated AZT showed no bone marrow toxicity with normal erythrocyte and leukocyte profiles. Also, enhanced localisation in the liver, spleen and lung was found with the AZT liposomes. Liposomal encapsulated AZT further reduced haematopoietic toxicity and resulted in enhanced antiretroviral activity in mice. Liposomal formulations have also been prepared for administration of AZT by the transdermal route [51]. The optimised liposomal formulation showed a transdermal flux of 98.8 ± 5.8 μg/cm^2 across rat skin as compared to 5.72 ± 0.3 μg/cm^2 for the free drug, and this should contribute to an improved bioavailability. These liposomes for the transdermal route were also able to target the RES organs more effectively.

Liposomes containing ddI were initially studied by Harvie et al. [52]. They found that the elimination plasma half-life of 112 and 83 nm liposomal ddI was 46 and 14 times higher than that of the free drug, respectively. They also reported efficient targeting of lymph nodes and macrophage-rich tissue with these conventional liposomes. In a subsequent study, they were able to extend further the ddI half-life in plasma from 3.9 h for conventional liposomes to 14.5 h by incorporating it into sterically stabilised liposomes. Following intravenous injection, the majority of the sterically stabilised liposomes also concentrated in the spleen with a peak level at 24 h (Fig. 3) [53].

Apart from AZT and ddI, zalcitabine (ddC) has also been investigated for encapsulation into liposomes by Makabi-Panzu et al. [54,55]. The ddC loaded liposomes were more rapidly taken up by the mouse macrophage cell line than the free ddC. They also reported that a high intracellular uptake of ddC was facilitated by the anionic nature of liposomes. To be pharmacologically active, dideoxynucleosides such as ddC must be phosphorylated into 5′-triphosphates by cellular kinases. Since some cell types have a low ability to phosphorylate these compounds, administration of the phosphorylated form of the drug would be most suitable. However, this would not be feasible as cell membranes are impermeable to the phosphorylated form, and phosphatases present in body fluids hydrolyse nucleotides into the corresponding nucleosides [56]. To overcome this limitation and to obtain site specific delivery, the antiviral effects of ddC and ddC-triphosphate (ddC-TP) and liposome encapsulated ddCTP (L(ddCTP)) were established and compared in cultured, human monocyte macrophages infected with HIV-1 [57]. ddCTP was dephosphorylated before entering the cells, while L(ddCTP) remained stable over days. These preparations

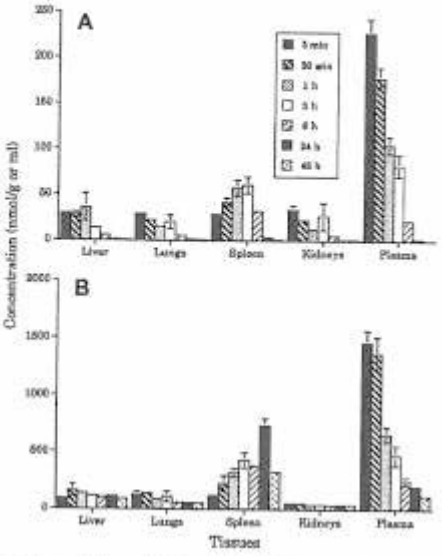

Fig. 3. Plasma and tissue distribution of ddI ([^{3}H]ddI) (A) and liposomal lipids ([^{14}C]DPPC) (B) from sterically stabilised liposome-encapsulated ddI after the administration of a single intravenous dose (3 mg of ddI per kg) to rats. Values are the means obtained for four to six animals per group per time point (reproduced from Harvie et al. [53]).

were also able to inhibit replication at nanomolar drug levels. Data obtained from liposome encapsulated ddCTP in a murine acquired immunodeficiency syndrome (MAIDS) model have also showed reduced proviral DNA in cells of the MPS in both spleen and bone marrow [58].

Liposomes have also been explored for the encapsulation and delivery of newly synthesized prodrugs. Lalanne et al. [59] synthesized two novel glycerolipidic ddI conjugates as prodrugs to avoid hepatic first pass metabolism. Liposomal formulations (1160 ± nm) of the prodrugs displayed antiviral activity and showed promise as formulations for enhancing drug bioavailability. Due to the low entrapment efficiency and high leakage of AZT from liposomes [48], AZT-myristate (AZT-M) has been synthesized as a prodrug and investigated for its potential for liposomal encapsulation. A high entrapment efficiency of 98% was achieved with higher plasma AZT being achieved with the AZT-M liposomes as compared to free AZT solution. Higher concentrations of AZT in organs of the RES and brain were also found with the liposomal preparation. This study could have been enhanced if AZT-M liposome preparations were compared not only with free AZT, but also with AZT entrapped liposomes. Prodrug liposomal preparations therefore offer the opportunity of not only more efficient targeting but also improved drug action and formulation processing.

In addition to liposomes having PEG chains attached to its surface, for increasing circulation time *in vivo* [60,61], active targeting of HIV infected cells can also be obtained by using liposomes that have surface attached ligands that specifically promote receptor interaction at the site of targeting [47] as well. Using the antibody, H-2-K(k), for Fc-mediated targeting, Betageri and Burrell [62] showed that the lipid composition of ddI–triphosphate liposomes influenced conjugation of antibodies and also retention of the

Table 2
Area under the curve for free and immunoliposomal indinavir in tissues after a single subcutaneous administration in mice (Reproduced from Gagne et al. [63])

Tissue	Immunoliposomal indinavir	Free indinavir	Ratio immunoliposomal/free indinavir
Cervical lymph nodes	523.2	7.6	68.8
Brachial lymph nodes	617	4.9	126.0
Mesenteric lymph nodes	192.8	6.4	30.1
Inguinal lymph nodes	144.5	4.1	35.2
Popliteal lymph nodes	134.2	4.5	29.8
Liver	731.1	35.0	21.0
Spleen	211.3	5.3	39.9
Plasma	77.6	2.3	33.8

encapsulated drug. Sterically stabilised immunoliposomes containing grafted anti-HLA-DR antibodies were effective in enhancing the concentrations of indinavir (INV) in all tissues leading to a 21- to 126-fold increased accumulation as compared to the unencapsulated drug (Table 2) [63]. Also, immunoliposomal INV was as efficient as the free agent to inhibit HIV-1 replication in cultured cells. Lectin receptors, which act as molecular targets for sugar molecules, are found on the surface of cells of the mononuclear macrophage system (MPS), and have also been included in the strategy to improve site specific drug delivery. Using a mannose binding protein, concanavalin A, maximum cellular drug uptake occurred when mannosylated liposomes containing stavudine (D4T) were used [64]. Other sugar molecules used for liposomal formulations to target cells of the MPS include galactosylated D4T and AZT liposomes [65,66]. Together, these studies confirmed enhanced targeting to tissues rich in galactose specific receptors, and confirmed their potential of providing sustained drug release characteristics. Slepushkin et al. [67] have also reported that synthetic peptides can bind specifically to HIV infected cells. The potential of various ligands for active targeting of ARV loaded liposomes has therefore been confirmed, and shows potential for formulation optimisation.

In addition to targeting liposomes to the phagocytic system, other areas in the body have also been of interest. Kompella et al. [68] evaluated the effect of neutral liposomes on corneal and conjunctival permeability of ddI. While the liposomal formulations were able to encapsulate ddI and permeate through the rabbit conjunctival mucosa, the permeability coefficient, initial flux and tissue levels of ddI at the end of the transport study were actually lower in the presence of liposomal formulations. These neutral liposomes failed to enhance the corneal or conjunctival transport or uptake of ddI.

One of the disadvantages of liposomes is the poor stability in terms of drug retention and poor encapsulation. When assessing the stability of ARVs incorporated into liposomes, Betageri [69] found that lipid composition influenced encapsulation and retention of ddI–triphosphate (ddITP); and that its retention in the DMPC:CHOL liposomes was maximum when stored at 4 °C.

A novel liposomal formulation, i.e., "emulsomes" for sustained and targeted delivery of AZT to the liver has recently been described by Vyas et al. [25]. Emulsomes are a novel lipoidal vesicular system with an internal solid fat core surrounded by a phospholipid bilayer. In addition to demonstrating a retarded drug release profile (12–15% after 24 h), studies in rats showed better uptake of the emulsomal formulations by the liver cells. We agree with the researchers that this proposed cationic emulsome-based system shows excellent potential for intracellular hepatic targeting.

Liposomes have clearly been more extensively investigated for their *in vitro* and *in vivo* properties than other NDDS for ARV delivery. A greater number of drugs and prodrugs have been encapsulated, and additional formulation optimisation techniques and in

vivo evaluations have been undertaken. These studies highlight and underscore the potential benefits of liposomes for improving ARV drug therapy.

3.4. Nanoparticles

Drug encapsulated nanoparticles are solid colloidal particles that range from 10 to 1000 nm in size [70]. Based on their size and polymeric composition, they are able to target drug to specified sites in the body, and have also shown potential for sustained drug delivery [71]. Nanoparticles have also been explored for improving the formulation and efficacy of drugs with physicochemical problems such as poor solubility and stability [72]. They are being increasingly investigated for targeted delivery of ARVs to HIV infected cells and to achieve sustained drug release kinetics. Their encapsulation into such systems may provide improved efficacy, decreased drug resistance, the reduction in dosage, a decrease in systemic toxicity and side effects, and an improvement in patient compliance.

Cells of the mononuclear phagocytic system (MPS), such as the monocytes/macrophages (Mo/Mac), act as a reservoir for the HIV virus [73]. Therefore, drug treatment of HIV infection should involve targeting drugs to these cells in addition to the lymphocytes. Several studies involving ARV loaded nanoparticles for targeting to the macrophages have consequently emerged. In an early preliminary study, Schafer et al. [74] prepared AZT loaded polyalkylcyanoacrylate (PACA), polymethylmethacrylate (PMMA) and human serum albumin (HSA) nanoparticles. This study confirmed uptake of the nanoparticles into macrophages isolated from HIV infected patients. The same group also later prepared and confirmed the potential of human serum albumin and poly(hexylcyanoacrylate) nanoparticles loaded with the nucleoside analogues, AZT and ddC for the targeting of macrophages. These *in vitro* studies were also undertaken using macrophages isolated from the peripheral blood of healthy blood donors and transmission electron microscopy [75]. Saquinavir (SQN) and ddC have also been loaded into poly(hexylcyanoacrylate) nanoparticles [76] by emulsion polymerization. While ddC showed no superiority to an aqueous solution of the drug in terms of reducing the HIV-1 antigen production, a significantly higher efficacy was observed for SQN loaded nanoparticles as compared to its aqueous solution. An *in vivo* study in rats to investigate the oral delivery of AZT bound to hexylcyanoacrylate nanoparticles for delivery to the reticuloendothelial cells was undertaken by Löbenberg, Araujo, and Kreuter [77]. The area under the curve (AUC) of [^{14}C] AZT in the liver was 30% higher when the drug was bound to nanoparticles than after administration of the solution. Higher AZT levels were also found in the blood and brain when nanoparticles were used as compared to the control solution. In an *in vivo* study a year later using the intravenous route instead, they showed that AZT concentrations were up to 18 times higher in organs of the RES if the drug was bound to nanoparticles as compared to unbound AZT [78]. Surface modification of nanoparticulate systems with hydrophilic groups such as polyethylene glycol has been shown to influence the biodistribution of nanoparticles [79]. Using THP-1 human monocyte/macrophage (Mo/Mac) cell line, Shah and Amiji [80] showed that a significantly higher percentage of the administered dose of nanoparticles was internalized within the cells when SQN was incorporated into poly(ethylene oxide)-modified poly (epsilon-caprolactone) nanoparticles (200 nm). Also, intracellular SQN concentrations were significantly higher when administered in the surface-modified nanoparticles as compared to its aqueous solution. A possible limitation of this study is that while aqueous solutions of SQN were compared to SQN PEG-modified nanoparticles, a comparative study with surface-unmodified SQN nanoparticles was not performed. This would have provided greater insight to the contribution of PEG specifically for ARV delivery. Most recently, the uptake of AZT loaded poly(lactic acid)–poly(ethylene glycol) nanoparticles by polymorphonuclear leukocytes *in vitro* was shown to be dependent on PEG and its ratio in the polymer [81].

Since the HIV virus can migrate to, multiply and localise in the CNS causing several neurological disorders, targeting of ARV drugs to the brain has become a significant goal for drug therapy. The blood–brain barrier (BBB) prevents access of ARVs to the brain due to the tight endothelial cell junctions of the brain capillaries and the presence of efflux transporters on the cell surface [81]. Nanoparticulate systems promote drug delivery in the brain, since they may gain entry by means of endocytosis/phagocytosis and are also moved away from the vicinity of efflux pumps [82,83]. Kuo [84] therefore loaded D4T into polybutylcyanoacrylate (PBCA) and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) nanoparticles for brain targeting. Drug loading of the nanoparticles (59.5–149.2 nm) was inversely proportional to particle size, and was also affected by freeze-drying and preservation as it influenced particle size. Similar to other studies [85], they also found pH to be critical, since variation in pH value of the loading medium from pH 7.2 led to a reduction in the loading efficiency of D4T. Kuo and Chen [86] then evaluated the effects of size of PBCA and MMA-SPM nanoparticles and alcohol on the permeability of AZT and lamivudine (3TC) across the BBB using blood–brain-microvascular endothelial cells model (BMEC). Both loading efficiency and permeability of AZT and 3TC decreased with an increase in the particle size of the two polymeric carriers. While PBCA nanoparticles increased the BBB permeability of AZT and 3TC 8- to 20- and 10- to 18-folds, respectively, the MMA-SPM nanoparticles led to a significant 100% increase in the BBB permeability of both drugs. A 4–12% enhancement in the BBB permeability of the two drugs with 0.5% ethanol was attributed to temporary unfolding of tight junctions among BMECs upon treatment with alcohol. In a subsequent paper, these authors compared the transport of D4T, delavirdine (DLV) and SQV across the *in vitro* BBB using (PBCA), (MMA-SPM) and also solid lipid nanoparticles (SLNs) [83]. These various polymeric systems investigated enhanced permeability of the drugs with higher permeabilities being reported with smaller particle sizes. In their most recent paper, Kuo and Kuo [87] showed that exposure to an electromagnetic field (EMF) could further enhance drug permeability across the BBB. The potential of SLNs for targeted brain delivery of another ARV, atazanavir, has also recently been confirmed [88].

More recently, a novel approach was proposed by Dou et al. [89,90]. They postulated that the mononuclear phagocytes, as the principal reservoir for viral dissemination, could also serve as a transporter of antiretroviral drugs themselves, since they are responsible for dissemination of HIV, i.e., macrophages can enter into tissues that limit entry of many ARV drugs. In these two papers, they describe a macrophage-based nanoparticulate system as a carrier itself for indinavir (INV). A nanoparticle indinavir (NP-INV) formulation was prepared and packaged into bone marrow-derived macrophages (BMMs). The effects of this drug carrier on drug distribution and disease outcomes were assessed in immune competent and human immunodeficiency virus type 1 (HIV-1) infected humanised immune-deficient mice [89]. Significant lung, liver and spleen BMMs and drug distribution were observed. This initial study also reported reduced numbers of virus infected cells in plasma, lymph nodes, spleen, liver and lung as well as CD4(+) T-cell protection when the NP-IDV BMMs were administered to HIV-1 challenged humanised mice. Later, a similar NP-INV formulation was prepared with Lipoid E80 [90]. They reported sustained drug release from the macrophages. The administration of NP-INV, when compared to equal drug levels of free soluble INV, also significantly blocked induction of multinucleated giant cells, production of reverse transcriptase activity in culture fluids and

cell associated HIV-Ip24 antigens after HIV-1 infection. This study proved that the use of a macrophage-based NP delivery system has potential for the treatment of HIV-1 infections.

The use of ligands on nanoparticles for receptor-mediated targeting has just been reported in the literature [91,92]. Since macrophages contain various receptors such as mannosyl and galactosyl, Jain et al. [91] prepared mannosylated gelatine nanoparticles (MN-G-NP) (248–325 nm) (Fig. 4) with a drug encapsulation of 40.2–48.5%. Via fluorescence and *ex vivo* studies using alveolar macrophages from rats, they showed a 18.0 and 2.7 times higher uptake by the macrophages from MN-G-NPs as compared to the free drug and uncoated G-NPs (Fig. 5).

The use of nanoparticles for targeting other areas such as the gastrointestinal mucosa and associated lymphoid tissues has also been reported by Dembri et al. [93]. As compared to the drug solution, AZT loaded isohexylcyanoacrylate nanoparticles were able to efficiently concentrate AZT in the intestinal mucosa. They also found that the nanoparticles were also able to control the release of free AZT.

Solid lipid nanoparticles (SLNs) are prepared from lipids that remain in a solid state at room and body temperature. Heiati et al. [94] initially prepared SLNs consisting of AZT-palmitate (AZT-P) and trilaurin (TL) as the solid core with dipalmitoylphosphatidylcholine (DPPC), and a mixture of DPPC and dimyristoylphosphatidylglycerol (DMPG). Their study concluded that the loading of AZT-P was proportional to the concentration of phospholipids content, and was independent of the amount of trilaurin used. Phospholipids with transition temperatures below 37 °C increased drug release. In a subsequent study, coating the SLNs with a PEG layer on its surface further increased the levels of AZT in the blood, since PEG creates a steric barrier that reduces particle uptake, thereby prolonging circulation [95]. They also found that the SLN–PEG nanoparticles were able to decrease the drug release rate in plasma as compared to SLN particles without PEG. The studies by this research group confirmed that surface modification with PEG could be used for controlling drug release and the pharmacokinetic behaviour of SLNs.

While the majority of studies have focused on targeted delivery of ARVs with nanoparticles, some studies have also focused on modifications to its preparation to enhance drug loading and decrease toxicity; and also to increase its absorption by facilitating pH-sensitive drug release. Boudad et al. [96] prepared SQN loaded poly(alkylcyanoacrylate) nanoparticles and showed that incorporation of cyclodextrins enhanced the entrapment of SQN. Studies on the Caco-2 cell line showed that incorporation of cyclodextrins with nanoparticles decreased cytotoxicity when compared to blank and SQN loaded nanoparticles. The ability of cyclodextrins to mask to some extent the cytotoxic effects of the aliphatic alcohols originating from the hydrolytic degradation of the polymers was proposed as a possible reason for this effect. The oral bioavailability of a poorly water soluble HIV-1 protease inhibitor (CGP 70726-Novartis) was also enhanced when incorporated into pH sensitive nanoparticles prepared from poly(methacrylic acid-co-ethacrylate) copolymer Eudragit L100-55 [72].

The surge of interest in nanoparticulate systems for ARV therapy has led to several drugs being studied for its incorporation. These *in vitro*/*in vivo* studies clearly confirm the ability of nanoparticles to enhance the therapeutic efficacy of ARVs, as well as addressing formulation problems.

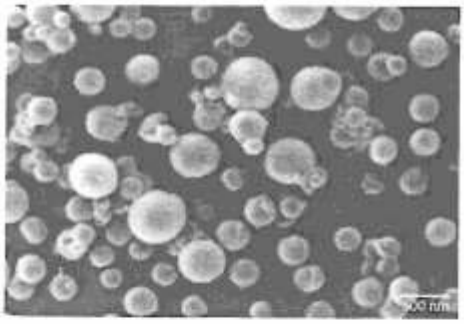

Fig. 4. Scanning electron micrograph of dill loaded mannose coupled gelatin nanoparticles (30,000×) (reproduced from Jain et al. [91]).

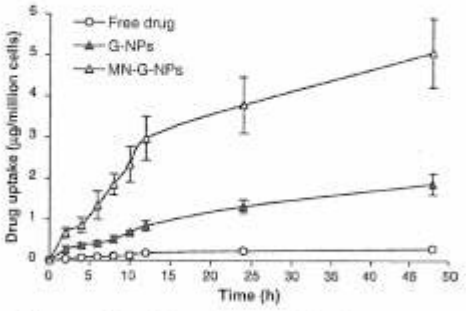

Fig. 5. Drug uptake from dill containing mannosylated gelatin nanoparticles by alveolar macrophages at different time points at 37 ± 2 °C (reproduced from Jain et al. [91]).

3.5. Nanocontainers

Dendrimer-based systems have also been explored for the concept of ARV targeting. Dendrimers are characterised as being synthetic, highly branched, spherical monodispersed macromolecules. Due to their unique architecture and macromolecular characteristics, they have emerged as an important class of drug carrier for targeted delivery [97,98]. Hence, not surprisingly, they have just been reported for targeting of ARV drugs. Recently, Dutta et al. [99] prepared poly(propyleneimine) (PPI) dendrimer-based nanocontainers for targeting of efavirenz (EFV) to Mo/Mac. Fifth generation PPI dendrimer, *t*-Boc-lycine conjugated PPI dendrimer (TPPI) and mannose conjugated dendrimers (MPPI) were synthesized and used to prepare "nanocontainers". Like a dendritic box, these molecules act as closed containers of nanoscopic size containing the entrapped drug, and are therefore called nanocontainers. The drug entrapment efficiency of the nanocontainers varied, with the mannose conjugated dendrimer being 47.4%, followed by that of the PPI dendrimer (32.15%) and *t*-Boc-glycine conjugated dendrimer (23.1%). While the PPI dendrimer released the drug by 24 h, the dendrimer-based nanocontainers of *t*-Boc glycine and mannose conjugated dendrimers prolonged the release rate up to 144 h. The authors found significant increase in cellular uptake of EFV by Mo/Mac with nanocontainers of the mannose conjugated dendrimer being 12 times higher than that of free drug and 5.5 times higher than those of *t*-Boc-glycine conjugated dendrimer. Further, PPI showed a very high toxicity on HEPG2 cells while TPPI and MPPI had negligible toxicity (Fig. 6). These differences were attributed to the free terminal amino groups in PPI which is masked in MPPI and TPPI. This study therefore showed that mannosylated

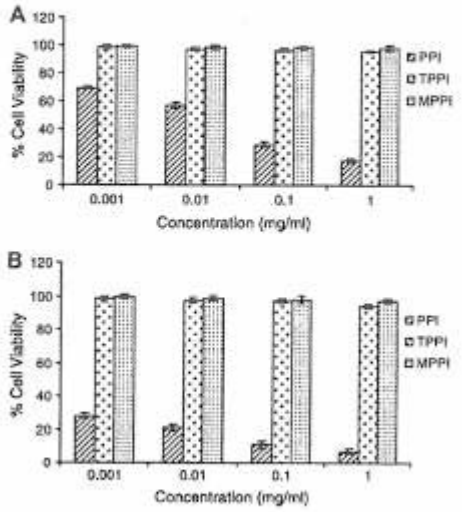

Fig. 6. Cytotoxicity of poly(propylenetmine) (PPI) dendrimer and its nanocontainers, t-Boc-lycine conjugated PPI dendrimer (TPPI) and mannose conjugated dendrimers (MPPI) (a) after 24 h and (b) after 48 h of incubation for targeting of efavirenz (EFV) to Mn/Mac (values = mean ± SD, n = 3) (Reproduced from Dutta et al. [99]).

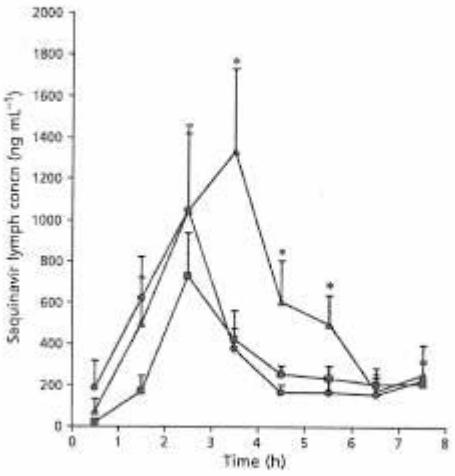

Fig. 7. Concentration of SQN in intestinal lymph versus time (mean ± SE, n = 5). SQN (5 mg) was administered intraduodenally to anaesthetized rats in a cremophor–oleic acid mixed micellar formation (closed circle), a TPGS–oleic acid mixed micellar formulation (closed circle) or as an oleic acid microemulsion (closed triangle) (Reproduced from Griffin and O'Driscoll [100]).

PPI dendrimers could be an effective carrier system for targeted delivery of EFV and possibly other ARVs.

3.6. Micelles and microemulsions

Microemulsions have been studied for ARV drug delivery as an approach to redirect the absorption of ARV from the portal blood to the HIV-rich intestinal lymphatics, thus enhancing the bioavailability of drugs that undergo extensive first pass metabolism and have poor oral bioavailability. Three formulations of SQN containing oleic acid have been studied [100] for targeted intestinal lymphatic transport using rats as the *in vivo* model: cremophor–oleic acid mixed micelles, D-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS)–oleic acid mixed micelles and an oleic acid microemulsion. The extent of lymphatic transport from the lipid vehicles was 0.025–0.5% of the dose administered. The microemulsion generated higher and more prolonged mesenteric lymph concentrations than the micellar formulations (Fig. 7). The systemic bioavailability was estimated to be 8.5% and 4.8% for the cremophor mixed micelle and the microemulsion, respectively. Since the cremophor mixed micelles produced higher bioavailability than TPGS mixed micelles, the researchers concluded that the nature of the surfactant can influence biodistribution of the drug between lymph and plasma.

3.7. Nanopowders

Most recently, nanopowders have been used as a delivery system for oral administration to enhance the dissolution rates of poorly soluble drugs. Tween 80/poloxamer 188 stabilised nanosuspensions of the hydrophobic ARV, loviride, were prepared by media milling, and sucrose co-freeze-dried to obtain solid nanopowders [101]. Morphological characterisation showed plate like structures in the nanopowder which was different from the morphology of untreated loviride crystals (Fig. 8). Loviride showed higher dissolution rates in nanosized products than in their respective physical mixtures, i.e., the amount of drug released after 15 min was 104.2% for the nanopowder prepared from freeze-drying with sucrose, 58% for the freeze-dried nanosuspension without sucrose, 54.8% for the physical mixture containing sucrose, 14.5% for the physical mixture without sucrose and 64.7% for the pure untreated loviride (Fig. 9). The addition of sucrose also further enhanced the dissolution rates. Caco-2 experiments revealed a significantly higher transport of loviride from the nanopowder formulation as compared to the physical mixture and the untreated loviride. Nanopowders were able to increase the dissolution rate due to its high surface area while sucrose had an additional enhancing effect due to its disintegrant properties.

3.8. Suspensions

Since studies with INV in HIV positive patients have indicated that drug concentrations in lymph node mononuclear cells were about 25–35% of mononuclear cells in blood, in a proof of concept study, Kinman et al. [102] showed that association of INV with lipids could enhance localisation in lymphoid tissues and also reduce the viral load. This was accomplished by preparing lipid associated complexes in suspension for subcutaneous injection to HIV-2287-infected macaques. They showed that INV concentrations in both peripheral and visceral lymph nodes were 250–2270% higher than plasma as compared with <35% with soluble lipid-free drug administration in humans. Also, administration of the INV–lipid complexes reduced significantly the viral RNA load and increased CD4 T cell number concentrations (Fig. 10).

3.9. Transdermal delivery

The advantages offered by drug administration via the transdermal route include avoidance of first pass effect and/or GI

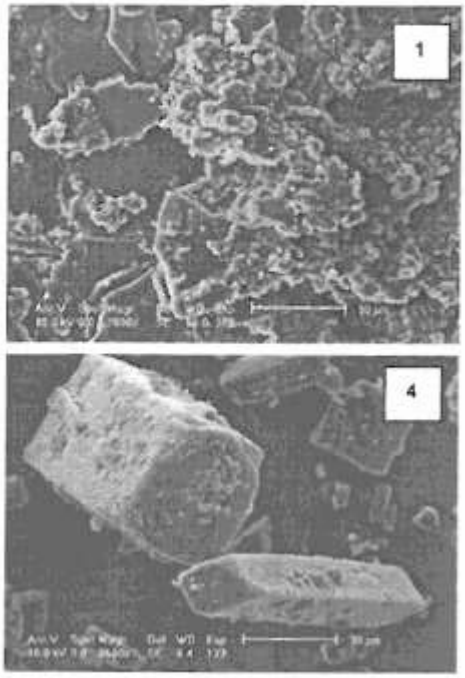

Fig. 8. Scanning electron micrographs of (1) nanopowder and (4) untreated loviride crystals (reproduced from Van Eerdenbrugh et al. [101]).

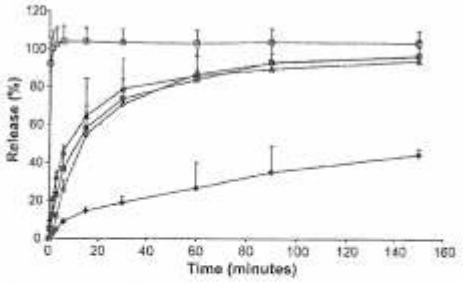

Fig. 9. Dissolution profiles: freeze-dried nanosuspension without sucrose (open diamond), physical mixture without sucrose (closed diamond), nanopowder (open square), physical mixture with sucrose (closed square), untreated lovicide (closed triangle) (reproduced from Van Eerdenbrugh et al. [101]).

degradation, reduced fluctuations in plasma drug concentrations, excellent targeting of the drug for local effect as well as improved patient compliance [103,104]. The potential of ARVs for transdermal administration has therefore been extensively reported. The various transdermal permeation studies with ARV drugs specifically in terms of the focus/foci of the particular investigation and

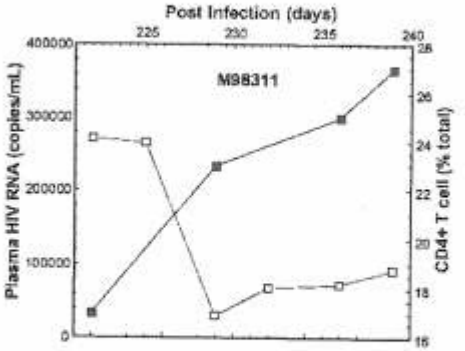

Fig. 10. Changes in plasma viral load of one HIV-2_{ja}-infected macaques at 25 weeks postinfection and treated with 10 daily 30 mg/kg (SC) doses of lipid associated indinavir over 14 days (reproduced from Kinman et al. [102]).

the main outcomes of the study are summarised in Table 3. The most commonly investigated drug thus far for transdermal delivery has been the AZT, although there are some studies that have also investigated ddC and ddI for transdermal delivery. One of the limitations of transdermal delivery of drugs is poor skin/percutaneous penetration/absorption of drugs. Hence, the majority of ARV transdermal studies have focused on permeation enhancement investigating, *inter alia*, various chemical enhancers, types of vehicles (solvents/cosolvents), as well as iontophoresis and anodal current application. Table 3 identifies specifically the various penetration enhancers and vehicles that have been specifically investigated thus far. These various permeation enhancement variables either alone or in combination have been found to be beneficial in promoting ARV drug permeation through the skin.

In addition to comparative permeation enhancement studies with drug solutions, some studies have developed and evaluated transdermal delivery systems of an ARV drug. Gels containing AZT [105,106] and AZT patches using a gum matrix [107,108] have been developed. Both were found to be capable of facilitating ARV permeation, and the gel formulations were also found to be more stable than drug solutions. One of the first vesicular carriers to be studied for transdermal delivery of AZT was aspasomes [109]. These are vesicles formed from ascorbyl palmitate (ASP) in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). Fig. 11 shows that aspasomal AZT (ASP-AZT) was able to significantly enhance transdermal permeation of drug as compared to the AZT solution. Although lower than ASP-AZT, the higher drug permeation of ASP-AZT dispersion as compared to AZT free drug solution showed that ascorbyl palmitate had skin permeation enhancing properties. An elastic liposomal formulation of AZT has also enhanced transdermal flux, provided sustained drug release and improved site specificity of the drug [51]. Pheroid™ is a patented submicron emulsion which has been shown to entrap, transport and deliver several pharmacological compounds for enhanced therapeutic action [110,111]. Pheroid™ comprises essential and plant fatty acids, i.e., ethyl esters of the essential fatty acids, oleic, linolenic and linoleic acids, which are emulsified in water and saturated with nitrous oxide. As shown in Table 2, oleic acid is an effective permeation enhancer due to its kinked structure that briefly disrupts the packed formation of the intercellular lipids [112]. Recently, the use of Pheroid™ was investigated for its potential to enhance the transdermal permeation of ddC, 3TC and

Table 1
Summary of transdermal delivery studies on ARVs

ARV drugs	Focus/foci of study	Summary of main findings	Reference
AZT	Investigated effect of *N*-methyl-2-pyrrolidone (NP) as a penetration enhancer and ethylene–vinyl acetate copolymer membrane for controlled-release	Permeation of AZT was significantly enhanced and plasma concentration of AZT maintained for 10 h after the application of NP controlled-release transdermal system	[120]
ddI	Explored transfollicular absorption route for ddI and investigated effect of penetration enhancers, i.e., azone and propylene glycol. Determined the pharmacokinetics of ddI after topical application	Systemic bioavailability in high and low follicular density rats was similar indicating unimportant role of the transfollicular route for ddI. Transdermal delivery of ddI exceeded the oral bioavailability and was further increased by pre-treatment with absorption enhancers	[121]
AZT	Investigated the effect of *p*-anethole, carvacrol, thymol, linalool and l-menthol. Determined the *in vitro* performance of AZT gel formulation	Transport of AZT was optimum with 5% enhancer concentrations. *In vitro* studies produced higher amount and rate of AZT transport than *in vivo* studies	[106]
ddC ddI AZT	Determined stability profiles of drugs in solution when in contact with hairless rat skin and identified the degradation mechanisms of ddC and ddI	AZT was found to be stable for 30 h at 37 °C. ddC and ddI degraded by bacterial and ddI by cutaneous enzyme-degradative mechanisms. ddC was stabilised with thiomersal or gentamicin, while ddI was stabilised with *para*-chloromercuribenzoic acid	[122]
ddC	Investigated the effects of ethanol/water and ethanol/tricaprylin cosolvents and other permeation enhancers such as oleic acid and *N*-methyl-2-pyrrolidone	Permeation rate across human cadaver skin was significantly lower than across hairless rat skin. Enhancement of ddC permeation using 1%w/v of oleic acid in ethanol/water (60:40) cosolvent was 4–5 times higher than target rate of 0.14 mg/cm²/h to maintain the therapeutic blood level	[123]
AZT	Determined drug release from AZT patches made from Karaya gum through excised hairless mouse skin and also investigated the effect of enhancers	Thickness of gum matrix and enhancers such as propylene glycol, oleic acid and sodium dodecyl sulphate influenced drug release from patches. Permeation was best enhanced with propylene glycol/oleic acid/sodium dodecyl sulphate ternary system	[108]
ddC ddI AZT	Investigated effects of ethanol/water and ethanol/tricaprylin as cosolvent systems and oleic acid as permeation enhancer on permeation rate of each of the drugs alone	Permeation rates of AZT, ddC and ddI increased with ethanol/water and ethanol/tricaprylin cosolvent systems. Addition of oleic acid to the ethanol/water system enhanced permeation but did not with the ethanol/tricaprylin system. Permeation rates reached the target for required therapeutic levels with ethanol/water (60:40) containing oleic acid at 1.0%v/v	[124]
ddC ddI AZT	Investigated effects of ethanol/water and ethanol/tricaprylin as cosolvent systems and oleic acid as permeation enhancer on the simultaneous skin permeation of the three drugs together using hairless rat skin	Permeation rates of AZT, ddC and ddI increased with ethanol/water and ethanol/tricaprylin. Addition of oleic acid in ethanol/water (40:20) significantly increased permeation but not in the ethanol/tricaprylin (50:50) solvent	[125]
ddC ddI AZT	Compared the skin permeation rates of ddC, ddI and AZT, alone or in combination with various compositions of ethanol/water and ethanol/tricaprylin cosolvent systems, across human cadaver and rat skins	Human cadaver skin permeation rates of the drugs alone, or in combination were lower than the rat skin. The addition of oleic acid at 0.1–1% v/v increased permeation rate of all three drugs. 5% v/v oleic acid increased permeation rate of ddC and ddI in combination and saturated in ethanol/water (80:20)	[126]
ddC ddI AZT	Compared permeation rates of drugs. Permeation enhancing effects of ethanol/water systems and oleic acid were investigated	Permeation increased as volume fraction of ethanol increased. For ddC, ddI and AZT, addition of oleic acid (>2.0%w/v) in ethanol/water (70:30) further enhanced skin permeation rate. Enhancement for hydrophilic drugs was greater than for lipophilic drugs	[127]
AZT	Investigated transdermal flux of AZT using iontophoresis and propylene glycol/oleic acid. Effect of flux enhancement by iontophoresis was also investigated using a karaya gum matrix formulation of AZT and compared with AZT solution	Enhancement of transdermal flux by iontophoresis was smaller with the karaya gum matrix containing AZT. The iontophoretic flux from AZT solution increased about 4- to 5-fold. Penetration enhancers increased the passive flux 2- to 50-fold and worked synergistically with iontophoresis	[107]
AZT	Investigated permeation of AZT using penetration enhancers such as menthol, cineole, linolenic acid, oleic acid, in combinations of cineole or menthol with either oleic acid or linolenic acid or anodal current application	Permeability enhancing properties of the penetration enhancers were in the order of linolenic acid > menthol > oleic acid > cineole > vehicle. Combination of cineole and oleic acids enhanced permeation. Simultaneous application of the current with menthol and cineole significantly increased AZT permeation	[128]
AZT	Compared permeation of a AZT gel formulation including penetration enhancers (menthol and oleic acid) with solutions	Gel formulation was found to be more stable than solutions. There was no retardation in permeability of AZT in the gel formulation across the rat skin compared to the AZT solution. Combination of penetration enhancers at 2.5% w/w enhanced permeation	[105]
AZT	Investigated effects of binary vehicles [ethanol/water; isopropyl alcohol/water; polyethylene glycol/water; and ethanol/isopropyl myristate (IPM)], penetration enhancers [*N*-methyl-2-pyrrolidone (NMP); oleic acid; and lauric acid] and polymer [microporous polyethylene (PE) membrane] on permeation	Ethanol/IPM (50:50, v/v) demonstrated highest transdermal flux. Use of vehicle and enhancer combinations (ethanol/IPM 20/80 plus 10% NMP and ethanol/IPM 30/70 plus 10% NMP) resulted in increased AZT solubility as well as high AZT flux values, when compared to vehicles without enhancers	[129]
AZT	Investigated permeation of AZT across human cadaver skin and the effect of terpenes [L-menthol and 1, 8-cineole] on phase behaviour and molecular organization of a model Stratum Corneum (SC) lipid system	Terpenes enhanced permeation of AZT by transforming SC lipids from a highly ordered orthorhombic perpendicular subcellular packing to a less ordered hexagonal subcell packing. Terpenes caused disruption/alteration in the barrier property of SC and enhanced permeation of AZT more than ethanol and water	[130]
AZT	Evaluated the formation and transdermal permeation properties of aspasomes containing AZT	Proportion of cholesterol affected drug release rate with maximum retardation achieved with 45 mol% of cholesterol. Aspasomes had better antioxidant activity than ascorbic acid. Aspasomal AZT enhanced transdermal permeation of the drug	[115]
AZT	Evaluated use of elastic liposomes for transdermal delivery of AZT	Elastic liposomes enhanced transdermal flux, provided sustained drug release and improved site specificity of AZT	[51]
3TC ddC *N*-acyl-3TC esters	Determined the *in vitro* transdermal permeation of ddC, 3TC and synthesized 3TC esters through human epidermis with or without Pheroid™ as drug delivery system	Drugs with higher aqueous solubilities displayed greater transdermal flux values both in PBS and in Pheroid™. Transdermal flux values of drugs in Pheroid™ were lower than in PBS	[113]

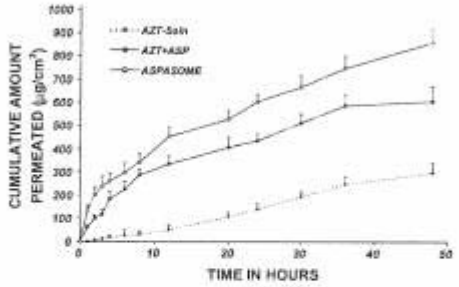

Fig. 11. In vitro permeation profiles of AZT across excised rat skin following treatment with various systems, i.e., aspasomal AZT (ASPASOME); AZT-ASP dispersion (AZT+ASP); free AZT solution (AZT-Soln) (reproduced from Gopinath et al. [109]).

several *N*-acyl lamivudine esters [113]. However, while the drugs were shown to be entrapped in the Pheroid™, the transdermal flux of the drugs in Pheroid™ was lower than in PBS. Hence, the Pheroid™ delivery system showed no practical advantage in terms of its transdermal application.

The various transdermal delivery studies with drugs such as ddI, ddC and AZT using various animal models such as the skin of rat, mouse, pig and human cadaver have confirmed the potential of ARV drugs for transdermal delivery.

3.10. Buccal delivery

Delivery of drugs via the buccal mucosa has received increased attention in the literature as an attractive alternative to the traditional oral and other conventional routes of drug administration. Use of the buccal mucosal route presents several advantages, such as the bypass of first pass hepatic metabolism and avoidance of gastrointestinal enzymatic degradation, thereby increasing the bioavailability of drugs [114]; higher permeability than that of the other routes such as the skin [115]; larger surface area for drug application, and good accessibility compared to other mucosal surfaces such as nasal, rectal and vaginal mucosa [116]. ARV drugs may therefore benefit from buccal mucosal administration instead of traditional oral administration.

Studies investigating the feasibility of the systemic buccal delivery of anti-HIV drugs have emerged. Shojaei et al. [117] initially investigated the use of a safe and effective permeation enhancer, i.e., menthol, on the buccal permeation of ddC. This study showed that the in vitro transbuccal permeation of ddC increased significantly in the presence of 1-menthol with an enhancement factor of 2.02 and a t_{lag} of 6 h. The permeation enhancement was not concentration dependent as no significant difference was observed between the permeation enhancement of ddC in the presence of 0.1, 0.2 and 0.3 mg/mL of 1-menthol [117]. Later, Xiang et al. [27] also studied the feasibility of transbuccal delivery of ddC using McIlvaine buffer solution (IMB). Their study focused on identifying the major permeation barrier within the epithelium of the buccal mucosa, the influence of sodium glycodeoxycholate (GDC) as a permeation enhancer as well as the histological effects of ddC on the buccal mucosa. These researchers reported that the basal lamina layer within the epithelium of buccal mucosa acted as an important barrier to the permeation of ddC. They also found that the permeability of ddC was significantly enhanced by GDC up to 32 times (Fig. 12). Histological studies revealed that the basal lamina remained intact, and no nucleated cell leakage was found within 24 h. These studies also showed that the thickness of epithelium was greatly reduced after buccal tissues were immersed in IMB solution for 12 and 24 h, and no difference was observed between the tissue samples incubated in the IMB and ddC IMP solutions. These two research groups concluded that transbuccal delivery is a potential route of administration of ddC, and hence for enhancing antiretroviral drug therapy.

Unlike the transdermal route, the buccal route for ARV permeation potential has not been comprehensively investigated. The reported studies to date have focused only on two different permeation enhancers, and no studies on the formulation and assessment of buccal delivery systems of ARVs could be found.

3.11. Rectal delivery

The rectal route has also been consdered for effective delivery of ARV drugs that undergo first pass hepatic metabolism and/or extensive GI degradation. Two studies were found to have been reported in the literature. Sustained-release AZT suppositories were prepared [118] using hydroxypropyl cellulose (HPC), and were assessed in rats. It was found that AZT suppositories at 10 mg/kg maintained constant plasma levels above 1 µM for more than 6 h, and they subsequently proposed suppositories as an alternative drug delivery system for AZT (Fig. 13). A further study of rectal

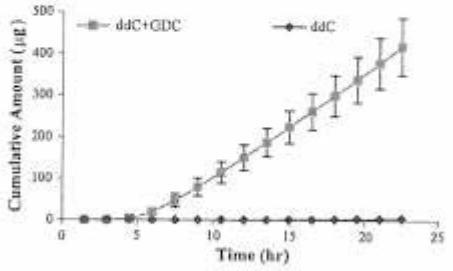

Fig. 12. Cumulative amount of ddC permeating through the porcine buccal mucosa without GDC (closed triangle) and with co-administration of GDC (closed square). Data are presented as means ± SD (n = 3) (Reproduced from Xiang et al. [27]).

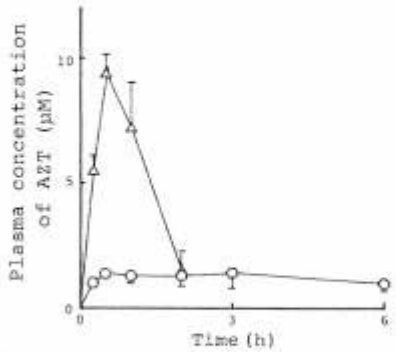

Fig. 13. Plasma concentration–time profiles following the administration of AZT suppositories: conventional (open triangle) and sustained release (open circle) (Reproduced from Kawaguchi et al. [118]).

administration of AZT [119] showed that the drug was considerably absorbed after rectal administration, with a pharmacokinetic profile that resembled that of a sustained-release delivery device. No further studies on this approach have since been identified in the literature. The work in this area appears to be limited, most probably due to patient inconvenience, as well as to the fact that HIV/AIDS patients often suffer from diarrhoea.

4. Conclusions and future studies

Despite significant advances that have been made in understanding the mechanism of HIV infection and in identifying effective treatment approaches, the search for optimum treatment strategies for AIDS still remains a major challenge. Results presented in this review indicate that novel drug delivery systems clearly present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral drug therapy. The use of such systems began in the early 1990s but it is only within the past 5 years that there appears to be a sudden surge of interest and publications in the use of novel drug delivery systems for ARV drugs. While several novel drug delivery systems have been investigated for ARV delivery, recently there appears to be greater interest and advancement in the use of liposomes and nanoparticles as compared to other systems. While the clinical potential for several NDDS has been reported from *in vitro* and animal studies, there is the lack of data on formulation optimisation and detailed physico-chemical/mechanical characterisation of these NDDS. Since HIV/AIDS treatment involves combination drug therapy, the potential of these novel drug delivery systems for simultaneous loading of various drug combinations needs to be investigated. While the potential of alternate routes of ARV drug administration such as transdermal and buccal has been confirmed, the design and development of drug delivery systems for these routes specifically are currently lacking. Correlations between the performances of these systems with their permeation potential need to be established. Although various papers report efficacy studies under *in vitro* conditions including experimental animal studies, there is the significant lack of data on the clinical applicability (human *in vivo* studies) and toxicity of these preparations. These therefore need to be extensively explored. Based on the complexity of the disease and the formulation optimisation and evaluation studies required, multidisciplinary research would be essential for eventual commercialisation of NDDS containing ARV drugs.

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APPENDIX IV

PUBLICATION TWO

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Investigating the Effect of *Aloe vera* Gel on the Buccal Permeability of Didanosine

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Key words
- buccal
- didanosine
- permeation enhancer
- histomorphology
- *Aloe vera* (L.) Burm. F.
- *Aloe barbadensis* Miller
- Asphodelaceae

Abstract

The buccal mucosal route offers several advantages but the delivery of certain drugs can be limited by low membrane permeability. This study investigated the buccal permeability properties of didanosine (ddI) and assessed the potential of *Aloe vera* gel (AVgel) as a novel buccal permeation enhancer. Permeation studies were performed using Franz diffusion cells, and the drug was quantified by UV spectroscopy. Histomorphological evaluations were undertaken using light and transmission electron microscopy. The permeability of ddI was concentration-dependent, and it did not have any adverse effects on the buccal mucosae. A linear relationship ($R^2 = 0.9557$) between the concentrations and flux indicated passive diffusion as the mechanism of drug transport. AVgel at concentrations of 0.25 to 2%w/v enhanced ddI permeability with enhancement ratios from 5.09 (0.25%w/v) to 11.78 (2%w/v) but decreased permeability at 4 and 6%w/v. Ultrastructural analysis of the buccal mucosae treated with phosphate buffer saline pH 7.4 (PBS), ddI/PBS, and ddI/PBS/AVgel 0.5%w/v showed cells with normal plasmalemma, well-developed cristae, and nuclei with regular nuclear envelopes. However, cells from 1, 2, and 6%w/v AVgel-treated mucosae showed irregular nuclear outlines, increased intercellular spacing, and plasmalemma crenulations. This study demonstrates the potential of AVgel as a buccal permeation enhancer for ddI to improve anti-HIV and AIDS therapy.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

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Introduction

Antiretroviral (ARV) drugs have revolutionized the treatment of HIV (human immunodeficiency virus) infection and AIDS (acquired immune deficiency syndrome) [1], widely acknowledged as being among the most serious public health problems [2]. However, several limitations exist with current ARV drug therapy via the oral route [3,4]. These drugs suffer from low bioavailability due to extensive first pass effects and gastrointestinal degradation. Also, short half-lives necessitate frequent administration of doses, and severe dose-dependent side effects may occur.

Buccal drug delivery, which is the administration of drug from a delivery system (e.g., films, patches, and gels) through the mucosae lining the cheeks of the mouth, has received increased interest as an alternative to the oral route. Drugs administered via the buccal route can bypass enzymatic degradation and hepatic first pass metabolism thereby improving bioavailability [5,6]. It has a high patient acceptability compared to other non-oral routes [7]. Buccal delivery systems offer an attractive approach for pediatrics and for patients with swallowing problems. Buccal delivery of ARV drugs can therefore contribute to overcoming some of their current disadvantages. While the potential of ARV drugs for administration via another non-oral route, namely the transdermal route, has been explored [8,9] their buccal delivery potential remains to be investigated.

The epithelium lining the oral cavity is a barrier to drug permeation. The use of permeation enhancers in many cases is decisive for efficient buccal drug delivery [10,11]. The discovery of new permeation enhancers is essential for optimizing drug delivery via the buccal route. Currently, there is an increasing interest for drug products that either are of natural origin or contain such components [12]. *Aloe vera* (*Aloe barbadensis* Miller) is a succulent plant with strap-shaped

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green leaves [12]. For medicinal applications, the aloe latex (or exudate), the aloe gel, and the whole leaf (or whole leaf extract) are the main parts used [13]. The inner pulp of the fresh leaves is used for gel extrusion [14]. The gel is composed mainly of water (> 99%), and the remaining 0.5–1% of solid material comprises several polysaccharides, vitamins, enzymes, lipids, as well as inorganic and small organic compounds [15]. It is recognized as an important medicinal plant that has effective anti-inflammatory, antifungal, and soothing effect on the mucosal lining as well as wound healing properties [16]. While it has recently been shown to be an effective transdermal [17] and intestinal [13] penetration enhancer for various drugs, its applicability for buccal permeation enhancement has not been investigated before. Those studies with AVgel as an enhancer for the intestinal and transdermal routes did not report its histomorphological effects [13, 17], which is important for assessing its preliminary suitability. Recently, it has been shown to have the potential to modify drug release profiles in dosage forms [18]. It appears that *Aloe vera* gel, with polysaccharides as a significant component, has the potential unlike several existing penetration enhancers, to also provide multifunctional properties in buccal drug delivery systems. A buccal controlled release product based on *Aloe vera* gel (AVgel) will therefore be an attractive system for the administration of ARV drugs.

The aim of this study was therefore to identify the buccal permeability potential of a model ARV drug, i.e., didanosine (ddI), in the absence and presence of a potential novel buccal permeation enhancer, namely AVgel. In addition, the study also aimed at evaluating the histomorphological effects of ddI and AVgel on the buccal mucosa.

Materials and Methods

Ethical clearance

Ethical approval was obtained from the University of KwaZulu-Natal Animal Ethics Committee in 2008 (001/08/Animal) and renewed annually in 2009 (028/09/Animal), 2010 (029/10/Animal), and 2011 (25/11/Animal).

Materials

Didanosine [ddI; chromatographic purity (HPLC) = 99.4%] was donated by Aspen Pharmacare. AVgel, in dry powder form, was received from the International Aloe Science Council (IASC, 051309, Texas, USA) and was the same sample used in our previously reported study in *Planta Medica* [13]. The ^{1}H-NMR spectrum of the AVgel and the quantities of chemical markers as determined by NMR spectroscopy are available as Supporting Information (**Fig. 1S** and **Table 1S**) and are discussed under the Results section. Disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride were purchased from Sigma-Aldrich. All other reagents used were of analytical grade.

Methods

Preparation of porcine buccal mucosae: Buccal mucosae harvested from pigs (30–40 kg) (Biomedical Resource Unit, UKZN) sacrificed by LECO euthanasia were appropriately excised; their thickness was 665 ± 72 µm (CV = 8.3%). Fresh buccal mucosae were used for histological evaluations. For buccal permeability studies, the buccal mucosae were snap frozen in liquid nitrogen, stored in a biofreezer (−85 °C) and used within three months [12].

In vitro permeation: Frozen buccal mucosae were allowed to thaw and equilibrated in phosphate buffer saline pH 7.4 (PBS). Franz diffusion cells (PermeGear, Inc.) with a diffusional area of 0.786 cm² were used for permeation experiments. The buccal mucosa was mounted to the diffusional area between the donor and receptor cells and was equilibrated with PBS at 37 °C. The donor compartment contained either varying concentrations of ddI in PBS alone (5, 10, 15, and 20 mg·mL^{-1}) or ddI (20 mg·mL^{-1}) in the presence of AVgel (0.25, 0.5, 1.0, 2.0, 4.0, and 6.0%w/v). The receptor compartments were filled with PBS. Samples were removed from the receptor compartments at predetermined time intervals and replaced with the same volume of ddI-free PBS. Each experiment was undertaken using a minimum of three replicates. Similar to permeation studies with other drugs [19,20], ddI was quantified by a validated UV spectrophotometry method at a λ_{max} of 250 nm (UV spectrophotometer 1650; Shimadzu).

Permeability data analysis: The cumulative amount of ddI permeated per unit surface area was plotted against time. The steady state flux (J_{ss}) was determined from the linear part of the permeability curve by linear regression analysis (Microsoft Excel 2007). The permeability coefficient (P) was calculated as follows [21]:

$$P = (dQ/dt) / A = Cd = Jss / Cd \quad (1)$$

Where dQ/dt is the cumulative amount permeated per unit time, A is the diffusion area, and C_d is the drug concentration in the donor compartment. The permeability of ddI was evaluated in the presence of various concentrations of AVgel. The enhancement ratio (ER) was calculated as follows [21]:

$$ER = \frac{\text{Permeability coefficient of drug in the presence of enhancer}}{\text{Permeability coefficient of drug in the absence of enhancer}} \quad (2)$$

Viscosity determination: The viscosities of ddI (20 mg·mL^{-1}) only and ddI (20 mg·mL^{-1}) in the presence of AVgel (0.25, 0.50, 1.0, 2.0, 4.0, and 6.0%w/v) were determined with a Modular Advanced Rheometer (ThermoHaake MARS Thermo Fischer Scientific), equipped with a titanium cone (C35/1° Ti) set at a sample gap of 0.051 mm and a Thermocontroller (UTC-MARS II). The relationships between the viscosity and shear stress as a function of shear rate were analyzed using HaakeRheoWin, 3.50.0012 software.

Light microscopy and transmission electron microscopy: Fresh buccal mucosa was cut into 1 × 1 × 0.1 cm cross sections. Mucosae were incubated in bottles containing either PBS only, or ddI/PBS (20 mg·mL^{-1}), or ddI/PBS (20 mg·mL^{-1})/AVgel in varying concentrations. The bottles were kept in a water bath at 37 °C over six hours. Untreated buccal mucosa was transferred from normal saline into 10% buffered formalin without incubation in PBS and served as the control. Both the control and treated buccal mucosae were fixed in formalin for seven days. They were dehydrated using an ethanol gradient and embedded in paraffin wax. The sections were collected on slides, dried and stained with hematoxylin and eosin (H&E). Semi-thin sections (1 µm) of the epoxy-embedded samples were also obtained and stained with toluidine blue. Sections were examined using a light microscope (Nikon 80i), and bright field images were captured using NIS Elements D software and a camera (Nikon U2).

Samples for transmission electron microscopy (TEM) were incubated as described above. They were cut into pieces not exceeding 0.5 mm³ and fixed for 24 hours (4 °C) using Karnovsky's fixa-

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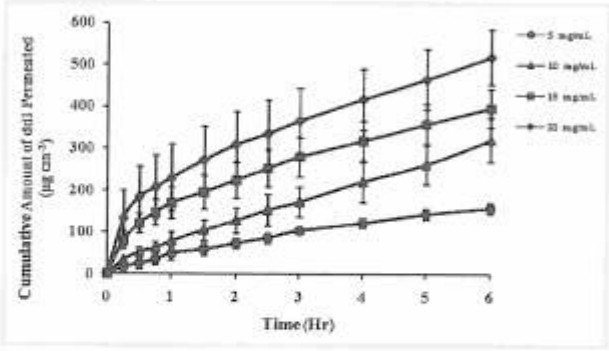

Fig. 1 Cumulative amount of ddI permeated per unit surface area vs. time profiles observed for ddI donor concentrations (mean values ± SD; n ≥ 3).

Table 1 Effect of ddI donor concentration on its permeability parameters.

Donor concentration of ddI ($mg \cdot mL^{-1}$)	Cumulative amount of ddI permeated ($\mu g \cdot cm^{-2}$)	Linear equation ($y = mx + c$)	Correlation coefficient (R^2)	Flux (J_{ss}) ($\mu g \cdot cm^{-2} \cdot hr^{-1}$)	Permeability coefficient (P) × 10^{-2} ($cm \cdot hr^{-1}$)
5	158.15 ± 13.17	$Q = 25.94_t + 15.65$	0.97	25.94 ± 1.35	0.52 ± 0.03
10	321.08 ± 52.82	$Q = 49.85_t + 22.23$	0.99	49.85 ± 8.99	0.49 ± 0.09
15	397.03 ± 46.01	$Q = 57.35_t + 85.14$	0.92	57.35 ± 5.88	0.38 ± 0.04
20	456.89 ± 57.11	$Q = 71.57_t + 128.70$	0.89	71.57 ± 3.12	0.36 ± 0.02

tive [22] buffered to pH 7.2, processed and embedded in epoxy resin using standard protocols. Ultrathin sections (90 nm) were cut, contrasted with uranyl acetate and lead citrate and viewed with a transmission electron microscope (JEOL 1010).

All experiments were performed using a minimum of three replicates.

Statistical analysis

The results, expressed as mean ± standard deviation (SD), were analyzed using one-way ANOVA followed by the Mann-Whitney test using GraphPad Prism® (Graph Pad Software, Inc., version 3). Differences were considered significant at $p < 0.05$.

Supporting Information

The chemical composition of the AVgel and its ^{1}H-NMR spectrum are available as Supporting Information.

Results and Discussion

The permeability potential of ddI in the absence of an enhancer was initially investigated. ⊙ **Fig. 1** shows the cumulative amount of ddI permeated at different donor concentrations. The flux values increased with an increase in ddI concentration and ranged from 25.94 ± 1.35 $\mu g \cdot cm^{-2} \cdot hr^{-1}$ to 71.57 ± 3.12 $\mu g \cdot cm^{-2} \cdot hr^{-1}$ (⊙ **Table 1**). There was a significant difference ($p = 0.001$) between all concentrations except between the flux values of 15 $mg \cdot mL^{-1}$ and 20 $mg \cdot mL^{-1}$ ddI, which were not significant ($p = 0.302$).

A linear relationship ($R^2 = 0.9557$) between the flux and ddI concentrations was obtained (⊙ **Fig. 2**), indicating passive diffusion as the main mechanism of ddI transport across the buccal mucosa [23,24]. Didanosine is hydrophilic, and its passive diffusion should favor the paracellular pathway [25,26].

Xiang et al. [3] highlighted the promising potential of zalcitabine (ddC), the only other ARV reported to date for buccal delivery. They reported a flux of 13.42 ± 6.35 $\mu g \cdot cm^{-2} \cdot hr^{-1}$ for ddC at 20 $mg \cdot mL^{-1}$ which is lower than the flux of ddI (71.57 ± 3.12 $\mu g \cdot cm^{-2} \cdot hr^{-1}$). Several drugs with similar and lower flux values have been reported as having the potential for improving drug therapy via the buccal route [23,24,27]. ddI may therefore be regarded as having the potential for improving HIV and AIDS drug therapy when administered by the buccal route.

The AVgel employed in this study to investigate its effect on ddI permeation was the same as used by Chen et al. [13] to study its effects on intestinal drug permeability. The ^{1}H-NMR spectrum of the AVgel is shown in **Fig. 1S** and the quantities of chemical markers as determined by NMR spectroscopy in **Table 1S**. The results indicate that the AVgel material contained all the essential markers especially aloverose.

The buccal permeability of ddI in the presence of AVgel (⊙ **Fig. 3**) was investigated. The flux of ddI in the absence of AVgel was 71.57 ± 3.12 $\mu g \cdot cm^{-2} \cdot hr^{-1}$. It increased significantly ($p < 0.001$) with an increase in AVgel concentration up to 2%w/v (⊙ **Table 2**), which demonstrated the highest permeability coefficient of 3.3×10^{-2} $cm \cdot hr^{-1}$ and an enhancement ratio (ER) of 11.78, thereby confirming for the first time the buccal permeation enhancement property of AVgel.

The permeation enhancing potential of AVgel from 0.25 to 2.0%w/v may have a similar mechanism to those proposed for other polysaccharides reported as permeation enhancers [28]. Polysaccharides such as chitosan are known to demonstrate mucoadhesivity, which causes prolonged drug retention on mucosae. It has been proposed that chitosan enhances buccal perme-

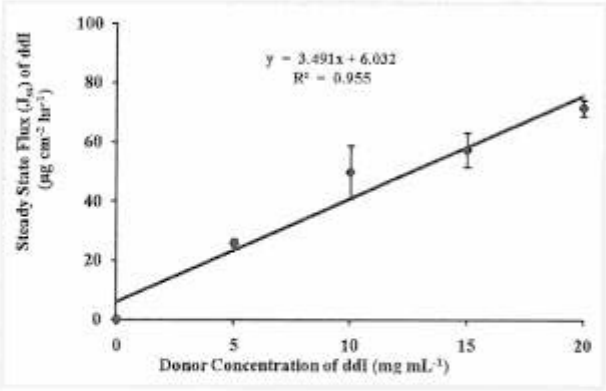

Fig. 2 Effect of donor concentration on the steady state flux of ddI at pH 7.4 (mean values ± SD; n ≥ 3).

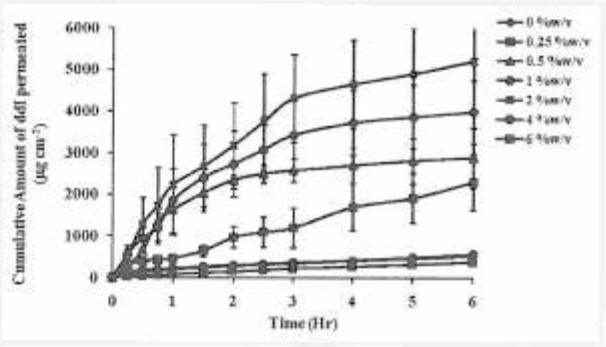

Fig. 3 Cumulative amount of ddI permeated per unit surface area vs. time profiles observed for AVgel concentrations (mean values ± SD; n ≥ 3).

Table 2 Effect of AVgel concentration on the permeability parameters of ddI.

Concentration of AVgel (%w/v)	Correlation coefficient (R^2)	Flux (Jss) ($\mu g \cdot cm^{-2} \cdot hr^{-1}$)*	Permeability coefficient (P) × 10^{-2} ($cm \cdot hr^{-1}$)	Enhancement ratio (ER)
0.0	0.89	71.57 ± 3.17[a]	0.36 ± 0.02	1
0.25	0.99	364.69 ± 92.59	1.82 ± 0.46	5.09
0.5	0.89	613.69 ± 292.49[b]	3.07 ± 1.46	8.58
1.0	0.85	650.07 ± 164.41[b]	3.25 ± 0.82	9.08
2.0	0.88	842.73 ± 129.24[b]	4.21 ± 0.65	11.78
4.0	0.95	83.95 ± 11.71[c]	0.42 ± 0.06	1.17
6.0	0.99	62.02 ± 5.41[c]	0.31 ± 0.03	0.87

* [(a vs. b; $p < 0.05$), (a vs. c; $p > 0.05$)]; [a] flux of the control; [b] statistically significant higher than control (ANOVA); [c] statistically non-significant compared to control (ANOVA)

ability by interactions with the epithelial barrier that may weaken it, partially dismantling the extracellular matrix structure and intercellular joint. Since the major components of AVgel are polysaccharides [18], a similar mechanism may apply. Also, AVgel is cationic, and its possible ionic interaction with sialic acid residues on the buccal mucosae could alter membrane permeability [25, 28].

Further increases in AVgel to 4.0 and 6.0%w/v led to a decrease in flux to 83.95 ± 9.24 and 62.06 ± 5.58 $\mu g \cdot cm^{-2} \cdot hr^{-1}$, respectively. Although there is a 10-fold reduction in the flux between 2 and 4%w/v AVgel, the flux at 4 and 6%w/v is reduced to a value which is statistically similar to the flux in the absence of AVgel (○ **Table 3**). The decrease may be attributed to a higher viscosity of AVgel at higher concentrations that can increase resistance to drug diffusion and hinder drug movement [18, 29]. Increasing the concentration of AVgel in the ddI/PBS/AVgel formulations led to an increased viscosity of the formulations (○ **Fig. 4**) and displayed a linear correlation ($R^2 = 0.972$). The viscosity of AVgel at 6.0%w/v

Concentration of AVgel (% w/v)	Viscosity (η) (mPa)	Percentage increase in viscosity (%)
0	0.84 ± 0.00	0
0.25	0.94 ± 0.05	12.19
0.5	1.05 ± 0.01	25.14
1	1.22 ± 0.04	45.51
2	1.86 ± 0.52	121.80
4	2.21 ± 0.14	163.38
6	2.84 ± 0.11	238.72

Table 3 Effect of AVgel concentration on the viscosity of ddI/PBS/AVgel formulations.

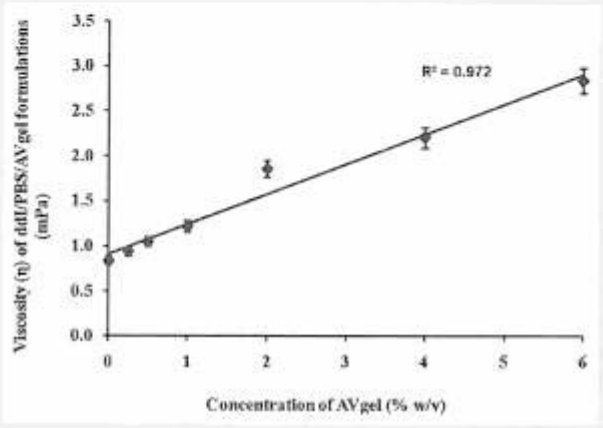

Fig. 4 Effect of AVgel concentration on the viscosity of ddI/PBS/AVgel formulations (mean values ± SD; n = 3).

(2.84 mPa) was almost three times (up to 240%) higher than that at 0.25%w/v (0.94 mPa) (○ **Table 3**). The viscosities of AVgel at 4.0 and 6.0%w/v may have been high enough to impede the buccal permeability enhancing potential of AVgel. Similar trends, with an initial increase in flux with an increase in enhancer concentrations (propylene glycol) but resultant flux decreases with further increases have been reported in another study [30], although possible reasons were not investigated.

The ER of ddI increased approximately 12-fold with AVgel 2.0%w/v but decreased to 0.87-fold with AVgel 6.0%w/v (○ **Table 2**). The ER values in this study are within the range of previous studies using AVgel at similar concentrations for other routes. The ER for colchicine through porcine skin was 11.2 (AVgel 3.0%w/v) [17] while that of insulin through the intestinal epithelial monolayer was 2.31 (2.5%w/v AVgel) [13]. A higher ER at a slightly lower concentration of 2%w/v is reported for the buccal mucosa in this study. One explanation is that the buccal mucosa is more permeable than skin. Also, insulin in the previous study is a larger molecule and may not permeate to a similar extent as ddI. The ER values of other buccal enhancers were found comparable to those observed with AVgel in this study. Other chemical enhancers such as sodium glycodeoxycholate (ER = 32), menthol (ER = 2.02), and sodium glycolate (ER = 9) have been reported as effective enhancers for buccal delivery [3, 21].

While permeation enhancing effects of substances are extensively reported, their effects on buccal mucosa morphology are limited [3, 31]. Since buccal delivery involves retention of a delivery system on the mucosae, an assessment of histological effects of a drug and or enhancer/s to evaluate their suitability is essential.

Histomorphological effects of the control/untreated and the treated porcine buccal mucosae (PBS alone and ddI/PBS in the absence or presence of AVgel) were assessed. The morphology of pig buccal mucosa has been described previously, and it closely resembles human buccal epithelium [32, 33]. In the control group, the buccal epithelium resembled that of a normal non-keratinized stratified squamous layer (○ **Fig. 5a**). Basal cells appeared oval and darkly stained in H&E (○ **Fig. 5b**) and toluidine blue (○ **Fig. 5c**) sections, reflecting their greater mitotic activity. The middle region showed large polygonal cells, and superficial cells showed desquamation (○ **Fig. 5d**). Basal cells were nucleated while some of the superficial cells were anucleate. The basal cell layer represents the germinal tissue from which new cells are produced and should form the focus of such studies. Damage to superficial layers can be rectified by renewed growth from the germinal layer, but chronic or severe damage to the basal cell layer is probably irreversible [34]. The appearance of the control, PBS, and ddI/PBS samples in H&E (○ **Fig. 5a–b**) and toluidine blue (○ **Fig. 5c–e**), respectively, were similar suggesting no influence of PBS or ddI (either alone or in combination) on tissue morphology. Therefore, ddI at the highest concentration had no adverse effects on the buccal mucosae.

The buccal mucosa upon treatment with ddI/PBS in combination with AVgel was examined. The addition of 0.5%w/v AVgel led to an increase in intercellular spaces and darker staining of the cytoplasm, resembling the structure of control samples (○ **Fig. 5f**).

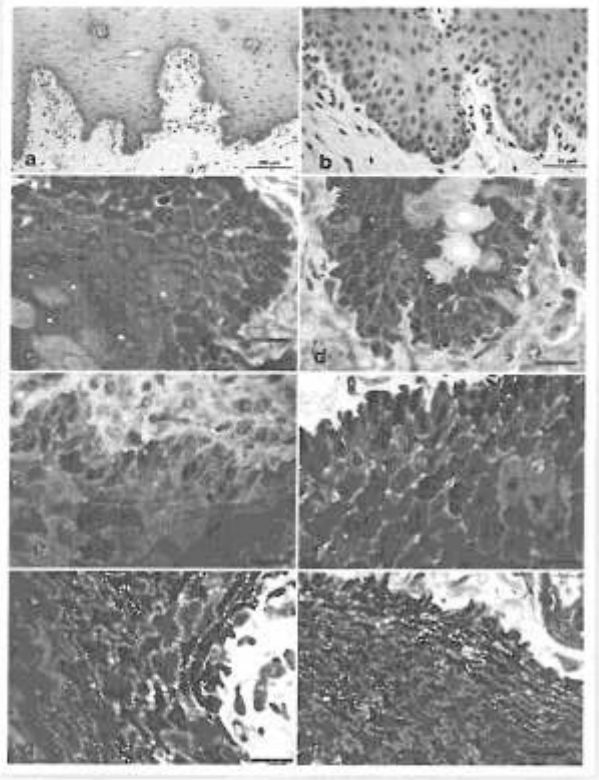

Fig. 5 Microphotographs of the control and treated buccal mucosal sections for light microscopy (LM) stained with H&E: **a** control/untreated, **b** ddI/PBS, and with toluidine blue: **c** control/untreated, **d** PBS, **e** ddI/PBS, **f** ddI/PBS/AVgel 0.5%w/v, **g** ddI/PBS/AVgel 1.0%w/v, **h** ddI/PBS/AVgel 6.0%w/v.

However, an increased AVgel concentration to 1%w/v showed a marked increase in intercellular spaces and distortion of cellular outlines (○ **Fig. 5g**). Cells appeared irregular and crenulated compared to controls. This was accentuated in 6%w/v AVgel samples where extreme compaction of cells in the basal region was observed (○ **Fig. 5h**). Although not shown, cells from the middle and superficial layers also appeared severely damaged. Furthermore, the epithelial surface and basal lamina of the mucosa in the H&E sections of the control, PBS alone, ddI/PBS, and ddI/PBS/AVgel 0.5%w/v still appeared intact after six hours, but extensive disordering of this cell layer was observed in toluidine blue sections of the ddI/PBS/AVgel 6%w/v. This disorder increased towards the epithelial surface and may be due to the higher concentration effect of AVgel on the buccal mucosa.

The ultrastructure of buccal mucosae was evaluated. The control buccal mucosae showed short profiles of endoplasmic reticulum, an abundance of ribosomes, and regular nuclei with evenly-dispersed chromatin (○ **Fig. 6a**). Mitochondria appeared dense with well-developed cristae suggesting normal cellular activity (○ **Fig. 6b**). Intercellular spaces were small, and clearly defined desmosomes between attachment plaques in neighboring cells were observed (○ **Fig. 6c**). PBS and ddI/PBS treated mucosae showed a similar ultrastructure to the saline control, confirming trends observed at light microscope level. Cells from 0.5%w/v AVgel samples also showed signs of active cellular metabolism and regular nuclear outlines (○ **Fig. 6d**). While electron translucent clearings within the mitochondria were occasionally observed, cellular damage was not evident (○ **Fig. 6e**). However, increasing AVgel concentration to 1%w/v led to cellular damage evident by irregular nuclear outlines, peripheral distribution of visibly-compacted chromatin, electron-lucent mitochondria containing little internal detail, and distended endoplasmic reticulum profiles (○ **Fig. 6f**). Increased intercellular spacing and crenulation of the plasmalemma also became evident (○ **Fig. 6g**). Further increases in AVgel concentrations to 2 and 6%w/v led to disruption of basal cell layers, severe cellular compaction, and larger intercellular spaces (○ **Fig. 6h** and **6i**).

Histomorphological evaluations showed that AVgel caused adverse effects on the mucosa at higher concentrations of 1, 2, and 6%w/v. Since the buccal mucosa was not adversely affected at lower concentration of 0.5%w/v, AVgel may therefore be considered as a safe permeation enhancer up to this concentration. At 0.5%w/v, AVgel showed an ER of 5.09 which is still higher than several other reported enhancers [3, 23, 27].

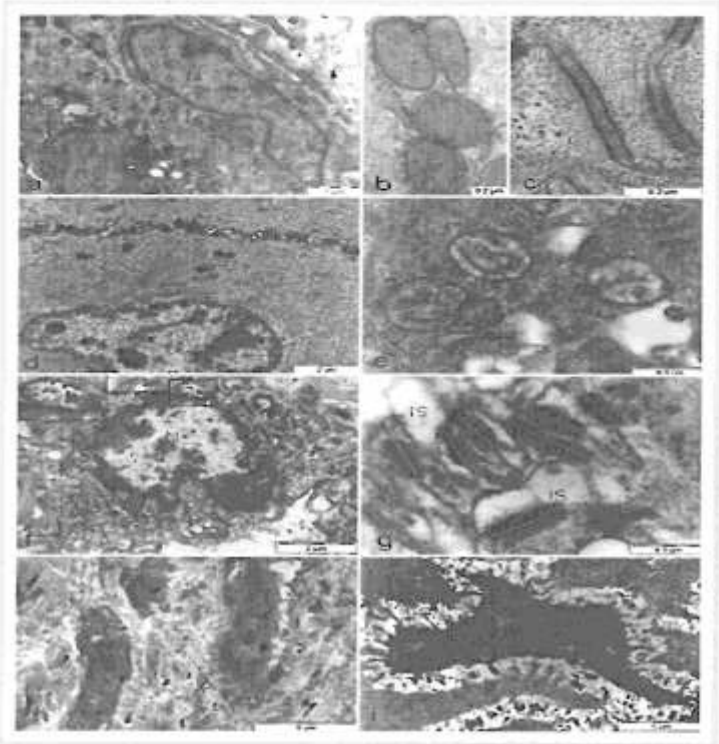

Fig. 6 Microphotographs of the control and treated ultra-thin buccal mucosa sections for transmission electron microscopy (TEM): **a** control/untreated, **b** PBS, **c** ddI/PBS, **d–e** ddI/PBS/AVgel 0.5%w/v, **f–g** ddI/PBS/AVgel 1.0%w/v, **h** ddI/PBS/AVgel 2.0%w/v, **i** ddI/PBS/AVgel 6.0%w/v.

The study has shown that ddI can permeate the buccal mucosa without adversely affecting its morphology. AVgel at concentrations up to 2%w/v was identified as an effective buccal permeation enhancer for ddI. Based on the findings it is proposed that AVgel be used in concentrations at or lower than 0.5%w/v due to adverse mucosal effects at higher concentrations. Histomorphological evaluations therefore proved useful in correlating the permeation enhancing properties of AVgel with its effects on the buccal mucosa. The results confirm the potential of developing a buccal drug delivery system containing ddI and AVgel as an enhancer for improving drug therapy.

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Conflict of Interest

The authors declare that there are no conflicts of interest for this study. The authors alone are responsible for the design, content, and writing of this paper.

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Supporting Information

Investigating the effect of *Aloe vera* gel on the buccal permeability of didanosine

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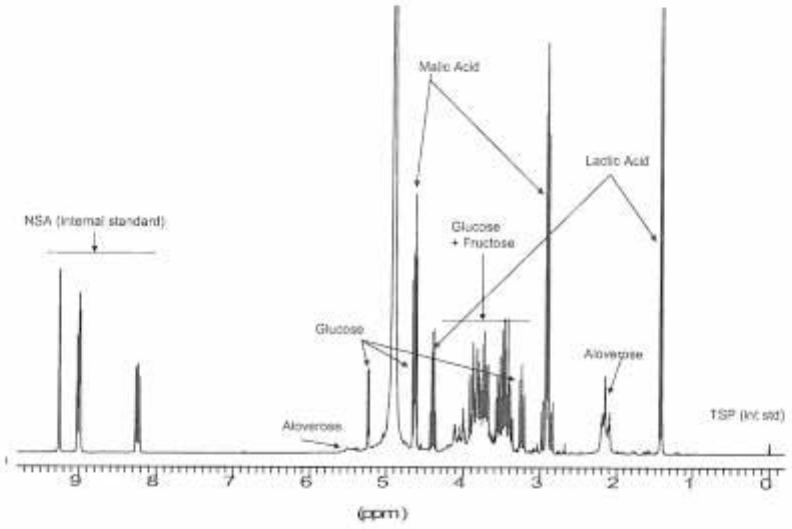
Table 1S Chemical composition of AVgel as determined by ^{1}H-NMR [15].

Chemical	AVgel	
	Content (%)	Content (mg/L)
Aloverose	12.7	892.1
Glucose	16.7	1171.2
Malic acid	20.0	1403.4
Lactic acid	5.1	359.2
Citric acid	not detected	
WLM	detected	
Maltodextrin	not detected	
Acetic acid	not detected	
Succinic acid	trace	
Fumaric acid	not detected	
Formic acid	not detected	
Sodium benzoate	not detected	
Potassium sorbate	not detected	

Fig. 1S ^{1}H-NMR spectrum of AVgel labeled with the main chemical constituents and markers [15].



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APPENDIX V

PUBLICATION THREE

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RESEARCH ARTICLE

Novel oleic acid derivatives enhance buccal permeation of didanosine

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Abstract

The aim of this study was to explore the potential of novel oleic acid (OA) derivatives as buccal permeation enhancers for the delivery of didanosine (ddI). The OA derivatives, i.e. ester derivative (OA1E), the dicarboxylic acid derivative (OA1A) and the bicephalous dianionic surfactant (OA1ANa) were synthesized and their effects were compared to the parent OA. OA, OA1E, OA1A and OA1ANa at 1% w/w all showed potential for enhancing the buccal permeability of ddI with enhancement ratio (ER) of 1.29, 1.33, 1.01 and 1.72, respectively. OA1ANa at 1% w/w demonstrated the highest flux ($80.30 \pm 10.37\ \mu g\,cm^{-2}\,h$), permeability coefficient ($4.01 \pm 0.57 \times 10^{-3}\ cm\,h^{-1}$) and ER (1.72). The highest flux for ddI ($144.00 \pm 53.54\ \mu g\,cm^{-2}\,h$) was reported with OA1ANa 2% w/w, which displayed an ER of 3.09 more than that with ddI alone. At equivalent concentrations, OA1ANa (ER = 3.09) had a significantly higher permeation-enhancing effect than its parent OA (ER = 1.54). Histomorphological studies confirmed that OA1ANa at all concentrations (0.5, 2.0 and 6.0% w/w) had no adverse effects on the mucosae. Morphological changes such as vacuoles formation and increased intercellular spaces were attributed to the buccal permeation-enhancing effect of OA1ANa. This study demonstrated the potential of novel OA derivatives as buccal permeation enhancers. OA1ANa at 2% w/w was also identified as the optimal novel OA derivative to widen the pool of fatty acid derivatives as chemical permeation enhancers for buccal drug delivery.

Keywords

Antiretroviral, buccal, didanosine, oleic acid derivatives, permeation enhancers

History

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Introduction

The buccal mucosa remains an attractive alternate and non-invasive site for the delivery of both locally and systemically active drugs[1,2]. It avoids the degradation of drugs by both the GI acids and enzymes and also bypasses hepatic first pass metabolism, thereby improving the systemic bioavailability of various drugs[3–5]. Furthermore, absorption following administration *via* the buccal route is not influenced by potential variations in the gastric emptying rate or the presence of food[6]. The permeability of the buccal mucosa is also higher than that of skin[7]. Hence, a lower loading dose in a transbuccal device could provide the same therapeutic effect as a transdermal patch. The buccal mucosa also has a larger area for drug application and has good accessibility compared to other mucosae such as the nasal, rectal and vaginal mucosae[1]. Various classes of drugs including zalcitabine (ddC) (antiretroviral; ARV)[8,9], peroxicam (non-steroidal anti-inflammatory)[10], morphine (opiate)[11], omeprazole (proton-pump inhibitor)[12], insulin (anti-diabetic hormone and blood glucose lowering agent)[13] and metoprolol (beta blocker)[14] have been studied for delivery *via* the buccal mucosa to exploit its above advantages. The buccal route therefore has wide applicability for diverse drugs and disease conditions.

One of the main challenges with buccal mucosal therapy is its limited mucosal permeability due to the epithelial lining of the membrane, which acts as a barrier to drug permeation[15]. The outermost layer of the stratified squamous epithelium is keratinized, covered by a thin layer of mucus and is comparatively thicker than the rest of the oral mucosal lining. The basement membrane lies directly underneath the epithelium, followed by the lamina propria and the submucosa. The mucosa is made up of about 40–50 cell layers, and a thickness of 500–800 μm has been reported[16,17]. The mucosal structure thus contributes to the challenges and factors that are responsible for the limited buccal permeability of drugs. Enhancing permeation of drugs across the buccal mucosa is therefore critical for optimizing bioavailability of various drugs[5]. Maximizing the bioavailability of several drugs after buccal administration for absorption through the mucosal lining will be beneficial to reducing intra and inter subject variability as well as side effects of the drugs[14,18]. Moreover, the cost of manufacture will be reduced by decreasing drug wastage owing to its low systemic bioavailability[19], especially where drugs have limited permeability and subsequently low bioavailability. Hence, the use of permeation-enhancing strategies in many cases is essential to overcome the limited permeability of the buccal mucosae for improved buccal drug delivery[20,21]. Recent advancements in the use of permeation-enhancing strategies have identified various approaches, including physical and chemical methods, to enhance the buccal permeability of drugs. It has been

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reported that chemical permeation enhancers (CPEs), for example, bile salts, surfactants and fatty acids (FAs), have proved promising for enhancing buccal permeability of drugs[22,23]. Other approaches include drug particle size reduction[24], ultrasound and electrical assisted approaches (iontophoresis and electroporation) as well as thermal enhancement[25–27]. More specifically, recent reports are emerging on the use of derivatives of common chemical enhancers for further maximizing mucosal drug permeation. For example, newly synthesized propanoyloxy derivatives of 5b-cholan-24-oic acid were more effective in enhancing permeation of theophylline as compared to its parent compound, cholic acid, thereby potentiating the efficacy of bile acids as a class of CPEs[28,29]. There is therefore a need to explore and identify new derivatives of chemical enhancers to widen the pool of available superior enhancers for buccal drug delivery.

FAs are widely used CPEs for various drugs[17]. Sodium caprate, caprylic acid, sucrose esters and lauric acid have been reported for enhancing the permeation of drugs such as lidocaine, ergotamine, insulin and sumatriptan across the buccal mucosa[25,30–33]. It has been reported that FAs can disrupt the lipid bilayer of the mucosal lining thereby increasing drug transport and bioavailability. Oleic acid (OA), in particular, has been reported as an effective CPE for drugs such as levothyroxine sodium *via* the intestinal route[34], caffeine and diclofenac sodium (DS) *via* the transdermal route[35] and 5-fluorouracil *via* the buccal route[3]. Novel derivatives of OA will therefore be useful for further improving their permeation-enhancing potential and will contribute to the pool of permeation enhancers for enhancing drug permeability.

In a previous study by our group, the synthesis of novel OA derivatives, known as oleodendrimers A1E, A2E, E1E and E2E were reported. In addition, the potential of these derivatives as transdermal permeation enhancers for the delivery of a non-steroidal anti-inflammatory drug, DS, was identified[36]. These derivatives, A1E, A2E, E1E and E2E are G1 and G2 Janus-type dendrimers in which the deodron moiety is linked with OA through ester and amide bonds. Oleodendrimers A1E and A2E have an amide linkage, whereas E1E and E2E have an ester linkage between the dendron and OA moiety. This study showed the OA derivatives, oleodendrimers, as being far more effective in enhancing the transdermal permeation of DS as compared to the parent OA. The specific mechanism of permeation enhancement was not cited in the previous study. However, as FAs, it could be proposed as the perturbation/disruption of the lipid bilayer of the stratum corneum, which could have led to increased drug permeation across the epidermis of the skin[35].

The potential of these novel oleodendrimers as permeation enhancers for buccal drug delivery has not been studied for any drug to date. The buccal permeation enhancement probability of the OA derivatives can expand its applicability in drug delivery systems. Therefore, in this study, it was decided to explore the potential of the oleodendrimer E1E (OA1E), its dicarboxylic acid derivative (OA1A) and a sodium salt of dicarboxylic acid (bicephalous dianionic surfactant, OA1ANa) as buccal permeation enhancers. OA1E has been studied previously as a transdermal enhancer only and derivatives OA1A and OA1ANa have not been studied as permeation enhancers for any drug by any route.

In the previously reported study, OA1E was synthesized by methods that involved the use of hazardous reagents such as thionyl chloride, harsh reaction conditions involving high reaction temperatures and it was furthermore a detailed multistep process[37]. In keeping with the current trends of applying green chemistry approaches[38], the synthesis of the OA1E derivative, from which OA1A and OA1ANa are obtained, by a modified method to eliminate the above drawbacks will be beneficial for its commercial application as enhancers for various routes.

In this study, didanosine (ddI) was selected as a model drug for *in vitro* buccal permeation investigations. ARV drugs have improved the treatment of human immunodeficiency virus infection and acquired immune deficiency syndrome, diseases that significantly affect the global population[39,40]. Most ARVs, including ddI, have low bioavailability due to the first-pass effect and gastrointestinal acidic and enzymatic degradation. In addition, they exhibit dose-dependent toxicities and adverse effects[41,42]. ARV drugs such as ddI may therefore benefit from buccal delivery. The permeability potential of ARV drugs *via* the buccal route has been investigated for ddC, ddI, TFV, AZT and SQV[7,9,43–45], and these ARVs have all shown potential for permeability *via* the buccal mucosa. There are limited studies on the identification of CPEs for ARV drugs. The latter have included polymeric excipients[44], aloe vera[43], bile salts[9] and menthol[8].

The aim of this study was therefore to synthesize novel OA derivatives by a greener chemistry approach and explore the potential of OA and its oleodendrimer derivatives as novel permeation enhancers for buccal permeability using ddI as a model ARV drug.

Materials and methods

Ethical clearance

Ethical approval (Reference 039/13/Animal) was obtained from the University of KwaZulu-Natal (UKZN) Animal Research and Ethics Committee.

Materials

OA (technical grade, 90%), *N*-Ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC.HCl), *p*-dimethylaminopyridine (DMAP) were obtained from Sigma (St. Louis, MO). 3-Amino-1-propanol and *tert*-butyl acrylate were purchased from Alfa-Aesar (Karlsruhe, Germany).

Acetyl chloride (AcCl) and dichloromethane (DCM) were from Merck Chemicals (Hohenbrunn, Germany). All other solvents used were of analytical grade and were procured from Merck Chemicals. Merck precoated Silica-gel $60F_{254}$ plates were used for thin layer chromatography. ddI [chromatographic purity (HPLC) = 99.4%] was purchased from Ruland Chemistry Co., Ltd (Nanjing, China). Disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl) and hydroxypropyl methyl cellulose (HPMC) were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were of analytical grade. Milli-Q purified water was obtained from the purification system (Millipore Corp., Billerica, MA) in our laboratories. Pigs (40–60 kg) were supplied by the Biomedical Resource Unit (BRU), UKZN (South Africa).

Methods

Synthesis and characterization of OA derivatives

Synthesis. The synthetic scheme in Figure 1 shows the reaction sequences involved in the synthesis of the different, three OA derivatives used in this study[37,46] i.e. ester derivative, oleodendrimer E1E (OA1E), the OA1A and the OA1ANa. The derivatives, i.e. OA1E, OA1A and OA1ANa as well as their molecular mass, formulae and chemical structures are presented in Table 1.

Synthesis of 3-N, N-di-(tert-butyloxycarbonylethyl)aminopropanol 2. Compound 2 was synthesized following the literature

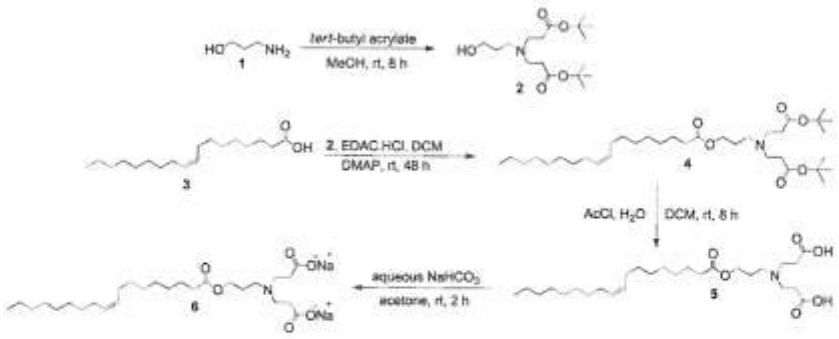

Figure 1. Scheme showing the reaction sequences involved in the synthesis of different OA derivatives.

Table 1. Molecular mass, molecular formula and chemical structure of ddI and OA, OA1A, OA1E and OA1ANa.

	Molecular mass (g mol^{-1})	Molecular formula	Chemical structure
ddI	236.23	$C_{10}H_{12}N_4O_3$	
OA	282.46	$C_{18}H_{34}O_2$	
OA1A	483.36	$C_{23}H_{45}NO_6$	
OA1ANa	527.32	$C_{23}H_{43}NNa_2O_6$	
OA1E	595.48	$C_{35}H_{65}NO_6$	

procedure. Briefly, to a solution of *tert*-butyl acrylate (19.2 g, 1.5 mol) in MeOH (200 ml), 3-amino-1-propanol **1** (3.75 g, 0.5 mol) in MeOH (100 ml) was added drop wise maintaining the reaction temperature below 30 °C. The reaction mixture was allowed to stand overnight after stirring at room temperature for 8 h. The solvent (MeOH) and excess *tert*-butyl acrylate were removed under vacuum to afford compound **2** as a colorless liquid (16.4 g, 99%).

Synthesis of oleodendrimer EIE (OA1E) 4. A mixture of compound **2** (2.84 g, 0.90 mol) and DMAP (0.68 g, 0.56 mol) in DCM (25 ml) was stirred at room temperature for 10 min.

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To this mixture, EDAC.HCl (2 g, 1.12 mol) was added followed by a mixture of OA (2 g, 0.75 mol) in DCM (25 ml). The formed clear solution was stirred at room temperature for 48 h. Solvent was evaporated under vacuo, and crude product obtained was purified by column chromatography using silica gel # 60–100 mesh with hexane/EtOAc, 9:1 as an eluent to obtain compound **4** as a colorless liquid (3.84 g, 91%).

OA1A 5 and its sodium salt (OA1ANa) 6. Literature-reported procedures[37] with slight modifications were followed for the synthesis of compound **5** and **6**. In short, to a mixture of compound **4** (5.95 g, 0.01 mol), H_2O (0.72 g, 0.04 mol) in DCM (100 ml), AcCl (3.14 g, 0.04 mol) was added drop wise over a period of 10 min and stirred for 8 h. The reaction mixture was washed with brine water and concentrated *in vacuo* after drying over anhydrous Na_2SO_4 to afford compound **5** as a viscous liquid (3.98 g, 82%). This compound **5** was utilized, without further purification, for the preparation of compound **6**. In short, a solution of **5** (5 g, 0.01 mol) in acetone (200 ml) was neutralized with 20% hot aqueous $NaHCO_3$ solution (8.35 ml, 0.019 mol) under vigorous stirring for 2 h. The precipitated white solid was further dried by removal of solvent under vacuum to afford compound **6** as an off-white solid (5.30 g, 97%).

Characterization of derivatives. The synthesized derivatives were characterized by standard analytical techniques for structural confirmation. FT-IR spectra were recorded using a Bruker Alpha spectrophotometer (Ettlingen, Germany). ^{1}H NMR and ^{13}C NMR were recorded using a Bruker NMR instrument operating at 400 and 100 MHz, respectively. HLB and log $P_{octanol/water}$ values of the OA derivatives were calculated using ChemSW® software (ChemSW Inc., Version 6.33, Fairfield, CA). The chemical structures, molecular formulae and mass of the derivatives are listed in Table 1.

Buccal permeation studies

Formulation and preparation of gels for permeation studies. To determine the effect of OA and its derivatives, simple gels containing ddI (2% w/w), HPMC (4% w/w), OA and its derivatives (OA1A, OA1E and OA1ANa) were prepared using 1% w/w of either OA or each of the derivatives. The compositions of the prepared gel formulations, in the absence of the enhancers and in the presence of either OA or its derivatives are described. Briefly, HPMC (4% w/w) was weighed and mixed in a beaker with sufficient quantity of purified water. Furthermore, ddI (2% w/w), weighed was separately mixed with a small quantity of purified water and added to the HPMC. The gel formulation was then made up to weight with further purified water and then stirred on a magnetic stirrer until all dissolved and was coded as formulation F1. Each enhancer, i.e. OA, OA1A, OA1E and OA1ANa was separately weighed and added to formulation F1 at a concentration of 1% w/w to form the drug/enhancer gels and were coded as formulations F2, F3, F4 and F5, respectively. For the concentration effects experiments, the compositions of the prepared formulations are described. Briefly, ddI gel in the absence of the enhancers (i.e. OA and its derivatives) was prepared as per formulation F1. OA at varying concentrations of 0.5, 1.0, 2.0, 4.0, 6.0% w/w were weighed, and separately incorporated with a sufficient quantity of formulation F1 to make the required formulations F6, F7, F8, F9 and F10, respectively. Similarly, OA1ANa in varying concentrations of 0.5, 1.0, 2.0, 4.0, 6.0% w/w was each added to a sufficient quantity of formulation F1 to make formulations F11, F12, F13, F14 and F15, respectively. Each prepared formulation was stored in an airtight amber container.

Preparation of porcine buccal mucosae. Porcine buccal mucosa has many similarities to the human buccal mucosa and was chosen as the biological membrane for the permeation experiments[47–49]. Pigs were sacrificed using the standard operating procedure at BRU, UKZN. Briefly, the pig was weighted and appropriate drugs were mixed to form a cocktail for intramuscular injection by the registered, licensed veterinary doctor at BRU, UKZN. The cocktail contained the following drugs, i.e. Domitor® (Zoetis, NSW, Australia), which contains medetomidine 1 mg/ml and dosed at 0.06 mg/kg; Zoletil® 100 (Virbac, Jalisco, Mexico), which contains a mixture of tiletamine 50 mg/ml; zolazepam 50 mg/ml and dosed at 1.5 mg/kg, and finally, butorphanol 10 mg/ml, (V-Tech Dispensing Pharmacy, South Africa) dosed at 0.17 mg/kg. The appropriate measured doses per kilogram body weight of these drugs were each placed in a sterile vial and subsequently mixed together and then drawn up into a single 5 ml syringe. The pig was restrained by placing it in a squeeze cage, and the cocktail was injected into the quadriceps muscle. The ear vein became easily accessible within 5–7 min such that the pig was at a stage of sedation and free of pain. The pig was then euthanized with Eutha-Naze® (Bayer, Isando, South Africa), containing sodium pentobarbitone 200 mg/ml, dosed at 1 ml/kg and administered intravenously at a rapid rate. Generally, a state of death was achieved with about 10–15 ml of Eutha-Naze. Buccal mucosae, harvested from euthanized pigs, were appropriately excised and prepared for the permeation experiments. The thickness of the buccal mucosa was 665 ± 72 µm (CV = 8.3%). For buccal permeability studies, the buccal mucosae were wrapped in foil, snap-frozen in liquid nitrogen and then stored in a bio freezer (–85 °C) until further use within three months according to previous reports[50,51].

In vitro *permeation.* Frozen buccal mucosae were allowed to thaw and equilibrated in phosphate buffer saline (PBS) pH 7.4. Franz diffusion cells (PermeGear, Inc., Bethlehem, PA) with a diffusional area of 0.786 cm^2 were used for permeation experiments. The buccal mucosa was mounted to the diffusional area between the donor and receptor cells and was equilibrated with PBS at 37 °C. Initially, just the ddI gel, in the absence of any enhancer (formulation F1) and presence of OA and the oleodendrimer derivatives, i.e. OA1A, OA1E and OA1ANa, formulated at only one gel concentration as formulations F2, F3, F4 and F5, respectively, were employed in the permeation experiments. Briefly, the donor compartment contained either ddI (2% w/w)/HPMC (4% w/w) gel alone or in the presence of 1% w/w of either OA or its oleodendrimer derivatives (OA1A, OA1E and OA1ANa). In the subsequent experiments, i.e. the concentration effect studies, ddI gel in the presence of either OA or OA1ANa, at varying concentrations, 0.5, 1.0, 2.0, 4.0 and 6.0% w/w, was placed in the donor compartment. The receptor compartments were filled with PBS. Samples were removed from the receptor compartments at predetermined time intervals and replaced with the same volume of PBS (drug-free). Each experiment was undertaken using a minimum of three replicates. ddI was quantified by a validated UV Spectrophotometry method at a λ_{max} of 250 nm (UV Spectrophotometer 1650, Shimadzu, Kyoto, Japan) as employed by previous buccal permeation studies with ARVs such as ddI[45] and ddC[6,9]; as well as other drugs including ondansetron[51], galantamine[52] and carbamazepine[53].

Permeability data analysis. The cumulative amount of ddI permeated per unit surface area versus time was plotted. The steady state flux (J_{ss}) across the mucosal membrane was determined from the linear part of the permeation graph by linear regression analysis (Microsoft Excel®, Microsoft Office

2010, Inc. Redmond, WA). The permeability coefficient (P) was calculated using the following equation[8]:

$$P = \frac{\frac{dQ}{dt}}{A} \times C_d = J_{SS} - C_d \quad (1)$$

where dQ/dt is the cumulative amount (Q) of ddI permeated per unit time (t), A is the active, cross-sectional diffusion area and C_d is the drug concentration in the donor compartment. The effects of OA, OA1A, OA1E and OA1ANa and the various concentrations of OA and OA1ANa on the permeability of ddI were evaluated. The enhancement ratio (ER) was calculated using the following equation[8]:

$$ER = \frac{P(Enhancer)}{P(No\ enhancer)} \quad (2)$$

Morphological evaluations using light microscopy and transmission electron microscopy. Histological evaluations were performed on freshly harvested, excised buccal mucosa. Untreated buccal mucosa was transferred directly after excision from normal saline into 10% buffered formalin without any equilibration in PBS and served as the control. Treated samples of the buccal mucosae comprised of those that were exposed to PBS only, or ddI gel formulation with and without OA1ANa at varying concentrations of 0.5, 2.0 and 6.0% w/w. Permeation experiments were performed as described in previous studies, without drug quantification[25,43,51]. At the end of the permeation experiments, the buccal mucosa was cut into cross sections. For light microscopy (LM), the samples were fixed in 10% buffered formalin for seven days, washed in water, dehydrated using an ethanol gradient and embedded in paraffin wax using previously described standard procedures[20,41,50]. The sections were collected on slides, dried and stained with hematoxylin and eosin (H&E). Sections (in 1 µm thick slices) were examined using a light microscope (Nikon 80i, Kanagawa, Japan), and bright field images were captured using NIS Elements D software (Nikon Instruments Inc., Melville, NY) and a camera (Nikon U2).

The samples for transmission electron microscopy (TEM) were obtained after the permeation experiments described above. They were cut into pieces not exceeding 0.5 mm^3, and fixed for 24 h (4 °C) using 4% glutaraldehyde fixative buffered to pH 7.2.[34] Samples were processed and embedded in epoxy resin using standard protocols. Ultrathin sections (90 nm) were cut and contrasted with uranyl acetate and lead citrate and viewed with a transmission electron microscope (JEOL 1010, Tokyo, Japan). All experiments were performed using a minimum of three replicates.

Statistical analysis

The results, expressed as mean ± standard deviation in values, were analyzed using one-way analysis of variance and followed by a Tukey's multiple comparison test using GraphPad Prism® (Version 5, Graph Pad Software Inc., La Jolla, CA). Differences were considered significant at $p < 0.05$.

Results and discussion

Synthesis and characterization of novel oleodendrimer derivatives

The OA derivatives, i.e. OA1E, the OA1A and the OA1ANa were successfully synthesized by a modified method that excluded harsh reaction conditions in keeping with the current trends of greener approaches to synthetic chemistry. The literature reported procedure for the synthesis of OA1E from which OA1A and OA1ANa were subsequently derived in this study[46] required a high reaction temperature and the use of thionyl chloride, a hazardous chemical. In this work, the synthetic methodology was successfully modified by the use of coupling agents, namely DMAP and EDAC.HCl. This synthetic modification enabled the avoidance of thionyl chloride to transform OA into oleoyl chloride synthesis at room temperature instead of 110 °C as well as a reduction in the number of steps involved in the synthesis of OA1E and the other OA derivatives.

The structures of the newly synthesized compounds by the modified method were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopic analysis and compared to the spectroscopic data for the OA derivatives synthesized by the original method from the literature[46]. The data below show that the compounds were identical to the compounds prepared by the previously reported method[77,48]. Therefore, the modified method in line with green chemistry approaches could be used to successfully prepare the OA derivatives.

3-N,N'-Di-(tert-butyloxycarbonylethyl)aminopropanol (compound 2)

^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 18 H), 1.72 (q, 2H), 2.42 (t, 4H), 2.64 (t, 2H), 2.77 (t, 4H), 3.75 (t, 2H).

OA1E (compound 4)

^{1}H NMR ($CDCl_3$) δ: 0.89 (t, 3H), 1.30 (m, 20H), 1.44 (s, 18H), 1.63 (q, 2H), 1.77 (m, 2H), 2.04 (m, 4H), 2.34 (m, 6H), 2.49 (t, 2H), 2.73 (m, 4H), 4.09 (t, 2H), 5.35 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.67, 24.98, 26.65, 27.20, 27.89, 28.09, 29.18, 29.31, 29.51, 29.70, 29.75, 31.89, 33.76, 34.34, 49.38, 50.13, 62.43, 80.31, 129.74, 129.97, 171.96, 173.85.

OA1A (compound 5)

^{1}H NMR ($CDCl_3$) δ: 0.89 (t, 3H), 1.30 (m, 20H), 1.45 (s, 18H), 1.64 (m, 4H), 1.77 (m, 2H), 2.01 (m, 6H), 2.35 (m, 6H), 2.95 (m, 4H), 3.32 (m, 4H), 4.16 (t, 2H), 5.34 (m, 2H), 6.25 (br s, 2H). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.67, 24.82, 27.15, 27.21, 29.06, 29.14, 29.31, 29.51, 29.58, 29.67, 29.76, 31.89, 33.99, 48.53, 50.68, 55.40, 60.93, 129.72, 130.02, 173.56, 179.50.

In vitro buccal permeation

Effects of OA, OA1E, OA1A and OA1ANa as permeation enhancers on the buccal permeability of ddI

In this study, the permeability enhancement potential of OA and its oleodendrimer derivatives, i.e. OA1A, OA1E and OA1ANa, as novel buccal permeation enhancers to enhance the buccal permeability of ddI is reported. The cumulative amounts of ddI permeated over six hours; its *in vitro* permeation parameters in the absence of any enhancer and in the presence of either OA or the novel oleodendrimer derivatives, i.e. OA1A, OA1E and OA1ANa, are shown in Figure 2 and Table 2, respectively. The flux of ddI in the absence of any of the enhancers was $46.57 \pm 10.15\ \mu g\ cm^{-2} h$. This flux was marginally increased to $60.08 \pm 10.34\ \mu g\ cm^{-2} h$ in the presence of OA, the parent compound with ER of 1.29, although the increase was not statistically significantly different from that of the control ($p = 0.0674$). The flux values for ddI in the presence of OA derivatives, i.e. OA1A, OA1E and OA1ANa, were $46.57 \pm 4.93\ \mu g\ cm^{-2} h$, $61.88 \pm 9.75\ \mu g\ cm^{-2} h$ and $80.30 \pm 10.37\ \mu g\ cm^{-2} h$, respectively, with ER values of 1.01, 1.33 and 1.72, respectively. Therefore, these OA derivatives were able to enhance permeation of ddI, with OA1E and OA1ANa specifically having higher ER values than the parent OA itself. OA1ANa with an ER of 1.72 had the highest permeation-enhancing effect as compared to OA, OA1A and OA1E.

Statistical analysis showed a highly significant difference ($p = 0.0004$) between the flux with OA1ANa when compared to the control (ddI without any enhancer). A highly significant difference was also observed between OA1ANa when compared to OA1A ($p < 0.0001$) as well as to OA ($p < 0.0001$). Moreover, there was an overall significant difference ($p = 0.0116$) between the permeability enhancing efficacy of OA1A and OA1E when compared with OA1ANa (Table 2). In comparisons to other studies, the permeability enhancements obtained in this study were consistent with data from the previously published literature sources with OA enhancers. The buccal mucosal permeability of fluorouracil in a gel formulation, incorporated with OA as an enhancer, was increased by 1–6-fold as compared to gels without enhancer[1,55,58]. However, lower permeability values have been reported where OA was used for drug permeability enhancement across the transdermal route[57,58]. In a recent study reported on OA's enhancing effect on DS, OA enhanced the transdermal permeation of DS (ER = 1.87)[36]. This is comparable to the ER obtained for OA's enhancing effect on ddI in this study (ER = 1.29).

Drugs generally can use either the paracellular or transcellular pathways or both for permeating the lipoidal membrane and enhancement across the buccal mucosa[59,60]. Hydrophilic drugs, in particular, can permeate the lipoidal membrane *via* the paracellular route. FAs as CPEs can improve the permeability of drugs *via* the paracellular pathway[60]. It has been postulated that OA enhances permeability of drugs across the membrane by disrupting the lipid structure of the membrane, causing solubilization by the formation of micelles to create aqueous channels. One other probable mechanism is that FAs cause extraction of the inter- and intra-cellular lipids and proteins of the membrane[31], thereby causing an increased fluidity in the membrane. Various reasons could be attributed to the observed permeation enhancement differences between the OA1ANa and the other two derivatives. With available literature findings on percutaneous and oro-mucosal permeation and the experimental observations in this study, a discussion to correlate the structural effects of OA derivatives on the permeation of ddI through porcine buccal mucosa is presented. The chemical structures, molecular mass, HLB, Log $P_{o/w}$ and ER_{flux} for the novel derivatives are shown in Tables 1 and 3. The basic structural difference in the three OA derivatives is that OA1E has a branched diester function, OA1A is a dicarboxylic acid and OA1ANa is a bicephalous diamionic surfactant. These structural differences of OA derivatives along with their physicochemical properties are taken into consideration to correlate structural effects on buccal permeability. Based on this, OA1E showed higher ER as compared to OA1A, which can be attributed to the higher lipophilicity of OA1E than OA1A[37]. The results correlate with previous findings where it has been observed that an increase in lipophilicity results in an increase in transdermal permeation of DS[36]. Furthermore, OA1E has a branched *tert*-butyl ester function at its periphery, which may have contributed to enhanced ddI permeation since the branched diesters can provide better permeation enhancement[61]. OA1ANa showed the highest ER amongst all the three derivatives although less lipophilic. Its higher ER as compared to the other two derivatives may be due to a combination of effects. It may be due to its amphiphilic nature and surfactant characteristics, which O1AE and OA1A lack. Like other surfactants such as sodium lauryl sulfate, OA1ANa might have showed effects like disorganization of the entire membrane architecture due to the extraction of the inter- and intra-cellular lipids and proteins of the membrane, the expansion of intercellular spaces and the insertion of OA1ANa molecules into the lipid structure[62], which facilitated better permeation of the drug through the lipid bilayer. These results are in good agreement with previous studies where oral mucosal absorption of lidocaine significantly increased in the presence of a surfactant derived from FA[63]. In addition to the disruption of the lipid bilayer by the FAs, the sodium derivative could have an added advantage of ionic interactions between the free Na^+ ions and the negatively charged sialic acid residues of mucin on the mucosae that may have further altered the membrane permeability[64]. This is proposed as an additional mechanism since in contrast, OA1A and OA1E are acidic and ester compounds, respectively, and lacked the Na^+ ions that may have caused the ionic interaction between sialic acid and the enhancer as in OA1ANa. Furthermore, similar interactions have

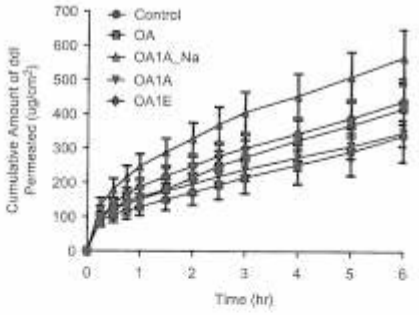

Figure 2. The effect of OA, OA1E, OA1A and OA1ANa on the cumulative amount of ddI permeated across the buccal mucosae.

Table 3. Log $P_{octanol/water}$, HLB and ER_{flux} of the OA derivatives, i.e. OA1E, OA1A and OA1ANa.

Derivative	HLB	Log $P_{octanol/water}$	ER_{flux}
OA1E	4.79	9.40	1.33
OA1A	7.41	6.00	1.01
OA1ANa	36.2	−2.96	1.72

Table 2. Permeability parameters of the OA, OA1A, OA1E and OA1ANa as novel buccal permeation enhancers for ddI (Mean ± SD; $n \geq 3$).

	Treatment	Amount permeated ($\mu g\,cm^{-2}$)	Flux, J_{ss} ($\mu g\,cm^{-2}\,h$)	Permeability, $P \times 10^{-3}$ ($cm\,h^{-1}$)	Enhancement ratio (ER)	p Value for flux, J_{ss}
Control	Didanosine	338.07 ± 76.6565	46.57 ± 10.15[a]	2.33 ± 0.56	–	–
Enhancer 1	OA	418.86 ± 77.31	60.08 ± 10.34[b]	3.00 ± 0.57	1.29	0.0674
Enhancer 2	OA1A	345.98 ± 57.96	46.57 ± 4.93[b]	2.34 ± 0.27	1.01	0.9747
Enhancer 3	OA1ANa	567.14 ± 80.46	80.30 ± 10.37[c]	4.01 ± 0.57	1.72	0.0004***
Enhancer 4	OA1E	438.56 ± 66.99	61.88 ± 9.75[b]	3.09 ± 0.53	1.33	0.0361*

"a versus b" demonstrates statistically non-significant difference ($p > 0.05$), in the flux values of the enhancers compared to the control.
"a versus c" demonstrates statistically significant difference ($p < 0.05$), in the flux values of the enhancers compared to the control.

been reported in previous studies where interaction of the enhancer with the sialic acid residues has been proposed as a mechanism of permeation enhancement, i.e. a cationic charged molecule with the mucosal layer[3,64].

The enhanced transbuccal permeation of ddI in the presence of OA1ANa is therefore presumably attributed to the disruption of the lipid bilayer as well as the ionic interaction between the sodium ions and sialic acid of the mucosal lining.

It has been reported that the log p values and HLB number can play an important role in differentiating ERs, flux and permeability coefficient values[28]. Moreover, it has been shown that the ester derivatives have better enhancement potential than the acid derivatives[16]. HLB and hydrophilicity order of OA derivatives was OA1ANa > OA1A > OA1E (Table 3). Significant increase ($p < 0.05$) in ER_{flux} with OA1ANa ($ER_{flux} = 1.72$) was observed than with OA1E ($ER_{flux} = 1.33$). From this observation, it can be concluded that an increase in HLB value and hydrophilicity of OA derivative, due to conversion into a surfactant molecule, resulted in increased ER_{flux} of ddI with OA1ANa. However, ER_{flux} value with OA1A was lower than with OA1E though the HLB and hydrophilicity of OA1A was more than OA1E. These contrasting results may be due to the fact that OA1E and OA1A as lipidic structures act by the same mechanism of action like other FA derivatives[61] and OA1ANa as a surfactant also acts by a mechanism of action similar to other surfactant like molecules[63]. It should be noted that the difference in ER_{flux} of OA1A and OA1E was not significant ($p > 0.05$).

Another observation is that there was no lag time in the permeation profiles obtained for the enhancing effect of OA and its derivatives on ddI permeability (Figure 2). By nature of the permeation-enhancing effect of the enhancers, the cell layers would have been disrupted, hence reducing and/or removing any lag time that the drug might experience. Furthermore, as ddI is hydrophilic, permeation may be *via* the paracellular pathway thereby further reducing the lag time despite the thickness of the mucosae. Similar lack of lag times during buccal permeation studies on various drugs including ddI, lidocaine and tenofovir, using porcine buccal mucosa has been reported previously[24,43,44,63].

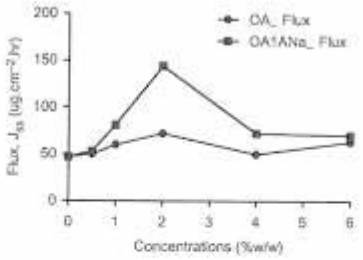

Figure 3. Concentration effects of OA and OA1ANa on the flux of ddI.

In this study, it has therefore been shown that OA and its novel derivatives, i.e. OA1E, OA1A and OA1ANa, to a different extent enhanced the buccal permeability of ddI. The oleodendrimer derivative OA1ANa further had the highest enhancement factor for ddI as compared to the parent OA and other derivatives.

Effects of varying concentrations of OA and OA1ANa on the buccal permeability of ddI

The above study identified OA1ANa as having the highest permeation-enhancing effect. The effects of varying concentrations of the OA1ANa derivative was then studied and compared to OA (Figure 3 and Table 4). This study also identified the concentration range at which it will be effective and potentially safe for buccal permeation enhancement of ddI. The permeation-enhancing potential of OA1ANa, as shown by the permeability properties of ddI, increased significantly ($p = 0.0014$) from 0.5% w/w (ER = 1.14) to 2% w/w (ER = 3.09), but decreased at 4% w/w (ER = 1.55) and at 6% w/w (ER = 1.50). Similar trends in the permeability properties of ddI in the presence of OA1ANa were also observed for OA at varying concentrations (Figure 3 and Table 4). Increase in OA1ANa concentrations from 0.5 to 2% w/w led to increasing ER values but further increases at 4 and 6% w/w led to subsequent decreases in ER values and they did not show any further enhancement in the ddI flux; and this was similarly observed for OA. Interestingly, the highest flux of ddI was obtained for both OA and OA1ANa at the same concentration of 2% w/w.

The results obtained in this study are similar to previous studies where increasing the concentration of the enhancers increased the buccal permeability enhancement initially, but further increases in concentration led to decreased permeability[31,43,57]. Reasons for the trend observed could be due to disruption of the lipid bilayer of the buccal mucosa occurring at the 2% w/w concentration to allow for optimum permeation, whilst increased concentrations to 4 and 6% w/w could have increased the viscosity at the mucosal layer, resulting in low drug movement[65]. Higher concentrations may have also decreased partitioning of ddI from the gel. Previous studies that used OA as a permeation enhancer have reported that the flux of piroxicam in the presence of OA increased from 0.3% w/w to 1% w/w but decreased at 3% w/w[57]. The reason attributed to this was that at a 5% w/w OA concentration, the presence of a large amount of the FA could have slowed down the partitioning of the drug out of the gel base, thereby reducing the permeability rate of piroxicam[57]. The maximum ER value for OA1ANa obtained (ER = 3.09) is similar to and in some cases higher than other reported CPEs stated as being promising enhancers for buccal permeation, e.g. 3.2 for propylene glycol and dodecyl-2-dimethylamino propionate HCl with ondasetron[25] and 1.8 for sodium dodecyl sulfate with caffeine[66]. It has been previously shown that the novel derivative,

Table 4. Effects of concentrations of OA and OA1ANa on the permeability parameters of ddI (mean ± SD; $n \geq 3$).

	Cum amt permeated, Q_{6h} (μg cm^{-2})		Flux, J_{ss} (μg cm^{-2} h)		Enhancement ratio (ER)	
Control	338.07 ± 76.66		46.57 ± 10.16 ± 10.15		–	
Concentrations [%w/w]	OA	OA1ANa	OA	OA1ANa	OA	OA1ANa
[0.5]	381 ± 63	392 ± 55	49.19 ± 5.90	52.78 ± 6.86	1.05	1.14
[1]	419 ± 77	567 ± 80	60.08 ± 11.51	80.50 ± 11.33	1.29	1.72
[2]	496 ± 93	970 ± 289	71.80 ± 12.03	144.00 ± 53.54	1.54	3.09
[4]	400 ± 105	508 ± 126	49.85 ± 11.50	72.25 ± 16.34	1.07	1.55
[6]	404 ± 36	440 ± 106	62.57 ± 6.38	69.91 ± 18.26	1.35	1.50

OA1E, used in this study could enhance the transdermal permeation of drugs[36]. The ER of the OA1ANa derivative compares with the oleodendrimers reported in that study. The ER ranged from 2.02 to 2.58 for oleodendrimers with the amide linkage. Interestingly, these ER values for transdermal permeation (2.02–2.58) are lower than the ER values obtained for OA1ANa (ER = 3.09). This can be due to the higher permeability of the buccal mucosa than that of the skin; since the skin unlike the buccal mucosa is keratinized and the application site in the skin layers could be thicker and greater thus posing more challenges for drug permeability[7]. In addition, possible interaction of the Na^+ ions with negatively charged sialic acid residues and the mucin molecules may have further led to the higher ER value for buccal permeation.

This study has identified for the first time a novel derivative of OA, OA1ANa, as an effective buccal permeation enhancer, thereby adding to the pool of CPEs for buccal delivery of ddI and other drugs.

LM and TEM for the histomorphological evaluations of the mucosa

Permeation enhancers can play a role in the improvement of the permeability of drugs; however, their suitability needs to be established as they may have membrane damaging effects[8,67]. Buccal delivery involves retention of the drug delivery system on the buccal mucosal site for diffusion to occur across the mucosa. Hence, the basal membrane of the mucosa must remain intact to ensure an effective diffusion of the drug. LM and TEM have been used in the literature to assess integrity of and histomorphological changes on the buccal mucosae after drug permeation studies[43,44,68].

In this study, the effects of OA1ANa, being the buccal permeation enhancer amongst the three OA derivatives studied with the highest permeability coefficient and flux values, were investigated using LM and TEM. It must be noted that the barrier function of the stratified epithelium lends itself to a "rebound" effect after prolonged exposure to a drug, which was not assessed in this study. However, adequate information can be obtained from LM and TEM images to determine whether the tissue suffered permanent/irreversible damage after exposure to the drug and enhancer treatment[64,68].

The morphology of the porcine buccal mucosa has been described previously, and its characteristics resemble that of the human buccal epithelium[49,69]. The LM- and TEM-treated sections were compared to the controls. In LM investigations, the untreated mucosae/controls resembled that of a normal non-keratinized, stratified squamous layer, and the basal cells appeared oval and darkly stained in H&E (Figure 4A). Hence, the cells observed in the control sections could be regarded as healthy cells. The cells observed in the ddI gel-treated mucosae (Figure 4B) resembles those seen in the control, therefore confirming that ddI gel and the excipients in the formulation did not adversely affect the mucosa.

The cells of the mucosa treated with ddI+ OA1ANa 0.5% w/w (Figure 4C) closely resemble that of the control (Figure 4A) and the ddI gel-treated mucosae (Figure 4B). This shows that no adverse effect was displayed in the cells of the mucosa treated with OA1ANa 0.5% w/w. Moreover, the appearance of the control, ddI and ddI+ OA1ANa 0.5% w/w mucosal sections

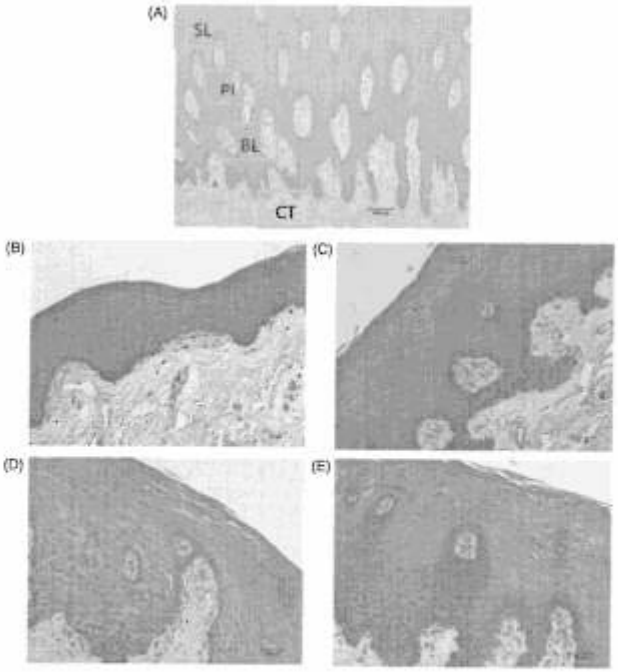

Figure 4. Photomicrographs of the control (untreated) and treated buccal mucosal sections stained with H&E (LM): (A) control/untreated (SL: superficial layer; PL: prickle layer; BL: basal layer; and CT: connective tissue); (B) ddI gel (in the absence of any enhancers); (C) ddI + 0.5% w/w OA1ANa; (D) ddI + 2% w/w OA1ANa and (E) ddI + 6% w/w OA1ANa.

were all similar suggesting that no adverse influence of ddI or OA1ANa at 0.5% w/w in the tissue morphology was evident. The outermost layer of the stratified squamous epithelium, basement membrane, lamina propria and submucosa were all intact in both the treated and untreated mucosae (Figures 4A–E). Therefore, OA1ANa 0.5% w/w had no adverse effect on the buccal mucosae. There were no morphological changes observed in the mucosae treated with ddI alone (Figure 4B) and those treated with ddI + OA1ANa 0.5% w/w (Figure 4C). The superficial, prickle and basal cells all remained intact, and no loss of the superficial cell layers and no formation of vacuoles in both the prickle and basal cells were observed.

However, the neatly aligned cells observed in Figure 4(A and B), which are the control and ddI gel-treated mucosae, respectively, appeared to be interspaced in Figures 4C–E, which are ddI + OA1ANa 0.5% w/w, ddI + OA1ANa 2% w/w and ddI + OA1ANa 6% w/w treated mucosae, respectively. The increased intercellular spaces (Figures 4C–E) could be attributed to the permeation-enhancing effect of OA1ANa. Furthermore, the intercellular spaces between the epithelial cells increased with an increase in the concentration of OA1ANa from 0.5% w/w to 2% w/w and further increased with ddI + OA1ANa 6% w/w (Figures 4C–E). Moreover, swellings and vacuoles formation were observed in the prickle layers of the enhancer-treated mucosae compared to the control and drug-treated mucosae. When compared to the ddI + OA1ANa 0.5% w/w treated mucosae, increased formation of vacuoles, swelling and increased intercellular spaces in both the basal and prickle cell layers were observed in the ddI + OA1ANa 2 and 6% w/w treated mucosae (Figures 4D–E). The progressive morphological changes observed in treated cells could therefore explain the increased permeation of ddI observed in this study with OA1ANa at these concentrations (Figure 3). Increase in swelling and vacuole formation may also be attributable to accumulation of both drug and enhancer in the mucosa cells without the loss of the superficial layer. Increasing permeation was observed from ddI + OA1ANa 0.5% w/w to ddI + OA1ANa 2% w/w, which correlates with the increased intercellular spaces, formation of vacuoles and swelling observed at these two concentrations.

There were clear distinctions between the cells treated with ddI + OA1ANa 2% w/w (Figure 4D) and with ddI + OA1ANa 6% w/w (Figure 4F). The cells treated with 6% w/w were not as darkly stained as in the control, 0.5% w/w and 2% w/w treated cells. They showed probable reduced mitotic activity in both the prickle and basal layers, which may prevent diffusion of the drug across the basal cuboidal cell layer. The partitioning of drug across the biological membrane is possibly due to the mitotic activity in the mucosal cells. Thus, if a disruption is shown in the cells, such that cell organelles are disorganized, there could be a reduced mitotic activity and hence reduced partitioning of drugs[70]. Evidence of disruption to the cell organelles could indicate lack of partitioning of drugs into the membrane, hence lack of drug transport by diffusion across the membrane. This slight reduction in the mitotic activity observed in the basal cell layers could have led to the reduced permeability observed at the higher concentration (ddI + OA1ANa 6% w/w). With ddI + OA1ANa 6% w/w treated mucosae, the basal cells were additionally not totally oval in shape, showing some distortions in the membrane. However, any damage to the mucosa is considered non-permanent as the mucosa can regenerate from its damaged status by renewed growth of the mucosa from the germinal layer[71]. The results of this study correlated with trends observed in the concentration effect of aloe vera gel on the enhancing properties of ddI that was reported previously[41].

In TEM investigations, an in-depth ultrastructural analysis of the cellular organelles of the buccal mucosa sections is made possible. TEM was therefore undertaken to confirm the effect of the novel derivative OA1ANa on the cellular organelles of the buccal mucosa that was observed at the LM level and reported above. The ultrastructure of the untreated buccal mucosa (control) showed regular nuclear profiles with closely packed cellular walls and well-arranged desmosomes at the gap junctions. The nuclear outlines appeared regular in the control buccal mucosal section (Figure 5A).

Both the ddI and OA1ANa 0.5% w/w treated mucosae resembled the control section by way of their regular nuclear outlines and absence of cellular distortions. The mitochondria appeared dense in both the ddI gel treated mucosa (Figures Bi and ii) and the OA1ANa 0.5% w/w treated mucosae (Figure Cii), although they showed slight electron translucent clearings within the mitochondria. An increase in the intercellular spaces was noted with ddI gel only (Figures 5B) as well as ddI + OA1ANa 0.5 and 2% w/w treated mucosae (Figures 5Ci and Di). The increased intercellular spaces observed could be attributed to the partitioning of the permeant (ddI) as well as the enhancing effect of OA1ANa.

A permeant can use either the paracellular, transcellular or both pathways mechanisms for permeability enhancement[7,69]. The increase in the intercellular spaces did not damage the gap junctions as evident by the presence of the desmosomes. Interestingly, the desmosomes appeared intact in the ddI + OA1ANa 2% w/w treated mucosa (Figure 5Dii). This may indicate that the highest permeability observed at this concentration level could be *via* the paracellular transport pathway. Surfactants can disrupt the lipid bilayer/the entire membrane architecture of the buccal mucosa, because an expansion in intercellular spaces can lead to an insertion of the OA1ANa molecules into the lipid structure[71], which can encourage the enhanced permeability of drugs. Though appearing slightly swollen, no evidence of damage to the desmosomes at the gap junction could be seen except for increased intercellular spaces. This can also be due to the enhancing effect of OA1ANa on the buccal permeability of ddI[17]. While the nuclear envelope appeared slightly irregular as observed in the ddI + OA1ANa 2% w/w treated mucosa, cellular damage was not evident. The nuclear membrane showed slight distortion in the outlines, which do not differ much from that of the ddI + OA1ANa 0.5% w/w treated mucosae. However, crenulation of the nuclear envelope was observed in the ddI + OA1ANa 6% w/w (Figure 5E).

In this study, LM confirmed that there was no adverse effect and no tissue damage as a result of the ddI gel when used alone and when in combination with OA1ANa 0.5 and 2% w/w except for the OA1ANa 6% w/w where adverse effects were observed. TEM revealed similar effects of OA1ANa on the mucosa as observed with the LM. Since the mucosa was not adversely affected at these concentrations, i.e. 0.5 and 2% w/w, OA1ANa could be used at these concentrations to enhance the permeability of ddI. In permeation studies, OA1ANa was identified as the enhancer with highest ER for ddI. Based on these results, OA1ANa at 2% w/w can be proposed as an enhancer in a delivery system since the exposure of OA1ANa concentrations up to 2% w/w did not show any adverse effects on the buccal mucosa and simultaneously displayed the highest permeability enhancement.

Conclusions

The derivatives of OA were successfully synthesized incorporating the use of a coupling agent, i.e. DMAP and EDAC.HCl, which

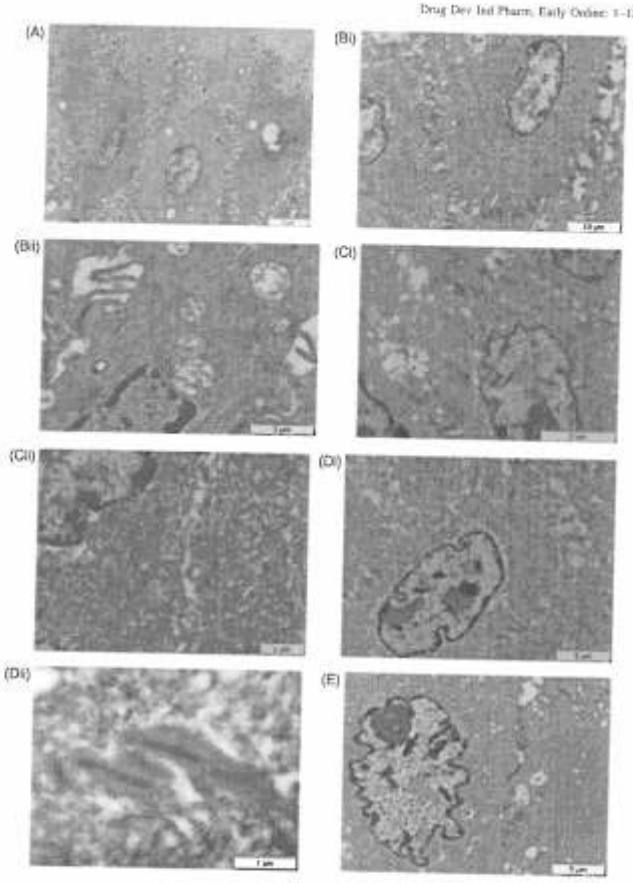

Figure 5. Electro-micrographs of the control (untreated) and treated ultra-thin buccal mucosal sections (TEM). (A) control/ untreated; (Bi and ii) ddI gel only (in the absence of any enhancers); (Ci and ii) ddI + 0.5% w/w OA1ANa; (Di and ii) ddI + 2% w/w OA1ANa; (E) ddI + 6% w/w OA1ANa.

eliminated the use of thionyl chloride and high reaction temperatures as well as reduced the number of synthetic steps. This study clearly demonstrated that OA and its novel derivatives, i.e. OA1E, OA1A and OA1ANa, could enhance the buccal permeability of ddI. All the novel derivatives of OA that were explored and reported in this study increased the buccal permeability of ddI, with the OA1ANa derivative having the best enhancing potential than other derivatives and its parent compound, OA. OA1ANa 2% w/w displayed the highest flux and permeability for ddI across the buccal mucosa with an ER of 3.09 more than that of the ddI alone. The permeability enhancing effects OA1ANa as a novel buccal permeation enhancer for ddI was shown to be concentration dependent. Interestingly, both OA and OA1ANa also showed a similar trend with flux values as their concentrations were increased. Maximum flux for both OA and OA1ANa were observed at 2% w/w with ER values of 1.54 and 3.09, respectively. The morphological changes reported in this study, i.e. vacuoles formation, increased intercellular spaces and swelling were attributed to, and correlated with the permeation-enhancing effect of the novel OA1ANa derivative. No adverse effects were observed in all treated and untreated mucosae in this study. The novel OA derivatives show potential for the enhancement of the buccal permeability of ddI and can widen the pool of CPEs for buccal delivery of various drugs for drug therapy optimization.

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Declaration of interest

The authors report no declaration of interest.

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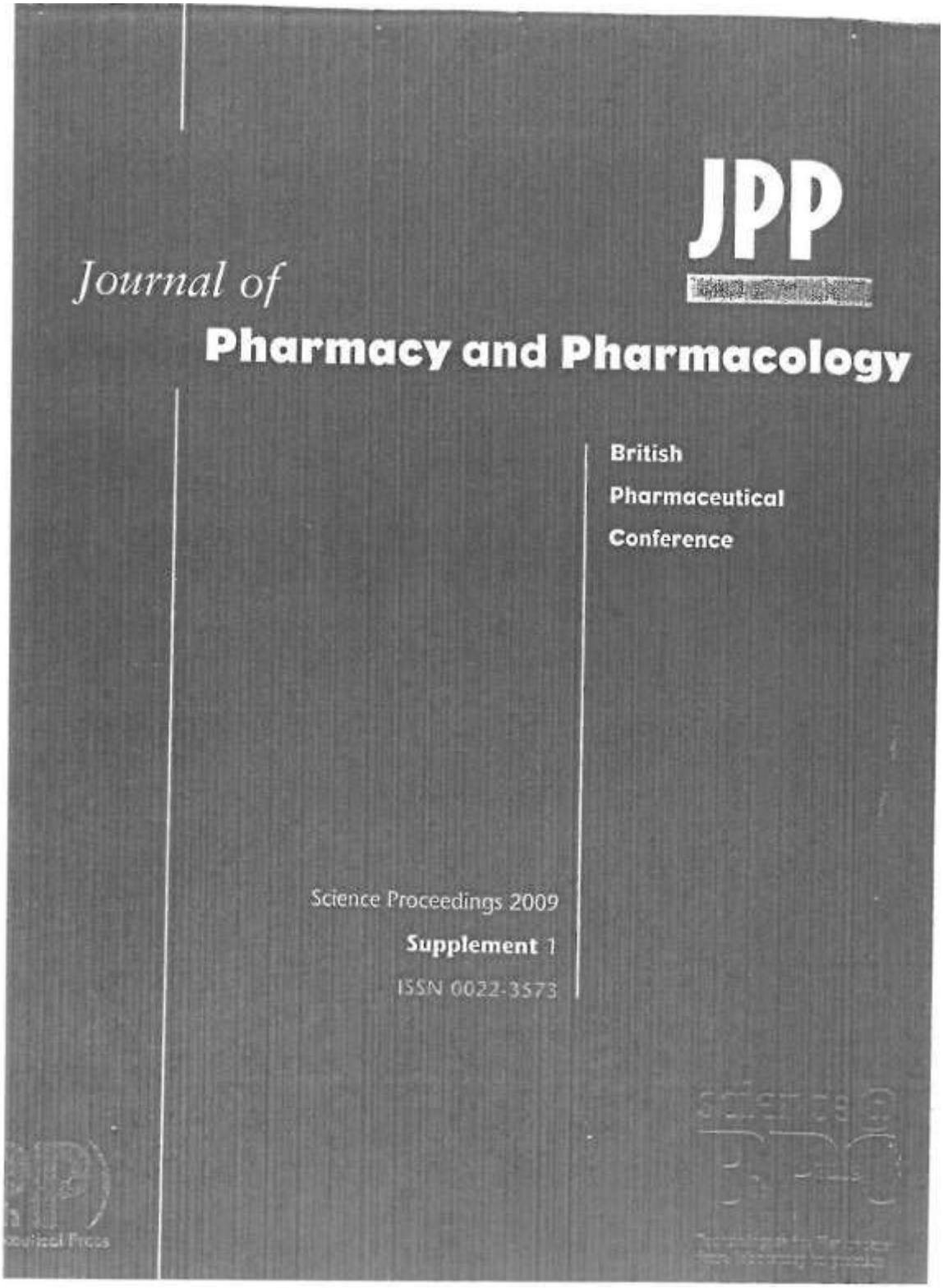
APPENDIX VI

PUBLISHED RESEARCH ABSTRACTS IN AN INTERNATIONAL ISI JOURNAL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Pharmaceutical
Conference
Science Proceedings 2009
Supplement 1
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Method

IVIVC is established by comparing the in-vitro dissolution curve with the drug input rate curve, which may be obtained by various methods of mass balance model techniques, such as Wagner–Nelson procedure (in case the absorption curve adjusts to a model of one compartment) and Loo–Riegelman method (in case the adjustment is significant for a model of two compartments), or by model-independent evaluation using pharmacokinetic parameters. The simplest way of demonstrating IVIVC is to plot the fraction absorbed *in vivo* versus the fraction released *in vitro*. The results of in-vitro and in-vivo drug release studies were correlated, and their regression coefficient was calculated.

Results

A correlation of results of in-vitro and in-vivo drug release studies was established; the correlation coefficient was found to be 0.985 and 0.949 for olanzapine and aripiprazole microspheres, respectively, and 0.949 and 0.947 for olanzapine and aripiprazole in-situ implant formulations, respectively. The above values confirmed a good correlation between in-vitro and in-vivo drug release data.

Conclusion

The results showed that there is a good correlation between in-vitro and in-vivo data. Therefore, it is strongly recommended by the authors that this method should be critically examined and validated and can be included in the regulatory guidelines for the prediction of in-vivo pharmacokinetic data from the in-vitro data of the depot formulation with different drug release pattern. This will help in minimising the in-vivo studies and will be helpful during the development of such kind of formulation.

Reference

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Drug Delivery

44
Exploring the effect of aloe vera gel on the buccal permeability of didanosine: permeability and histomorphological studies

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Introduction and Objectives

The aim of the study was to determine the effect of aloe vera gel (AVgel) on the buccal permeability of didanosine (ddI) and to assess its histomorphological effects on the buccal mucosa. Buccal permeability can be improved by the use of penetration enhancers; thus, effective and safe enhancers need to be identified. AVgel has been reported as a potential enhancer for intestinal[1] and skin[2] permeability; however, data on its buccal permeability are yet to be reported.

Methods

Ethical approval was obtained from University of KwaZulu-Natal (UKZN) Ethics Committee (Ref: 006/09/Animal). In-vitro permeation of ddI was studied using Franz diffusion cells and porcine buccal mucosa with phosphate-buffered saline (PBS) pH 7.4 at 37°C. Varying concentrations of AVgel from 0.25 to 6.0% w/v were investigated. ddI was quantified by ultraviolet (UV) spectrophotometric analysis. Flux values were calculated using linear regression analysis. Histological investigations were undertaken using light microscopy. Data were analysed using one-way analysis of variance (ANOVA) with Bonferroni post-hoc tests.

Results and Discussion

The amount of ddI permeated with an increase in AVgel is shown in Figure 1. The initial flux of ddI was 293 $\mu g/cm^2h$ and was increased significantly ($P < 0.001$) with an increase in AVgel concentrations from 0.25 to 2% w/v. However, the flux values decreased to 84 and 62 $\mu g/cm^2h$ with further increases in AVgel concentrations of 4.0 and 6.0% w/v, respectively. Similar trends with other enhancers have been reported.[3] No major differences were observed in the thickness of the epithelium, cell architecture and cellular alignment of the mucosa in both the control (normal saline) and the treated (PBS/ddI or ddI/PBS/AVgel) mucosae.

Conclusion

The permeability of ddI is dependent on the concentration of AVgel. AVgel in combination with ddI does not adversely affect the epithelium and basal lamina of the buccal mucosa. AVgel can be considered as a potential buccal permeation enhancer.

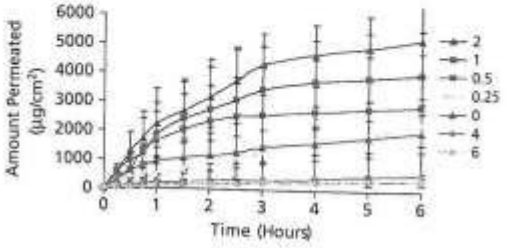

Figure 1 The effect of aloe vera gel concentrations on didanosine permeation.

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45
Preparation and evaluation of mucoadhesive polymeric films for buccal delivery of anti-HIV/AIDS drug (didanosine)

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Introduction and Objectives

Antiretroviral (ARV) drugs, such as didanosine (ddI), are available for the oral route of administration only. Its buccal administration may improve bioavailability by avoiding hepatic first-pass metabolism and gastrointestinal degradation. The incorporation of an ARV into a buccal delivery system has not been reported. This study aimed to prepare and evaluate ddI containing homopolymeric and monolayered multipolymeric films (MMFs) with polymers of similar and opposing solubilities for buccal delivery.

Method

ddI-loaded monopolymeric films with hydroxypropylmethylcellulose (HPMC) or Eudragit® RS 100 (Evonik Rohm GmbH, Darmstadt, Germany) were prepared in varying ratios using a silicone-moulded tray with individual wells. HPMC films were prepared by casting/solvent evaporation and EUD films by emulsification casting/solvent evaporation. MMFs comprising of ddI : HPMC : EUD in varying ratios were prepared by emulsification/casting/solvent evaporation. Films were characterised in terms of drug content (UV spectrophotometry) and drug release (shaking water bath). Film thickness was measured with an electronic digital micrometer and surface morphology assessed using scanning electron microscopy (SEM).

Results and Discussion

ddI : HPMC (1 : 0.5)-only films were homogenous and exhibited immediate release profiles. ddI : EUD (1 : 2.5)-only films were homogenous, elastic and flexible and showed controlled release profiles. The incorporation of ddI into MMFs with polymers and drug of opposing solubilities ddI : HPMC : EUD (1 : 0.5 : 2.5) resulted in homogenous, elastic and flexible films with immediate release profiles. Increasing the EUD concentrations led to controlled release profiles (Figure 1).

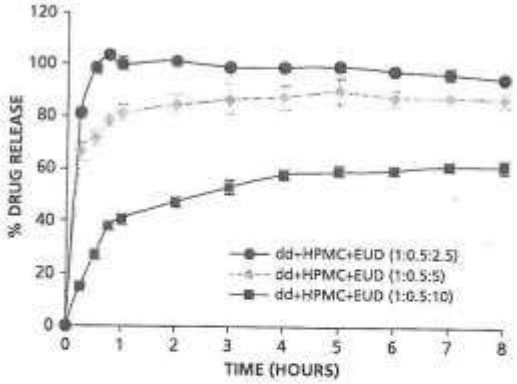

Figure 1 Effect of EUD on drug release profiles from MMFs.

Drug content, size, thickness and weight of the MMFs ddI : HPMC : EUD (1 : 0.5 : 2.5) were 97.65 ± 5.78%, 2 × 3 cm^2, 0.187 ± 0.023 mm and 108.65 ± 6.71 mg, respectively. SEM showed the films to have a smooth and homogenous compact surface before dissolution and changes in texture and pore formation after dissolution.

Conclusion

ddI can be incorporated into HPMC monopolymeric films for immediate ddI release applications. Homogeneous MMFs with drug and polymer (EUD) of opposing solubilities could also be prepared for controlled ddI release applications. MMFs with immediate and controlled ddI release profiles, with improved flexibility as compared with the monopolymeric films, can be prepared. The various films prepared in this study are potential candidates for optimisation of ddI films as a buccal delivery system.

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Specific swelling behaviour of bacterial cellulose composite as potential candidate for drug carriers

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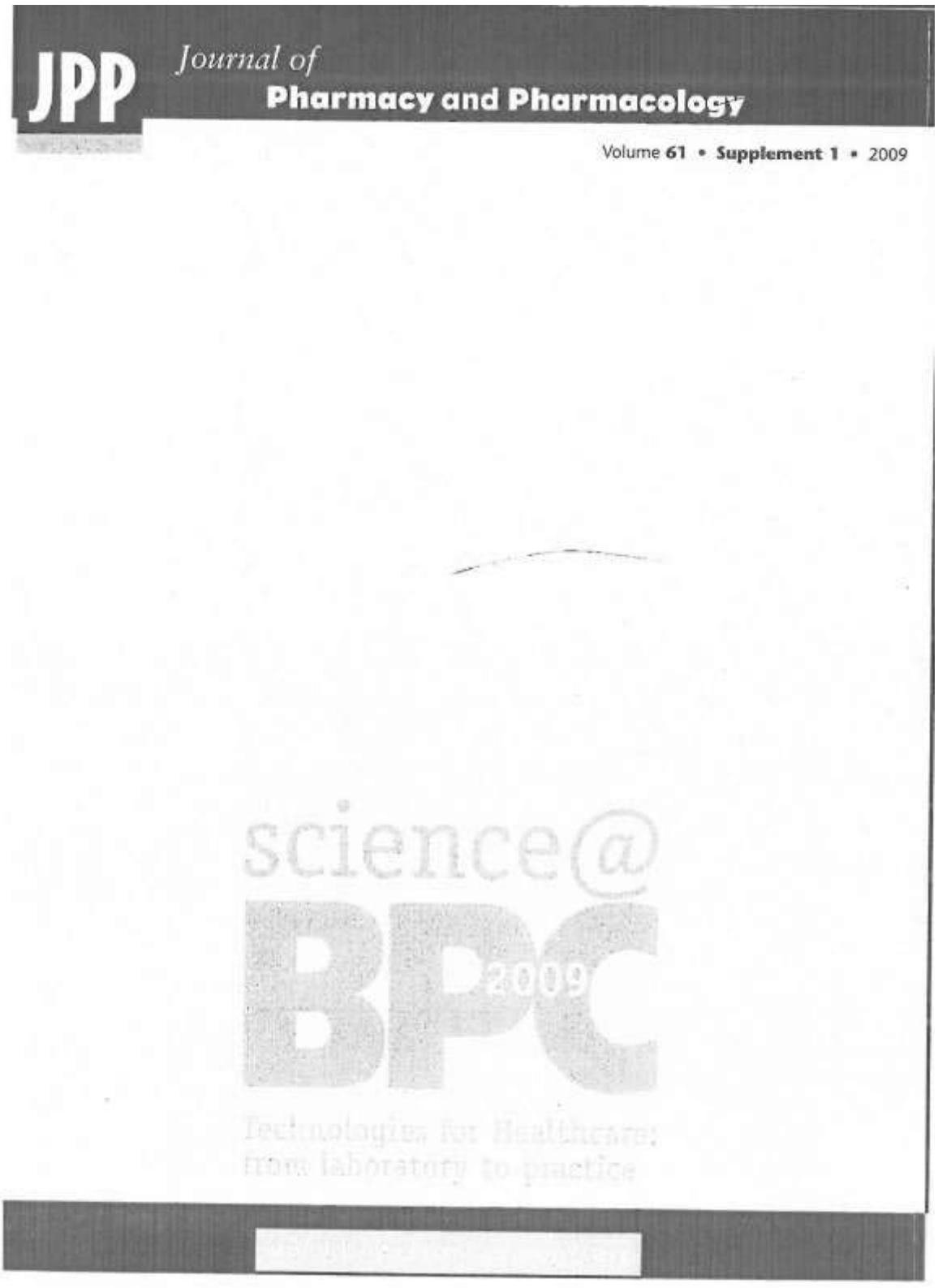
Introduction and Objectives

The aim of this study was to produce a biocompatible hydrogel using bacterial cellulose as a natural filler in the composite. Intelligent hydrogels are being synthesised and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Technologies for Healthcare:
from laboratory to practice

APPENDIX VII

CONFERENCE PRESENTATIONS

Comparing the buccal delivery potential of two antiretroviral drugs: Permeability and histological studies on didanosine and zalcitabine

Elizabeth B. Ojewole[1], Irene Mackraj[2], Kamil Akhundov[3] and Thirumala Govender[1]

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INTRODUCTION

- The buccal mucosal route presents several advantages such as the bypass of first pass hepatic metabolism and avoidance of gastrointestinal enzymatic degradation, thereby increasing the bioavailability of drugs, and higher permeability than that of the other routes such as skin.
- Disadvantages of antiretroviral (ARV) drugs include limitations such as low bioavailability due to extensive first pass metabolism and/or degradation in the gastrointestinal environment, which necessitates increased dosage and frequency of administration. ARV drugs also undergo severe dose-dependent adverse effects [1-3].
- ARV drugs, such as didanosine (ddI) and Zalcitabine (ddC) may therefore benefit from buccal instead of oral administration.

AIM

- To determine the buccal permeability properties of two model ARVs, i.e. didanosine (ddI) and zalcitabine (ddC) and to asses their histological effects on the mucosal tissue.

METHODS

Ethical Clearance for the study was obtained from University of KwaZulu-Natal Ethics Committee (001/08/Animal).

Permeability studies

In vitro permeation of ddI and ddC, each at 20 mg/mL was studied using porcine's buccal mucosa and Phosphate Buffer Saline, pH 7.4. The studies were undertaken using modified vertical Franz diffusion cells at 37 °C. ddC and ddI were quantified by UV Spectroscopy at 270 nm and 250 nm respectively.

Histological studies

Preliminary investigations were performed using Light Microscopy of the Haematoxylin & Eosin stained sections of the porcine's buccal mucosa exposed to drugs and relevant controls.

RESULTS AND DISCUSSION

PERMEABILITY STUDIES

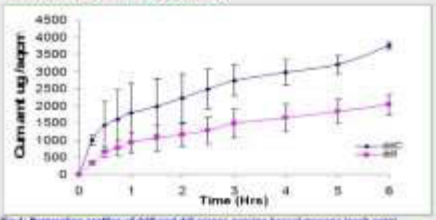

Fig 1: Permeation profiles of ddC and ddI across porcine buccal mucosa (each point represents the mean ±SD of 6 experiments).

Table 1: Permeation parameters of ddC and ddI. (Donor concentration was 20 mg/mL, data represent Mean ±SD, N = 6).

Drug	Cum Amt Permeated (µg/cm²)	R^2	Flux, J_{ss} (µg/cm².hr)	Permeability Coefficient P x 10^{-2} (cm/hr)
ddC	3736.84 (±112.17)	0.8695	490.88	2.4543
ddI	2028.78 (±301.82)	0.9015	293.91	1.4696

- Figure 1 shows that both ddC and ddI permeated the buccal mucosa. This was confirmed by previous studies on transbuccal delivery of ddC [4-6].
- Permeability coefficient, P and steady state flux, J_{ss} were calculated from a straight line obtained with figure 1.
- The permeability coefficient of ddC was higher than that of ddI (Table 1).
- The steady state flux for ddC was higher than for ddI. This could be attributed to the lower molecular weight, pKa and thus increased solubility of ddC that promote permeation across the mucosa.

HISTOLOGICAL STUDIES

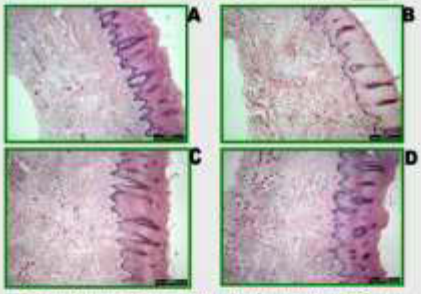

Fig 2 Microphotographs of porcine buccal mucosa: A: control untreated; B: sample subjected to diffusion in PBS; C: sample subjected to diffusion in ddC D: sample subjected to diffusion in ddI. Untreated mucosa was fixed in formalin straight from normal saline, while treated mucosae were incubated in PBS, ddC and ddI at 37 °C, over 6 hours. (Magnification 40).

- Figure 2 shows the H&E sections of the excised buccal mucosae following formalin fixation and paraffin embedment of the control and treated tissues.
- The sections revealed that the epithelial surface and basal lamina of the mucosae were still present and not greatly affected after 6 hours.
- No major differences were observed in the histology of treated (PBS, ddC or ddI) and untreated buccal mucosa over six (6) hours.

CONCLUSION

Didanosine and Zalcitabine therefore show potential for administration via the buccal route.

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2. Shojaei A.H., Khan M., Lim G., Khosravan R., (1998) Int J Pharm 192: 139-146.
3. Xiang J., Fang X., Li X., (2002) Int J Pharm., 231: 57-66.

ACKNOWLEDGEMENTS

- ASPEN Pharmacare and University of Kwa-Zulu Natal, for financial support.
- Biomedical Resource Unit, UKZN, for mucosal harvesting and surgical microscope.
- Electron Microscope Unit, UKZN, for Light Microscopy & image analysis.
- Dr Vorster, Vetdiagnostix, for Pathological interpretation.

In Vitro Transbuccal Delivery of An Antiretroviral Drug: Effect of Donor Concentrations on Didanosine Permeation.

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[1]School of Pharmacy and Pharmacology, [2]School of Medical Sciences, [1,2]University of KwaZulu-Natal, Durban, South Africa [3]Ogori Daiichi Hospital, Yamaguchi, Japan

INTRODUCTION

- Didanosine (ddI) is an antiretroviral (ARV) drug, with limitations such as low bioavailability due to severe degradation in the gastrointestinal (GI) environment and extensive first pass hepatic metabolism which necessitates increased dosage and frequency of drug administration (Li and Chan 1999., Xiang et al 2002).
- The buccal route presents several advantages which include bypass of first pass hepatic metabolism and the avoidance of GI degradation, thereby increasing the bioavailability of drugs. Buccal administration, is thus, an alternative route for ARV such as ddI.
- A need for studies on the buccal permeability properties of ddI is essential for eventual formulation into a suitable buccal delivery system.

AIM

- To examine the effect of donor concentrations on the transbuccal permeation of ddI.

METHODOLOGY

- *Ethical* Approval (Ref: 028/09/Animal) was obtained from the Animal Ethics Committee of the University of KwaZulu-Natal (UKZN).
- *Permeation Study* was undertaken using porcine buccal mucosa and Franz diffusion cell (PermeGear Inc. USA), with phosphate buffer saline (PBS) pH 7.4 at 37°C. Varying concentrations of ddI (5, 10, 15 and 20 mg/mL) were investigated over six hours. ddI was quantified using UV Spectroscopy (λ_{max} 250nm, UV1650PC Shimadzu Japan).
- *Data Analysis* was performed by plotting the amount of ddI permeated per unit surface area against the permeation time. Flux value was calculated using linear regression analysis (Microsoft Excel 2003). Permeability coefficient was determined using the calculated flux value. A minimum of three replicates was conducted for all experimental conditions.
- Statistical analysis was performed using one-way ANOVA with Bonferroni Post Hoc test (SPSS 15 for Windows® USA).

RESULTS AND DISCUSSION

EVALUATION OF THE PERMEABILITY PARAMETERS OF DIDANOSINE

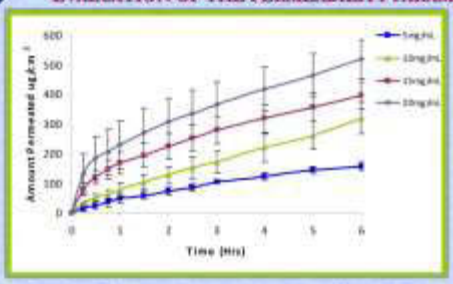

Fig 1: The effect of donor concentration on ddI permeation (Mean ±SD, N ≥ 3)

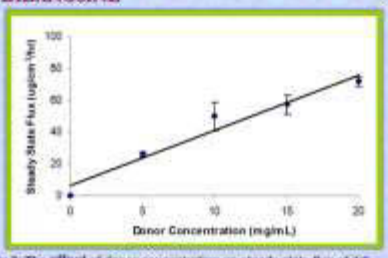

Fig 2: The effect of donor concentration on steady state flux of ddI (Mean ±SD N ≥ 3 y = 3.492x + 6.032 R^2 =0.9557)

Table 1: Effect of donor concentration on the permeability parameters of ddI. (Mean ±SD, N ≥ 3)

Donor Conc (mg/mL)	Correlation coefficient (R^2)	Amount per unit area ($\mu g/cm^2$)	Flux, J_{ss} ($\mu g\ cm^{-2}hr^{-1}$)	Permeability Coefficient $P \times 10^{-3}$ ($cmhr^{-1}$)
5	0.9742	158.15 (±13.17)	25.94 (± 1.35)	5.19 (±0.27)
10	0.9924	321.08 (± 52.82)	49.85 (± 8.99)	4.99 (± 0.91)
15	0.9177	397.03 (± 46.01)	57.35 (± 5.89)	3.82 (± 0.33)
20	0.8973	456.89 (± 57.11)	71.57 (± 3.12)	3.58 (± 0.16)

- The flux value of ddI at pH 7.4 increased with the increase in donor concentrations. The initial flux was 25.94 ± 1.35 $\mu g/cm^2$ hr for the 5 mg/mL donor concentration. Increase in the donor concentrations from 5 to 20 mg/mL led to a significant increase ($p < 0.05$) in the flux values for ddI (Table 1).
- Permeability coefficient decreased with increase in the donor concentrations. Permeability coefficients were 5.19 ±0.27, 4.99 ±0.91, 3.82 ±0.33 and 3.58 ±0.16 $x10^{-3}$cm/hr for donor concentrations of 5, 10, 15 and 20 mg/mL respectively.
- Results showed a linear relationship (R^2 = 0.9557) between the steady state flux and the donor concentrations of ddI and the observed trend suggested a passive transport mechanism for ddI. Similar trend has been reported for another antiretroviral drug (zalcitabine) by Xiang et al 2002.

CONCLUSIONS

- Didanosine successfully permeated the buccal mucosa at all the investigated concentrations and thus has the potential for administration via the buccal route.
- The buccal permeability of didanosine is concentration-dependent.

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2. Shojaei A.H., Khan M., Lim G., Khosravan R., (1999) Int J Pharm 192: 139-146.
3. Xiang J., Fang X., Li X., (2002) Int. J. Pharm., 231: 57-66.

ACKNOWLEDGEMENTS

- ASPEN Pharmacare and University of Kwa-Zulu Natal for financial support.
- Biomedical Resource Unit, UKZN, for mucosal harvesting and use of surgical microscope.
- Electron Microscope Unit, UKZN for Light Microscopy and Image analysis.

BUCCAL PERMEABILITY ENHANCEMENT OF DIDANOSINE USING ALOE VERA GEL: HISTOLOGICAL AND MICROSCOPICAL EVALUATIONS

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[1]School of Pharmacy and Pharmacology, [2]School of Medical Sciences, [3]EM Unit [1,2,3]University of KwaZulu-Natal, Durban, [4]Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa, [5]Ogori Daiichi Hospital, Yamaguchi – Japan

INTRODUCTION

- Didanosine (ddI) suffer several limitations including low bioavailability due to extensive first pass metabolism and/or degradation in the gastrointestinal environment, which necessitates increased dosage and frequency of administration (Li and Chan 1999., Xiang et al 2002).
- The buccal mucosal route presents several advantages such as the bypass of first pass hepatic metabolism and avoidance of gastrointestinal enzymatic degradation, thereby increasing the bioavailability of drugs. ddI may therefore benefit from buccal administration.
- The epithelium of the buccal mucosa acts as a barrier to the permeation of drugs for buccal delivery. Buccal permeability can be improved by the use of penetration enhancers; thus effective and safe enhancers need to be identified.
- Aloe Vera gel (AVGel) has been reported as a potential intestinal (Chen et al 2009) and skin (Cole et al 2007) permeability enhancer; however data on its buccal permeability enhancing potential remains to be reported.

AIM

- To determine the buccal permeability enhancement properties of AVGel on ddI and to evaluate its histomorphological effects on the buccal mucosal tissue.

METHODOLOGY

➢**Ethical Clearance:** Ethical approval was obtained from the Animal Ethics Committee of the University of KwaZulu-Natal (008/09/Animal and 028/09/Animal).

➢**Permeability Enhancement Study:** ddI (20mgmL) and AVGel in varying concentrations (0.5, 1.0 and 2.0 %w/v) were studied using porcine buccal mucosal tissue and modified Franz diffusion cells with phosphate buffer saline (PBS) pH 7.4 at 37°C. All experiments were performed in triplicates over six hours. The amount of drug transferred from the donor to the receptor compartment was determined by UV Spectrophotometeric analysis at λ_{max} of 250 nm (UV Spectrophotometer 1650 Shimadzu Japan). Flux value was calculated using linear regression analysis (Microsoft Excel 2007, USA). Permeability coefficient and enhancement ratio were determined. Data was analyzed using one-way ANOVA with Bonferroni Post Hoc test (SPSS 15 for Windows®, USA).

➢**Histological and Microscopical evaluations:** Buccal mucosal tissue was divided into three portions, each treated with either PBS, ddI/PBS or ddI/PBS/AVGel at concentrations stated above. Transmission Electron Microscopy (TEM) was employed to evaluate histomorphological changes. Each mucosal sample was processed and embedded in epoxy resins for TEM using standard protocols. Ultrathin sections were contrasted with uranyl acetate and lead citrate and viewed with a JEOL 1010 TEM.

RESULTS AND DISCUSSION

PERMEABILITY EVALUATION

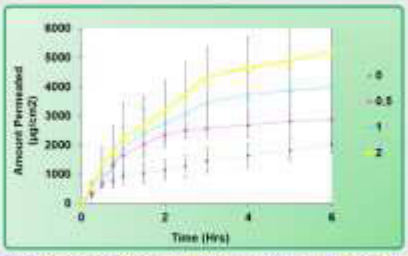

Fig 1: The effect of AVGel concentration on ddI permeation profiles (Mean ±SD N ≥ 3).

Table 1: Effect of AVGel concentration on the permeability parameters of ddI. (Mean ±SD N ≥ 3).

Concentration of AVGel (%w/v)	Amount per unit area (µg/cm²)	Flux, J_{ss} (µg cm⁻²hr⁻¹)	Permeability Coefficient P x 10⁻³ (cmhr⁻¹)	Enhancement Ratio (ER)
0	2026.76 (±301.82)	293.91	1.47	1.0
0.5	2986.21 (±719.32)	451.86	2.26	1.5
1.0	3990.44 (±755.60)	650.57	3.25	2.2
2.0	5266.82 (±1169.27)	660.73	3.30	2.3

TRANSMISSION ELECTRON MICRSOCOPY

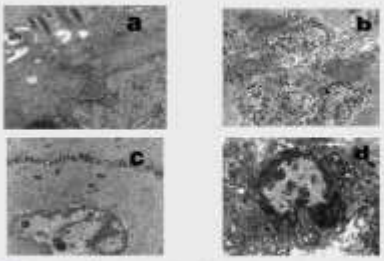

Fig 2: Micrographs of the ultrathin sections of the treated mucosa viewed and captured with TEM (a): Control, (b): ddI/PBS/AVGel 0.5%w/v (c): ddI/PBS/AVGel 1.0%w/v (d): ddI/PBS/AVGel 2.0%w/v (Magnification 4X).

➢The buccal permeability enhancement of ddI increased with increase in AVGel concentrations (Fig. 1). The flux, permeability coefficient and enhancement ratio increased significantly ($p < 0.05$) from their initial/baseline values to 661 µg cm⁻²hr⁻¹, 3.3cmhr⁻¹ and 2.3 respectively (Table 1). Results showed that 2.0 %w/v AVGel enhanced ddI permeability by almost 2.5 times its initial flux value.

➢The preliminary ultrastructural analysis of the mucosa (control) showed nucleus with normal chromatin distribution, dense mitochondria and desmosomes (Fig. 2a). However, some cells from the mucosa treated with 0.5%w/v AVGel showed increased endoplasmic reticulum profiles and abundance of ribosomes activity indicating increased cellular activity in response to treatment (Fig. 2b).

➢Signs of cellular damage were evident in mucosae treated with 1.0 %w/v and 2.0 %w/v AVGel . These signs include increased intercellular spaces, mitochondrial electron-lucency with few cristae (Figs. 2c and 2d). Nuclear envelopes appeared distended and chromatin compacted and unevenly dispersed. Signs of deleterious impact of AVGel 1 and 2 %w/v on the buccal mucosae may be attributed to a possible stress response of the mucosae to the higher AVGel concentrations. Interestingly, the desmosomal structure remained normal and intact throughout all treatments.

CONCLUSIONS

➢AVGel at concentrations 0.5, 1.0 and 2.0 %w/v enhanced the buccal permeability of ddI. Its enhancement ability was highest at 2.0 %w/v with an enhancement ratio of 2.3. AVGel is therefore a potential enhancer for buccal permeability of didanosine.

➢AVGel at 0.5 %w/v caused changes in the cellular structures of the buccal mucosa, but cellular damage was evident with AVGel at both 1.0 and 2.0 %w/v. Further histomorphological investigations at lower concentrations of AVGel are thus required to determine its safety.

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4. Xiang J., Fang X., Li X., (2002); Int.J Pharm., 231: 57-66.

ACKNOWLEDGEMENTS

- ASPEN Pharmacare and University of Kwa-Zulu Natal for financial support.
- Biomedical Resource Unit, UKZN for buccal mucosa.
- Electron Microscope Unit, UKZN for LM, TEM and Image analysis

PERMEABILITY AND MUCOSAL ULTRASTRUCTURAL ANALYSES FOR TRANSBUCCAL DELIVERY OF DIDANOSINE

UNIVERSITY OF KWAZULU-NATAL

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[1]School of Pharmacy and Pharmacology, [2]School of Medical Sciences, [3]Electron Microscope Unit [1,2,3]University of KwaZulu-Natal, Durban,
[4]Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa

UNIVERSITY OF KWAZULU-NATAL

INTRODUCTION AND AIM

- Antiretroviral (ARV) drugs, such as didanosine (ddI), are mostly available for the oral route of drug administration and suffer from low bioavailability due to gastric acid and enzymatic degradation, increased dosage and frequency of administration.
- The buccal route may improve bioavailability of ddI by avoiding hepatic first pass metabolism and gastrointestinal degradation (Li and Chan 1999., Xiang et al 2002). Therefore, its administration via the buccal route may be an advantage in the treatment of HIV/AIDS.
- Buccal permeability of drugs can be improved by the use of permeation enhancers. However, the epithelium of the buccal mucosa acts as a barrier to the permeation of drugs for buccal delivery. It is therefore important to identify effective and safe enhancers for buccal permeability of drugs.
- Aloe vera gel (Avgel) has been reported as a potential intestinal (Chen et al 2009) and skin (Cole et al 2007) permeability enhancer; however data on its potential as a buccal permeability enhancer is yet to be reported.
- This study aimed to examine Avgel as a buccal permeation enhancer for ddI and to assess its histomorphological effects on the buccal mucosa.

METHODOLOGY

- **Ethical Clearance:** Approval (Ref. 029/10/Animal) was obtained from the Ethics Committee, University of KwaZulu-Natal (UKZN).
- ***In vitro* Permeation experiments:** Permeability of ddI (20mgml^{-1}) in phosphate buffer saline (PBS) pH 7.4 at 37°C in the absence and presence of Avgel at varying concentrations (0.5 to 6.0 % w/v) was studied using porcine buccal mucosa and vertical Franz diffusion cells (PermeGear® Inc. USA). The amount of drug transferred from the donor to the receptor compartment was determined by UV Spectrophotometric analysis at λ_{max} 250 nm (UV Spectrophotometer 1650 Shimadzu Japan). All experiments were performed with a minimum of three replicates over six hours. The permeability parameters (flux, permeability coefficient and enhancement ratio) were calculated using linear regression analysis. Data were analyzed using one-way ANOVA with Bonferroni Post Hoc tests (SPSS version 15 for Windows®, USA).
- **Histomorphological Evaluation:** Buccal mucosa was divided into three portions, each incubated in PBS, ddI/PBS or ddI/PBS/Avgel at concentrations stated above. Each sample was processed and embedded in epoxy resins for transmission electron microscopy (TEM) using standard protocols. Ultrathin sections were contrasted with uranyl acetate and lead citrate and viewed with a TEM (JEOL 1010®, Japan).

RESULTS AND DISCUSSION

PERMEABILITY PARAMETER ANALYSIS

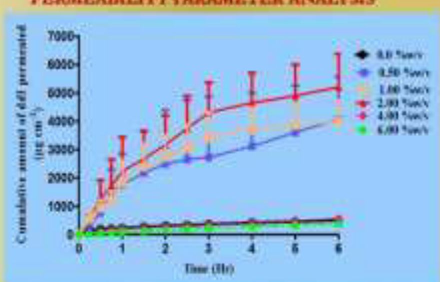

Fig 1: The effect of Avgel concentration on ddI permeation (Each value is Mean ±SD; N ≥ 3)

Table 1: Effect of Avgel concentration on the permeability parameters of ddI (Mean ±SD, N ≥ 3)

Avgel Concentration (% w/v)	Correlation coefficient (R^2)	*Flux (J_{ss}) (µg cm^{-2}hr^{-1})	Permeability Coefficient (P) x 10^{-2} (cm hr^{-1})	Enhancement Ratio (ER)
0.0	0.89	71.57 ± 3.12^a	0.36 ± 0.02	1
0.5	0.89	613.69 ± 292.49^b	3.07 ± 1.46	8.58
1.0	0.85	650.07 ± 164.41^b	3.25 ± 0.82	9.08
2.0	0.88	842.73 ± 129.24^b	4.21 ± 0.65	11.78
4.0	0.95	83.95 ± 11.71^c	0.42 ± 0.06	1.17
6.0	0.99	62.02 ± 5.41^c	0.31 ± 0.03	0.87

*[(a,b,c $p < 0.05$) , (a,b,c $p > 0.05$)]

MUCOSAL ULTRASTRUCTURAL ANALYSIS

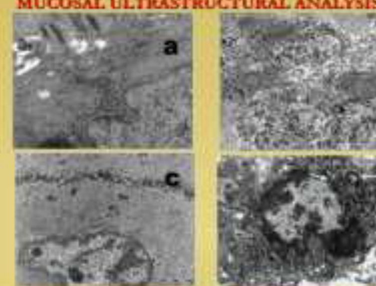

Fig 2: TEM micrographs of the ultrathin section of the buccal mucosa (a): PBS, (b): ddI/PBS/AVGel 0.5%w/v (c): ddI/PBS/AVGel 1.0%w/v (d): ddI/PBS/AVGel 2.0%w/v

- Avgel enhanced the permeability of ddI at concentrations 0.5 to 2 %w/v (Fig. 1). Enhancement ratios ranged from 8.58 (0.5 %w/v) to 11.78 (2 %w/v) (Table 1). There was a significant increase in ddI flux from 71.57 ± 3.12 to 842.73 ± 129.24 (R^2 = 0.77; ANOVA p = 0.0001). This could be due to the disruption of aqueous channels and desmosomal gap junctions thereby promoting the movement of drug across the mucosa. This phenomenon suggests paracellular mechanism of enhancement. Similar phenomenon has been reported for chitosan as a buccal penetration enhancer (Senel et al 2000). Further increases in Avgel concentration from 2.0 to 4.0 and 6.0 %w/v led to a decrease in ddI flux from 842.73 ± 129.24 (2.0 %w/v) to 83.95 ± 11.71 (4.0%w/v) and 62.02 ± 5.41 (6.0%w/v). This could be attributed to higher viscosity of Avgel at these high concentrations which may provide resistance to drug diffusion across the mucosa (Jani et al 2007; Shin and Kim 2000).
- Ultrastructural analysis of the PBS, ddI/PBS and ddI/PBS/Avgel 0.5 %w/v treated mucosa showed cells with normal plasmalemma, nucleus with normal chromatin distribution, dense mitochondria and desmosomes (Fig. 2a). However, cells from the mucosa treated with 0.5 %w/v Avgel showed increased endoplasmic reticulum and abundance of ribosomes indicating increased cellular activity (Fig. 2b). Signs of cellular stress were evident in mucosa treated with 1, 2 and 6 %w/v. These signs include enlarged intercellular spaces, mitochondrial electron-lucency, cellular compaction, plasmalemma crenulation and distended nuclear envelopes (Figs 2c and 2d). The enlargement of intercellular spaces could be due to disarrangement of the desmosomal junctions thus indicating possible enlargement of paracellular pathway(Sandri et al 2006), and suggesting paracellular mechanism.

CONCLUSIONS

- Avgel can enhance the buccal permeability of ddI and it is concentration-dependent.
- The mucosal cells remained unchanged at Avgel 0.5%w/v, but showed signs of deleterious effects at higher concentrations.
- Based on the mucosal ultrastructural analyses noted in this study, the preferred mechanism of enhancement of Avgel, its safety and effectiveness at concentrations above 0.5 % w/v require further investigation.

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- ASPEN Pharmacare and University of KwaZulu-Natal for financial support.
- Biomedical Resource Unit, UKZN for porcine buccal mucosa.
- Electron Microscope Unit, UKZN for TEM and image analysis

Preparation and evaluation of mucoadhesive polymeric films for buccal delivery of anti-hiv/aids drug (didanosine)

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INTRODUCTION

- HIV/AIDS remains the most serious cause of death in SubSahara Africa and especially in South Africa.
- Anti-HIV/AIDS drugs, such as didanosine (ddI), are available for the oral route of administration only.
- Its buccal administration may improve bioavailability by avoiding hepatic first pass metabolism and gastrointestinal degradation.
- The incorporation of an ARV into a buccal delivery system has not been reported.

AIM

- This study aimed to prepare and evaluate ddI containing homopolymeric and monolayered multipolymeric films with polymers of similar and opposing solubilities (MMFs) for buccal delivery.

METHODS

Film preparation

ddI loaded monopolymeric films with Hydroxypropylmethylcellulose (HPMC) or Eudragit® RS-100 (EUD) were prepared in varying ratios using a Silicone Moulded Tray with individual wells. HPMC films were prepared by casting/ solvent evaporation and EUD films by emulsification/casting/ solvent evaporation. MMFs comprising of ddI:HPMC:EUD in varying ratios were prepared by emulsification/casting/solvent evaporation.

Film evaluation

Films were characterised in terms of drug content (UV Spectrophotometry) and drug release (Shaking Water Bath). Film thickness was measured with an Electronic Digital Micrometer and surface morphology assessed using Scanning Electron Microscopy (SEM).

RESULTS AND DISCUSSION

DRUG RELEASE

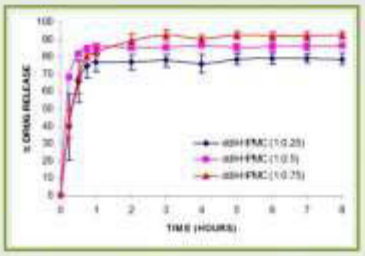

Fig 1: Drug Release profiles of HPMC films containing ddI.

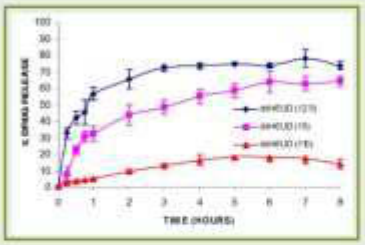

Fig 1: Drug Release profiles of HPMC films containing ddI.

- ddI:HPMC (1:0.5) only films were homogenous and exhibited immediate release profiles. ddI:EUD (1:2.5) only films were homogenous, elastic and flexible and showed controlled release profiles.
- The incorporation of ddI into MMFs with polymers and drug of opposing solubilities ddI:HPMC:EUD (1:0.5:2.5) resulted in homogenous, elastic and flexible films with immediate release profiles.
- Increasing the EUD concentrations led to controlled release profiles (Figure 1).
- Drug content, size, thickness and weight of the MMFs ddI:HPMC:EUD (1:0.5:2.5) were 97.65 ± 5.78%, 2x3cm2, 0.187 ±0.023 mm and 108.65 ±6.71 mg respectively.
- SEM showed the films to have a smooth and homogenous compact surface prior to dissolution and changes in texture and pore formation after dissolution.

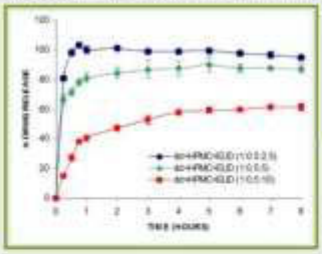

Fig 3: Effect of EUD on drug release profiles from MMFs.

Fig 4: SEM photograph of MMF, prior to dissolution studies

Fig 5: : SEM photograph of MMF, after dissolution studies .

CONCLUSIONS

- ddI can be incorporated into HPMC monopolymeric films for immediate ddI release applications.
- Homogeneous monolayered multipolymeric films with drug and polymer (EUD) of opposing solubilities could also be prepared for controlled ddI release applications. MMFs with immediate and controlled ddI release profiles, with improved flexibility as compared to the monopolymeric films can be prepared.
- The various films prepared in this study are potential candidates for optimization of ddI films as a buccal delivery system.

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- ASPEN Pharmacare and University of Kwa-Zulu Natal, for financial support.
- Electron Microscope Unit, UKZN, for Light Microscopy & image analysis.

EFFECT OF *ALOE VERA* GEL ON THE BUCCAL POLYMERIC FILMS OF DIDANOSINE

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[2]Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa.

INTRODUCTION

- The buccal route can improve the bioavailability of drugs such as didanosine (ddI) which are susceptible to degradation in the gastrointestinal tract. The lining epithelium of the buccal mucosa constitutes the main cellular barrier to drug permeability, hence the need for permeation enhancers. The buccal permeability properties of ddI has been identified (Ojewole E et al 2009). Drug-containing films can act as buccal delivery systems (Hearnden et al 2011).
- Aloe vera gel (Avgel) is a suitable excipient in pharmaceutical modified-release formulations (Jani et al 2007). Its potential to enhance the buccal permeability of ddI has been reported (Ojewole E et al 2009). A delivery system which can also incorporate Avgel as a permeation enhancer could be an attractive modified-release system for the buccal administration of ddI. However, the incorporation of Avgel into a buccal delivery system has not been reported.

AIM

To determine the formulating effects of Avgel and its concentration on the buccal polymeric films containing didanosine.

METHODS

Preparation of Films

Films containing ddI, hydroxypropyl methylcellulose (HPMC) and Eudragit® RS-100 (EUD) in a fixed ratio of 1:0.5:10 (ddI films) were formulated using silicone molded tray (SMT) with teflon coated perspex inserts by solvent casting / evaporation method. ddI/Avgel films comprising ddI:HPMC:EUD (1:0.5:10) and Avgel in varying concentrations of 25, 50 and 75 %w/w were formulated using the above method.

Evaluation of Films

Routine evaluations of both ddI and ddI/Avgel films were performed in terms of appearance (Samsung digital camera, Japan), thickness (Digital micrometer, Mitutoyo), and weight (Mettler Toledo AB204-S). Drug assay and drug released were determined using UV Spectrophotometer. Film morphology using scanning electron microscope (LEO, Germany) as well as pH of film surface (Hannah pH meter 211, Portugal) were assessed

RESULTS AND DISCUSSIONS

Assay

Table 2: Assay values of ddI and ddI/Avgel films (Mean ± SD, N=6)

Films	*Assay (%)*	*CV %*
Control	94.58 ± 9.89	10.46
25%w/w	91.25 ± 13.18	14.44
50% w/w	88.93 ± 11.29	12.69
75%w/w	87.21 ± 9.49	10.89

- Drug assay and *in vitro* drug release for ddI/Avgel 50% films, 88.93 ± 11.29 % (assay) and 45.13 ± 2.25 % (drug release) were lower as compared to those of ddI films, 94.58 ± 9.89 % (assay) and 62.39 ± 6.11 % (drug release).
- Assay values of the films formulated in this study were generally low, and this could be attributed to inadequate solvent extraction of the drug.

Film Morphology

Table 1: Effect of Avgel on the morphology of buccal films containing ddI

Avgel (% w/w)	Appearance	Before drug release	After drug release
Control			
25			
50			
75			

- ddI films (control) were homogenous, smooth and flexible with thickness and weight of 0.33 ± 0.02 mm; 305.55 ± 3.83 mg respectively.
- ddI/Avgel 50%w/w films were thicker (0.55 ± 0.02 mm, p = 0.210), heavier (313.78 ± 5.33 mg, p = 0.09), and stickier with patchy surfaces compared to ddI films.

Drug Release

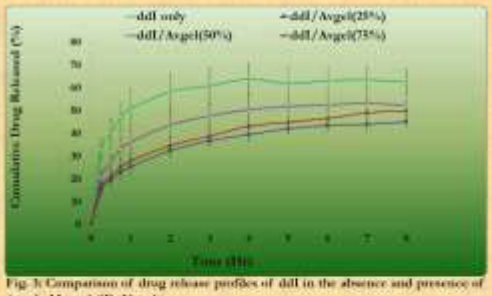

Fig. 3: Comparison of drug release profiles of ddI in the absence and presence of Avgel. Mean ± SD, N = 6)

- Drug released from ddI/Avgel films were generally lower than the ddI films. The lower percentage drug released from 62.39 ± 6.11% (control) to 45.13 ± 2.25% (50%) could be due to diffusion of ddI being retarded by Avgel. A similar study has reported the modifying effect of Avgel in a sustained release matrix tablets (Jani et al 2007).
- Therefore, the overall decrease in drug release over the eight hours of study could be credited to the modifying effects of Avgel.

CONCLUSIONS

- Results showed that Avgel can be incorporated as an excipient in the buccal polymeric films of ddI.
- Avgel retarded and controlled the release of ddI from the ddI/Avgel buccal films.
- Based on the results in this study, the assay of the ddI and ddI/Avgel films require further investigations to ensure values comply with compendia specifications.

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ACKNOWLEDGEMENTS

- UKZN, Aspen Pharmacare, MRC and NRF for financial support
- Electron Microscope Unit for SEM studies

APPENDIX VIII

WEB OF SCIENCE CORE COLLECTION - CITATION REPORT

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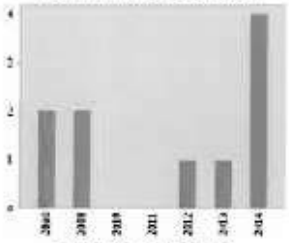

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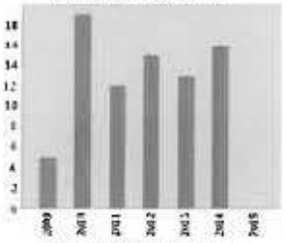

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	2011	2012	2013	2014	2015	Total	Average Citations per Year
	12	15	13	16	0	80	13.33
1. **Exploring the use of novel drug delivery systems for antiretroviral drugs** By: Ojewole, Elizabeth; Mackraj, Irene; Naidoo, Panjasaram; et al. EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS Volume: 70 Issue: 3 Pages: 697-710 Published: NOV 2008	9	12	10	2	0	48	6.86
2. **Polymeric Nanoparticles for Enhancing Antiretroviral Drug Therapy** By: Govender, T.; Ojewole, E.; Naidoo, P.; et al. DRUG DELIVERY Volume: 15 Issue: 8 Pages: 493-501 Published: 2008	3	3	2	0	0	17	2.43
3. **Investigating the Effect of Aloe vera Gel on the Buccal Permeability of Didanosine** By: Ojewole, Elizabeth; Mackraj, Irene; Akhundov, Kamil; et al. PLANTA MEDICA Volume: 78 Issue: 4 Pages: 354-361 Published: MAR 2012	0	0	1	5	0	6	2.00
4. **High-energy ball milling of saquinavir increases permeability across the buccal mucosa** By: Rambharose, Sanjeev; Ojewole, Elizabeth; Branham, Michael; et al. DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY Volume: 40 Issue: 5 Pages: 639-648 Published: MAY 2014	0	0	0	3	0	3	3.00
5. **Comparative buccal permeability enhancement of didanosine and tenofovir by potential multifunctional polymeric excipients and their effects on porcine buccal histology** By: Rambharose, Sanjeev; Ojewole, Elizabeth; Mackraj, Irene; et al.	0	0	0	3	0	3	3.00

PHARMACEUTICAL DEVELOPMENT AND TECHNOLOGY Volume: 19 Issue: 1 Pages: 82-90 Published: FEB 2014

6.	Monolayered multipolymeric buccal films with drug and polymers of opposing solubilities for ARV therapy: Physico-mechanical evaluation and molecular mechanics modelling By: Jones, Elsabe; Ojewole, Elizabeth; Pillay, Viness; et al. INTERNATIONAL JOURNAL OF PHARMACEUTICS Volume: 456 Issue: 1-2 Pages: 197-212 Published: OCT 15 2013	0	0	0	3	0	3	1.50
7.	Novel oleic acid derivatives enhance buccal permeation of didanosine By: Ojewole, Elizabeth; Kalhapure, Rahul; Akamanchi, Krishnacharya; et al. DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY Volume: 40 Issue: 5 Pages: 657-668 Published: MAY 2014	0	0	0	0	0	0	0.00
8.	In vitro comparative evaluation of monolayered multipolymeric films embedded with didanosine-loaded solid lipid nanoparticles: a potential buccal drug delivery system for ARV therapy By: Jones, Elsabe; Ojewole, Elizabeth; Kalhapure, Rahul; et al. DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY Volume: 40 Issue: 5 Pages: 669-679 Published: MAY 2014	0	0	0	0	0	0	0.00
9.	Exploring the effect of aloe vera gel on the buccal permeability of didanosine: permeability and histomorphological studies By: Ojewole, E.; Mackraj, I.; Akhundov, K.; et al. JOURNAL OF PHARMACY AND PHARMACOLOGY Volume: 61 Pages: A34-A35 Meeting Abstract: 44 Published: 2009	0	0	0	0	0	0	0.00
10.	Preparation and evaluation of mucoadhesive polymeric films for buccal delivery of anti-HIV/AIDS drug (didanosine) By: Ojewole, E.; Mackraj, I.; Jones, E.; et al. JOURNAL OF PHARMACY AND PHARMACOLOGY Volume: 61 Pages: A35-A35 Meeting Abstract: 45 Published: 2009	0	0	0	0	0	0	0.00

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APPENDIX IX

PUBLICATIONS CO-AUTHORED / CO-SUPERVISED

DURING THIS STUDY

PUBLICATIONS CO-AUTHORED / CO-SUPERVISED DURING THIS STUDY

1. PUBLICATION CO-AUTHORED

Below is a publication (review article) from the literature search generated during this study.

1.1 T Govender, **E Ojewole**, P Naidoo, I Mackraj Polymeric nanoparticles for enhancing antiretroviral drug therapy. Drug Delivery, **2008**; 15: 493 – 501.

2. PUBLICATIONS FROM CO-SUPERVISION

Below is a list of publications co-supervised during this study. The two Masters Students generated the data for these publications, submitted their disstertations and graduated during this study.

2.1 Elsabe Jones, **Elizabeth Ojewole**, Viness Pillay, Pradeep Kumar, Sanjeev Rambharose, Thirumala Govender. Monolayered multipolymeric buccal films with drug and polymers of opposing solubilities for ARV therapy: physico-mechanical evaluation and molecular mechanics modeling. International Journal of Pharmaceutics, **2013**; 455: 197 – 212.

2.2 Sanjeev Rambharose, **Elizabeth Ojewole,** Irene Mackraj, Thirumala Govender. Comparative buccal permeability enhancement of didanosine and tenofovir by potential multifunctional polymeric excipients and their effects on porcine buccal histology. Pharmaceutical Development and Technology, **2014;** 19: 82 – 90.

2.3 Sanjeev Rambharose, **Elizabeth Ojewole,** Michael Branham, Rahul Kalhapure, Thirumala Govender. High-energy ball milling of saquinavir increases permeability across the buccal mucosa. Drug Development and Industrial Pharmacy, **2014**; 40: 639 – 648.

2.4 Elsabe Jones, **Elizabeth Ojewole,** Rahul Kalhapure, Thirumala Govender. 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 Pharmacy, **2014**; 40: 669 – 679.