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A NOVEL CONTROLLED RELEASE INTRAVAGINAL BIOADHESIVE POLYMERIC DEVICE

VALENCE MATHIAS KESSY NDESENDO

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree of Doctor of Philosophy

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2009

DECLARATION

I, Valence Mathias Kessy Ndesendo, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

……

Signature

This ……. day of ……....., 2009

DEDICATION

This thesis is dedicated to my wife Christine, my daughter Karen and my son Collins for their love, prayers and much-needed support towards the completion of this study. I also dedicate this thesis to my father Mathias, for being a great inspiration towards the pursuance of this work, my mother Angela and my mother-in-law Bertha, for their endeavored support, prayers and love throughout my studies.

RESEARCH OUTPUTS, PATENT AND ACCOLADES

A. Publications

1. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Leith C.R. Meyer, Eckhart Buchmann and Uwe Rosin (2009). *In vitro* and *Ex Vivo* Bioadhesivity Analysis of Polymeric Intravaginal Caplets Using Physicomechanics and Computational Structural Modeling. *International Journal of Pharmaceutics,* 370, 151-159. (Abstract in Appendix A1).

2. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Eckhart Buchmann, David N. Bayever and Leith C.R. Meyer (2008). A Review of Current Intravaginal Drug Delivery Approaches Employed for the Prophylaxis of HIV/AIDS and Prevention of Sexually Transmitted Infections. *AAPS Pharmaceutical Sciences and Technology,* 9, (2), 505-520. (Abstract in Appendix A2).

3. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Leith C.R. Meyer , Riaz A. Khan, Eckhart Buchmann, and Uwe Rosin (2009). Investigation of the Physicochemical and Physicomechanical Properties of an Optimized Intravaginal Bioadhesive Polymeric Device in the Pig Model. *AAPS Pharmaceutical Sciences and Technology,* submitted. (Abstract in Appendix A3).

4. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Leith C.R. Meyer , Riaz A. Khan, Eckhart Buchmann, and Uwe Rosin (2009). *In vivo* Drug Content Analysis and Histopathological Evaluation of an Intravaginal Bioadhesive Polymeric Device in Pig Tissue. Submitted. (Abstract in Appendix A4).

5. Valence M. K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Riaz A. Khan, Eckhart Buchmann and Uwe Rosin (2009). Application of Artificial Neural Networks for the Elucidation of an Optimized Synergistic Polymer Combination for Effective Performance of an Intravaginal Bioadhesive Polymeric Device. Submitted. (Abstract in Appendix A5).

6. Valence M. K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Riaz A. Khan, Eckhart Buchmann and Uwe Rosin (2009). Preformulation Investigations for the Development of a Lead Intravaginal Bioadhesive Polymeric Device. Submitted. (Abstract in Appendix A6).

B. Conference Proceedings

1. Valence M. K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Leith C.R. Meyer, Eckhart Buchmann and Uwe Rosin. *In Vitro* and *Ex-vivo* Evaluation of a Polystyrene Sulfonate-loaded Vaginal Polymeric Platform through Porcine Vaginal Tissue. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 8-12, 2009, Los Angeles Convention Center, Los Angeles, Califonia, USA. (Abstract in Appendix B1).

2. Valence M. K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Leith C.R. Meyer, Eckhart Buchmann and Uwe Rosin. Development of a Highly Sensitive Assay Method for the Determination of AZT in Porcine Plasma. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 8-12, 2009, Los Angeles Convention Center, Los Angeles, Califonia, USA. (Abstract in Appendix B2).

3. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Leith C.R. Meyer, Eckhart Buchmann and Uwe Rosin. *In Vivo* Morphological and Histopathological Evaluation on a Vaginal Polymeric Device in a Porcine Model. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 8-12, 2009, Los Angeles Convention Center, Los Angeles, Califonia, USA. (Abstract in Appendix B3).

4. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Leith C.R. Meyer, Eckhart Buchmann and Uwe Rosin. *In vivo* Drug Content Analysis and Histopathological Evaluation of an Intravaginal Polymeric Platform in a Pig Model. Fifth International Conference of South African Pharmaceutical and Pharmacological Sciences Society (ICPPS). 23rd-26th September, 2009, North-West University, Potchefstroom Campus, South Africa. (Abstract in Appendix B4).

5. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Leith C.R. Meyer, Eckhart Buchmann and Uwe Rosin. Development of an X-ray Detection Method for Hydroxyapatite-Loaded Matrices for Intravaginal Insertion in a Pig Model. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix B5).

6. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Leith C.R. Meyer, Eckhart Buchmann and Uwe Rosin. Evaluation of the Bioadhesive Potential of A Multiconfigured Polymeric Matrix for Intravaginal Drug Delivery. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix B6).

7. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit and Eckhart Buchmann. Combination of Biosorbable, Relatively Low and High Compressible Polymers in Controlled Drug Delivery. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix B7).

8. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Leith C.R. Meyer, Eckhart Buchmann, Uwe Rosin, and Lisa C. du Toit. Release Rate Modulation and *Ex Vivo* Diffusion of Zidovudine from a Stealth Polymeric Matrix through Porcine Vaginal Tissue. 29th Annual Conference of the Academy of Pharmaceutical Sciences of South Africa (APSSA). 22nd to 26th September, 2008, Magaliesberg, S. Africa. (Abstract in Appendix B8).

9. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Leith C.R. Meyer, Eckhart Buchmann, Uwe Rosin, and Lisa C. du Toit. The Influence of an Innate Moisture Protective Shell on Drug Release from a Composite Polymeric Platform. 29th Annual Conference of the Academy of Pharmaceutical Sciences of South Africa (APSSA). 22nd to 26th September, 2008, Magaliesberg, South Africa. (Abstract in Appendix B9).

10. Ndesendo, V.M.K., Pillay, V., Choonara, Y.E., Buchmann, E. and Meyer, C.R.L. A Comparison between the Bioadhesivity Potential of Two Novel Polymer-Based Matrices for Intravaginal Drug Delivery. 28thAnnual Conference of the Academy of Pharmaceutical Sciences of South Africa (APSSA), Cape Town, South Africa, 2007. (Abstract in Appendix B10).

11. Ndesendo, V.M.K., Pillay, V., Choonara, Y.E., Buchmann, E. and Meyer, C.R.L. A Development of a Method to Investigate the *Ex Vivo* Bioadhesivity of a Novel Polymer-Based Platform on Freshly Excised Rabbit Vaginal Tissue. 28thAnnual Conference of the Academy of Pharmaceutical Sciences of South Africa (APSSA), Cape Town, South Africa, 2007. (Abstract in Appendix B11).

12. Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Leith C.R. Meyer, Eckhart Buchmann, Uwe Rosin and Lisa C. du Toit. A Resident Homeo-Prophylactic Intravaginal Polymeric Caplet for the Prevention of HIV/AIDS and STIs. Presented at the Faculty of Health Sciences Research Day. University of the Witwatersrand, 20th August, 2008. (Abstract in Appendix B12).

13. Valence MK Ndesendo, Viness Pillay, Yahya E Choonara, Leith C.R. Meyer, Eckhart Buchmann, Uwe Rosin and Lisa C du Toit. An *In vitro, Ex-vivo, In vivo* Analysis as well as Histopathological and Histomorphological Evaluation of an Intravaginal Microbicidal Bioadhesive Polymeric Device for the Prevention of HIV and STIs. Presented at the Faculty of Health Sciences Research Day. University of the Witwatersrand, 15th August, 2009. (Abstract in Appendix B13).

C. Patent

1. Valence M.K. Ndesendo, Viness Pillay and Yahya E. Choonara. A Novel Controlled Release Intravaginal Bioadhesive Polymeric Device. South Africa Patents and Trade Mark Office, 2008. Filed.

D. Additional Outputs from Collaborative Research

D.1 Publications

1. Girish Modi, Viness Pillay, Yahya E. Choonara, Valence M. K. Ndesendo, Lisa C. Du-Toit and Dinesh Naidoo (2009). A review of nanotechnological applications for the treatment of neurodegenerative disorders. *Journal of Progress in Neurobiology*, 88, (4), 272-285. (Abstract in Appendix C1).

2. Yahya E. Choonara, Viness Pillay, Lisa C. du Toit, Girish Modi, Dinesh Naidoo, Valence M.K. Ndesendo and Sibongile R. Sibambo (2009). The Molecular Pathogenic and Clinical Therapeutic Trends in Common Neurodegenerative Disorders. *International Journal of Molecular Sciences,* 10, (10)*,* 2510-2557. (Abstract in Appendix C2).

3. Samantha Pillay, Viness Pillay, Yahya E. Choonara, Dinesh Naidoo, Riaz A. Khan, Lisa C. du Toit, Valence M. Ndesendo, Girish Modi, Michael P. Danckwerts, Sunny E. Iyuke (2009). Design, Optimization and Biometric Simulation of a Nano-enabled Scaffold Device for Enhanced Delivery of Dopamine to the Brain. *International Journal of Pharmaceutics,* 382, 277-290. (Abstract in Appendix C3).

4. Y.E. Choonara, V. Pillay, N. Singh, R.A. Khan and V.M.K. Ndesendo (2008). Chemometric, physicomechanical and rheological analysis of the sol-gel dynamics and degree of crosslinking of glycosidic polymers. *Biomed. Mater.* 3, (2), 1-15. (Abstract in Appendix C4).

5. A Kolawole, V Pillay, Y E Choonara, L C du Toit and V M K Ndesendo (2009). The influence of polyamide 6,10 synthesis variables on the physicochemical characteristics and drug release kinetics from a monolithic tablet matrix. *Journal of Pharmaceutical Development and Technology*, accepted. (Abstract in Appendix C5).

6. Yahya E. Choonara, Viness Pillay, Valence M.K. Ndesendo, Lisa C. Du Toit, Riaz A. Khan, and Caragh S. Murphy (2009). The simultaneous in vitro characterization of poly(lactic co-glycolic acid) and poly(glucuronide)-rich nanoparticles employing various sol-gel synthetic wet chemical processing strategies. *Biomedical Materials* (BMM), submitted. (Abstract in Appendix C6).

7. Valence M.K. Ndesendo, Viness Pillay, Girish Modi, Yahya E. Choonara, Lisa Du Toit. Applications of Nanotechnology in Parkinson's and Motor neurone diseases. 2009, submitted. (Abstract in Appendix C7).

8. Pius Fasinu, Viness Pillay, Valence M.K. Ndesendo, Lisa du Toit, Yahya E. Choonara. Advances in the approaches for enhancement of oral drug delivery. 2009, submitted. (Abstract in Appendix C8).

9. Rubina Shaikh, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo, Priya Bawa and Shivaan Cooppan. "Smart" (stimuli-responsive) polymers: Applications in membranous drug delivery systems (and the formulation thereof). *AAPS Pharmaceutical Sciences and Technology,* submitted. (Abstract in Appendix C9).

10. Rubina Shaikh, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo and Riaz A. Khan. A Modified Crosslinked Oramucosal Lyophilized Wafer System for Gradual Drug Delivery, submitted. (Abstract in Appendix C9).

11. Ndidi Ngwuluka, Viness Pillay, Lisa C. du Toit, Valence M.K. Ndesendo, Yahya E. Choonara, Priya Bawa and Shivaan Cooppan. Progress in the Design of L-dopa-Loaded Delivery Systems for the Therapeutic Management of Parkinson's disease. *Expert Opinion on Drug Delivery,* submitted. (Abstract in Appendix C11).

12. Ameena Wadee, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo and Caragh S. Murphy. Recent Advances in the Design of Drug-loaded Polymeric Implants for the Treatment of Solid Tumors, submitted. (Abstract in Appendix C12).

13. Kovanya Moodley, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo, Priya Bawa and Shivaan Cooppan. Oral Drug Delivery Systems Comprising Altered Geometric Configurations for Controlled Drug Delivery, submitted. (Abstract in Appendix C13).

14. Clare Dott, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo and Caragh S. Murphy. Pharmaceutical Applications of Electro-Spinning, submitted. (Abstract in Appendix C14).

15. Farina Loonat, Valence M.K. Ndesendo, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit and Riaz A. Khan. Design and Development of Pediatric Wafers for the Treatment of Pediatric HIV/AIDS, submitted. (Abstract in Appendix C15).

16. B Sibeko, V Pillay, YE Choonara, RA Khan, G Modi, SE Iyuke, D Naidoo and VMK Ndesendo. Fabrication, characterization and optimization of MTX-loaded PLA/MAA nanoparticles, submitted. (Abstract in Appendix C16).

17. Neha Singh, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit and Valence M.K. Ndesendo. Modulation of Nicotine Release from a Gelisphere–Loaded Compressed External Polymeric Matrix. 2009, submitted. (Abstract in Appendix C17).

D.2 Conference Proceedings

1. Valence M. K. Ndesendo, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. The simultaneous *in Vitro* analysis of poly (Lactic-co-glycolic acid) and poly (glucuronide) - rich alginate nanoparticles: A formulation approach. International Conference on Nanoscience and Nanotechnology (NanoAfrica, 2009), 1-4 February, 2009, CSIR, Pretoria, South Africa. (Abstract in Appendix C18).

2. Y.E. Choonara V. Pillay, M.P. Dankwerts, T. Carmichael, C. Wanblad, S. Naylor and V.M.K. Ndesendo. A Biodegradable Doughnut-Shaped Minitablet for Sustained Intraocular Release of Ganciclovir. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix C19).

3. O.A. Kolawole, V. Pillay, Y. E. Choonara L.C. du Toit and V. M. K. Ndesendo. Design and Characterization of a Pore-Regulated Matrix for Transmucosal Drug delivery. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix C20).

4. O.A. Kolawole, V. Pillay, Y. E. Choonara L.C. du Toit and V. M. K. Ndesendo. The Influence of Pore Morphology on the Matrix Integrity, Drug Loading and Drug Release from a Porous Delivery Platform. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix C21).

5. P. Bawa, V. Pillay, Y. E. Choonara L.C. du Toit and V. M. K. Ndesendo. Formulation and Evaluation of a Buoyant Prolonged Release Drug Delivery System. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS),

November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix C22).

6. D. Reddy, V. Pillay, Y. E. Choonara L.C. du Toit and V. M. K. Ndesendo. Evaluation of the Disintegration Characteristics of a Multi-Configured Oramucosal Drug Delivery System. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix C23).

7. Z. Khan, V. Pillay, Y.E. Choonara, L.C. du Toit and V.M.K. Ndesendo. *In Vitro* Evaluation of the Drug Release, Swelling and Erosion Dynamics of a Layered Multi-Disk Tablet Matrix. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix C24).

8. S. Cooppan, V. Pillay, Y. E. Choonara, L.C. du Toit and V. M. K. Ndesendo. Physical Characterization of a Novel Composite Membranous System for Rate-Modulated Drug Delivery. Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), November 16-20, 2008, Georgia World Congress Center, Atlanta GA, USA. (Abstract in Appendix C25).

E. Accolades

1. Postgraduate Merit Award (PMA), University of the Witwatersrand, South Africa, 2008 and 2009.
2. Bradlow Postgraduate Award (BPA), University of the Witwatersrand, South Africa, 2008 and 2009.

3. University Council Postgraduate Award (UCPA), University of the Witwatersrand, South Africa, 2008 and 2009.
4. Novartis BioCamp Seminar Award, Novartis International Biotechnology Leadership Camp, Cambridge, Massachusetts, USA, 2009.
5. Novartis excellence award for the outstanding performance at Novartis BioCamp 2009, Novartis International Biotechnology Leadership Camp, Cambridge, Massachusetts, USA, 2009.

F. Grants and Funding

1. A scholarship from Norwegian Agency for Development Co-operation (NORAD)-NORWAY, August 2006-August 2009.
2. Medical Faculty Research Endowment Fund (MFREF), University of the Witwatersrand, South Africa, 2008 and 2009.
3. International conference travelling grant, University of the Witwatersrand, South Africa, 2008
4. Academy of Pharmaceutical Scientists of South Africa (APSSA) conference grant, APSSA, South Africa, 2008.

SUMMARY

HIV/AIDS was discovered almost a quarter of a century ago and has so far claimed the lives of more than 25 million people worldwide. Developing countries remain disproportionately affected, with sub-Saharan Africa contributing more than two-thirds of infections globally. Sexual transmission is the primary route of HIV/AIDS acquisition, and women bear the greatest burden of this pandemic. We are now at a stage where biotechnological advances are needed that can either cure HIV/AIDS, stimulate the immune system to produce anti-HIV-antibodies by vaccination, or prevent HIV infections. One of these advances has been the development of various microbicides. However, a lack of effective drug delivery systems for these agents has remained as a rate-limiting step towards successful HIV prevention. In an attempt to overcome this problem, this study aimed at designing and developing a novel intravaginal bioadhesive polymeric device (IBPD) as a delivery system to effectively deliver a microbicide {polystyrene sulfonate (PSS)} and antiretroviral (ARV) {3'-azido-3'-deoxythymidine (AZT)} combination to the vagina. The development of a successful intravaginal microbicidal drug delivery system requires the design of a formulation to deliver the microbicide-ARV combination in a safe, effective, and consistent manner. The first step therefore was to undertake extensive preliminary screening studies on various polymeric materials using a one variable at a time (OVAT) approach to find suitable polymers for developing an IBPD. Initially 18 biodegradable and biocompatible polymers were employed to produce 62 formulations that were further screened through the OVAT approach to result in 15 lead formulations.

Two major concerns of this study were the attainment of satisfactory residence time of the IBPD in the vagina as well as the ability of the IBPD to contain and release the microbicide-ARV in a controlled manner. Therefore, optimization of the IBPD was based on these two requirements for which proper matrix integrity was a pre-requisite. Artificial neural networks (ANNs), a computational technique that is able to simulate the neurological processing ability of the human brain through mathematical modeling, was employed for optimization. The ANN approach confirmed that 5 of the 18 studied polymers could be suitable for the development of an optimized IBPD. To finally attain good vaginal retention for the developed delivery system, extensive bioadhesivity testing was undertaken on the optimized device. Thorough *in vitro* and *ex vivo* bioadhesivity analysis was conducted using physicomechanics and computational structural modeling. Allyl penta erythritol-crosslinked poly acrylic acid (APE-PAA) appeared to contribute most to the bioadhesivity. Apart from being employed as a matrix component, PAA was further used as a coating agent to achieve extended bioadhesivity within the posterior fornix of the vagina. Since prolonged release and suitable permeation of the microbicide-ARV across the vaginal tissue was a critical requirement of this study, the device was designed to provide a controlled and prolonged drug release. Prolonged release for up to 72 days was achieved. Furthermore, the design was constructed to ensure that the released drug could permeate into the vaginal tissue and be retained substantially. This was determined by measuring drug flux through *ex-vivo* permeation studies using freshly excised pig vaginal tissue in a Franz Diffusion Cell (FDC) apparatus.

The ultimate aim of the study was to have the IBPD well accommodated in the vagina for successful prevention of STIs and HIV infection. Achievement of this aim was ensured by undertaking extensive *in vivo* studies in Large White pig model. The IBPDs were inserted under anaesthesia into the posterior fornix of the vagina, using a novel applicator. To detect the retention of the IBPDs and determine their sequential biodegradation pattern in the vagina, X-ray imaging was employed, using radio-opaque Barium Sulphate ($BaSO_4$). To demonstrate that the developed drug delivery system acted locally and was only minimally absorbed systemically, blood samples were taken from the jugular vein of each pig at pre-determined time intervals and subjected to UPLC analysis. The drug content in the vaginal tissue at the end of the study was also determined. Histopathological evaluation was carried out on vaginal epithelium to access the potential for toxicity of the IBPDs. The drug content analysis revealed that greater amounts of AZT and PSS were retained in the vaginal tissue with relatively small quantities (AZT:17%; PSS:13%) crossing into the systemic circulation. The results from the toxicity studies showed that the IBPDs were safe for use. This suggests that the developed drug delivery system (the IBPD) may be suitable for application in the prevention of STIs and HIV infections.

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Coming to this end, I would like to express my special gratitude, thanks and gratefulness to my wife Christine, my daughter Karen and my son Collins, for being pillars of strength during my study period, for accepting to undergo the endurance of staying without me for the whole period of my studies and for their constant love, persistent prayers, support and encouragement during this meandering arduous journey. May this achievement be a reward for their endurance. I am also highly endeavored to my Dad, Mum and my Mother-in-law for their constant prayers, wisdom, love, encouragement and support throughout my studies. I am sure that this work makes them very happy and proud. I also extend my thanks to my brothers, sisters, brother-in-laws and sister-in-law for their love and support towards this achievement.

Last but certainly not the least, I would like to thank the Almighty God and his son Jesus Christ for what They have bestowed upon me.

ANIMAL ETHICS DECLARATION

I hereby confirm that the following study entitled “A Novel Controlled Release Intravaginal Polymeric Bioadhesive Device” had received the approval from the Animal Ethics Committee of the University of the Witwatersrand with ethics clearance number 2007/25/05. (Appendix D).

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Chapter One

Background, Rationale and Motivation for this Study

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CHAPTER ONE

BACKGROUND, RATIONALE AND MOTIVATION FOR THIS STUDY

1.1 Introduction

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) and other sexually transmitted infections (STIs) are a burgeoning problem globally (Nicoll et al., 2000; Liu et al., 2005; Pusch et al., 2005; Todd et al., 2006; Becker et al., 2008; Lazarus et al., 2008; Bártolo et al., 2009; Nijhawan et al., 2009). AIDS has caused the mortality of more than 25 million people since it was first recognized in 1981 and has become one of the most destructive pandemics in history (UNAIDS/WHO, 2006a). With reference to the recent global estimates, more than 42 million people have contracted HIV/AIDS (90% of these in developing countries), 5 million became infected in 2005 and more than 3 million deaths occurred from HIV-related diseases in 2005 (Malcolm et al., 2006; Nikolic et al., 2007). According to a UNAIDS report, more than 6,800 people become infected with HIV daily (UNAIDS, 2007a and b). Furthermore, it has been estimated that heterosexual transmission of HIV accounts for over 90% of adult infections worldwide and that male-to-female transmission is eight times more likely to occur than female-to-male transmission. The biological reason is that women are more susceptible to STIs and HIV/AIDS than men due to larger mucosal exposure to seminal fluids as a result of the anatomical structure of the vagina. According to the recent UNAIDS/WHO global report, over 19 million women worldwide are currently living with HIV/AIDS, meaning that the proportion of HIV/AIDS occuring in women is now nearly 50% (UNAIDS/WHO, 2006b; UNFPA, 2006; Ambrose et al., 2008). The impact of HIV/AIDS on women continues to gain significance in terms of the number of women that become infected everyday and the implications thereof to their children and sex partners (Weeks et al., 2004; Chakraborty,

2008; Skoler-Karpoff et al., 2008). Once infected with an STI, many women are asymptomatic and may remain untreated, thus increasing their susceptibility to HIV infection. The effects of untreated STIs in women tend to be more serious than in men. For instance, it may result in the development of pelvic inflammatory disease (PID), which can subsequently lead to infertility or a life-threatening ectopic pregnancy. In addition, women living in low socio-economic settings are more vulnerable to STIs and HIV/AIDS than men because of their lower social status that may be related to their lower income and gender discrimination, which may render them the inability to refuse unsafe sexual practices in some cultures. In view of the above, coupled with a lack of effective protective measures, it is apparent that many women are at high risk of acquiring STIs or HIV/AIDS.

Infection with HIV remains an incurable condition (Division of HIV/AIDS Prevention, 2003; Frieden et al., 2005; Amet et al., 2008; Sing and Govender, 2009; Xu, 2009). For the foreseeable future the control of HIV/AIDS will depend on the prevention of infection and alleviation of the disease process in affected persons. HIV is transmitted through direct contact of the systemic circulation or mucous membranes with bodily fluids infected with HIV, such as blood, semen, vaginal fluid, pre-seminal fluid and breast milk (San Francisco AIDS Foundation, 2008). Transmission of HIV may occur during anal, vaginal or oral sex, contaminated blood transfusions, hypodermic needle pricks, exchange between mother and child during pregnancy, childbirth or breastfeeding. However, the highest rate of HIV transmission is through the exposure of the vaginal mucosal surface to HIV during sexual intercourse (San Francisco AIDS Foundation, 2008). To date, vaginal epithelial mucosal surfaces are the major route of HIV infection worldwide (Greenhead et al, 2000; Shattock, 2001; Wira et al., 2004; Lackner and Veazey, 2007; Belyako and Ahlers, 2008). This mode of transmission is increasing in prevalence more rapidly than any other (Royce et al., 1997; Wortley and Flemming, 1997). The transmission of HIV is said to be either by selective penetration or selective amplification within the infected host (Verhofstede et al., 2003). Interruption of cell-associated transmission requires an understanding of the

processes by which a virus moves from infected donor cells to cells of the recipient. The migration of HIV-infected cells and the movement of assembled virus particles out of the infected donor cell are critical to this process. Studies have shown that budding of HIV-1 occurs selectively through lipid rafts on the epithelial cell surface (Nguyen and Hildreth, 2000; Chazal and Gerlier, 2003; Herzberg et al., 2006). In addition, the ability of lipid rafts to act as adhesion platforms has been shown to facilitate cell-cell interactions and migration, which may be important for cell-to-cell transfer of the virus and for entry of infected cells through genital tract epithelia (Krauss and Altevogt., 1999). Therefore, understanding of the first critical events in genital mucosal transmission of HIV infection is important in developing strategies to block or limit such transmission.

The majority of deaths from HIV/AIDS have occurred in sub-Saharan Africa where the prevalence rate for sexually active adults is greater than 35% (Pisani et al., 2003; Berger, 2004; Malcolm et al., 2006). This culminates in causing the death of economically active adults with the accompanied macro-economic challenges. Much of the debates and the perpetual carnival of meetings addressing the HIV/AIDS epidemic that have taken place have ignored the truth about human sexual behavior which includes the sexual desire of women, but rather focuses on the 'vulnerability of women' and thus is an 'incomplete" explanation for higher rates of HIV infection among women and girls (Berger, 2004). At present, the current use of ARV treatment reduces both the mortality and morbidity associated with HIV infection, but routine access to antiretroviral medication is not available in all countries. Furthermore, the stigma accompanying HIV/AIDS is more severe than that associated with other life-threatening conditions and extends even to healthcare providers and volunteers involved with the care of people living with the disease.

Although the immunopathogenetic events that culminate in HIV transmission during unprotected sex are still poorly understood, it is now clear that STIs enhance both HIV

susceptibility and secondary transmission (Staff, 2002; Rebbapragada and Kaul, 2007 Levinson et al., 2009). STIs may enhance HIV transmission through the following mechanisms: i) the impairment of innate mucosal defenses, including the integrity of the mucosal epithelium itself; ii) the local induction of pro-inflammatory cytokines that enhance HIV replication; iii) the recruitment of activated immune cells that might serve as targets for initial HIV infection and enhance local HIV replication; iv) enhanced susceptibility to other STIs and/or alterations in the normal vaginal microflora; and v) impairment of systemic HIV immune control with an increased viral load in both blood and the genital tract. STIs affect men and women of all backgrounds, with approximately two-thirds of cases occurring in individuals less than 25 years of age (Brown et al., 1999; Drug Digest, 2004). Based on a WHO report that was last revised in 2007, 340 million new cases of curable STIs (syphilis, gonorrhoea, chlamydia and trichomoniasis) occur annually worldwide in adults aged 15-49 years (Reddy et al., 2002; Facts sheets, WHO, 2007). The same report has indicated that STIs and their associated complications are among the top five disease categories for which adults seek health care (Facts sheets, WHO, 2007). According to WHO, STIs, excluding HIV, rank second to maternal factors as causes of disease and death in women of childbearing age in the developing countries (WHO, 2007).

Practising of unsafe sex by individuals infected with HIV infection predisposes them to the risk of acquiring STIs. Such co-infection leads to both ulcerative and non-ulcerative STIs that ultimately promote HIV transmission by augmenting HIV infectivity and host susceptibility by a variety of biological mechanisms (Royce et al., 1997). Thus, regular screening and prompt treatment of STIs should be part of the routine care of HIV-positive patients. The management of some STIs such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis* is normally the same for HIV-positive and negative individuals. However, many of the sexually transmitted pathogens result in more prolonged or severe infections in association with HIV/AIDS, and treatment may be

required for longer periods and may have a higher failure rate (Royce et al., 1997). For instance, infection with herpes simplex virus may require higher doses of antiviral therapy to suppress recurrences and resistance. Infection with human papillomavirus (HPV) causes genital warts that may respond less to treatment and may recur more frequently. The incidence of HPV-associated neoplasia such as anal cancer and cervical squamous intraepithelial lesions is also increased (Cohen, 2004).

The management of syphilis in HIV-infected individuals can be a challenge (Butsashvili et al., 2001; Malow et al., 2009). Interpretation of serological tests for syphilis can be difficult, as titers can be unusually high, low, or fluctuating. False-negative results have also occurred (Baughn and Musher, 2005; Amy et al., 2007). HIV-infected patients may have a higher risk of neurological complications in early syphilis, therefore making it a necessity to have a cerebrospinal fluid examination before treatment. Although such patients may have a higher rate of treatment failure, this is likely to be minimal (Rolfs et al., 1997), thus allowing clinicians to recommend the same treatment for primary, secondary, and early latent syphilis regardless of HIV status. However, some clinicians prefer to treat all HIV-infected patients with the regimen recommended for neurosyphilis (Thompson and Samuels, 2002; Goh, 2005). In any case, regardless of the treatment regimen, careful long-term follow-up is essential.

Despite the magnitude of the HIV pandemic, it is surprising that the probability of HIV transmission during a single episode of unprotected sex ranges from 0.1–1.0% only, irrespective of the type of sexual practice or biological factors in each partner (Royce et al., 1997). Furthermore, studies have shown that HIV acquisition and secondary transmission is highly influenced by the presence of genital tract co-infections in either partner, particularly STIs (Cohen, 2004; Kaul et al., 2008; Sheung et al, 2008; Spinillo et al., 2008). This may have accelerated spread of the HIV/AIDS epidemic worldwide and might serve to explain the profound regional and gender-based differences in HIV/AIDS

epidemics. Thus, there is a need to elucidate the immunopathogenetic events that underpin HIV sexual transmission at a mucosal level. Although studies on HIV immunopathogenesis have traditionally focused on systemic immune events, it is now evident that HIV transmission occurs mostly across the penile, oral, cervico-vaginal or rectal mucosa (Kaul et al., 2008). What is required is a better understanding of how HIV transmission occurs, and how it is influenced by genital co-infections and their effect on the mucosal micro-environment. This could contribute significantly in developing new technologies for HIV prevention modalities, such as HIV/STI microbicidal drug delivery systems.

1.1.1 Current status of the HIV/AIDS treatment modalities

The treatment of HIV/AIDS was revolutionized by the introduction of highly active anti-retroviral therapy (HAART) in the 1990s (Glare, 2001; Greenberg, 2007). Use of HAART has resulted in a dramatic decrease in the incidence of HIV-associated morbidity and mortality (Palella et al., 1998; Farmer et al., 2001; Thirlwell et al., 2003; Battaglioli-DeNero, 2007; Marin-Muller et al., 2009). However, cohort studies have found that regimens fail in many patients basically due to virological rebound or because initial suppression could not be achieved (Greub, et al., 2001, Fischer et al., 2003). A rise in plasma viral load indicates treatment failure which may be due to several reasons including low level of adherence to the therapy, emergence of drug resistance, suboptimal drug levels, and use of less potent regimens. The cluster of differentiation 4 (CD4) cell count is normally used as the main determinant in deciding when to start treatment since there is an increased mortality in patients with CD4 counts below 200/mm^3 (WHO, 2006). Treatment is therefore indicated in all patients with a CD4 count <200/mm^3 and in symptomatic patients (those with AIDS according to the 1993 Centers for Disease Control and Prevention classification as well as patients with wasting, thrush, or unexplained fever for >2 weeks) (Peritt et al., 1999). However, there is a lack of data to determine at which CD4 level antiretroviral therapies should be started for asymptomatic patients with CD4

counts >200/mm^3. Quite often, an asymptomatic patient with a CD4 count >350/mm^3 presents with a high pre-treatment viral load (>50,000-100,000), which is another predictor of clinical progression (Bartlett, 2003; Kassaye and Katzenstein, 2003; Benson et al., 2004). Therefore, the best option is to individualize treatment decisions by taking into account diverse factors such as the CD4 count and its rate of decline, viral load, patient interest and potential to adhere to therapy, individual risks of toxicity, drug interactions, as well as the risks and benefits of early versus late initiation of treatment. Adherence is a key determinant factor for any successful treatment regimen.

To date, current treatment regimens have not been able to eradicate HIV and therefore the strategy is to: i) maximize inhibition of viral replication; ii) restore and/or preserve immunologic function; iii) improve quality of life; and iv) reduce HIV-related morbidity and mortality. This must also consider associated problems such as expensive regimens, pill burdens, complicated dosing schedules, drug-specific toxicities, drug interactions, viral resistance, and long-term complications, especially lipodystrophy and metabolic changes. Thus far, at least 19 antiretroviral (ARV) agents have been approved for the treatment of HIV infection (Pulido and Arribas, 2003; Marks and Gulick, 2004; Stanic and Grana, 2009), some of them available through expanded-access programs to allow a large variety of potential combinations. However, no data exists to demonstrate the superiority of one regimen over another and therefore it is considered that the first regimen may afford the best opportunity for long term control of viral replication if administered and monitored properly (Vrijens and John 2005; WHO, 2006). Furthermore, the importance of individualization of therapy should always be emphasized. This is a complex clinical area which therefore requires proper management by experts in the field.

1.1.1.1 Combination therapy in HIV/AIDS

It is now more than a decade since it became apparent that treatment of HIV infection with only one ARV agent is associated with rapid development of viral drug resistance (Richman et al., 1994; Brenner et al., 2002; Martinez-Picado and Wai, 2007; Marconi et al., 2008). Studies have shown that resistance is becoming more prevalent in ARV-naive patients and therefore ARV drug resistance testing is now an integral part of the management of the HIV-infected persons (Dam et al., 2001; Little et al., 2002). Clinical trials have shown that combining two antiretroviral agents leads to improved virological and immunological responses, when compared to the use of a single agent. A three-drug combination has proven to be even more effective than a two-drug combination (Bulgheroni et al., 2004: Youle et al., 2006; Greenberg, 2007). Therefore, ARV monotherapy is not recommended unless under special cases such as in pregnancy, to reduce perinatal transmission. Combination of two or more ARVs has now been adopted as standard treatment for maintaining viral suppression and minimizing the emergence of resistant strains, thereby reducing the risk of disease progression and death. The recommended three drug regimen of HAART usually include two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) plus a Protease Inhibitor (PI), or a Non-nucleoside Reverse Transcriptase Inhibitor (NNRTI), or three NRTIs.

PI drugs may be used singly or in combination with low-dose ritonavir as a boosted regimen that increases trough levels, minimizing opportunities for viral replication and potentially allowing for activity against moderately resistant strains of virus (Stanic, 2005; Lucas, 2008). Boosting also affords a more convenient regimen in terms of pill burden, scheduling, and elimination of food restrictions. Hepatotoxicity is the commonest adverse effect of PIs, especially ritonavir (Lucas, 2008). An NNRTI-based regimen can be as effective as a PI-based regimen with the additional advantage of reserving PIs for a later date. Hepatotoxicity may be caused by both, but the risk with nevirapine (an NNRTI), is twice that of efavirenz (also an NNRTI) after adjustment for PI use and hepatitis C virus

co-infection (Pulido and Torralba, 2002). An advantage of efavirenz is its once-daily dosing, but central nervous system side effects can cause hyperexcitability, vivid dreams, mood disturbances, and personality changes. Both may cause severe skin rashes (Pulido and Torralba, 2002). For triple NRTI regimens, the most promising are the ones containing abacavir (Kaufmann, and Cooper, 2000; Kessler, 2005; Dieterich, 2006). Advantages of NRTI regimens include a twice-daily schedule, low pill burden (i.e. available in a fixed-dose combination), relatively few adverse effects, and lack of undesirable pharmacokinetic interactions (Keiser, 2002; Sharma and Chakraborty, 2009). Furthermore, there is the advantage of reserving the other two classes of drugs which may be used at a later stage. It has been observed that hypersensitivity reactions to abacavir occur in 5% of patients subjected to this regimen (Hervey and Perry, 2000; Khanlou et al., 2005). A disadvantage of NRTI regimens is that they are considered ineffective in patients with high baseline viral loads or low CD4 counts (Khanlou et al., 2005; Gallant, 2008). Studies have shown that despite the introduction of HAART, multiple treatment failures still occur (Ledergerber et al. 1999; Lucas et al., 1999; Moyle, 2003; Flint et al., 2006; Wilcox and Saag, 2008). Many strategies have been proposed to deal with repeated treatment failures, but with dissapointing results (Lucas et al., 1999; Idemyor, 2004; Lemiale and Korokhov, 2009). Thus, despite the emergence of ARVs, HIV infection is still incurable. Therefore, the mainstay in the control of the pandemic remains primary prevention.

1.1.2 Anatomy and physiology of the vagina in relation to drug delivery

The human vagina is a tubular, fibromuscular organ that extends from the cervix of the uterus to the vaginal vestibule with a length of about 9 cm (das Neves and Bahia, 2006). The vagina wall is composed of 4 layers namely the stratified squamous epithelium, lamina propria, muscular layer, and tunica adventitia (Figure 1.1). The epithelial thickness is about 200–300μm, and it is not significantly affected by the reproductive cycle (Song et al., 2004). The epithelial layer forms a series of transverse folds called rugae which

dramatically increase its surface area. Vaginal ecology is normally influenced by factors such as the glycogen content of epithelial cells, glucose, pH, hormonal levels, trauma during sexual intercourse, birth-control method, age, antimicrobial treatment and child delivery (Budtz-jorgensen, 2000; Verman and Garg, 2000; Justin-Temu et al., 2004; Valenta, 2005). The vaginal flora is a dynamic system composed of facultative and obligate aerobes and anaerobes with *Lactobacillus* (Döderlein's bacilli) as the most prevalent organism (He et al., 2004). *Lactobacilli* produce sufficient lactic acid to acidify vaginal secretions (including the seminal fluid) to a pH of 3.5–5.5 which covers the pH range that keeps the vagina healthy (4.0-5.0) by inactivating HIV and other pathogens. *Lactobacilli* maintain this pH range by converting glycogen from exfoliated epithelial cells to lactic acid (Boskey et al., 2001). Thus, *Lactobacilli* play a vital role in maintaining the healthy vaginal acidic environment. Normally, vaginal pH changes with age, stages of the menstrual cycle, infections and sexual arousal. Therefore, in most women, a pH gradient exists in the vagina (Valenta, 2005). Possible causes of alterations in vaginal pH are various bacterial infections, yeast infections particularly of the *Candida* species type, STIs, premature rupture of fetal membranes, hormonal imbalance, postoperative infected wounds and excessive intimate care (Vitali et al., 2007). A disturbance in the micro-ecological balance in the vagina (e.g. dysbiosis) is often connected with an increase in the pH level (Mastromarino et al., 2009). Semen, menstrual flow, cervical and uterine secretions act as alkalizing agents in the vagina changing the pH from acidic to near-alkaline. (Desphande et al., 1992; Richardson and Illum, 1992). The vagina may also act as a site for drug absorption and action. The anterior and posterior vaginal fornixes are recesses (along with the other fornices) that exist due to the projection of the cervix to the vagina. If there is no cervix, there are no fornices. The deepest of these fornices is the posterior fornix, which is the most isolated from any activities. For instance, it is the only part of the vagina that is unlikely to be disturbed by sexual intercourse. It also happens to be out of the way of the menstrual flow, because it is behind and above the cervix. Thus, it is an ideal site for drug delivery application. Vaginal pH is an important parameter for

the efficacy of a drug delivery system (Ramsey et al., 2002). For instance, it may influence drug absorption and must therefore be considered during the development of any such drug delivery system. It has been observed that changes in hormonal levels (especially estrogen) during the menstrual cycle may lead to pH changes as well as alterations in the thickness of the epithelial cell layer, width of intercellular channels and secretions (Cajander and Rylander, 1988; Owen, 1975). Variations in enzyme activity (endopeptidases and aminopeptidases) with hormonal changes may further complicate the problem of achieving consistent drug delivery (Furuhjelm et al., 1980; Pschera et al., 1989). During the post-menopausal period, women may experience progressive atrophy of the vaginal epithelium with elevation of the pH (6.0–7.5), and a decrease in the quantity of vaginal secretions (das Neves, 2006; Simon et al., 2008). These features may significantly impair the performance of a drug delivery system and therefore should be considered during development and evaluation of vaginal drug delivery systems.

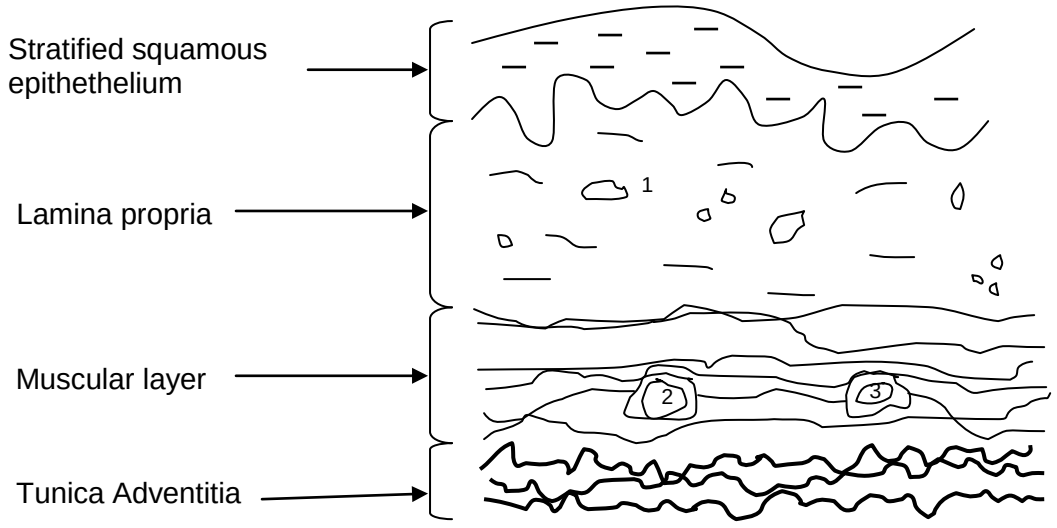


Figure 1.1: A schematic of the vaginal wall showing 1) capillary vessels, 2) artery and 3) vein, (Adapted: das Neves, 2006).

1.1.3 The vaginal mucus in relation to bioadhesion

The target for the interaction of bioadhesive agents in the vagina is the mucus. Vaginal mucus is highly viscous and forms a protective coating over the lining of the vaginal epithelium in contact with external media. The vaginal mucus coating has several important physiological functions. It acts as a physical barrier to pathogenic organisms (Eggert-Kruse et al., 2000). It is a mixture of large glycoproteins (mucins), water, electrolytes, epithelial cells, enzymes, bacteria, bacterial products and various other materials depending on the source and location of the mucus (Junginger, 1990). Several mechanisms have been proposed to explain the interaction between a bioadhesive polymer and a biological surface, most being based on the structure of the mucin (Ch'ng et al., 1985; Lehr, 1995). The force and work of adhesion of a given bioadhesive system on a biological tissue is a good indicator of its bioadhesive capacity (Lejoyeux et al., 1989; Valenta et al., 2001).

Vaginal secretions are a mixture of fluids produced from various sources (Stechever et al., 2002). Although the vaginal epithelium is often considered to be a mucosal surface, it lacks goblet cells and therefore cannot release vaginal secretions such as mucin directly (Valenta, 2005). Vaginal mucus originates mostly from the endocervix, with small contributions from the endometrium and fallopian tubes. Vaginal discharge is comprised of a mixture of endocervical mucus, transudates through the epithelium, exfoliating epithelial cells, secretions of the Bartholin's glands, leukocytes, and endometrial and tubal fluids (Desphande et al., 1992).

Studies have shown that contact of synthetic polymers that have ionic characteristics with the cervical mucus may cause significant changes in the structure of mucus gels (Willits and Saltzman, 2001; Espinosa et al., 2002; Valenta, 2005). Mucin is negatively charged due to the presence of sialic acid residues on its carbohydrate-rich regions (Valenta, 2005). It has been observed that anionic {e.g. poly(acrylic acid) (PAA)}, non-ionic {e.g.

poly(ethylene)glycol} and cationic (e.g. polyvinyl pyridine) molecules may interact with the mucin gel to form regions with higher fiber density (Valenta, 2005). The fiber aggregation is mainly a result of electrostatic interactions between the polymer and the mucin. The ability to modify the structure of mucus gels by adding biocompatible synthetic polymers may provide new prevention measures for pathogenic organisms that infect the vaginal mucosa such as STI and HIV pathogens (Valenta, 2005; das Neves, 2006). Thus, the vagina has been targeted as a potential site for delivery of various drugs. The delivery of compounds is almost exclusively through the vaginal epithelium as described below. One of the most useful applications is the delivery of drugs that destroy or render dysfunctional spermatozoa and pathogens such as viruses and bacteria.

1.1.4 The vagina as an application site for drug delivery

The vagina, much as it has been proven to be a potential route for systemic drug delivery and uterine targeting, also has huge potential for the administration of drugs intended to confer a local effect, such as microbicides (Richardson and Illum, 1992; Jiménez-castellanos et al., 1993; Vermani and Garg, 2000; Knox, 2004; Schiffman, 2004; Hussain and Ahsan, 2005; Valenta, 2005; das Neves and Bahia, 2006; Sitruk-Ware, 2007; das Iyer et al., 2008; das Neves et al., 2008; Nelson, 2008; Lai et al., 2009). The epithelium, normal flora, immune cells and acidic pH contribute to the vaginal defences which together with vaginal vascularity provide a potential route for local drug delivery (Li et al., 2000; Vermani and Garg, 2000; Ogra et al., 2001; Valenta, 2005; Andrews et al., 2009). The vaginal commensals release organic acids, inorganic compounds and antimicrobial peptides into the milieu of the vaginal fluid which includes hydrogen peroxide, lysozyme, lactoferrin, bacteriocin-like substances, biosurfactants, secretory leukocyte protease inhibitor (SLPI), human neutrophil peptides (HNP-1, -2, and -3), and human ß-defensins (HBDs) which aid in killing pathogenic invaders (Wagner and Levin, 1978; Huggins and Preti, 1981; Cohen et al., 1984; Boris and Barbes, 2000; Valore et al., 2002; Venkataraman et al., 2005; Cole and Cole, 2007). Furthermore, it has been demonstrated

that the anatomical position of the vagina in humans, favors secure retention of vaginal formulations (Holmes, 2000; Iyer et al., 2008). Thus far, there has been considerable progress in the field of vaginal drug delivery in the recent years but with some challenges due to various factors (Merabet et al., 2005 Dobaria et al., 2007). To date, only a limited number of vaginal dosage forms are available, but increasing efforts are being made to make studies in this area of research.

Apart from the novel drug-loaded inserts which are currently available on the market, there are also hydrogel drug delivery systems which may display higher retention times (Kumar et al., 2002; Lin and Metters, 2006). Most vaginal drug delivery systems are based on bioadhesive polymers since bioadhesitivity is an intrinsic pre-requisite for drug retention on the vaginal epithelium (Chang et al., 2002; Francois et al., 2003; Valenta, 2005; Perioli et al., 2009). Bioadhesive polymers are able to swell while eroding slowly when exposed to an aqueous environment and therefore may well facilitate controlled drug release (Gavin et al., 2002; Kumar et al., 2002; Lin and Metters, 2006; Sudhakar et al., 2006). Significant efforts have been made to improve the adhesive properties of local drug delivery systems resulting in better retention and consequently improved availability and increased therapeutic efficacy (Gavin et al., 2002; Asane et al., 2008; Madhavlal and Manordas, 2009).

Permeation of drugs through the vaginal epithelium is influenced by the physicochemical properties of the drug carrier and the tested drug, in terms of molecular weight, solubility, dissolution rates, lipophilicity, ionization, chemical stability, thermal stability, and also the characteristics of the membrane (Williams, 2003, Justin-Temu et al., 2004; Sandri et al., 2004). In addition, physiological factors also play a role in the permeation process, for example cyclic changes in the thickness of the vaginal epithelium, fluid production volumes and composition as well as vaginal pH (Hussain and Ahsan, 2005). For instance, an increase of vaginal fluid volume may increase the absorption rate of poorly water-

soluble drugs. Changes in the vaginal pH may also have an influence on the release of pH-sensitive drugs from vaginal delivery systems (Hussain and Ahsan, 2005). Lipophilic molecules permeate faster than hydrophilic molecules (van Eyk and van der Bijl, 2005). Studies have shown that for the vaginal epithelium, the molecular weight cut-off points for permeation may be higher than those found in other epithelial surfaces (Williams, 2003, Justin-Temu et al., 2004). In addition, ionization may affect the permeation of drugs across the vaginal epithelium (Valenta, 2005; Nicolazzo and Finnin, 2008). Drugs that are in an unionized state penetrate epithelial membranes more freely (Williams, 2003, Justin-Temu et al., 2004). Generally, chemical compounds cross the epithelial cell layers by two major pathways, namely the intercellular (paracellular) pathway where compounds pass between cells and the intracellular (transcellular) pathway where compounds pass through the cells. In both cases, drug permeation takes place either through passive diffusion, carrier-mediated transport or endocytosis (Meijer et al., 1990; Kapitza et al., 2007). For mucosal delivery of most drugs, the intercellular route of diffusion predominates (Ghate and Edelhauser, 2006). Furthermore, studies have shown that two types of pathways exist within the intercellular space (Wertz and Squier, 1991, Harris and Robinson, 1992; Williams, 2003). The first one is the hydrophobic pathway through the lipid bilayers while the second is the parallel hydrophilic pathway along the narrow aqueous regions that are associated with the polar head groups of the lipids. The penetration of the compound through the epithelial barrier increases as the hydrophobicity of a permeant molecule increases (Wertz and Squier, 1991, Harris and Robinson, 1992; Williams, 2003; van Eyk and van der Bijl., 2005).

1.1.4.1 Advantages and disadvantages of vaginal drug delivery

The vaginal route of drug administration offers advantages over other routes such as the avoidance of hepatic first-pass metabolism, reduction in the incidence and severity of side effects within the gastrointestinal tract (GIT), and reduction in hepatic side effects of steroids used in hormone replacement therapy or contraception (Patel, 1984; Cedars et

al., 1987; Vermesh et al., 1998; Dezarnaulds and Fraser, 2002). The vaginal route overcomes the inconvenience caused by pain, tissue damage and possible infection by parenteral routes. Furthemore, it has the potential for uterine targeting of active agents such as progesterone and danazol (Cicinelli et al., 1998, 2000; Einer-Jensen et al., 2002). Compared to the oral route, the vagina might prove better for the delivery of hormonal contraceptives due to a lack of drug interactions often encountered in the GIT. Another advantage is the possibility for self-administration of single-dose drug delivery systems that may suffice in releasing drugs over a period of weeks or months and simultaneously provide optimal drug pharmacokinetic profiles (Cohen et al., 1984; Woolfson et al., 2000; Benkop-Schnurch and Hornof, 2003; Iyer et al., 2008).

Further advantages with vaginal administration include convenient access, prolonged retention of formulations, an extensive region for drug permeation, high vascularisation and relatively low enzymatic activity. Therefore, intravaginal drug delivery has exploitable advantages compared to other routes of administration in the area of bioavailability and controlled drug delivery (Carrington et al., 1944; Goldberger et al., 1947; Mishell et al., 1972; Fried et al., 1973; Kirton et al., 1973; Nuwayser and Williams, 1974; Johansson et al., 1975; Verman and Garg, 2000; Valenta et al., 2001; Yoo et al., 2006; Bonferoni et al., 2007; Wang and Tang, 2008). A contributing factor to the exploitable advantages conferred by the vaginal route of drug administration is the fact that the adult vaginal cavity has an extensive surface area (100-150cm^2) that is adequately accessible for self-administration purposes (Valenta, 2005). Furthermore, the vaginal route provides room for continuous programmed administration of drugs while simultaneously preventing the superfluous peaking of blood levels that is often seen with the use of oral drug delivery systems administered as discrete discontinuous doses (Valenta, 2005; das Neves and Bahia, 2006).

The vaginal route is less preferable in terms of convenience depending on the type of dosage form. The influence of the estrogen concentration on the permeability of the vaginal membrane, which can influence the pharmacokinetics of drugs designed for systemic action, is yet another disadvantage (Okada et al., 1983; Acaturk and Robinson, 1996). The quantity of vaginal fluid in the vagina of an adult woman is in the range of 2–3g/24h (Müller, 1986). This quantity however decreases as the age increases. This volume of fluid may also affect the vaginal absorption of drugs. Furthermore, high quantity of vaginal fluid may lead to the expulsion of the dosage form. Studies have also shown that typical drug delivery systems such as foams, gels, and rings may be removed by the self cleansing action of the vaginal track (Vermani et al., 2002; Alam et al, 2007; Bilensoy et al., 2007; Romano et al., 2008). Since drugs must be in solution before they can be absorbed, the presence of a film of moisture might be an advantage but contrary to this, the presence of thick cervical mucus may also act as a barrier to drug absorption. This may however be an advantage for drugs administered for the purpose of exerting a local effect as is the case with microbicidal agents. In either case, for successful vaginal drug delivery, factors such as changing physiology of the cervix and vagina, the menstrual cycle, coitus, as well as leakage of the formulation from the vagina may grossly affect performance of an intravaginal drug delivery system. These factors must be recognised during design and development of an intravaginal drug delivery system (Garg et al., 2003).

1.2 Statement of the Problem

Several therapeutic agents for curing HIV/AIDS have been developed, but, to date none has been proven to be ideally successful (Norris, 2008). Other than therapeutic agents use, other strategies proposed and adopted over the years to prevent sexually-acquired HIV infection include the promotion of abstinence, monogamy, condom use, reduction in the number of sexual partners and treatment of sexually-related infections. Yet, cultural, social and economic factors, particularly in the developing world, have resulted in these

strategies not being as successful as expected. Condom use has been the last and often preferred preventative measure advanced to combat the spread of HIV and STIs, but youth in particular do not respond appropriately to such initiatives (Fauci, 2005). When used consistently and correctly, male condoms can reduce the risk of pregnancy and many STIs, including HIV by about 80-90% (Weller and Davis; 2002; Hatcher,et al., 2004; Hearst and Chen, 2004; Holmes et al., 2004; Warner et al. 2004). Furthermore, condoms (including female condoms), are contraceptive methods that are substantially effective at reducing the risk of both STIs and pregnancy (USAID Health, 2009). There is the perception that HIV and STIs only affect 'others' and despite the significant attempts to counter the belief that only certain groups are vulnerable and at risk of infection, studies show that some young men see HIV as a disease of atypical behavior associated with rape and commercial sex workers or as a result of excessive alcohol consumption (Pettifor et al., 2005). A Reproductive Health Research Unit study by Pettifor and co-workers (2005) showed an extremely high percentage of respondents (93%), identified condom use as being an effective preventative measure but 67% did not use condoms consistently and as many as 31% had never used condoms. There is the belief that the way to protect 'innocent victims' is to stop only those who are infected from infecting others, perpetuating the view that their own conduct is unproblematic. As much of the research has failed to advance any explanation for the failure to transform knowledge into positive action, many of the crucial questions remain unasked and unanswered. In the wake of this denial, other effective female controlled measures that can protect both parties from exposure to this fatal disease are needed.

The most compelling solution to HIV/AIDS is an effective vaccine. However, after 25 years of research, development of an effective vaccine has remained unsuccessful due to various obstacles including inadequate resources, regulatory capacity concerns, intellectual property issues and mainly the scientific challenges (Malcolm et al., 2006; Lau et al., 2007; Gurunathan et al., 2009; Katsnelson, 2009). Despite major efforts and new

approaches, it is unlikely that an HIV vaccine will be available for human use in the near future (Weber et al., 2005; Hayden, 2009). This scenario, and the global estimate number of 5 million new cases of people suffering from HIV infection annually, coupled with the rising prevalence rate in women especially in resource-limited settings, identifies the core problem that this study seeks to address through a drug delivery perpective.

1.3 Rationale and Motivation for this Study

Given the devastating effects of the HIV/AIDS epidemic and the continuing difficulties in developing an effective HIV vaccine, there is a clear scientific rationale for developing alternative strategies to prevent STIs and the transmission of HIV infection. Thus, there is a need of searching for accessible, inexpensive and female-controlled pre-exposure prophylaxis strategies to prevent mucosal transmission of the virus. The most promising of these preventative approaches is the development of effective intravaginal microbicidal delivery systems (Uckun and D'Cruz, 1999; Bob, 2000; Best, 2000; van Damme, 2002a and b); Moore and Shattock, 2003; Balzarini and van Damme 2004; Malcolm et al., 2004; Uckun and D'Cruz, 2004, Mantell et al., 2005; Penttinen, 2005; Weber et al., 2005; Dhawan and Mayer, 2006; Balzarini and van Damme 2007; Eaton and Kalichman, 2007; Holmes, 2007; Nikolic et al., 2007; Brouwers et al., 2008; Kathambi, 2008; Klasse et al., 2008; Ndesendo et al., 2008; Obioha 2008; Baloglu et al., 2009; Ndesendo et al., 2009). These are bioactive agents that when administered in the vagina prior to coitus, have the potential to either prevent or reduce HIV/AIDS and STI transmission through well established mechanisms (D'Cruz et al., 2003b; D'Cruz and Uckun, 2004; D'Cruz et al., 2004a; Weber et al., 2005; Balzarini and Van Damme, 2007).

A microbicide as a preventive strategy may thus function as follows:

i) Maintenance and mobilization of normal physiological vaginal defence mechanisms in the cervicovaginal environment. A healthy, intact vaginal mucosa is an ideal natural

protective barrier against infection. This barrier may be supported by gel-forming agents that provide lubrication and an additional barrier to infectious organisms.

ii) Enhancement of the natural vaginal defence mechanisms by maintaining an acidic pH environment, commensal vaginal microflora, antibodies and antimicrobial peptides.

iii) Direct inactivation of sexually transmitted pathogens (HIV and STIs) by disrupting the pathogens' protective membranes or envelopes.

iv) Inhibition of early phases in the viral cycle of HIV and entry into the mucosal cells of the vaginal wall by specifically blocking surface proteins on the virus or by binding to receptors on human cells or by non-specifically coating the virus or human cells.

v) Interruption of the viral life cycle at a post infection stage. ARVs already used for therapeutic use may also be employed as topical microbicides. These agents act by inhibiting viral replication or local spread of HIV infection.

As opposed to the use of condoms, there may be greater potential for microbicidal drug delivery systems to empower women to protect themselves from HIV and STIs. An effective microbicide would be a valuable woman-controlled means to slow down the pace of the HIV/AIDS epidemic particularly in sub-Saharan Africa, where women aged between 15 to 24 years are three times more likely to be infected with HIV-1 compared to the same age group of men (Fauci, 2005; Quinn and Overbaugh, 2005). To date, the development of an ideal microbicide (i.e. safe, effective, acceptable, affordable and accessible) has not been entirely successful (Weeks et al., 2004; Das Neves et al, 2006; Klasse et al., 2006; Balzarini and Van Damme 2007; Nuttall et al., 2007). Only a limited number of microbicidal drug delivery systems are available. The currently available vaginal delivery systems have several limitations which include leakage, messiness, low residence time and poor bioavailability, contributing greatly to poor compliance (Justin-Temu et al., 2004; Valenta, 2005; Biradar et al., 2009). Furthermore, the results of early clinical trials for the already developed microbicides have not been very encouraging (McCormack et al.,

2001; Lazarus et al., 2008; Skoler-Karpoff et al., 2008). Therefore, efforts need to be directed at the development of novel microbicidal intravaginal drug delivery systems.

The ultimate success of any intravaginal drug delivery system depends on the formulation and the bioactive agent. This requires consideration of several variables including the bioactive agent, vaginal physiology and the design of the delivery system. For these drug delivery systems, the challenge is to design a delivery system that provides a high drug concentration in the vagina over a prolonged period of time (Benkorp-Schnurch et al., 2003). For controlled, zero-order release, sustained over prolonged periods (days extending to months), solid polymeric systems may be most suitable provided they are compatible with the physicochemical nature of the drug to be delivered and the body.

To date, drug release durations for intravaginal delivery systems are either too rapid which ultimately requires the drug to be applied several times daily (typical of gels and tablets) (Gavin et al., 2002; El-Kamel et al., 2002; Wang and Lee, 2004; Bilensoy et al., 2006) or the release period is adequate but they have been formulated for preventing HIV infection only, as is the case with vaginal rings (van Laarhoven et al., 2002; Malcolm and Woolfson., 2006). This research therefore proposes the development of a novel drug delivery system that will be active against both HIV and STIs with a controlled release effect of at least 1 month. This is a caplet-shaped device referred to as an "*Intravaginal Bioadhesive Polymeric Device "(IBPD)"* throughout this study. The delivery system will have desirable bioadhesive properties and will be capable of inducing an acidic pH environment in the vagina upon degradation, thus maintaining a wide spectrum of activity against pathogens. The main advantages of the developed IBPD are: i) it is easier and more convenient to apply a caplet than a gel or ring; ii) the developed bioadhesive caplet has desirable vaginal retention as opposed to poor retention conferred by gel formulations (Gavin et al., 2002); iii) controlled release of the drug will mean less frequent application and therefore enhanced patient compliance; iv) the developed IBPD is non-messy and

can reside in the vagina long before coitus releasing drug gradually; the IBPD will be produced at low manufacturing costs and v) the IBPD can utilize more than one preventive mechanism of action unlike existing vaginal delivery systems which utilizes single mechanism of action. The IBPD has been designed to have a dual action by releasing an ARV and microbicide simultaneously. A formulation that simultaneously delivers an ARV and microbicide was sought because it has been suggested that a vaginal drug delivery system combining several mechanisms of prevention would be more effective or have less side effects than one with a single mechanism of action as is the case with preparations currently being researched (D'Cruz and Uckun, 2004; Ramjee, 2006; Balzarini and Van Damme 2007; Cutler and Justman, 2008; Ndesendo et al., 2008, Gupta and Garg, 2009), partly due to inadequate attention to formulation, design and delivery technology development (Panttinen, 2005). The model microbicidal agent employed was Polystyrene sulfonate (PSS) (as a sodium salt) while the ARV agent was 3'-azido-3'-deoxythymidine (AZT). The dimensions of the IBPD were 22×9×5mm for insertion into the posterior fornix of the vagina using an applicator (Figure 1.2). The posterior fornix location was purposely chosen to avoid any interference with the formulation during menses and sexual intercourse (Section 1.1.2). The anterior fornix was considered to be inappropriate for this purpose because it is right in the line of entry for a male sexual organ, and the in and out movement might disrupt the adhesion of the device. The formulation was designed in such a manner that a steady-state concentration of bioactive agents could be maintained locally (within the vaginal membrane and in the vaginal inner mucosal muscularated stratum).

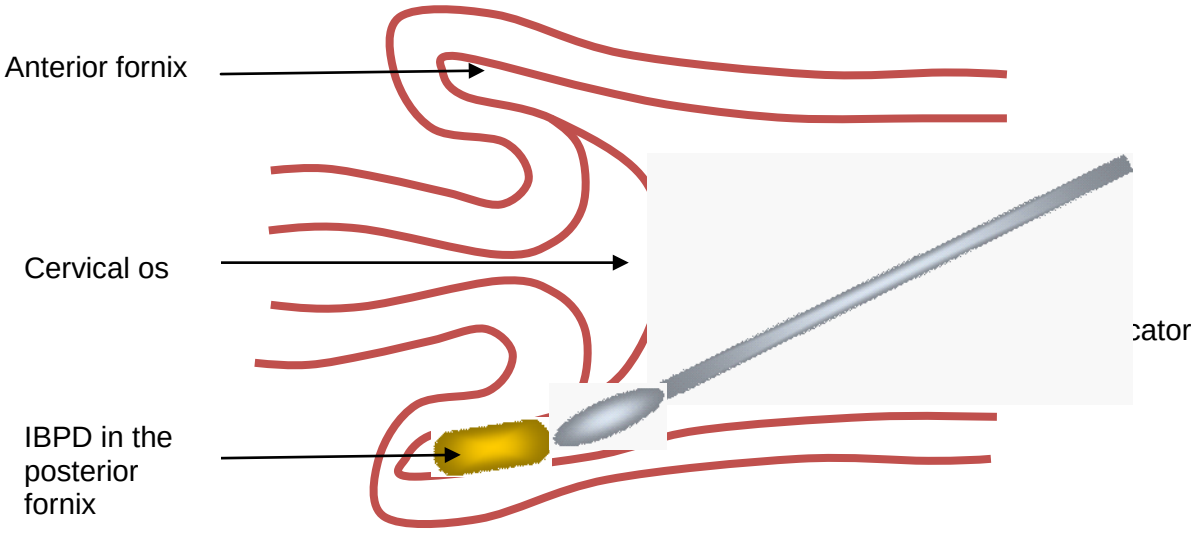


Figure 1.2: A schematic of the upper vagina region depicting the posterior fornix as the site of the IBPD application.

1.4 Aim and Objectives of this Study

The aim of this study was to design and develop an IBPD for the potential prevention of STIs and HIV infection. In order to achieve this aim, the following objectives have been outlined:

1. To conduct extensive preformulation screening work on various biodegradable and biocompatible FDA-approved polymers for the selection of lead formulations that would ultimately provide an ideal bioadhesive polymeric set which is also capable of providing controlled drug delivery.
2. To optimize the IBPD using the Artificial Neural Networks (ANN) approach.
3. To assess the bioadhesivity of the drug-free IBPD that was developed from the blends of the selected polymers both *in vitro* and *in vivo* and optimize for bioadhesivity thereof.
4. To perform *in vitro and ex vivo* analysis on the dug-loaded optimized IBPD in terms of physicomechanical and physicochemical properties, bioadhesivity, permeation and drug release characteristics.
5. To perform computational and chemometric molecular structural modeling studies on the native polymers, drug-free IBPD and dug-loaded optimized IBPD.

6. To conduct *in vivo* studies on the drug-loaded optimized IBPD in the pig model.

1.5 Overview of this Thesis

Chapter 1 provides the study protocol which contains the introduction, rationale, motivation for the study and objectives. The introduction provides a concise description of the current situation regarding the HIV/AIDS epidemic, proposing the way forward for which the enaction is taken care of by the highlighted aim and objectives. A clear description on the role played by STIs in enhancing both HIV susceptibility and secondary transmission is provided. An overview of the treatment modalities that have been employed in the management of HIV/AIDS is given. In addition, a description is provided on the anatomy and physiology of the vagina in relation to drug delivery, and the vagina as an application site for drug delivery, as well as the advantages and disadvantages of this route of adminstration. The characteristics of an ideal vaginal drug delivery system are described, and the concept of a microbicidal-Intravaginal Bioadhesive Polymeric Device is introduced.

Chapter 2 provides a descriptive review of the current intravaginal drug delivery approaches that have been employed for the prophylaxis of HIV/AIDS and prevention of STIs. The current status of various microbicidal drug delivery systems, particularly in the context of their stage within clinical trials and their potential cervicovaginal defence successes are described. Furthermore, approaches that may lead to the successful design and development of more effective intravaginal microbicidal delivery systems for preventing the transmission of STIs and HIV are suggested.

Chapter 3 explains thoroughly how various natural and synthetic biodegradable and biocompatible polymers with hydrophilic and hydrophobic properties were screened in an attempt to find suitable polymers for developing a novel intravaginal bioadhesive

polymeric device (i.e. IBPD). The key requirements for an intravaginal delivery system are: i) ease of manufacture; ii) low manufacturing costs; iii) ease of insertion; iv) high retention rate; v) minimal adverse effects such as damage to the vaginal epithelium; and vi) the potential for attaining controlled drug release and suitable lateral permeation (i.e. across the vaginal tissue). To satisfy all these requirements, it was necessary that the materials used for the development of such systems should have a multitude of essential properties. These include among others, biodegradability, biocompatibility, bioadhesivity, permeation capacity, compressibility and high matrix resilience. To achieve this goal, a One Variable at a Time (OVAT) approach was employed.

Chapter 4 discusses how Artificial Neural Networks (ANNs) have been successfully employed in the optimization of the IBPD. For successful design and development of novel drug delivery systems careful selection and optimization of several formulation and process related variables is of paramount importance. This avoids wastage of materials and time, with the subsequent benefit of reducing the production costs. It is important to know exactly how preparation compositions determine the formulation characteristics and in particular how formulation factors and potential interactions between them affect the performance of a given delivery system. Generally, the impact of each variable can be assessed by varying one variable at a time while keeping others constant. The OVAT approach employed in Chapter 3 in the extensive investigational screening of the polymers has thus enabled the selection of the best 15 lead formulations from 62 formulations. However, the OVAT approach cannot take into account interactions between the formulation factors. ANNs have been shown to be uselful in dealing efficiently with this difficulty and have therefore been used in this study.

Chapter 5 provides an evaluation of the bioadhesive capacities of the various polymers that have been employed in this study for developing the IBPD. For any given intravaginal drug delivery system, its retention to the vagina is an absolute requirement.

Good retention allows for the bioactives to be maintained at an optimal level locally and for extension of their residence time at the site of administration. To meet this requirement, an intravaginal device needs to be highly bioadhesive.

Chapter 6 provides a thorough description of the evaluation of bioadhesivity, *in vitro* drug release, and permeation of the drugs from the IBPD. It also seeks to explain the physicochemical and physicomechanical properties of the developed drug delivery system. One of the main objectives of this study was to design and develop an intravaginal drug delivery system in which the formulation would initially swell in order to facilitate bioadhesion, and thereafter erode gradually to release the drug in a controlled manner, to finally permeate laterally into the vaginal tissue and provide the needed preventive effect against HIV and STIs.

Chapter 7 describes the analysis of the drugs (AZT and PSS) in the vaginal tissue and plasma of a pig model admistered with an IBPD. It also provides a thorough histopathological toxicity evaluation of the IBPD in the pig model. This was in line with the ultimate goal of the study which required an intravaginal delivery system that could be retained in the vagina and release an ARV and microbicide simultaneously. Furthermore, the intention was that most of the released drugs would be retained in the vaginal tissue without causing toxicity. Assessment of the adherence, degradation and distribution patterns of the IBPD was also undertaken in this Chapter.

Chapter 8 concludes the thesis, assimilating the significant issues addressed regarding the design and development of the novel controlled release IBPD, with recommendations for future investigations in terms of system applicability.

1.6 Concluding Remarks

The current status of the HIV pandemic including the successes and failures of the therapeutic approaches which therefore identified the research problem has been well addressed. The aim and objectives of this study were pragmatically selected to realize the goal of developing a bioadhesive controlled release/prolonged release microbicidal drug delivery system. The synergy between STIs and HIV which enhances the susceptibility to secondary transmission of HIV have been elucidated and emphasized as being important entities to consider for the rational development of novel HIV prevention strategies. The anatomy and physiology of the vagina in relation to drug delivery has been addressed. Although the vagina exhibits a degree of dynamism, the benefit of exploiting it as a potential route for drug delivery still holds. Thus, the vagina still has its own place as a unique site for drug administration. Vaginal drug delivery can be engineered in such a way that maximum benefit is obtained as long as factors such as cyclic changes and physiological changes during menopause, are taken into consideration. Currently, the availability of microbicides is not a challenge as several are already in existence. The greatest challenge is finding the most effective way of delivering them to the required sites. It is anticipated that the design and methodological approach that has been undertaken in this study, will offer significant progress to this end, with the added benefit of the inclusion of an ARV into the formulation.

CHAPTER TWO

LITERATURE REVIEW OF CURRENT INTRAVAGINAL DRUG DELIVERY APPROACHES EMPLOYED FOR THE PROPHYLAXIS OF HIV/AIDS AND PREVENTION OF SEXUALLY TRANSMITTED INFECTIONS

2.1 Introduction

Intravaginal drug delivery systems have traditionally been used to deliver contraceptives and drugs such as antibacterials, antifungals, antiprotozoals, antivirals, labor-inducing and spermicidal agents, prostaglandins, steroids, peptides and proteins to treat vaginal infections (Katz et al., 1997; Verman and Garg, 2000; Weber et al., 2005). Formulations have included pessaries and tablets designed after the advent of rectal suppositories. The first intravaginal controlled drug delivery system was developed in 1970, using a vaginal ring for the delivery of medroxyprogesterone acetate for contraception (Verman and Garg, 2000). However, tablets, creams, and suppositories are now the most conventional formulations in vaginal drug delivery while vaginal rings are more commonly employed for long-term drug delivery**.**

Recent advances have been made in the area of bioadhesive gels, microparticles and tablets, which show great promise for use as controlled intravaginal microbicide delivery systems (Tang et al., 2005; Valenta, 2005; Bonferoni et al., 2006; Al-Tahami and Singh, 2007). Numerous hydrophilic polymers and hydrogels have been used in a number of vaginal products (Lew, 1994; Bogentoft and Carlsson, 1996; Robinson and Bologna, 2002; Al-Tahami and Singh, 2007). Natural hydrogels include among others, starch, collagen, various proteins, gelatin and dextrans. Swollen cellulose derivatives such as hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC) and sodium carboxymethylcellulose (NaCMC) have also been used, especially in tablet formulations.

Among the standard synthetic hydrogels, poly(ethylene oxide) and poly(acrylic acid) have been used in vaginal microbicide delivery formulations (Lee et al., 2000). The thick cervical mucus in the vagina may assist in the bioadhesion of various delivery systems, at the same time serving as a diffusion barrier to subsequent microbicidal delivery (Lard-Whiteford et al., 2004). Vaginal gels such as carageenan and PRO 2000™ Gel (Indevus Pharmaceuticals, Inc. Lexington, MA, USA) formulations when employed for the prevention of HIV and herpes virus transmission may be effective for up to one hour after sexual intercourse (Phillips and Maguire, 2002; Cadena, 2006; Rando et al., 2006; Brache et al., 2009).

Thus, there is a need to develop affordable and easy to apply microbicidal delivery systems. Among potential intravaginal delivery systems about which researchers at the WHO, UNAIDS, and other organizations have expressed hope in terms of safety and effectiveness, are vaginal gel formulations such as Buffergel™ and Carraguard™ (Maguire, 2001; Thom, 2007). It is anticipated that future research will focus on the development of combination products capable of delivering microbicides that violate the HIV life-cycle at multiple steps, have increased efficacy and have limited cross-resistance and which can minimize microbicide-induced host toxicity (Penttinen, 2005). In the absence of an effective prophylactic anti-HIV vaccine or therapy, current efforts are aimed at developing topical intravaginal formulations of anti-HIV agents or microbicides to reduce vaginal and perinatal virus transmission and cure and prevent STIs which may enhance HIV transmission. Therefore the purpose of this Chapter is to provide an overview and description of recent developments in intravaginal delivery systems for microbicides employed for the prophylaxis of HIV/AIDS and prevention of STIs.

2.2 The Rationale for the Development of Intravaginal Microbicidal Delivery Systems

Currently, much research on intravaginal drug delivery focuses on the prevention of HIV infection and STIs. Development of vaccines for the prevention of HIV transmission is still a challenge (Bourinbaiar et al., 2006; Lau et al., 2007; Hayden, 2009; Katsnelson, 2009). Microbicides circumvent many of the immunological difficulties associated with HIV vaccine development and make topical formulations such as gels a more realistic goal, especially in the short term. On the other hand, male condoms are not always accepted and correctly used. Unlike condoms, microbicidal delivery systems may be controlled by women, and do not require the cooperation, consent or even knowledge of the partner. Microbicidal delivery systems can provide an option to thousands of women who are at risk of contracting HIV and other STIs but are unable to negotiate condom use because of gender inequality, illiteracy, cultural resistance and poverty in many developing countries (Joshi et al., 2005). Thus, microbicidal delivery systems may be a solution to the prevention of STIs and sexual transmission of HIV infection (Moore et al., 2003; Malcolm et al., 2005).

The potential for developing controlled release formulations for long-term intravaginal delivery of microbicides has recently gained momentum and may overcome challenges relating to compliance, acceptability and efficacy associated with current gel-based formulations (Woolfson et al., 2000; Moore et al., 2003; Nikolic et al., 2007; Klasse et al., 2008; Obioha 2008; Baloglu et al., 2009). Furthermore, the emergence of resistant HIV strains and toxic side-effects of current ARVs require intravaginal delivery systems with superior safety profiles and potential for improved patient compliance. An intravaginal microbicidal delivery system combining several mechanisms of preventing the transmission of STIs and HIV would need to possess added effectiveness and less side-effects than a delivery system having a single mechanism (Penttinen, 2005). Figure 2.1 depicts various mechanisms of preventing the transmission of sexually transmitted

infections and HIV by employing microbicide delivery systems. The challenge is to design a delivery system that is able to provide high concentrations of the microbicidal compound in the vagina over a prolonged period of time (Benkorp-Schnurch et al., 2003). Studies have indicated that dosages in first-generation microbicides remain effective only for a few hours and therefore necessitate administration shortly before coitus (Kiser, 2006a).

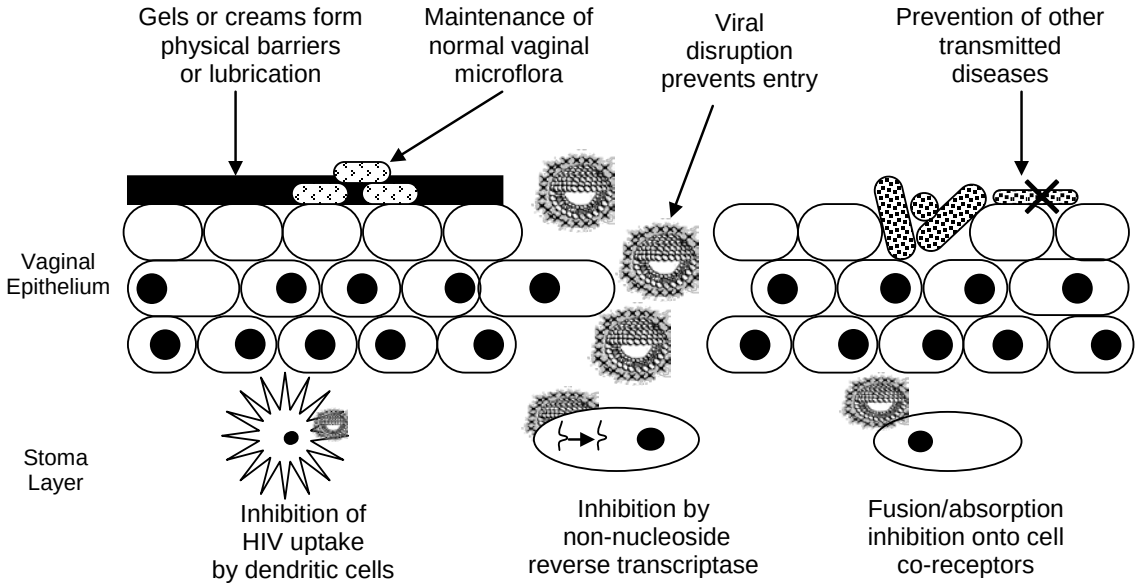


Figure 2.1: Schematic depicting the various mechanisms of preventing the transmission of sexually transmitted infections and HIV by employing microbicide delivery systems, (Adapted: Stone, 2002).

2.3 Current Intravaginal Microbicide Delivery Approaches for Preventing the Transmission of STIs AND HIV

The majority of microbicidal delivery systems for intravaginal administration that have been developed and evaluated in ongoing clinical trials are conventional semi-solid aqueous gels and vaginal ring formulations. These have been designed to provide a single dose of a microbicidal agent (Di Fabio et al., 2003; Herrewege et al., 2004; Alliance for Microbicide Development, 2005; Mitchel, 2006). Prior to widespread use, an intravaginal microbicide delivery system should meet several criteria (Table 2.1) in that the microbicide employed should be effective against a diversity of HIV strains.

Table 2.1: Desirable criteria for ideal intravaginal microbicidal delivery systems

i) Simple to manufacture, cost effective and easy to apply thus facilitating patient compliance.
ii) Non-irritative and free from producing any physical discomfort.
iii) Provide immediate and sustained protection by releasing the microbicide in a controlled manner over a prolonged period of time.
iv) Have suitable vaginal retention and distribution.
v) Be versatile against various pathogens encompassing STIs and HIV.

Currently, the maximum duration of drug release for intravaginal microbicidal delivery systems is as follows: i) Vaginal gels (6 hours) (Wang and Lee, 2004; Bilensoy et al., 2006), ii) vaginal tablets (8 hours) (El-Kamel et al., 2002; Gavin et al., 2002), and iii) vaginal rings (71 days) (van Laarhoven et al., 2002; Malcolm and Woolfson., 2006). Vaginal gels and tablets have rapid release rates which, for effective use, ultimately require administration several times a day. Vaginal rings have adequate release rates but have only been formulated for preventing the transmission of HIV and as a contraceptive. Several intravaginal delivery systems for microbicides have been formulated and traditionally include a large variety of pharmaceutical dosage forms such as semi-solids, tablets, capsules, pessaries, liquid preparations, vaginal films, vaginal rings, foams, and tampons. The most widely used semi-solid preparations for vaginal drug delivery include creams, ointments, and gels (Verman and Garg, 2000). These systems could be used to reverse the rising rates of STIs such as external genital warts, gonorrhea, chlamydia, and syphilis, which significantly predispose individuals to acquiring HIV (Penttinen. 2005; Kiser, 2006a)

Over the past decade, there have been major advances in the field of microbicidal delivery with diverse types of delivery systems in various stages of development (Harrison, 2003). The ultimate success of an intravaginal microbicide delivery system requires consideration of variables including the microbicidal agent, vaginal physiology and the design of the delivery system. Targeting HIV entry into the body is a favored

preventative approach as it is the initial step in the process of infection. Several readily available anionic polymers interfere with the entry processes of HIV, and hence these polymers qualify as primary candidates for designing various microbicidal delivery formulations (Bernkop-Schnurch et al., 2003). However, few of these formulations have been tested in Phase I/II clinical trials, in HIV-uninfected, and HIV-infected populations (D'Cruz and Uckun, 2004). Current multi-center Phase I/II safety and Phase II/III efficacy studies are being conducted or planned in different geographical locations, by various groups which aim to design systems for rapid clinical applications (Bentley et al., 2002; Morin et al., 2008).

2.4 Design of Intravaginal Microbicide Delivery Systems

Intravaginal delivery may be formulated for administration of microbicides by using an applicator or specifically designed systems for intravaginal administration. In general, based on the delivery system or the microbicide used, drug absorption, distribution and residence time in the vagina may vary. Early studies by Johnson and Masters (1962) showed that the microbicide distribution in the vaginal tissue varies considerably with the nature of the delivery system. Solutions, suspensions and foams displayed greater superiority over oral dosage forms. Ideally, a vaginal delivery system that is intended for localized microbicidal delivery should distribute uniformly throughout the vaginal cavity. Thus, for a local effect to occur in the vagina, semi-solid, or fast dissolving solid systems are required. Bioadhesive delivery systems or intravaginal ring systems are more suited for topical effects. However, it has been difficult to measure the distribution of a microbicide after intravaginal administration (Di Fabio et al., 2003; Hussain and Ahsan, 2005; Scorpio et al., 2008; Hendrix et al., 2009). Currently there is no data available regarding the bioavailability of most intravaginal microbicide delivery systems after extended vaginal exposure (D'Cruz and Uckun, 2004; Cutler and Justman, 2008; Hendrix et al., 2009). Furthermore, most of the intravaginal drug delivery systems for contraception and the prophylaxis against HIV lack efficacy (Katz et al., 1997).

Engineering and materials science may provide critical and new information to the process of designing and developing superior intravaginal microbicide delivery systems. First-generation microbicidal compounds that are currently under investigation are expected to be available within a few years. These systems may only be 50-60% effective in delivering microbicides (Kiser, 2006a), but even with this efficacy if they are used by only 20% of women, in 73 low-income countries, they may still lead to the prevention of 2.5 million new infections during a three-year period (Penttinen. 2005; Kiser, 2006a).

2.4.1 Creams and gels

Although creams and gels are commonly used for the topical intravaginal delivery of microbicides (Bernkop-Schnürch and Hornof, 2003; Hardy et al., 2007), these systems are messy, uncomfortable and may not provide an exact dose due to non-uniform distribution and their leakage (DuBouchet, et al., 1998; Hussain and Ahsan, 2005). To evaluate the efficacy of a 3-day course of clindamycin vaginal cream in the treatment of bacterial vaginosis, some researchers performed a randomized, placebo controlled trial in pregnant women (Broumas and Basara, 2000; Lamont et al., 2003). They found that the clindamycin cream was well tolerated and more efficacious than placebo (Broumas and Basara, 2000; Lamont et al., 2003). Vaginal creams and gels are based on the principle of emulsion or hydrogel-based drug delivery. During the past few years, considerable work has been done on the development of hydrogel controlled release microbicide delivery systems (Owen, et al., 1999; Verman and Garg, 2000; Alvarez-Lorenzo and Concheiro, 2002; Tang et al., 2005; Valenta, 2005; Bonferoni et al., 2006; das Neves and Bahia, 2006; Al-Tahami and Singh 2007; Alvarez-Lorenzo and Concheiro, 2008). For example, a 3% alginate gel of nonoxynol-9 has been investigated for intravaginal spermicidal activity (Owen et al., 1999). The study found that the spermicidal activity and the diffusion of the agent changed with the pH and osmolarity of the formulation. Recently, a gel microemulsion-based spermicide formulation, phenyl phosphate derivative of zidovudine, with anti-HIV effect, has been developed (Yiv, 2006; D'Cruz and Uckun, 2006a and b;

D'Cruz and Uckun, 2007). Multiple intravaginal applications of this drug as a microemulsion gel formulation did not cause any damage in the vaginal epithelium in the pig model (D'Cruz, 2003b; D'Cruz et al., 2004b; D'Cruz and Uckun, 2006a; Yiv, 2006; D'Cruz and Uckun, 2007).

2.4.2 Tablets and suppositories

Some authors use the terms pessaries and suppositories interchangeably and consider vaginal tablets as a separate dosage form. These formulations are designed to melt in the vaginal cavity and release the microbicide over several hours. Suppositories are most commonly used to administer drugs for cervical ripening prior to child birth and for local delivery of various drugs. Vaginal tablets may contain binders, disintegrants and other excipients that are used to prepare conventional oral tablets. Bioadhesive polymers are sometimes used in tablet formulations to increase the vaginal residence time of the microbicide been delivered. The presence of hydrophobic and release retarding materials may decrease the absorption of microbicides from a vaginal delivery system. Highly hydrophobic microbicides may not be suitable for vaginal tablets. The presence of penetration enhancers such as surfactants or bile salts can also significantly enhance systemic absorption if required. Other vaginal tablet-like formulations are extrapolations of silicone-based vaginal rings. Research groups have studied the release of microbicides from silicone matrices (Katz and Dunmire, 1993; Klavinskis, 1999). Release studies were performed *in vitro* for up to 1 year and *in vivo* in pigs for up to 52 days. Both *in vitro* and *in vivo* studies showed consistent release profiles over time, showing that microbicide delivery is controlled by diffusion from the silicone delivery device and was not limited by absorption through the vaginal epithelium. Further analysis of this diffusion process was performed by the development of a composite physical model. This involved the simultaneous receding boundary release of drug from a cylindrical silicone matrix delivery system, diffusion across an aqueous layer (vaginal fluid) and transport across the vaginal membrane by way of parallel lipoidal and aqueous pore pathways. The important

parameters in the analysis of this simulation are the solubility of the drugs in the silicone matrix, the solubility of the drugs in water, diffusion coefficients, silicone/water partition coefficients, and the permeability coefficient of the vaginal membrane to the microbicide.

2.4.3 Vaginal rings

Vaginal rings are circular ring-type drug delivery devices designed to release microbicides in a controlled manner after insertion, to prevent the transmission of STIs and HIV (Malcom et al., 2004 and 2006; Woolfson et al., 2006). The advantages of such a device are that they are controlled by the user; they do not interfere with coitus and they allow for the continuous delivery of microbicidal compounds. Vaginal rings are approximately 5.5cm in diameter with a circular cross section diameter of 4–9mm. In simple vaginal rings, the microbicide is homogeneously dispersed within a polymeric ring with the surface of the ring releasing the microbicide faster than the inner layers. The key challenge in development of these systems is finding the optimum dose that will deliver the least amount of microbicide necessary to ensure protection. Advances have been made on the original two-layer ring system by adding a third, outer, rate controlling drug-free elastomer layer (Washington, et al., 2001). This formulation design minimizes the drug concentration spike which was often observed during the first treatment cycle using a new ring. The rings are usually designed so that a new device is re-inserted at a time interval which is dependent upon the precise design of the delivery system and the microbicide being delivered. To obtain constant microbicide release from the vaginal ring, sandwich or reservoir-type rings have also been developed. Sandwich type devices consist of a narrow microbicide-loaded layer located below the surface of the ring and positioned between a central core and an outer band. In reservoir type ring systems, microbicides are dispersed in a centralized core, which is then encapsulated by a polymeric layer. In a single ring, it is possible to have several cores of different microbicidal compounds, thereby allowing administration of several microbicidal compounds from the same device. The rate of drug release can be modified by changing

the core diameter or thickness of the polymeric coating. Much of the vaginal ring literature relates to the commonly used polymer, poly(dimethylsiloxane) or silicone devices, although other elastomeric polymers such as ethylene vinyl acetate and styrene butandiene block copolymers have been tested (Roumen and Dieben, 1999; and van Laarhoven, et al., 2002; Airey et al., 2008; Hamid et al., 2008). The addition of vinyl acetate units in the polyethylene provides increased flexibility and greater adhesion. In a study by Roumen and Dieben (1999), evaluating the tolerability of ethylene vinyl acetate placebo vaginal rings of diameter 54mm, there was 91% acceptability among subjects involved in the study. The ring remained inserted for 21 consecutive days after insertion, with temporary removal during coitus (Roumen and Dieben, 1999). Most women judged the ring easy to insert and remove, and no side-effects were experienced among the test group during the study period (Roumen and Dieben, 1999; Ballagh, 2001; Harwood and Mishell, 2001; Dezarnaulds and Fraser, 2002; Novák, et al., 2003).

2.5 Bioadhesive Intravaginal Systems

Most conventional intravaginal formulations however are associated with disadvantages of low retention to the vaginal epithelium, leakage and messiness, thereby causing poor patient compliance. To circumvent these challenges, bioadhesive microbicidal delivery systems are being promoted. A bioadhesive polycarbophil gel, Replens® (One Nil Trade Ltd, Swanbridge, Caerphilly, UK), is currently available on the market, which is used to retain moisture and lubricate the vagina. The formulation remains in the vagina for 2–3 days and maintains the vagina at a healthy acidic pH (Richardson and Armstrong, 1999). Bioadhesive polymers that have been used for intravaginal formulations include among others polycarbophil, hydroxypropylcellulose and PAA (Saettone et al., 2002; Hussain and Ahsan, 2005; Andrews et al., 2009). The first bioadhesive systems for vaginal drug delivery were in the form of tablets which could be placed on a diseased area for delivery of bleomycin, an anti-cancer agent (Hwang, et al., 1977; Brannon-Peppas, 1992). These tablets contained hydroxypropylcellulose and PAA (Carbopol® 934). Release rates *in vitro*

increased with increasing quantities of hydroxypropylcellulose and water absorption increased with increasing quantities of PAA. Studies *in vivo* showed that after 5 days of treatment with a total of 150mg of bleomycin, in some cases, cancerous lesions had necrosed and the normal mucosa was unaffected (Hwang, et al., 1977; Brannon-Peppas, 1992). Bioadhesive tablets have been studied for the delivery of zinc sulphate as a model drug from compressed tablets of PAA and sodium carboxymethylcellulose, as bioadhesive polymers and cellulose (Avicel® PH102) (FMC BioPolymer, Philadelphia, USA) as a diluent (Sanders and Matthews, 1990). Experiments were performed *in situ* using bovine vaginal tissue. The force necessary to detach the tablets from bovine tissue was measured and correlated with the tablet composition. It was found that tablets containing only polyacrylic acid and zinc sulphate exhibited the optimal integrity. The presence of Avicel® in the formulations increased the tablet swelling in all cases but did not substantially decrease the force of detachment nor alter the drug release behavior. Increasing the quantity of polyacrylic acid increased the bioadhesion of the tablets to the vaginal epithelium (Woodley, 2001; Knuth, et al., 1993; Ceschel, et al., 2001).

Attempts have also been made to deliver microbicides using bioadhesive microparticulate vaginal systems (Vasir et al., 2003; Hussain and Ahsan, 2005). These systems may be a multi-phase liquid or a semi-solid, but they have been designed so as not to seep from the vagina like pessary formulations. The system is designed to provide controlled microbicidal delivery for at least 3 hours. The units of the release system have a lipoidal external phase and a non-lipoidal internal phase, providing bioadhesion when applied. A bioadhesive controlled release delivery system for nonoxynol-9 was reported by Lee and co-workers (1996) consisting of varying levels of nonoxynol-9 and ethylenediamine tetra-acetic acid (EDTA), a chelating agent, formulated using the polymer Carbopol® 934P. Carbopol® 934P provided a high burst release of nonoxynol-9 in the first 2 minutes and controlled release for up to 6 hours (Lee et al., 1996). Ceschel and co-workers (2001) developed a new bioadhesive vaginal dosage form by incorporating bioadhesive polymers

namely polycarbophyl, hydroxypropylmethylcellulose and hyaluronic sodium salt, into pessaries made of semi-synthetic solid triglycerides. The authors of this study argued that these polymers hold the pessaries in the vagina for longer periods of time, without side-effects, thereby prolonging the permanence of the drug on the vaginal epithelium. The presence of bioadhesive polymers largely modulated the behavior of pessaries in terms of adhesive force, liquefaction time and permanence of the drug in the simulated application site. However, their presence did not alter the release of the drug. The developed formulations showed good technological and adhesion properties and the ability to hold the delivery system at the application site.

2.6 Compounds Delivered Intravaginally as Microbicides

The numerous intravaginal compounds delivered intravaginally as microbicides are listed in Table 2.2 for which a concise description is provided hereafter.

Table 2.2: Classification of the numerous intravaginal compounds delivered intravaginally.

Classification	Compound
Polymeric Compounds:	Carageenan, Monocaprin, Polyacrylic Acid, *Lactobacillus*, Cellulose Acetate Phthalate, Cellulose Sulfate, Polystyrene Sulfonate, Naphthalene Sulfonate, Sulfated Polyvinyl Alcohol and Lactic Acid
Non-Polymeric Compounds:	Cetyl Betaine, Myristamine Oxide, Stampidine, Cyanovirin-N, Monoclonal Antibodies, Lyposomes, Thrombospondin-1, Lime Juice, Yoghurt, Tenofovir and Zidovudine
Nano-Structured Compounds:	Dendrimers, Thiourea, Silver, Polystyrene and Sodium Lauryl Sulfate

2.7 Dual-function Polymeric Gel-based Formulations Employed in the Design of Intravaginal Microbicide Delivery Systems: As Excipients with Potential Therapeutic Activity

Microbicidal gel formulations are likely to be only partially effective, but can produce a significant difference in places such as Africa, where most gels can be produced at reasonable costs. The properties of the gel in which microbicides are included are known to affect the epithelial permeation rate of microbicides and their microbicidal properties (Wang and Lee, 2002; Geonnotti and Katz, 2006; Hendrix et al., 2009). A formulation comprising Carbopol® 974 (Noveon Inc, Cleveland, OH, USA) 1.0–1.5%$^{w}/_{w}$ and HPMC 1.0–1.5%$^{w}/_{w}$ displayed a desirable gel-base intravaginal microbicide delivery system (Garg et al., 2001). Furthermore, the development of a "universal" placebo gel is required in order to assure that the placebo formulation does not distort either safety or efficacy assessments of microbicides. Recently, a hydroxyethylcellulose (HEC) placebo gel has been proposed as an adequate "universal" placebo, which is safe and sufficiently inactive for use in the clinical study of investigational microbicides (Wang and Lee, 2004a).

Patient compliance is one of the main attributes that excipients should provide to gel-based vaginal delivery systems for microbicides. Classes of excipients usually added to vaginal gels include gelling agents, humectants, preservatives and vehicles (Garg et al., 2004b; Oshlack et al., 2009). Various polymers are also commonly used as gelling agents in the formulation of hydrophilic vaginal gels. By definition, excipients are usually chosen from among materials that are very nearly pharmaco-toxicologically inert, and they usually have no therapeutic activity. However, some sulphated polymers, such as the carageenans, that are usually used as gelling agents; present themselves as one of the most promising classes of potential microbicidal compounds (Perotti, et al 2003; Smit, 2004; Buck et al., 2006; das Neves and Bahia, 2006; Blakemore et al., 2008; Andrews et al., 2009).

2.7.1 Carageenan-based gel formulations

Carageenan is a mixture of highly sulfated polysaccharides, comprised mainly of polygalactans such as gums, agar and fruit pectins. It is a hydrocolloid obtained from various members of the *Gigartinaceae* or *Solieriaceae* families or the red seaweed, *Rhodophyceae.* It is a yellow to white powder and is odorless and tasteless. The extracted product consists of three types of carageenan, known as *iota*, *kappa* or *lambda* (Table 2.3). Carageenan formulations may be effective against the spread of HIV infection because they appear to prevent the trafficking of HIV-infected mononuclear cells from vaginal fluids into the epithelium. These polymers form a negatively charged coat around HIV-infected mononuclear cells preventing their adhesion to the vaginal epithelial surface (Perotti et al., 2003). Data from a Phase I preliminary safety study indicates that a Carbopol® 974 Gel containing carageenans as microbicidal agents do not cause significant irritation to the female reproductive tract (Spencer et al., 2004). This may be an advantage over other microbicides or formulation excipients that are irritant to the vagina (Spencer et al., 2004).

Before usage, excipients that are used to formulate vaginal drug delivery systems must prove their biocompatibility. These tests are usually performed in cell culture models. Biocompatibility can be determined by cell growth/cell viability assays, cell proliferation assays, cytotoxicity assays, nitrite assays, and by protein assays and identification. Carageenan has been classified as "Generally Recognized as Safe" (GRAS) material by the United States Food and Drug Administration (US FDA). Further extensive oral pharmacokinetic studies have been conducted in various animal models. These have shown that the breakdown of carageenans in the gastrointestinal tract is minimal and that absorption is virtually non-existent (Blakemore et al., 2008). Sulfated polysaccharides that are useful in inhibiting cell-to-cell transmission of HIV and thus the sexual transmission of HIV as well as STIs include *iota* carageenan, dextran sulfate, *kappa* carageenan, *lambda* carageenan, heparin mimetics, heparin sulfate, pentosan polysulfate, chondrotin sulfate,

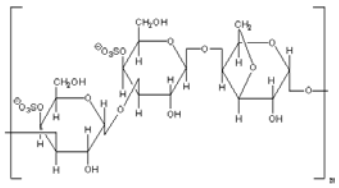
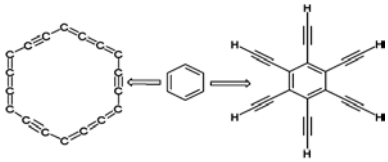
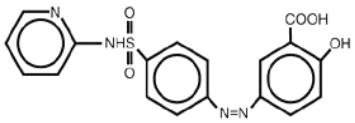
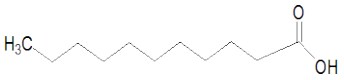
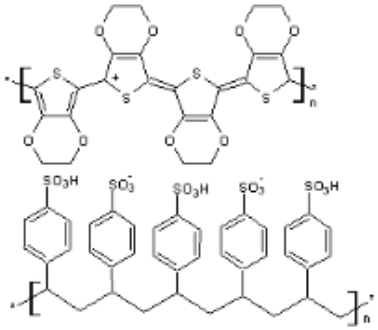
lentinan sulfate, curdlan sulfate, de-N-sulfated heparin and fucoidan (Smit, 2004). It has been shown that certain carageenans, or permutations of various carageenans, possess specific physicochemical properties which enable them to provide a prolonged antimicrobial effect and inhibit or reduce the possibility of STIs. Carageenan is one of the most active microbicides among the sulfated polysaccharides (Pearce-Pratt and Phillips, 1996). *Iota* carageenan is the most efficacious of the commercially available sulfated carageenans, in preventing HIV infection and in blocking chlamydia infection *in vitro* and *in vivo* (Blakemore et al., 2008).

2.7.1.1 A carageenan vaginal gel formulation for HIV and HPV inhibition

Carraguard™ (The Population Council Co., New York, USA) is a gel-like delivery system obtained from *lambda* and *kappa* carageenan that acts as an absorption inhibitor by coating the vagina. This may prevent HIV from entering the vaginal epithelium (Forbes and Harrison, 2000) (Table 2.3). The system forms a three-dimensional polymeric matrix with a high degree of physicochemical reticulation. Long, disordered chains are connected at specific points, but the connections are reversible. The molecular mechanisms of gelation are poorly understood, but researchers are attempting to design and enhance molecules with these properties. Carraguard™ has several advantages over other vaginal microbicide delivery systems such as a higher bioavailability, safety, versatility, and economic saving (Justin-Temu et al., 2004). There appears to be no effect on sperm motility (Coetzee et al., 2006; Fernandez-Romero et al., 2007). Furthermore, carageenan has shown the potential of being a potent inhibitor of sexually transmitted external genital warts. Certain sexually transmitted HPV types are causally associated with the development of cervical cancer (Perotti, et al., 2003; Smit, 2004; Buck et al., 2006). Recent development of high-titer HPV pseudoviruses has made it possible to perform high-throughput *in vitro* screening to identify HPV infection inhibitors. Comparison of a variety of compounds has revealed that carageenan is an extremely potent infection inhibitor for a broad range of sexually transmitted HPVs and it acts primarily by preventing

the binding of HPV virions to cells (Buck et al., 2006). Clinical trials revealed that carageenan-based intravaginal microbicide delivery systems are effective against genital warts (Hartmann et al., 2006). However, phase 3 clinical trials did not show that Carraguard™ is effective in preventing HIV transmission during vaginal sex (Population council, 2009).

Table 2.3: Chemical structures of polymeric microbicide compounds

Compound	Structure	Key Function	Reference
Kappa Carageenan		Absorption inhibitor	(Forbes and Harrison, 2000)
Carbomer		Interruption of HIV cell binding	(D'Cruz and Uckun, 2004)
Cellulose Acetate Phthalate		Inactivates HIV and HSV	(Manson et al., 2000)
CapricAcid		Inactivates HIV and HSV	(Neyts et al., 2000)
Polystyrene Sulfonate		Activity against HIV and HSV	(Simoes et al., 2002)

2.7.1.2 A combinatory gel formulation of carageenan and a non-nucleoside reverse transcriptase inhibitor

PC-815 gel is an intravaginal delivery system combining a microbicide containing carageenan with MIV-150. MIV-150 is a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) which binds to reverse transcriptase enzymes that in turn prevents HIV-infected cells from replicating. The safety and activity of carageenan against STIs has already been proven (Finley et al., 2006; Fernandez-Romero et al., 2007). Pre-clinical tests of MIV-150 have shown a significant increase in activity against HIV-1 primary isolates. Extensive pharmacological and toxicological profiles of MIV-150 have shown that the compound is non-toxic *in vitro* and *in vivo* (Fernandez-Romero et al., 2007). However, it has a low oral bioavailability which makes it an ideal compound for intravaginal delivery, since the chances of causing systemic side-effects become diminished (Fernandez-Romero et al., 2007). *In vitro* pharmacological studies have indicated that the PC-815 delivery system has significantly higher activity against HIV than that of the Carraguard™ system (Fernandez-Romero et al., 2007). MIV-150 and carageenan have different mechanisms of action, which when combined, have a synergistic effect. The anti-HIV activity of MIV-150 is not affected by the presence of seminal fluid or vaginal secretions and the activity of carageenan is not affected by the addition of MIV-150 to the formulation.

2.7.1.3 A topical non-contraceptive carageenan gel formulation

PC-515 gel is a topical gel formulation containing 3%$^{w}/_{w}$ carageenan, benzyl alcohol as a preservative, and hydrochloric acid as pH adjuster. It is under development as a non-contraceptive microbicidal delivery system that may offer disease protection while allowing women to conceive. Zacharopoulos and Phillips (1997) measured the protective properties of PC-515 in the vagina of mice inoculated with Herpes Simplex Virus (HSV), a standard model used to test microbicidal formulations. It was shown that PC-515 protected against HSV, with an effect superior to many other microbicidal delivery

systems. Even at 100 times the lethal dose of HSV, 40% of mice were protected from infection. The protective effect was seen across a wide range of pH levels and lasted up to 18 hours (Zacharopoulos and Phillips, 1997). PC-515 has undergone developmental trials in humans to ascertain the overall performance of the formulation (Maria-Elisa et al., 2003; Hart and Evans-Strickfaden, 2007; Ramjee, 2007).

2.7.2 A polyacrylic acid-based gel formulation

A PAA polymeric gel (BufferGel™, ReProtect, LLC, Baltimore, MD, USA), was subjected to Phase II clinical trials as an intravaginal spermicidal delivery system for contraception and prevention of HIV in 2006 (Malcolm et al., 2006). Its principal mechanism of action involves maintaining the natural acidity of the vagina, even in the presence of semen, by buffering the vagina to a pH of less than 5 due to the presence of PAA which ensures the survival of *lactobacilli* (Boskey et al., 2000). Ideally vaginal delivery systems should not be detrimental to *lactobacilli* because they are responsible for producing lactic acid and hydrogen peroxide which inactivate many pathogens that cause STIs (Boskey et al., 2000; Quayle, 2002; Lohr, 2007). The pH of a healthy human vagina ranges from 4-5 (~pH 4.5), an environment too acidic for STI pathogens and HIV to survive (Forbes and Harrison 2000). However, semen is alkaline and therefore during intercourse the vaginal pH increases, allowing the survival of STI pathogens and HIV (Forbes and Harrison 2000). The BufferGel™ system acidifies semen, thereby destroying sperm cells, HIV and a wide range of STI pathogens and keeps the vagina acidic even during sexual intercourse and thus creates a physical barrier that inhibits the passage of pathogens into the vaginal and cervical epithelium (Forbes and Harrison, 2000; Boskey et al., 2000 and 2001; van de Wijgert, 2001; D'Cruz and Uckun, 2004). A similar system includes Acidform™ gel (CONRAD/CDC, Atlanta, Georgia, USA), an acid buffer anti-HIV vaginal gel formulation which is currently under Phase III clinical trials (Amarl et al., 2004; O'Brien, 2006; Mumper et al., 2009). However, recent clinical trials have revealed that BufferGel™ has no effect on HIV prevention (PENN Medicine News, 2009).

2.7.3 A *Lactobacillus crispatus* soft-gel capsule formulation

A *Lactobacillus crispatus (LC)* microbicidal agent (Lactin Vaginal Capsules™, Gynelogix, Louisville, CO, USA) which acts by re-colonizing the vagina with hydrogen peroxide producing *lactobacillus* once released from the soft-gel capsule has been developed (Harrison et al., 2003). *LC* (Figure 2.2) is a normal commensal bacteria found in healthy vaginas (a sister species of lactobaccilli is found in yoghurt). *LC* assists in keeping the vagina free from infection by producing hydrogen peroxide, a substance that is weakly acidic. Normally, upon disturbing the ecology of the vagina either through infection, douching, or poor hygiene is when the vaginal pH rises above 4.5. This leads to the loss of *lactobacilli* resulting in the overgrowth of other bacteria and the proliferation of bacterial vaginosis which is a significant risk for pre-term labor, amnionitis, PID and HIV acquisition (Paavonen, 1983; Boskey et al., 2000; Forbes and Harrison, 2000).

Maggi and co-workes (2000) conducted a study on ten strains of *lactobacilli* by evaluating their viability in restoring the normal indigenous flora for the treatment of urogenital tract infections (UTI) in women. The microorganisms were formulated in single- and double-layer vaginal tablets. Each layer was characterized by different release properties. The first one was an effervescent composition that ensured a rapid and complete distribution of the active ingredient over the whole vaginal surface while the second one was a sustained release composition capable of releasing the *lactobacilli* over a longer period of time (Maggi et al., 2000). Three different retarding polymers were tested and all formulations were evaluated in terms of technological processability, bacterial viability and stability, and cell adhesion properties of the microorganisms. The results indicated that three out of ten strains appeared to be suitable for the application in the treatment of UTI.

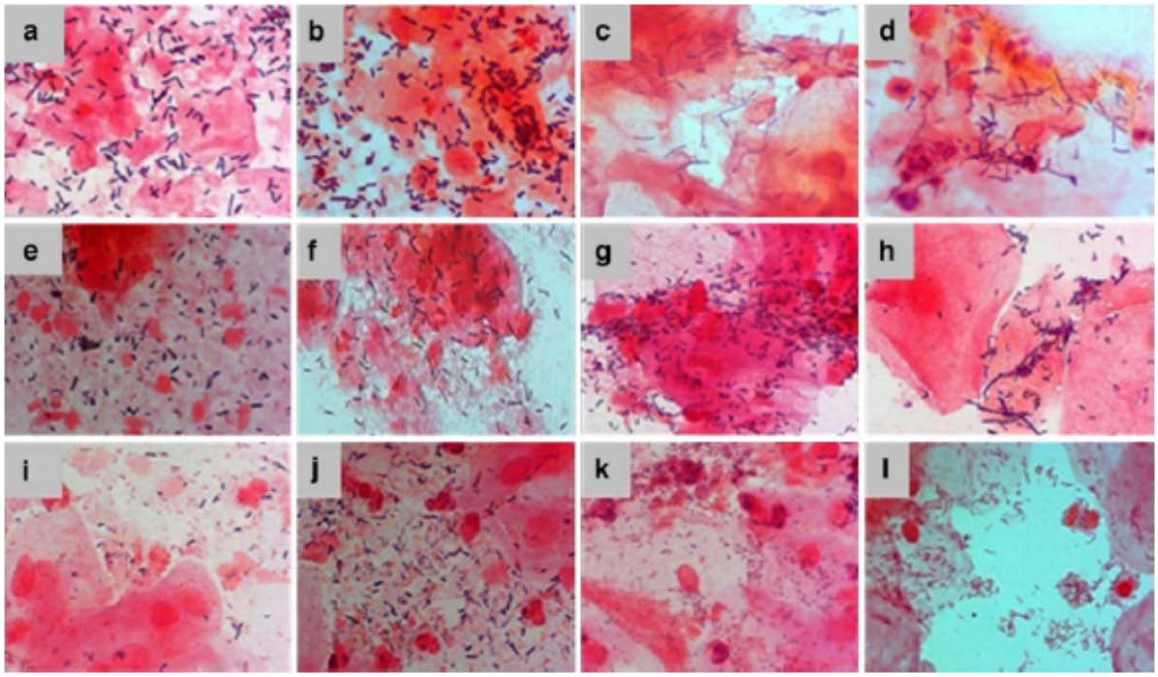


Figure 2.2: Microscopic images of gram-stained vaginal smears depicting different categories of vaginal microflora. a) and b), grade 1a *Lactobacillus crispatus* cell types; c) and d), grade 1b non-L. crispatus cell types; e) and f), grade 1ab mixtures of L. crispatus and non-L. crispatus cell types; g) and h), grade 1-like Gram positive rods; i) and j), grade 2 mixture of *Lactobacillus* cell types and bacterial vaginosis-associated bacteria cell types; k) and l), grade 3 bacterial vaginosis. (Source: Verhelst et al., 2005).

2.7.4 Thermosensitive gel formulations

Thermosensitive gels are systems that alter their physical characteristics with exposure to environmental changes within the vagina. This may be advantageous for intravaginal drug delivery due to the dynamic changes continually occurring in the vagina. These systems are capable of gelling in response to temperature changes, generally from ambient temperature to body temperature. Usually, the gelation temperatures have been considered suitable if they are in the range of 25–37ºC (Chang et al., 2002). The thermo-gelation mechanisms include partial crystallization, coil-to-helix transition, hydrophobic association, and micelle packing, which serve as reversible physical crosslinking points to form a gel (Jeong et al., 2002). The thermo-reversible properties of gels such as carageenan can be evaluated by rheological parameters such as the shear stress changes upon shear rates, sol–gel transition temperature, and viscoelasticity moduli

(Chang et al., 2002). Commonly used thermosensitive polymers employed for intravaginal microbicide delivery include the polysaccharides, N-isopropylacrilamide copolymers, poloxamers and its copolymers, poly (ethylene oxide)/poly(D,L-lactic acid-co-glycolic acid), and a few liposome-based systems. Poloxamer hydrogels perhaps represent the most extensively studied thermosensitive polymeric systems, while polysaccharides usually demonstrate good biocompatibility and/or biodegradability, with their solutions being thermosensitive at low polymeric concentrations (Ruel-Gariepy and Leroux, 2004). Chang and co-workers (2002) managed to produce thermosensitive vaginal gels containing clotrimazole. Results indicated that the gels were good potential candidates for safe, convenient, and effective intravaginal treatment. These gels, which also presented bioadhesive behavior, were prepared with mixtures of poloxamers and polycarbophil (Chang et al., 2002).

2.7.5 Long-chain sulfated polysaccharides and sulfonated polymeric formulations

A microbicidal delivery system that is a byproduct of non-cytotoxic poly(sodium 4-styrene sulfonate) (T-PSS) and cellulose sulfate (Ushercell™, Polydex Pharmaceuticals, Toronto, Canada) have reached Phase I and III clinical trials respectively (Bourne et al., 2003; Rohan and Sassi, 2009). Ushercell™ is a long-chain sulfated polysaccharide (~1,900 kDa) and T-PSS is a long-chain sulfonated polymer (Table 2.3). Both have broad-spectrum antimicrobial activity and act as adsorption inhibitors when released (Simoes et al., 2002). They are known to inhibit multiplication or activity of HIV, HPV, HSV-1, HSV-2, *Chlamydia trachomatis,* and *Neisseria gonorrhea in vitro* (Anderson et al., 2000; Herold et al., 2000; Christensen et al., 2001; Anderson et al., 2002; Simoes et al., 2002; Zanevelde et al., 2002). T-PSS gel formulations have shown promising *in vivo* activity as topical microbicidal delivery systems. Gel formulations tested contained 5–10%$^{w}/_{w}$ T-PSS, hydroxyethylcellulose, glycerine, propylene glycol, benzoic acid, methylparaben, and sodium hydroxide (Bourne et al., 1999). These systems do not inhibit *Lactobacilli* and have been proven safe in a variety of animal studies (Anderson et al., 2000; Anderson et

al., 2002; Zanevelde et al., 2002). As a result, the US FDA approved an Investigational New Drug (IND) application for both products. However, recently the Contraceptive Research and Development Program (CONRAD) reported that from interim data, from a trial where more than 1300 women in South Africa, Benin, Uganda and India that received Ushercell™, showed a greater prevalence of HIV infection compared to those who received the placebo gel (Highleyman, 2007; Thom, 2007). The Independent Data Monitoring Committee (IDMC, Rockville, MA, USA) overseeing this trial has agreed to conduct a detailed review of the data so as to better understand the findings and help determine their implications. Thus, these clinical trials have been halted pending the outcome of these investigations.

Other, novel synthetic sulfated polymers, namely, sulfated polyvinyl alcohol (PVAS) and sulfated copolymers of acrylic acid with vinyl alcohol (PAVAS) have proved to be potent and selective inhibitors of HIV-1 and HIV-2 *in vitro* (Baba et al., 1990; Balzarini and van Damme, 2007). These polymers have been found to completely inhibit HIV-1-induced cytopathogenicity and HIV-1 antigen expression at a concentration of 0.8µg/mL and equally effective against HIV-2 replication (Baba et al., 1990). As is the case with other sulfated polysaccharide formulations, the mechanism of action of PAVAS and PVAS resides in the inhibition of virus entry into vaginal epithelial cells once released from the delivery system (Baba et al., 1990; De Clercq, 2004).

2.7.6 A carbomer, lactic acid and naphthalene sulfonate gel formulation

A water-based gel comprising a synthetic carbomer, a lactic-acid/lactate buffer system (pH 4.5) (Table 2.3), common preservatives and naphthalene sulfonate as an antiviral agent (PRO 2000™ Gel, Indevus Pharmaceuticals, Inc. Lexington, MA, USA) is an intravaginal microbicide delivery system currently under development for the prevention of STIs and HIV (Morrow et al., 2003; Dhawan and Mayer, 2006; Joshi et al., 2006). The gel is clear, with a slight yellow tint and has a viscosity twice that of KY® Jelly (Johnson and

Johnson, New Brunswick, NJ, USA). The mechanism of action of the microbicide released from the PRO 2000^{TM} Gel formulation is by binding to the HIV virus, thereby disrupting any interaction of the virus with target cells. Similarly it is able to prevent chlamydia and HSV-2 infections (Forbes and Harrison, 2000). Results from *in vivo* animal studies have shown that PRO 2000™ Gel is safe and well tolerated (Ruscnis et al., 1996; Bourne et al., 1999; van Damme et al., 2000; Morrow et al., 2003; Smita et al., 2006). Currently it is under Phase III clinical trials where it has displayed promising results in low-risk women (Mayer et al., 2003 and Smita et al., 2006; MTN microbicide trials network, 2009). A recent clinical trial that involved more than 3,000 women in Southern Africa and USA has demonstrated for the first time the potential of PRO 2000™ gel for preventing HIV infection in women. According to the findings, PRO 2000™ Gel was found to be 30% effective (MTN microbicide trials network, 2009). However, additional evidence is required to thoroughly ensure the effectiveness of PRO 2000™ Gel. PRO 2000™ Gel is also compatible with the use of condoms, and therefore its further development may even prove to provide more benefits for women worldwide (Cadena, 2006).

2.7.7 A micronized cellulose acetate phthalate gel formulation

Cellulose Acetate Phthalate (CAP) has been used for several decades in the pharmaceutical industry for enteric coating of oral tablets and capsules (Table 2.3). Micronized CAP, is available commercially as an aquateric system, and contains excipients required for micronization and tablet coating. It has shown to adsorb and inactivate HIV-1, HSV and other STIs (Manson et al., 2000). Studies have indicated that a gel formulation of micronized CAP has the potential to be used for topical intravaginal delivery of microbicides for prevention of STIs and HIV (Neurath et al., 2003; Fichora et al., 2005). It is converted into a gel and therefore does not have to be removed following its application (Neurath et al., 2003). CAP is one of the potential anti-HIV vaginal gel formulations that are under Phase II clinical trials (Neurath et al., 2003; Fichora et al., 2005).

2.7.8 A monocaprin-loaded hydrogel formulation

Hydrogel formulations containing a 1-monoglyceride of capric acid (monocaprin) possess potent *in vitro* microbicidal activity against HIV, HSV, *Chlamydia trachomatis* and *Neisseria gonorrhea* (Thormar et al., 1999; Neyts et al., 2000) (Table 2.3). *In vitro* studies have shown that gels containing monocaprin, formulated using gel-forming agents such as either sodium carboxymethylcellulose and polyvinylpyrrolidone (pH 7.0) or carbomer and hydroxypropylmethylcellulose (pH 5.0), are highly virucidal to HSV-1 and less cytotoxic than nonoxynol-9 (Kristmundsdottir et al., 2000). In addition, the gels had spermicidal properties, and thus they may have potential contraceptive activity (Thormar et al., 1999). *In vivo* studies in mice showed that these gels were non-irritant and non-toxic in the vagina (Neyts et al., 2000). The results from these studies also indicated that 20mM monocaprin hydrogel could prevent HSV-2 infection and mortality in mice (Neyts et al., 2000). Hydrogel formulations of monocaprin could be further pursued in the development of intravaginal microbicide delivery systems for the prevention of STIs and HIV.

2.7.9 Polystyrene sulfonate vaginal tablets

Polystyrene Sulfonate (PSS) is also a microbicide that has been developed as an intravaginal tablet formulation (Garg et al., 2004a; Romano et al., 2008) (Table 2.3). When PSS intravaginal tablets were used as a microbicide delivery system they showed that they provided superior antimicrobial activity against HIV and HSV (Garg et al., 2004a). This formulation did not immobilize sperm, was not cytotoxic and did not inhibit normal vaginal microflora. This demonstrates its potential as a safe and effective vaginal microbicide delivery system for the prevention STIs and HIV (Garg et al., 2004a; Romano et al., 2008).

2.8 Non-polymeric Intravaginal Microbicide Delivery Systems for Preventing the Transmission of STIs and HIV

2.8.1 A cetyl betaine and myristamine oxide combinatory cream formulation

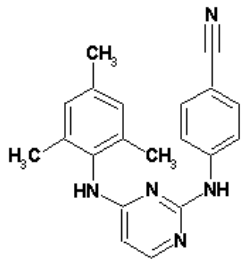
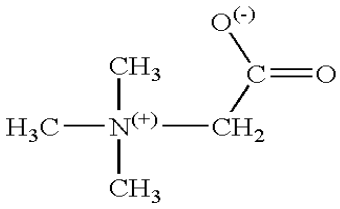
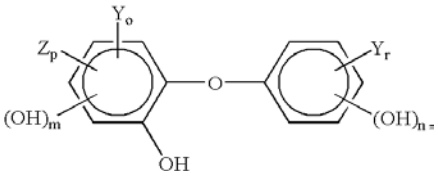
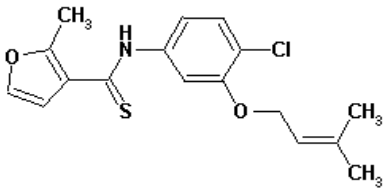
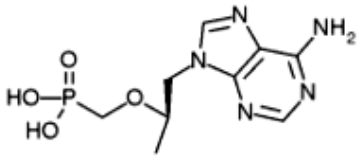
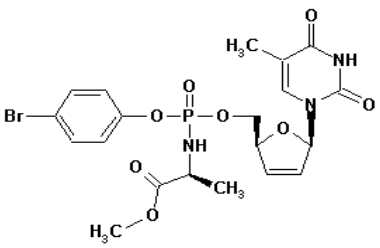
C31G cream (Savvy™, Cellegy Pharmaceuticals, Inc, San Francisco, CA, USA) comprises a broad-spectrum antimicrobial and spermicidal agent with two surface-active compounds, cetyl betaine (a C_{16} alkyl betaine) and myristamine oxide (a C_{14} alkyl amino oxide) (Table 2.4). The formulation is an equimolar mixture of the two amphoteric, surface-active compounds and displays activity against gram-positive and gram-negative bacteria, fungi, yeasts, and enveloped viruses (Krebs et al., 2000). Savvy™ has shown activity against numerous micro-organisms that cause STIs, including chlamydia, HSV-1, HSV-2 and gonorrhea. These effects occur once the microbicidal agent diffuses through the cream, which is formulated with HEC and also acts as a surface-active microbicide. In Phase III clinical trials, the delivery system has shown the ability to prevent HIV-1 and HIV-2 transmission and also has a desirable contraceptive activity (van Damme et al., 2002). Other studies have shown that Savvy™, when used alone, may also be approximately 85% effective in the prevention of pregnancy (van Damme et al., 2002; Dhawan and Mayer, 2006).

2.8.2 A non-nucleoside reverse transcriptase inhibitor-loaded gel formulation

UC781 (Cellegy Pharmaceuticals, Inc South San Fransisco, CA, USA) is a tight-binding Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) that is incorporated into gel formulations (Table 2.4). It has significant potential for use as an anti-HIV microbicide. Short pre-treatment of both isolated cells and human cervical tissue explants, with low concentrations of UC781 provided a strong barrier to subsequent virus infection by cell-free or cell-associated HIV-1 (Gadi and Parniak, 2001; Kerr, 2007). UC781 is readily formulated as an appropriate carrier for vaginal application in the form of Replens® Gel (Anglian Pharma, Hertfordshire, UK). Furthemore, UC781 has shown to have a favorable

toxicity profile (Gadi and Parniak, 2001). UC781 warrants further clinical assessment for its use as a topical intravaginal delivery system for the prevention of STIs and HIV.

Table 2.4: Structures of non-polymeric microbicide compounds

Compound	Structure	Key Function	Reference
Cetyl Betaine		Broad-spectrum antimicrobial	(Krebs et al., 2000)
Myristamine		Spermicidal agent	(Krebs et al., 2000)
UC781		Tight binding NNRTI	(Kerr, 2007)
Tenofovir		Blocks HIV replication	(De Clercq, 2006)
Stampidine		Broad-spectrum anti-HIV agent	(D'Cruz, 2003a)
Dapirivine		Potent NNRTI	Malcolm and Woolfson, (2006)

2.8.3 A nucleotide analogue-loaded gel formulation

PMPA Gel (Tenofovir™, Gilead Sciences, Foster City, CA, USA) is a microbicide delivery system that blocks HIV from replicating once it has entered cells (De Clercq, 2006). The hope is that Tenofovir™ could be absorbed by the vaginal epithelium to prohibit the replication and penetration of the virus in the outer cells of the vaginal wall (Table 2.4). Numerous ARVs initially explored for potential AIDS therapy were later abandoned due to inadequate delivery systems that provided poor systemic absorption. These may be suitable for re-formulation into newer effective intravaginal microbicide delivery systems (Forbes and Harrison, 2000). Preliminary results from studies of Tenofovir™ are encouraging and indicate that it may be a new microbicide delivery system to consider as part of a potent anti-HIV regimen. Tenofovir™ may require less frequent dosing than currently available therapies (only once daily) and appears to be active against resistant strains of HIV, including protease resistance (Forbes and Harrison, 2000).

2.8.4 A novel synthetically derived aryl phosphate-loaded gel formulation

Stampidine is a novel aryl phosphate derivative of stavudine (Table 2.4). It is a potent, broad-spectrum anti-HIV agent with potential to be used as a newer class of non-spermicidal microbicides (D'Cruz, 2003a). A vaginal gel formulation of stampidine was tested for its potential to cause vaginal mucosal toxicity in New Zealand white pigs (D'Cruz, 2003a; D'Cruz et al., 2003a). Application of 0.5-2.0%$^{w}/_{w}$ of this formulation, produced only minimal-to-mild vaginal irritation, of which is in an acceptable range for a clinical trial (total score <4 out of a possible 16). Repeated intravaginal treatment with stampidine gel was shown to cause no inflammation or hyperplasia in the vaginal epithelium and sub-epithelial tissue (D'Cruz et al., 2003a). Stampidine and its major metabolites were not detectable in the plasma of pigs after they received a 2%$^{w}/_{w}$ stampidine-containing gel intravaginally. Thus, this finding coupled with the favorable toxicity profile of intravaginally administered stampidine-containing gel may provide the

foundation for its clinical development as a safe and effective broad-spectrum anti-HIV microbicide delivery system.

2.8.5 A cyanovirin-N gel formulation for chemotherapeutic and immunoprophylactic prevention of HIV

Cyanovirin-N (CV-N) is a novel protein, originally isolated from cultures of the cyanobacterium (blue-green algae) called *Nostoc ellipsosporum* that has generated interest as a natural product for the chemotherapy of HIV infection (Boyd et al., 1997; De Clercq, 2000). A possible combined chemotherapeutic and immunoprophylactic approach for preventing HIV may be based upon the concept that continuous exposure of HIV to gp120-binding agents from a gel formulation may diminish glycosylation sites, thus triggering the production of specific neutralizing antibodies to previously hidden gp120 epitopes (Boyd et al., 1997; De Clercq, 2000). Both natural and recombinant CV-N are highly potent as virucidal agents (Boyd et al., 1997; De Clercq, 2000; Tsai et al., 2003; Balzarini, 2005; Xiaowen et al., 2006). CV-N inhibits the *in vitro* fusion of HIV-infected and non-infected cells as well as cell-to-cell transmission of HIV-1 infection. These effects make CV-N a promising candidate as a microbicide gel formulation for intravaginal delivery (Boyd et al., 1997; O' Keefe, 2001). In cell cultures it is non-toxic, it is remarkably resistant to physical degradation and it is water-soluble and stable in solution with a shelf life of at least 6 months (Boyd et al., 1997).

2.8.6 Sodium lauryl sulfate as an Invisible Condom™ gel-like formulation

Sodium lauryl sulfate (SLS) which is an anionic surfactant that denatures membrane proteins of pathogens is currently under Phase II clinical trials and is classed as an entry fusion inhibitor (Malcolm et al., 2006). It is also called an Invisible Condom™ (Invisible Condom™, Laval University, Infectious Diseases Research Center, Quebec City, QC, Canada). Ward and Ashley (1979) were the first to demonstrate that SLS at low concentrations is a potent inactivator of rotavirus and poliovirus. Previous *in vitro* studies

have demonstrated the ability of SLS to inhibit the infective capacity of different enveloped viruses such as HSV-1, HSV-2 and HIV-1 (Roy et al., 1999; Piret et al., 2000). SLS has also been reported to inhibit the infective capacity of non-enveloped bovine HIV and HPV (Howett et al., 1999). This suggested that SLS could be a candidate for use as an intravaginal microbicide gel formulation to prevent the sexual transmission of STIs and HIV. As previously mentioned, thermosensitive gels are recognized to allow advantageous application of active substances in the vagina. A thermoreversible gel composed of polyoxypropylene and polyoxyethylene in citrate buffer (pH 4.0) presents a phase transition temperature of 28ºC. This showed to be an ideal vehicle to deliver microbicidal substances, such as SLS or *n*-lauroylsarcosine, when applied in a murine model of HSV-2 (Roy et al., 1999).

2.8.7 Dapivirine anti-HIV vaginal rings

As mentioned earlier vaginal rings are suitable for the delivery of microbicides to prevent the transmission of STIs and HIV (Malcolm and Woolfson, 2006). While most research on microbicides has focused on single-dose and semi-solid topical gels, ring-based delivery systems could reduce the burden of patient compliance. These can provide long-term, controlled release of microbicides that may confer continuous protection against STIs and heterosexually transmitted HIV, and rule out the need for application of the intravaginal microbicide delivery system near the moment of sexual intercourse (Smith, 2006; Woolfson et al., 2006). Dapivirine, which is also known as TMC120, is a potent non-nucleoside reverse transcriptase inhibitor and is the only vaginal ring system used as an intravaginal microbicide delivery system for preventing the transmission of STIs and HIV (Malcolm and Woolfson, 2006; Smith, 2006; Nutall et al., 2007) (Table 2.4).

2.8.8 Polyherbal anti-HIV praneem-loaded vaginal tablets

Praneem has been developed as an anti-HIV vaginal tablet formulation. Praneem contains purified extracts of *Azadirachta indica* also known as the Neem tree. Praneem

has preventative activity against STIs *in vitro* and therefore it has been developed as a possible intravaginal microbicide delivery system. Phase I safety studies on Praneem tablets, revealed that the formulation is safe for once daily intravaginal use, for 14 consecutive days, in sexually active uninfected women (Joshi et al., 2005).

2.9 Nanotechnology-based Intravaginal Microbicide Delivery Systems

2.9.1 A Dendrimer-based microbicide formulation

SPL7013 (VivaGel™, Starpharma Ltd., Melbourne, Victoria, Australia) is a dendrimer with a polyanionic outer surface that allows multiple interactions with target sites. It is the most advanced dendrimer-based microbicide delivery system in which the dendrimer is not a carrier but an active ingredient (Short, 2007). These substances are large highly branched macromolecules synthesized from a polyfunctional core (Bourne et al., 1999). SPL7013 emerged as the most promising dendrimer after pre-clinical studies, and which was formulated as a gel that has been undergoing clinical trials (Jiang, et al., 2005; Nikolic et al., 2007). Bernstein and co-workers (2003) showed that a $5\%^{w}/_{w}$ Carbopol® 974 Gel (pH 4.5) can also be a suitable vehicle for the intravaginal administration of SPL7013. SPL7013 binds and blocks HIV-1, and chimeric simian/HIV-1 (SHIV) replication, thereby preventing STIs, including HIV and genital herpes (Okada, 1991; Highleyman, 2007; Short, 2007). It works by making use of its branched structure to bind viral surface proteins thereby preventing attachment of the virus to human cells. Gels containing different concentrations of SPL7013 were formerly used as intravaginal topical microbicide systems in female pigtailed macaques to study their ability to prevent vaginal transmission of SHIV. All untreated macaques (8/8) and seven (out of eight) macaques treated with a placebo gel formulation were infected with SHIV within 2 weeks after exposure. They showed a low plasma viremia and dramatic $CD4^{+}$ cell decline within 4 weeks (Short, 2007). Extrapolation from these results suggests that VivaGel™ may be a promising anti-HIV microbicide formulation. Phase I trials have shown that VivaGel™ is safe and well-tolerated (Rupp et al., 2007; Short, 2007; Fairley, 2009a). Further trials are

being conducted to test its efficacy against genital herpes and HIV (Highleyman, 2007; Short, 2007; Fairley, 2009b).

2.9.2 Silver nanoparticles employed as microbicide delivery systems

Various studies are currently investigating the production of a novel class of nanomaterials called protein-conjugated noble metal nanoparticles (Cho et al., 2005; Yacaman et al., 2006; Pal et al., 2007). The production involves direct conjugation of noble-metal nanoparticles with proteins that may be used in numerous forms including antiviral and antibacterial delivery systems. The process includes one or more targeting molecules (e.g. cytokines) that bind specifically to target cells expressing the cognate receptor for the cytokine. Researchers initially developed a method for synthesizing water-soluble noble metal nanoparticles, grown within and directly functionalized by bovine serum albumin (BSA), a globular protein, without the use of additional protecting or linking agents. Both gold and silver nanoparticles have been produced using this method, and the approach can easily be extended to other noble metals, such as the platinum group (Yacaman et al., 2006). Other globular proteins such as human serum albumin (HSA) may also be substituted for BSA and include, immunoglobulins, cytokines, receptors, lectins, glycoproteins, lipoproteins, toxins, toxoids, collagen, bacterial proteins, viral proteins, parasitic proteins, and fusion proteins (Richardson and Illum, 1992). The method of synthesis includes chemical reduction of an ionic metal precursor at room temperature in aqueous solution in the presence of a globular protein. Under certain pH conditions, disulfide bonds of the protein are available for direct bonding with the noble metal nanoparticles. The polypeptide backbone of the protein remains intact and the method does not affect the functional groups of the constituent amino acid residues. Silver nanoparticles take advantage of the microbicide properties of silver with different materials to produce effective microbicide delivery systems for preventing STIs and HIV transmission (Cho et al., 2005; Barbara et al., 2006; Yacaman et al., 2006).

2.9.3 Polystyrene nanospheres as microbicide delivery systems

Mucosal secretory IgA may have an important role in the prevention of HIV-1 transmission during sexual intercourse. Therefore, substances that induce HIV-1-specific IgA antibodies in the genital tract have shown promise for use as prophylactic vaccines against HIV-1 infection. It has been reported that Concanavalin A-immobilized Polystyrene Nanospheres (Con A-NS) could efficiently capture HIV-1 particles and gp120 antigens on their surface and that intravaginal immunization with these inactivated HIV-1-capturing nanospheres (HIV-NS) induce vaginal anti-HIV-1 IgA antibodies in mice (Agaki et al., 2003). Thus, application of HIV-NS by intravaginal delivery is a practical approach to promote HIV-1-specific IgA responses as an effective immunization strategy. Likewise, lectin-immobilized polystyrene nanospheres have been synthesized and examined for their HIV-1 capturing abilities (Hayakawa et al., 1998). When Con-A is immobilized on the surface of polystyrene nanospheres (400nm in diameter) with polymethacrylic acid branches and incubated with HIV-1 suspensions at room temperature for 60min, the Con A-NS achieves greater reduction in viral infectivity of HIV-1 (III_B strain) than does Con A-free nanospheres (Hayakawa et al., 1998). The combination of Con A-NS treatment followed by *in vitro* filtration with a microporous membrane efficiently removes virion-free gp120 as well as infectious viral particles from HIV-1 suspensions. Electron microscopic examination has demonstrated that HIV-1 virions may be entrapped on the surface of Con A-NS (Hayakawa et al., 1998; Carmen and Angel, 2003). Thus, Con A-NS is able to capture HIV-1 virions and gp120 with a high affinity, and may potentially be an effective nanotechnology-based delivery system for the prevention of HIV-1 transmission (Hayakawa et al., 1998; Carmen and Angel, 2003).

2.9.4 A gel-like molecular condom formulation as a barrier for STIs and HIV transmission

The molecular condom is a recently developed anti-HIV vaginal gel (Kiser, 2006a and b). It is a polymeric construct designed, to incorporate controlled release characteristics, to

allow for enhanced tissue coating, to reduce viral transport and to allow for triggered release of anti-HIV bioactives by interacting with semen. The term “molecular condom” arises from the concept that the polymer construct is liquid at room temperature and, when applied intravaginally, spreads and converts to a gel that effectively coats the vaginal wall (Kiser, 2006a and b). The gel is designed to release the anti-HIV bioactives upon contact with semen during sexual intercourse. It is an aqueous-based gel or "hydrogel" sensitive to body temperature and pH, and serves as a "smart semen-triggered vaginal microbicide delivery vehicle." This system is nanotechnology-based and explores the use of bioresponsive drug delivery by tailoring the physiological and mechanical requirements essential for intravaginal application. This is a type of a prophylactic agent that women may use without relying on their partners. It is of particular interest in areas such as Sub-Saharan Africa and South Asia where HIV-infection rates are substantially high while use of condoms is not well accepted. It is believed that the bio-distribution and bioavailability of antiviral bioactives can be modulated to increase potency and allow for burst release of free HIV inhibitors at the initial onslaught of viral exposure. Thus far, *in vitro* studies have demonstrated that the substance converts from liquid to gel at body temperature and a vaginal pH of 4-5 and then reverts to liquid and releases bioactives, such as microbicides, when exposed to semen, with a pH of 7.5 (Kiser, 2006a and b). The ultimate hope for this technology is to protect women and their unborn or nursing children from HIV. It is estimated that if all goes well, clinical trials in humans will start in three to five years, and the gel is expected to reach the market in several more years (Kiser, 2006a and b). The future plan is to incorporate an ARV into the gel so that it can cater both for blocking HIV movement and preventing the virus from replicating.

2.10 Other Microbicidal Compounds Currently under Investigation

2.10.1 A secretory leukocyte protease inhibitor

Secretory leukocyte protease inhibitor (SLPI) is an endogenous antimicrobial agent found in mucosal tissue and saliva, most prominently in mucosal sites open to the external

environment. Its original function is believed to be an anti-protease defense against neutrophils at the mucosal surfaces (Bob, 2000). *In vitro* laboratory studies have shown that SLPI can inhibit HIV entry into $CD4^+$ cells by interacting with targeted cells and blocking access as a barrier function. This may provide a reason as to the low rate of HIV infection through oral sex (San Francisco AIDS Foundation, 2008) due to the presence of SLPI in saliva. Data from studies of various mucosal tissues such as the mouth, gut, rectum and endocervix, suggest an inverse relationship between infectious HIV and the presence of SLPI (Bob, 2000; Zuckerman et al., 2003; Campo et al., 2006; Odaka and Ding, 2009). In breast milk, levels of SLPI rise dramatically at birth and begin to drop two to three weeks later, to a point where they are not sufficient to inhibit HIV infections in infants (Bob, 2000; Cheshenko et al., 2004). Further studies of SLPI as "a natural endogenous product" need to be performed since it has been shown to have the potential to function as an effective microbicide (Bob, 2000; Cheshenko et al., 2004; Keller et al., 2005).

2.10.2 Thrombospondin-1 as a barrier to mucosal transmission of HIV-1

During an investigation of the physiology of HIV inhibition by human saliva, laboratory studies identified thrombospondin-1 (TSP1) to have potential as a barrier agent against local mucosal transmission of HIV-1 (Crombie et al., 1998, 2000, 2001). TSP1 is a trimeric-sulfated glycoprotein that belongs to a family of five distinct extracellular matrix adhesion molecules (Bornstein, 1995; Adams, 1997; Crombie, 2000). Unlike other multifunctional extracellular matrix glycoproteins, TSP1 is found in lower concentrations in plasma and most body fluids (Mosher, 1990) (Figure 2.3) but not in saliva (Crombie et al., 1998). Since TSP1 is a high molecular mass compound, difficult to purify, and labile in the presence of tissue proteases, it is an impractical candidate to be formulated as a localized intravaginal delivery system. Investigations have therefore focused on the activity of peptides prepared from different regions of this molecule for which the attention has been on type 1 properdin/malaria-like repeat sequence (Crombie et al., 1998, 2000, 2001).

Characterization of alternative TSP1 peptide modifications currently under design may identify conformations that are more potent and stable and thus better for localized intravaginal therapy (Crombie et al., 2001).

2.10.3 Short interfering RNA as potential liposomal microbicide delivery systems

Short interfering RNA (siRNA) is a type of microbicide based on RNA interference (RNAi), a natural selective process for turning off genes, which holds great potential in its ability to treat disease at the genetic level (Dykxhoorn and Lieberman, 2006; Palliser et al., 2006; Soifer et al., 2007). In contrast to other classes of therapeutics, such as proteins, antibodies and small molecules, RNAi do not require either "drugable" domains on the molecular target, or specific locations at the cell membrane nor an extracellular site for their effective action. RNAi is triggered by siRNA molecules that engage a group of cellular proteins, known as RNA-induced silencing complexes (RISC). RISC guides the siRNA to its target mRNA (messenger RNA) by complementary base pairing and splits it in a selective fashion, thus halting protein expression or viral replication (Roumeliotis, 2006). Currently, *in vitro* vaginal tissue culture systems have been investigated and work continues with *in vivo* vaginal xenograft models to determine the optimum dose required for maximum down-regulation of virus replication. *In vitro* tests utilizing fluorescent tagged siRNA in liposomal delivery systems indicate that siRNAs are absorbed throughout the vaginal tissue (Cristofaro and Ramratnam, 2005; Holmes, 2007).

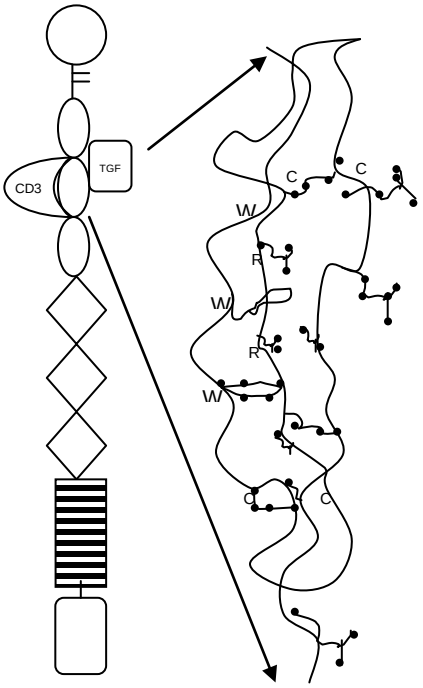


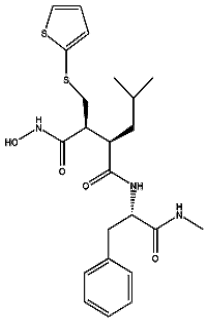
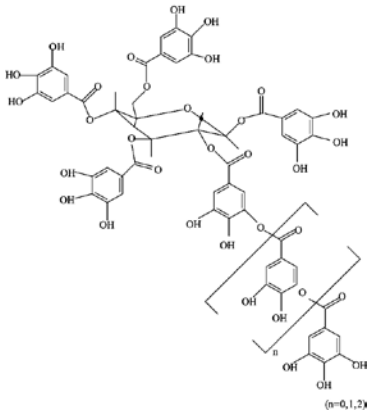
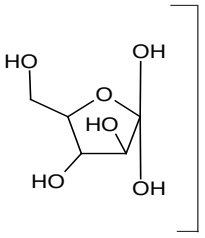
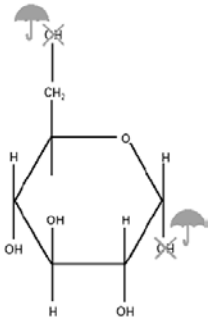
Figure 2.3: Schematic of the trimeric-sulphated glycoprotein Thrombospondin-1 with five distinct extracellular matrix adhesion molecules, (Adapted: Mosher, 1990).

2.10.4 Thiophen-Thiourea: A non-nucleoside reverse transcriptase inhibitor

PHI-443 (N′-[2-(2-thiophene) ethyl]-N′-[2-(5-bromopyridyl)] thiourea) is a rationally designed novel thiophene-thiourea NNRTI with potent activity against the wild-type and drug-resistant primary clinical HIV-1 isolates (D'cruz et al., 2004a; D'cruz and Uckun, 2005c) (Table 2.5). Its potential as a non-spermicidal microbicide for preventing the sexual transmission of HIV has been examined by testing its effect on *in vitro* sperm function where it has shown to inactivate viruses in human cells. It completely prevents the vaginal transmission of a genotypically and phenotypically drug-resistant HIV-1 isolate in the humanized severe combined immunodeficient mouse model of sexually transmitted HIV (D'cruz et al., 2004a). Exposure of human sperm to PHI-443 at doses 30,000 times greater than those that yield effective concentrations against HIV has shown that PHI-443 has no effects on sperm motility, kinematics, cervical mucus penetrability, or the viability of vaginal and cervical epithelial cells. Exposure of pig semen to PHI-443 either *ex vivo* or *in vivo* resulted in no side-effects on the *in vivo* fertilizing ability. Repeated intravaginal administration of 0.5– 2%$^{w}/_{w}$ of PHI-443 as a gel has been found to be safe in pigs

(D’cruz et al., 2004a). Thus, PHI-443 has potential as a prophylactic broad-spectrum anti-HIV microbicide without contraceptive activity.

Table 2.5: Chemical structures of other novel microbicide compounds

Compound	Structure	Key Function	Reference
Thiophen-Thiourea		NNRTI against drug resistant HIV	(D'Cruz et al., 2004a)
Monoclonal Antibodies		Passive immunization	(Cairns, 2007)
Lime Juice	 n-1	Retards HIV transmission	(Hemmerling et al., 2007)
Yoghurt		Inhibits transmission of HIV	(Turpin, 2006; Ramratnam, 2006)

2.10.5 Monoclonal antibodies as microbicides

Monoclonal antibodies are Y-shaped molecules synthesized by the immune system as 'mirror images' of foreign substances (Cairns, 2007). A long-range goal in vaginal protection is to develop monoclonal human antibodies in a microbicidal gel for protecting genital skin and epithelia against infections by topical passive immunization. Mucus secretions contain large quantities of antibodies that are highly specific and potent agents for preventing the entry of pathogens (Workman, 2003) (Table 2.5). Secreted mucosal antibodies also help maintain healthy bacterial flora on mucosal surfaces. Mucosal antibodies are polyvalent and hence bind with high affinity to pathogens. Monoclonal synthetic antibodies against sperm, HIV, and other STI pathogens can be applied directly to genital skin and epithelia for protection (Cone and Whaley, 1994; Sherwood et al., 1996). This may closely mimic the normal function of antibodies in the mucosal immune system, e.g. antibodies in breast milk help protect the surfaces of the mouth, nose, eyes, and digestive tract of the baby. To date, the results of studies performed indicate that monoclonal antibodies delivered to the vagina may help prevent pregnancy as well as sexual transmission of genital herpes and HIV (Castle et al., 1997; Zeitlin, et al., 1999; Castle 2002; Perotti et al., 2003; Whaley and Zeitlin, 2005). Topical application of these synthetic antibodies protects mice from HSV infection (Briggs and Zeitlin, 2000; Yim et al., 2005; Pedras-Vasconcelos et al., 2006). The end objective is to develop a protective topical gel containing these synthetic antibodies for human use (Briggs and Zeitlin, 2000).

2.10.6 Lime juice as a microbicide

Lime juice has been known for long in history as a contraceptive and vaginal douche (Thaineua et al 2003; Clarke et al., 2006; Hemmerling et al., 2007) (Table 2.5). *In vitro* studies that were conducted by Potts and co-workers (2004) demonstrated that lemon/lime juice is an effective microbicide and in *in vivo* primate studies, repeated use of lime juice did not damage the vagina. Hemmerling and co-workers (2007) further conducted a study on lime juice that involved twenty five sexually abstinent women. Tests

for genital infections, measurement of inflammatory biomarkers and a colposcopy were conducted, before and after treatment. Results indicated that no participant showed severe vaginal irritation. The reported side effects were mostly singular, mild and transient events. Thus, lime juice up to 20% concentration proved to have an acceptable safety profile for vaginal use (Potts et al., 2004; Hemmerling et al., 2007). Recent *in vitro* research has shown that, for effective prevention of HIV transmission the concentration of lime juice should be at least 50% which is associated with some toxicity uncertainties (Hemmerling et al., 2007). Therefore, there is an urgent need for a systematic study to determine the best ways of delivering lime juice effectively without any toxicity risks. If the use of lime juice can retard HIV transmission, then lime juice could prove to be a life-saving microbicide. If the effect is modest, lime juice might still prove to be a 'gold standard' microbicide with which artificial microbicides could be compared, short circuiting the need for time-consuming expensive placebo controlled trials (Potts et al., 2004). However, thorough research still needs to be conducted on the efficacy of lime juice as an inexpensive and ubiquitous intravaginal microbicide drug delivery system.

2.10.7 Yoghurt as a microbicide

It has been shown that yoghurt may soon be enlisted in the battle against HIV/AIDS (Turpin, 2006) (Table 2.5). *Lactobacillus* which is a harmless bacterium that helps to turn milk into yoghurt has been engineered to produce HIV-fighting microbicides (Turpin, 2006). Consuming yoghurt containing *Lactobacillus* may provide a way for women to fend off HIV if no other means are available (Turpin, 2006). Apart from being present in yoghurt, *lactobacillus* is a natural inhabitant of the human vagina. It survives the passage through the gastrointestinal tract and can easily cross from the anus to the vagina and colonize there. Once in the vagina, the bioengineered bacteria can produce compounds that inhibit transmission of HIV and maintain a normal vaginal pH, thus helping prevent HIV and STIs infection (Turpin, 2006). Similarly, Ramratnam (2006) showed that it was possible to genetically manipulate bacteria present in yoghurt to release an anti-HIV drug.

This could successfully alter the genetic constitution of *Lactobacillus lactis* to enable it to release cyanovirin which has already been found to confer protection against HIV infection in monkeys and human cells. Although successful results (followed by clinical trials) are yet to be obtained, there is optimism that yoghurt may eventually pave a way for the development of cost-effective microbicide delivery systems (Ramratnam, 2006). .

2.11 Concluding Remarks

Intravaginal microbicide delivery systems are providing a new option for preventing the transmission of STIs and HIV. This could have considerable public health and economic impact especially in resource-poor countries. As described in this Chapter, numerous safe and effective anti-HIV intravaginal microbicide delivery systems are currently being evaluated at various stages in clinical trials that include the dual-function polymeric compounds, non-polymeric compounds and nano-structured compounds. To date, none of the delivery systems described, which comprise mainly gel formulations, have been found to be fully effective at preventing the transmission of STIs and HIV. Several clinical trials have demonstrated that intravaginal microbicide delivery systems have the ability to protect against the transmission of STIs and HIV but numerous challenges still remain before these systems are used commercially. These challenges include: i) the need for the development of newer and innovative intravaginal microbicide delivery technologies that can completely prevent the transmission of STIs and HIV; ii) securing a cost-effective and efficient production and distribution of these compounds and systems; iii) securing wide access to these compounds and systems that takes into account cultural sensitivities of the most affected countries; and iv) securing extensive education in the use of the intravaginal microbicidal delivery system. It is thus anticipated that future research will focus on the design and development of more effective intravaginal microbicidal delivery systems for preventing the transmission of STIs and HIV employing a wide variety of dosage forms with multiple mechanisms of action and microbicidal delivery.

CHAPTER THREE

PREFORMULATION INVESTIGATIONS FOR THE DEVELOPMENT OF LEAD INTRAVAGINAL BIOADHESIVE POLYMERIC DEVICES

3.1 Introduction

Major efforts have been directed towards designing intravaginal drug delivery systems that can deliver drugs to the vagina at a desired rate to maximize drug efficacy while minimizing side effects (Garg and Tambwekar, 2003; das Neves and Bahia, 2006; D'Cruz and Uckun, 2006a; Andrews et al., 2009). However, successful design of intravaginal drug delivery systems still poses numerous challenges (Justin-Temu et al., 2004; Ndesendo et al., 2008; Rohan and Sassi, 2009). It is imperative that the type and properties of the drug and the drug carrier (e.g. polymer) be thoroughly understood if successful intravaginal delivery is to be achieved. In the case of a polymer as the carrier agent, appropriate selection is of paramount importance. The polymer employed can be either degradable or non-degradable. Biodegradable polymers that are biocompatible to the body are most frequently chosen for the design of intravaginal drug delivery systems. Their value is attributed to the fact that they do not require to be removed from the body after application. The type, physicochemical and physicomechanical properties, proportions of the polymers in the formulation, as well as the degree of swelling and /or erosion for the polymers employed in formulating an intravaginal delivery system, play an important role in matrix integrity and retention capacity. These are the determinant factors for bioadhesion and release characteristics of a given system (Vueba et al., 2004; Ravi et al., 2008). Figure 3.1 depicts a biometric simulation undertaken in our laboratories on ACD/I-Lab, V5.11 (Add-on) software (Advanced Chemistry Development Inc., Toronto, Canada, 2000), indicating the different levels of coiling that polymers may undergo to produce the highest degree of matrix integrity. A polymer's physicochemical and

physicomechanical properties contribute substantially to diffusion, swelling, degradation and erosion dynamics, which play an important role in terms of drug release kinetics (Siepmann and Peppas, 2001; Avgoustakis, 2004; Arifin et al., 2006). Other phenomena such as osmotic, magnetic or electric effects may also be involved. The chemical nature of polymers commonly used for pharmaceutical purposes differs significantly. Some are freely water-soluble, while others are completely water-insoluble. Thus, they vary in the rate and extent of swelling, bioerosion/biodegradation and their potential to interact with the drug.

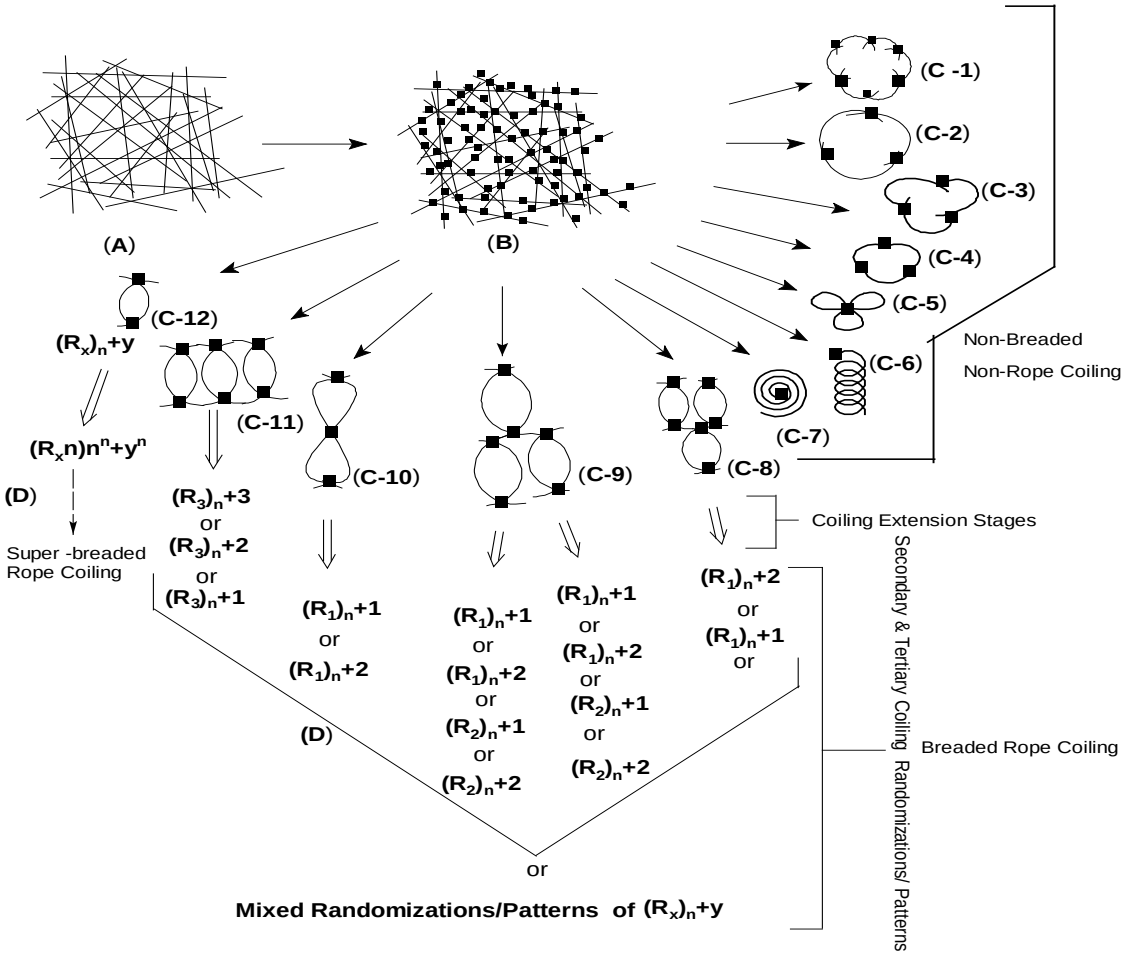


Figure 3.1: A computationally-derived model predicted in our laboratories depicting the polymer coiling stages a) polymer strands, b) identification of molecular sites, c) initial stages of coiling, d) secondary and tertiary coiling randomizations and patterns where (C-1)-(C-7) is non-breaded non-rope coiling and (C-8)-(C-12) is breaded rope coiling. The number of coil arm may vary in (C-1)-(C-5). R_1, R_2 and R_3 are functional groups. The lines represent polymeric strains while the blocks represent polymer strain interactions.

In the current study, both natural and synthetic biodegradable and biocompatible polymers have been employed, some with hydrophilic properties, others with hydrophobic properties and yet others with bioadhesion properties. Hydrophilic polymers can control drug release because of their compressibility and swelling properties, as well as the ability to accommodate high levels of drugs (Pinto et al., 2004; Hiremath and Saha, 2008; Jin et al., 2008). However, hydrophilic matrix systems generally result in nearly first-order release profiles (Hassan et al., 2003; Chopra et al., 2007; Rahman et al., 2009). For this reason, use of both hydrophilic and hydrophobic polymers have been considered to be an absolute necessity in this study since the ultimate aim was to achieve zero-order delivery (Einmahl et al., 2001; Breton et al., 2008; Barakat et al., 2009). Studies have shown that an increase in hydrophobicity may substantially alter the release kinetics of a drug delivery system (Nirmal, 2006; Khoee et al., 2007; Wu and Jin, 2008; Smith et al., 2009). This is attributable to its ability to hinder the penetration of the solvent molecules into the polymer matrix thereby leading to a reduction in the drug release rate (Sanchez-Lafuente et al., 2002; Sant et al., 2008; Yu et al., 2009). Furthermore, an increase in hydrophobicity often assists to control the rate of polymer disentanglement (Khoee et al., 2007; Smith et al., 2009). This occurs through various mechanisms, but mostly through a decreased tendency for the polymer to undergo hydrolysis due to decreased exposure to water (Liu et al., 2001; Khoee et al., 2007; Wu and Jin, 2008; Smith et al., 2009). In addition, hydrophobilic polymers may also provide several advantages, ranging from differing stability at varying pH and moisture levels, to well-established safe applications (Tiwari et al., 2003).

In this study a novel caplet-shaped device was considered to be the most appropriate based on the route of application. Conventional vaginal delivery systems including solutions, semi-solids (ointments, creams, gels), tablets and pessaries suffer in one way or another from the problems of retention, spreadability and drug release control (Garg and Tambwekar, 2003; das Neves and Bahia, 2006; Garg et al., 2007). Semi-solids in

particular are perceived as messy in use and prone to leakage (Justin-Temu et al., 2004; Valenta, 2005). Furthermore, the conventional vaginal drug delivery systems do not provide sufficient design flexibility in the control of drug release over periods extending from days to months. In this regard, there is then a great need for designing more specific formulations that can circumvent the problems associated with the conventional vaginal drug delivery systems.

Polymer swelling is one of the factors that determines the matrix stability and subsequently the drug release kinetics from polymer matrices (Patel and Patel, 2007; Barakat et al., 2008; Sibeko et al., 2009). Normally in swellable polymer matrices, polymer relaxation and erosion mechanisms may coexist (Bettini et al., 2001). Swelling and erosion front domains specifically define the gel layer thickness, which is considered to be the key factor in drug release kinetics and in most cases occur simultaneously (Christ et al., 1998; Bettini et al., 2001; Al-Taani and Tashtoush, 2003). Thus, the extent of polymer swelling, relative mobilities of dissolution medium and drug, and matrix erosion dictate the kinetics as well as mechanism of drug release from polymeric matrices (Dahlberg et al., 2007). Generally, swelling–controlled release systems consist of a drug, which is molecularly dispersed in a polymeric matrix. Swelling occurs with subsequent formation of a thin gel layer as soon as water begins to penetrate the polymer matrix. In addition, it has been shown that the porosity of a polymeric system is another dominant factor that may control the swelling behavior (Mehta et al., 2000; Vlachou et al., 2001). Increased porosity leads to fast initial rates of water uptake and therefore high extent of swelling equilibrium. For a better outcome in terms of matrix stability and therefore optimal drug release kinetics, low swelling and slow erosion are the most desirable features.

The aim of this Chapter was therefore to screen various polymers through an extensive preformulation investigation so as to ultimately obtain polymer combinations for the design of lead Intravaginal Bioadhesive Polymeric Devices (IBPDs). A one-dimensional

search with successive variation in variables i.e. OVAT approach was employed. Although it is practically impossible for a one-dimensional search to accomplish an appropriate optimum in a finite number of experiments (Haltrich et a., 1994; He et al., 2005), it does successfully provide a lead formulation/s upon which mathematical optimization can be used for further refinement.

3.2 Materials and Methods

3.2.1 Materials

The following polymers were employed in this study: Modified polyamide 6,10 ($_m$PA 6,10) was synthesized using hexamethylenediamine, sebacoyl chloride, anhydrous n-hexane and cyclohexane, all purchased from Sigma-Aldrich Chemie (Sigma-Aldrich Chemie, Steinheim, Germany). The remainder of the polymers employed were commercially available. These were poly(acrylic acid) (Carbopol® 734 and 974) (Noveon Inc Cleveland, OH, USA), carageenan (Type one-kappa and alpha), ethylcellulose (Ethocel-10), xanthan gum, tragacanth, bovine serum albumin (BSA) (Sigma-Aldrich Chemie, Steinheim, Germany); poly(ethylene oxide) (Union Carbide Corporation, Danbury, CT, USA); poly(lactic-co-glycolic) acid (Resomer® RG504; Boehringer Ingelheim, Ingelheim, Germany); poly(vinyl alcohol), polyvinyl povidone (Merck-Schuchardt, Hohenbrunn, Germany); gelatin, beeswax (Saarchem (Pty) Ltd., Krugersdorp, South Africa); methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose (Merck-Schuchardt®, Hohenbrunn, Germany); poly(ethyl acrylate, methyl-methacrylate, and chlorotrimethyl-ammoniumethylmethacrilate) S 100 (Eudragit® S-100) and poly(ethyl acrylate, methyl-methacrylate, and chlorotrimethyl-ammoniumethylmethacrilate) RS 100 (Eudragit® RS-100) (Rohm & Co., Pharma Polymers, Darmstadt, Germany); calcium hydroxide, glycerol, acetic acid (Associated Chemical Enterprises (Pty) Ltd., Southdale, South Africa). All other reagents used were of analytical grade and employed as received.

3.2.1.1 Synthesis of modified polyamide 6,10

The modified polyamide 6,10 ($_m$PA 6,10) was synthesized in our laboratory using a method developed by Kolawole and co-workers (2007) employing hexamethylenediamine (HMD), sebacoyl chloride (SC), hexane (HXN), cyclohexane (C-HXN), sodium hydroxide (NaOH) and deionized water (DW). The overall chemical reaction is illustrated in Figure 3.2. These researchers focused on exploring the effect of volume ratio, stoichiometric variations and the addition of solvent phase modifiers such as sodium hydroxide and cyclohexane on the physicochemical and physicomechanical properties of the $_m$PA 6,10. In the present study, it is only the optimized $_m$PA 6,10 that was synthesized. Briefly two sets of solutions were prepared. The first solution comprised SC dissolved in a mixture of HXN and C-HXN while the second solution comprised of specific quantities HMD and NaOH dissolved in DW. The optimized formulation comprised of HMD (1.5g), SC (0.63), HXN 40 (mL), C-HXN (40mL), NaOH (0.1g) and DW 10 (mL).

The first solution was gradually added to the second to form two immiscible phases which resulted in a polymeric film being formed at the interface (i.e. by an interfacial polymerization process). The polymeric film was collected as a mass by slowly rotating a glass rod at the interface. Upon collection of the polymeric mass, it was thoroughly washed, with HXN to remove any unreacted SC and then with DW (3×300mL) to remove any un-reacted NaOH. The polymeric mass was then lightly rolled on filter paper (diameter 110mm, pore size 20µm) to remove any excess solvent and dried to a constant weight at 40°C over 48 hours.

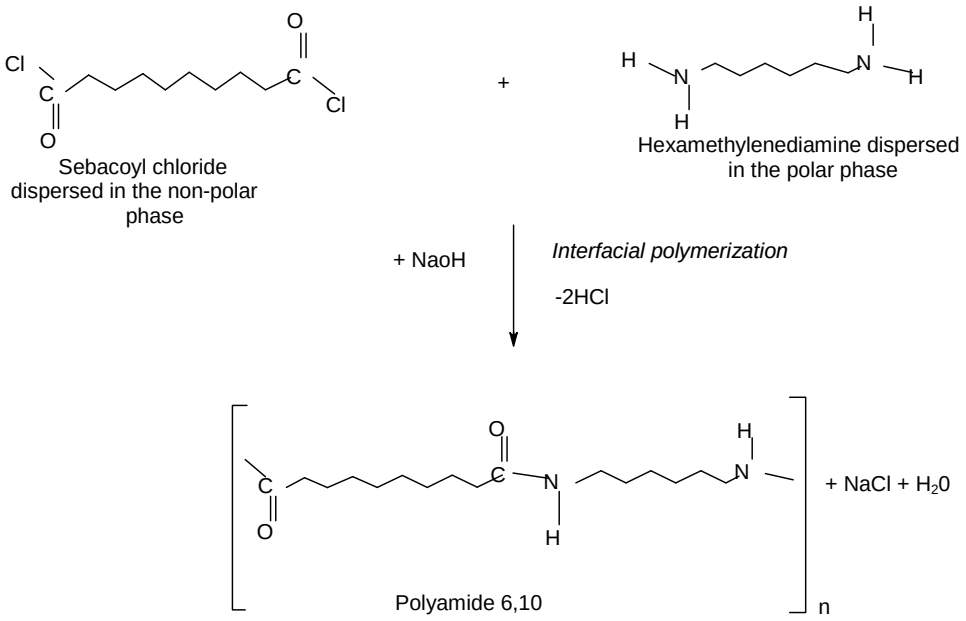


Figure 3.2: Synthesis of modified polyamide 6,10 by interfacial polymerization.

3.2.2 Methods

3.2.2.1 Selection of formulation components

In polymeric drug delivery systems, controlled drug delivery occurs when a polymer, whether natural or synthetic, is combined with a drug or other bioactive agent in such a manner that the bioactive agent is released from the polymeric material in a pre-determined manner. Initially 18 polymers were investigated. These were modified polyamide 6,10 ($_m$PA 6,10), poly(lactic-co-glycolic acid) (PLGA), polyethylene oxide (PEO), poly(acrylic acid) (PAA), carrageenan (CG), ethycellulose (EC), polyvinylalcohol (PVA), polyvinyly polidone (PVP), tragacanth (TG), xanthan gum (XG), gelatin (GL), methylcellulose (MC), Hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), hydroxypropymethylcellulose (HPMC), beeswax (BWX), poly(ethyl acrylate, methyl-methacrylate, and chlorotrimethyl-ammoniumethylmethacrilate) S 100 (Eudragit® S100) (ED-S100) and poly(ethyl acrylate, methyl-methacrylate, and chlorotrimethyl-ammoniumethylmethacrilate) RS 100 (Eudragit® RS 100) (ED-RS100). Out of the 18 polymers, 5 of them namely modified polyamide 6,10 ($_m$PA 6,10), poly(lactic-co-glycolic acid) (PLGA), ethycellulose (EC), polyvinylalcohol (PVA) and poly(acrylic acid) (PAA)

have finally been employed to encapsulate PSS and AZT (Figure 3.3) with the purpose of attaining controlled release within the vagina (see Chapter 6 later). These polymers have their own inherent properties when used alone. However, this study has aimed at using their combination, a move that is expected to provide superior physicochemical and physicomechanical properties suitable for development of the IBPD. Furthermore, these polymeric materials are FDA approved and are biocompatible and biodegradable. Table 3.1 depicts the polymer type, properties and the rationale for selection of each polymer.

Table 3.1: Polymers, properties and rationale for selection

Polymer	Properties	Rationale for selection
$_{m}$PA 6,10	• High matrix resilience • Abrasion resistance • Chemical inertness • High modulus • Thermoplasticity	• To control drug release • To control permeation • To intensify the robustness of the IBPD
PLGA	• Highly hydrophobic • Degrades into two acidic units namely lactic acid and glycolic acid	• To produce an acidic pH environment within the vagina upon degrading into lactic and glycolic acid. • Acidic pH maintains the normal vaginal ecology by favoring the growth of *Lactobacilli*-containing microflora which prevents bacterial vaginosis. • To control drug release
EC	• Highly hydrophobic • Easily compressible	• To control drug release • To control permeation
PVA	• Hydrophilic • Compressible • Mildly bioadhesive	• To control drug release • To foster bioadhesivity
PAA	• Hydrophilic • Possesses hydrogen bonds and strong anionic/ cationic charges • High molecular mass and chain flexibility • Surface energy interactions favoring spreading onto mucus	• Confers bioadhesiveness to the IBPD, thus favoring extension of drug residence time in the vagina • To control drug release • To control permeation

For an intravaginal drug delivery system to be effective as an anti-HIV microbicidal agent, the drug employed should be able to inactivate HIV replication in lymphocytes, epithelial cells and sperm cells (D'Cruz and Uckun, 2002). Given the fact that the passage of HIV-infected mononuclear cells in semen contributes to the sexual transmission of HIV (Zhang et al., 1998; D'Cruz and Uckun, 2002), the anti-HIV microbicide should be metabolized

with equal efficiency by both the seminal cells and the epithelial cells of the cervico-vaginal region (D'Cruz and Uckun, 2002). AZT (Figure 3.3a) is one such a compound and therefore the reason for being employed in this study. It undergoes intracellular hydrolysis yielding monophosphate derivatives which further become phosphorylated by thymidylate kinase to produce a bioactive triphosphate derivative. PSS (Figure 3.3b) is a microbicidal polymer which is also homogenous and viscous, and forms bioadhesive dispersion itself, thus facilitating its retention in the vagina for prolonged periods of time (Garg et al., 2004a). Furthermore, It has been FDA approved as an alternative vaginal topical microbicide following the drawbacks found with the Nonoxynolol-9 when it was once used as a spermicidal and contraceptive (i.e. it potentiated bacterial vaginosis as well as HIV transmission) (Richardson et al., 1998; Rosenstein et al., 1998; Anderson et al., 2000; Simoes et al., 2002). In addition, PSS has been shown to possess some ARV effect (Simoes et al., 2002), and it has gelling properties which may help in preventing HIV transmission by acting as a physical barrier

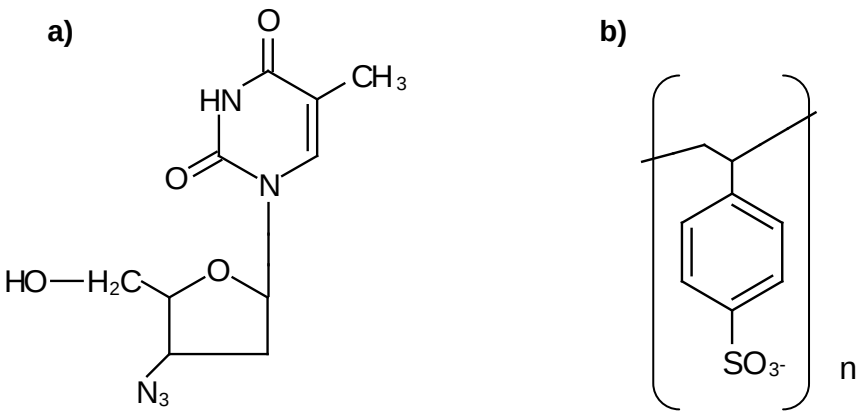


Figure 3.3: a) 3'-azido-3'-deoxythymidine (AZT), molecular weight 267.24 and a solubility 20.1 mg/mL, b) Polystyrene Sulfonate (PSS) molecular weight 70,000, water soluble.

3.2.2.2 Preparation of preliminary lead intravaginal bioadhesive polymeric devices

In the search for a suitable combination of polymers which will finally have the suitable bioadhesivity, matrix integrity and controlled release effect for the development of a novel controlled release intravaginal bioadhesive device, vast *in vitro* preformulation work was

carried out on 18 different polymers as mentioned above (Section 3.2.2.1). From these polymers, 62 different formulations weighing 800mg each were obtained through different polymer combinations at varying quantities of each polymer (Table 3.2). All polymer combinations were thoroughly blended using an Erweka cube blender (Erweka Apparatebau, Heusenstamm, Germany) and then directly compressed into caplet devices using a Carver Press (Carver Inc. Hydraulic Laboratory Press, Wabash, IN, USA) at a force of 5 tons. Each device was subjected to in-process validation tests that involved longitudinal crushing force, friability and mass uniformity analysis to ensure manufacturing reproducibility. A sample of 10 devices from each formulation was used for each test. Longitudinal crushing force test was conducted on a Hardness Tester (Pharma Test, Hainburg, Germany) while friability was conducted on a Friabilator (Erweka D-63150, Heusenstamm, Germany) at 25rpm for 4 minutes with 1% set as the upper limit of acceptability. The weight of each device was determined using an electronic balance (Mettler, Model AE 240, Griefensee, Switzerland) with readings recorded to 2 decimal places.

Table 3.2: The sixty two formulations obtained from different polymer combinations

	Polymeric Composition (mg)																							
F	**P6**	**PL**	**PE**	**PA**	**CG**	**EC**	**PV**	**XG**	**GL**	**ML**	**HE**	**HP**	**BW**	**ES**	**ER**	**MA**	**MB**	**MC**	**MD**	**ME**	**MF**	**MG**	**MH**	**MI**	**WT**
1	150	150	100	200	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
2	100	150	150	200	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
3	100	100	200	250	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
4	130	155	170	205	140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
5	120	180	160	210	130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
6	200	150	150	200	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
7	105	155	105	205	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
8	110	165	120	230	175	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
9	140	160	110	240	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
10	230	120	100	110	240	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
11	210	105	130	205	250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
12	205	150	145	200	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
13	150	300	150	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
14	250	200	150	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
15	50	300	125	125	-	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
16	50	200	125	125	-	300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
17	50	150	125	125	-	350	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
18	50	100	125	125	-	400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
19	150	250	-	125	-	275	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
20	150	225	-	175	-	250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
21	150	200	-	225	-	225	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
22	160	250	-	190	-	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
23	170	250	-	205	-	175	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
24	180	250	-	220	-	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
25	190	250	-	235	-	125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
26	195	250	-	225	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
27	200	250	-	25	-	300	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
28	175	250	-	25	-	300	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
29	150	250	-	25	-	300	75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
30	125	250	-	25	-	300	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
31	100	250	-	25	-	300	125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
32	220	275	-	25	-	250	25	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
33	215	275	-	25	-	250	25	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
34	210	275	-	25	-	250	25	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
35	205	275	-	25	-	250	25	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
36	200	275	-	25	-	250	25	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
37	195	275	-	25	-	250	25	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
38	190	275	-	25	-	250	25	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
39	185	275	-	25	-	250	25	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
40	180	275	-	25	-	250	25	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
41	175	275	-	25	-	250	25	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
42	195	300	-	25	-	250	25	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
43	185	300	-	25	-	250	25	-	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
44	175	300	-	25	-	250	25	-	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
45	165	300	-	25	-	250	25	-	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
46	155	300	-	25	-	250	25	-	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
47	175	300	-	25	-	250	25	-	-	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800
48	175	300	-	25	-	250	25	-	-	-	25	-	-	-	-	-	-	-	-	-	-	-	-	-	800
49	175	300	-	25	-	250	25	-	-	-	-	25	-	-	-	-	-	-	-	-	-	-	-	-	800
50	175	300	-	25	-	250	25	-	-	-	-	-	25	-	-	-	-	-	-	-	-	-	-	-	800
51	175	300	-	25	-	250	25	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	-	-	800
52	175	300	-	25	-	250	25	-	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	-	800
53	150	400	-	25	-	150	25	-	-	-	-	-	-	-	-	50	-	-	-	-	-	-	-	-	800
54	150	400	-	25	-	150	25	-	-	-	-	-	-	-	-	-	50	-	-	-	-	-	-	-	800
55	150	400	-	25	-	150	25	-	-	-	-	-	-	-	-	-	-	50	-	-	-	-	-	-	800
56	150	400	-	25	-	150	25	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-	-	-	800
57	150	400	-	25	-	150	25	-	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-	-	800
58	150	400	-	25	-	150	25	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-	800
59	75	400	-	25	-	200	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75	-	-	800
60	75	400	-	25	-	200	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75	-	800
61	75	400	-	25	-	200	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75	800
62	150	400	-	25	-	200	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800

F: *Formulatio number;* ***P6:*** *Modified polyamide 6,10;* ***PL:*** *Poly(lactic-co-glycolic acid);* ***PE:*** *Polyethylene oxide* ***PA:*** *Polyacrylic acid;* ***CG:*** *Carrageenan;* ***EC:*** *Ethycellulose;* ***PV:*** *Polyvinylalcohol;* ***XG:*** *Xanthan gum;* ***GL:*** *gelatin;* ***ML:*** *Methylcellulose;* ***HE:*** *Hydroxyethylcellulose;* ***HP:*** *Hydroxypropylcellulose;* ***BW:*** *beeswax;* ***ES:*** *Eudragit S100;* ***ER:*** *Eudragit RS 100;* ***MA, MB, MC, MD, ME, MF:****Mixture of two polymers among gelatin, beeswax, xanthan gum and Eudragit S 100 25mg each;* ***MG, MH, MI:*** *Mixture of three polymers among gelatin, beeswax, xanthan gum and Eudragit S 100 25mg each;* ***WT:*** *Weight*

For the mixture of two polymers, MA was GL+ES; MB: GL+BW; MC: ES+BW; MD: ES+XG; ME: BW+XG and MF: GL+XG while for the mixture of three polymers, MG was GL+ES+BW; MH: GL+BW+XG and MI: ES+BW+XG.

3.2.2.3 Equilibrium swelling studies conducted on the preliminary formulations as a critical indicator of the formulation's matrix stability

A polymer combination selected as the working formulation onto which the OVAT approach was applied in an attempt to find lead IBPDs was: $_m$PA 6,10 (50mg), PLGA (300mg), PEO (125mg), PAA (125mg) and CG (200mg). A series of swelling tests were conducted on various sets of the formulations shown in Table 3.2 to investigate which device would have the optimal swelling at both human and pig vaginal pH (i.e. ~4.5) and human/pig seminal pH (i.e. ~7.4) by employing the OVAT approach. Due to the physiological similarity between the human and pig (Buddhikot et al. 1999; Quintanar-Guerrero et al., 2001; Sandri et al., 2004; D'cruz et al., 2005b), simulated human vaginal and seminal fluids (Owen and Katz, 1999, 2005) prepared as indicated in Table 3.3, were employed for the tests. Each of the tested devices was weighed, immersed into both simulated fluids and then placed in an orbital shaking incubator (MRC Laboratory Instruments Ltd., Hahistadrut, Holon, Israel), maintained at 20rpm and a temperature of 37°C for 24 hours. After 24 hours each device was removed from the orbital shaking incubator, gently blotted on a filter paper and then re-weighed. The swelling behavior was determined in terms of the equilibrium swelling ratio (ESR) which was calculated using Equation 3.1. The ESR (after 24 hours) which was the critical indicator of the formulation's matrix stability (i.e. the degree of matrix robustness), was used as a screening parameter for each formulation.

$$ESR = \frac{W_t - W_o}{W_o} \qquad \textbf{\textit{Equation 3.1}}$$

where ESR is the equilibrium swelling ratio, W_o is the initial weight of the device and W_t is the weight of the device after 24 hours.

Table 3.3: Constituents used to prepare the simulated human vaginal and seminal fluids

SHVF[1]		SHSF[2]	
Component	**Quantity(g/L)**	**Component**	**Quantity(g/L)**
NaCl	3.510	$NaH_2PO4{\cdot}H2O$	16.974
KOH	1.400	Na_2HPO_4	17.466
$Ca(OH)_2$	0.222	$Na_3C_3H_5O(CO_2)_3$	8.130
Bovine serum albumin	0.018	KCl	0.908
Lactic acid	2.000	KOH	0.881
Acetic acid	1.000	$CaCl_2$	1.010
Glycerol	0.160	$MgCl_2$	0.920
Urea	0.400	$ZnCl_2$	0.344
Glucose	5.000	Glucose	1.020
		Fructose	2.720
		Urea	0.450
		Lactic acid	0.620
		Bovine serum albumin	50.400

[1] *Simulated human vaginal fluid according to Owen and Katz, (1999)*
[2] *Simulated human seminal fluid according to Owen and Katz, (2005)*

3.2.2.4 Effect of changing the formulation components of the IBPD on the equilibrium swelling ratio

The formulations components of the IBPD were changed sequentially (changes highlighted in grey color) to see the effect on equilibrium swelling ratio.

3.2.2.4.1 Effect of the elimination of carrageenan

Carrageenan is a highly hydrophilic compound which is substantially water-soluble with the capacity to transform into a gel that presents with a high degree of swelling in an alkaline pH (Campo et al., 2009). It was thus eliminated to see the effect in terms of the composite swelling of the devices. After elimination of the carrageenan an adjustment of the quantities of the remaining polymers was undertaken to maintain a constant weight of 800mg (Table 3.4).

Table 3.4: Formulations without carrageenan

Polymeric Composition (mg)				
F #	**$_m$PA 6,10**	**PLGA**	**PAA**	**PEO**
1	50.0	400.0	250.0	100
2	100.0	350.0	250.0	100
3	200.0	250.0	250.0	100
4	250.0	200.0	250.0	100
5	300.0	150.0	250.0	100
6	350	100.0	250.0	100

3.2.2.4.2 Effect of the addition of ethylcellulose

Due to its hydrophobic nature and high compressibility, EC was employed to replace CG. The quantities of PAA and PEO were substantially reduced as they enhanced matrix swelling, due to their high degree of hydrophilicity. The quantities of EC employed ranged from 240mg-460mg as shown in Table 3.5.

Table 3.5: Formulations in which carrageenan was substituted with ethylcellulose

Polymeric Composition (mg)					
F #	**$_m$PA 6,10**	**PLGA**	**PEO**	**PAA**	**EC**
1	90.0	340.0	65	65.0	240.0
2	90.0	240.0	65	65.0	340.0
3	90.0	190.0	65	65.0	390.0
4	90.0	140.0	65	65.0	440.0
5	90.0	130.0	65	65.0	450.0
6	90.0	120.0	65	65.0	460.0

3.2.2.4.3 Effect of the elimination of polyethylene oxide

PEO is a hydrophilic polymer with a high tendency to undergoing swelling (Varma et al., 2004). PEO as a bioadhesive and a rate-controlling polymer was removed to reduce the number of hydrophilic polymers so as to reduce the extent of swelling of the devices. However, PAA remained included throughout all formulations due to its substantial bioadhesive imparting (Table 3.6).

Table 3.6: Formulations without polyethylene oxide

Polymeric Composition (mg)				
F #	**$_m$PA 6,10**	**PLGA**	**PAA**	**EC**
1	100	300.0	75.0	325
2	100	300.0	100	300
3	100	300.0	150	250
4	100	300.0	200.0	200
5	100	300.0	250.0	150
6	100	300.0	300.0	100

3.2.2.4.4. Effect of the addition of polyvinyl alcohol

PVA has high tensile strength and flexibility, as well as adhesive and high oxygen barrier properties (Yan et al., 2006; Yang et al., 2008; Mukherjee, 2009). It is also an atactic material but exhibits crystallinity as the hydroxyl groups are small enough to fit into the lattice without disrupting it. It was therefore incorporated into the formulation to enhance the control of drug release as well as the bioadhesivity of the devices. Quantities ranging from 25mg-150mg were employed in formulations to produce a total weight of 800mg/device while keeping the quantities of PLGA, EC and PAA constant (Table 3.7).

Table 3.7: Formulations containing polyvinyl alcohol

Polymeric Composition (mg)					
F#	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**
1	200.0	250.0	300.0	25.0	25.0
2	175.0	250.0	300.0	25.0	50.0
3	150.0	250.0	300.0	25.0	75.0
4	125.0	250.0	300.0	25.0	100.0
5	100.0	250.0	300.0	25.0	125.0
6	75.0	250.0	300.0	25.0	150.0

3.2.2.4.5 Effect of the addition of polyvinyl povidone

PVP is a water-soluble material with a Newtonian-type viscosity. When dry it is a light flaky powder, capable of readily absorbing up to 40% of its weight in atmospheric water. In solution, it has excellent wetting properties and readily forms films which make it a good coating agent. It was therefore added to the formulations ranging from 25-150mg with the intention of fostering the bioadhesivity of the devices. The quantities of PLGA, EC and PAA were kept constant as shown in Table 3.8.

Table 3.8: Formulations containing polyvinyl povidone

Polymeric Composition (mg)					
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVP**
1	200.0	250.0	300.0	25.0	25.0
2	175.0	250.0	300.0	25.0	50.0
3	150.0	250.0	300.0	25.0	75.0
4	125.0	250.0	300.0	25.0	100.0
5	100.0	250.0	300.0	25.0	125.0
6	50.0	250.0	300.0	25.0	150.0

3.2.2.4.6 Effect of the addition of xanthan gum

XG is a water-soluble polysaccharide produced by *Xanthomonas campestris*. It forms a single or double-stranded helix in dilute solution that transforms into molecular assemblages which show liquid crystallinity in the presence of water (Rodd et al., 2000; Iseki et al., 2001). XG can form hydrogels upon annealing its solution in the sol state and then cooling (Quinn et al., 1994; Song et al., 2006). XG was therefore added to the formulations due to its gel-forming and viscosity-enhancing properties in an attempt to facilitate adhesion of the device to the vaginal epithelium. The quantities added ranged from 5-50mg. The quantities of PLGA, EC, PAA and PVA were kept constant (Table 3.9).

Table 3.9: Formulations containing xanthan gum

Polymeric Composition (mg)						
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**	**XG**
1	220.0	275.0	250.0	25.0	25.0	5.0
2	210.0	275.0	250.0	25.0	25.0	15.0
3	200.0	275.0	250.0	25.0	25.0	25.0
4	190.0	275.0	250.0	25.0	25.0	35.0
5	180.0	275.0	250.0	25.0	25.0	45.0
6	175.0	275.0	250.0	25.0	25.0	50.0

3.2.2.4.7 Effect of the addition of guar gum

GG is a polysaccharide comprising galactose and mannose. In water it is nonionic and hydrocolloidal and remains stable in solution over a pH range of 5-7. Guar gum presents with high low-shear viscosity but is strongly shear-thinning. It is very thixotropic above 1%$^w/_v$ with a low degree of thixotropy below a concentration of 0.3%$^w/_v$ (Soukoulis et al., 2007). It shows viscosity synergy with xanthan gum. Guar gum has almost eight times

the water-thickening potency of other thickening agents such as starch, and therefore can be used in the preparation of various multi-phase formulations (Billa et al., 2000). It was therefore added to the current formulations so as to enhance the bioadhesivity. The quantities added ranged from 5-50mg (Table 3.10).

Table 3.10: Formulations containing guar gum

Polymeric Composition (mg)						
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**	**GG**
1	220.0	275.0	250.0	25.0	25.0	5.0
2	210.0	275.0	250.0	25.0	25.0	15.0
3	200.0	275.0	250.0	25.0	25.0	25.0
4	190.0	275.0	250.0	25.0	25.0	35.0
5	180.0	275.0	250.0	25.0	25.0	45.0
6	175.0	275.0	250.0	25.0	25.0	50.0

3.2.2.4.8 Effect of the addition of gelatin

GL is a translucent, brittle, solid substance derived from the collagen obtained from skin and bones of animals. It forms a solution of high viscosity in water, which sets to a gel on cooling. Gelatin solutions present with viscoelastic flow and streaming birefringence (Ward and Courts, 1977). It qualifies as a binder, and therefore was used to substitute xanthan gum in an attempt to improve the bioadhesivity of the devices. The quantities added ranged from 5-50mg while the quantities of the other polymers remained constant (Table 3.11).

Table 3.11: Formulations in which xanthan gum was substituted with gelatin

Polymeric Composition (mg)						
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**	**GL**
1	195.0	300.0	250.0	25.0	25.0	5
2	185.0	300.0	250.0	25.0	25.0	15
3	175.0	300.0	250.0	25.0	25.0	25
4	165.0	300.0	250.0	25.0	25.0	35
5	155.0	300.0	250.0	25.0	25.0	45
6	150.0	300.0	250.0	25.0	25.0	50

3.2.2.4.9 Effect of the addition of beeswax

Beeswax is comprised of a mixture of many organic compounds, including hydrocarbons, wax esters, and fatty acids. It has emollient, soothing and softening properties. Furthermore, it is very stable over prolonged periods. It is resistant to hydrolysis and natural oxidization and is completely insoluble in water. Thus, beeswax was added to the formulations ranging from 5-50mg (Table 3.12) so as to increase the matrix stability of the devices.

Table 3.12: Formulations in which gelatin was substituted with beeswax

	Polymeric Composition (mg)					
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**	**BW**
1	195.0	300.0	250.0	25.0	25.0	5
2	185.0	300.0	250.0	25.0	25.0	15
3	175.0	300.0	250.0	25.0	25.0	25
4	165.0	300.0	250.0	25.0	25.0	35
5	155.0	300.0	250.0	25.0	25.0	45
6	150.0	300.0	250.0	25.0	25.0	50

3.2.2.4.10 Effect of the addition of tragacanth

Tragacanth is a translucent and horny fracture short material. It swells in water forming smooth nearly uniform stiff opalescent mucilage that is free of cellular fragments. It imparts great viscidity to water, a property that renders it useful for the suspension of heavy insoluble powders. Pharmaceutically, it is has good applications particularly in imparting consistence to troches and emulsions. It was added to the formulations for its effect on the matrix stability. The quantities added ranged from 5-50mg (Table 3.13).

Table 3.13: Formulations containing tragacanth

	Polymeric Composition (mg)					
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**	**TG**
1	195.0	300.0	250.0	25.0	25.0	5
2	185.0	300.0	250.0	25.0	25.0	15
3	175.0	300.0	250.0	25.0	25.0	25
4	165.0	300.0	250.0	25.0	25.0	35
5	155.0	300.0	250.0	25.0	25.0	45
6	150.0	300.0	250.0	25.0	25.0	50

3.2.2.4.11 Effect of the addition of methylcellulose, hydroxyethylcellulose, hydroxyl-propylcellulose, hydroxypropylmethylcellulose, Eudragit® S100 and Eudragit® RS 100 as substitutes for gelatin

MC, HEC, HPC and HPMC are cellulose ethers that differ in their type and degree of substitution. These polymers are hydrophilic in nature with pharmaceutical attributable properties that favor their use in the preparation of controlled release formulations. They are also used as bulking agents in the tabletting process. When exposed to aqueous media, they swell forming a gel layer around the tablet core (Baumgartner et al., 2002). Chain dissolution may take place at the gel surface depending on the type of cellulose ester. These polymers were added to the formulations to investigate their effect on the equilibrium swelling ratio of the devices with the intention of finally employing them to control drug release.

ED-S100 and ED-RS100 are poly(ethyl acrylate, methyl-methacrylate, and chlorotrimethyl-ammoniumethylmethacrilate) co-polymers. They are insoluble at physiological pH but undergo swelling in water (Pignatello et al., 2002). ED-S100 and ED-RS100 are commonly employed for the enteric coating of tablets and in the preparation of controlled release formulations. Both are good materials for the dispersion of drugs and have been used successfully to obtain appropriate controlled release matrix formulations (Ndesendo et al., 1996; Wong et al., 1999; Trapani et al., 2007). They were therefore added to the formulations for the purpose of modulating the release kinetics. The quantity of each polymer added to the formulations was 25mg. The quantities of the other polymers were kept constant (Table 3.14).

Table 3.14: Formulations containing methylcellulose, hydroxyethlycellulose, hydroxyl-propylcellulose, hydroxypropylmethylcellulose, Eudragit® S100 and Eudragit® RS100

Polymeric Composition (mg)						
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**	**Additional polymer**
1	175.0	250.0	300.0	25.0	25.0	25 (MC)
2	175.0	250.0	300.0	25.0	25.0	25 (HEC)
3	175.0	250.0	300.0	25.0	25.0	25 (HPC)
4	175.0	250.0	300.0	25.0	25.0	25 (HPMC)
5	175.0	250.0	300.0	25.0	25.0	25(ED-S100)
6	175.0	250.0	300.0	25.0	25.0	25 (ED-RS100)

3.2.4.12 Effect of the addition of a binary polymer combination to the formulation

Combinations of 2 polymers in equal quantities of 25mg each (1:1), from among gelatin, beeswax, xanthan gum and ED-S100 were added to the formulations to investigate their effect on the matrix stability. The quantities of the other polymers were kept constant (Table 3.15).

Table 3.15: Formulations with a binary polymer combination among gelatin, beeswax, xanthan gum and Eudragit® S100

Polymeric Composition (mg)						
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**	**Combination of 2 polymers**
1	150.0	400.0	150.0	25.0	25.0	50 (GL+ED-S100)
2	150.0	400.0	150.0	25.0	25.0	50 (GL+BWX)
3	150.0	400.0	150.0	25.0	25.0	50 (ED-S100+BWX)
4	150.0	400.0	150.0	25.0	25.0	50 (ED-S100+XG)
5	150.0	400.0	150.0	25.0	25.0	50 (BWX+XG)
6	150.0	400.0	150.0	25.0	25.0	50 (GL+XG)

3.2.2.4.13 Effect of the addition of a tertiary polymer combination to the formulation

Combinations of 3 polymers in equal quantities of 25mg each (1:1:1), from among gelatin, beeswax, xanthan gum and ED-S100 were added to the formulations to investigate the effect on the matrix stability of the devices. The quantities of the other polymers were kept constant (Table 3.16).

Table 3.16: Formulations with a tertiary polymer combination among gelatin, beeswax, xanthan gum and Eudragit® S100

	Polymeric Composition (mg)					
F #	**$_m$PA 6,10**	**PLGA**	**EC**	**PAA**	**PVA**	**Combination of 3 polymers**
1	75.0	400.0	200.0	25.0	25.0	75(GL+ED-S100+BWX)
2	75.0	400.0	200.0	25.0	25.0	75 (GL+BWX+XG)
3	75.0	400.0	200.0	25.0	25.0	75 (ED-S100+BWX+XG)

3.3 Results and Discussion

3.3.1 Physical properties of $_m$PA 6,10 and in-process validation tests

The $_m$PA 6,10 product synthesized presented as a firm white crystalline spherical compact. The unrefined $_m$PA 6,10 powder was subsequently sieved through an aperture size of 1mm in keeping with the other polymeric components of the caplet matrix to obtain a particle size ranging from 0.8-1.2mm which facilitated desirable powder compression properties. The compressed caplets (Figure 3.4) were sufficiently strong and robust (i.e. sturdy and with high resilience) with an average longitudinal crushing force of 286±0.03N, a uniformity mass of 800±0.48mg and average friability of 0.31±0.04% which was within the set limits (Table 3.17).



Figure: 3.4: The intravaginal bioadhesive polymeric devices (IBPDs) a) Planar view, b) Side view, c) Oblique view and d) Longitudinal view.

Table 3.17: Mass, friability and longitudinal crushing force of the sixty two caplet formulations

F#	Mass (mg)	±SD	% Friability	±SD	Longitudinal Crushing Force (N)	±SD
1	789.22	3.633	0.39	0.018	284.81	1.311
2	803.65	4.109	0.41	0.019	285.76	1.317
3	788.11	2.998	0.44	0.020	284.28	1.309
4	799.06	3.678	0.42	0.019	284.82	1.313
5	800.09	4.003	0.40	0.018	284.92	1.315
6	801.89	4.006	0.40	0.018	285.61	1.318
7	798.09	3.593	0.42	0.019	285.90	1.319
8	788.72	3.701	0.41	0.020	285.82	1.317
9	804.29	4.015	0.39	0.017	285.09	1.312
10	803.22	4.012	0.39	0.015	284.41	1.308
11	804.21	4.018	0.46	0.024	280.63	1.309
12	790.32	3.456	0.37	0.013	285.71	1.326
13	800.33	4.009	0.35	0.011	285.92	1.328
14	803.43	4.013	0.36	0.013	286.03	1.330
15	805.21	4.127	0.33	0.010	286.41	1.338
16	802.44	4.018	0.32	0.010	286.65	1.339
17	801.29	4.003	0.32	0.012	286.91	1.400
18	801.29	4.003	0.30	0.009	287.25	1.403
19	803.64	4.012	0.29	0.009	287.07	1.401
20	789.08	3.987	0.28	0.007	287.38	1.328
21	789.00	3.967	0.26	0.005	286.82	1.330
22	805.78	4.134	0.27	0.006	286.71	1.338
23	788.99	3.556	0.31	0.011	286.52	1.339
24	801.98	4.007	0.33	0.013	286.33	1.400
25	800.37	4.001	0.32	0.013	287.18	1.403
26	789.98	3.897	0.30	0.010	288.97	1.401
27	810.02	5.004	0.24	0.005	289.36	1.428
28	801.99	4.120	0.29	0.009	287.66	1.429
29	788.89	3.898	0.30	0.010	287.94	1.407
30	802.77	4.108	0.31	0.010	286.84	1.330
31	807.22	4.130	0.32	0.012	286.62	1.338
32	789.69	3.789	0.34	0.012	285.51	1.339
33	803.11	4.015	0.37	0.015	285.42	1.400
34	801.25	4.001	0.35	0.014	285.08	1.403
35	800.66	4.002	0,33	0.013	284.93	1.381
36	798.76	3.972	0.30	0.009	287.19	1.328
37	792.53	3.864	0.32	0.010	287.28	1.330
38	801.48	3.999	0.33	0.013	287.39	1.338
39	790.98	3.870	0.26	0.010	288.14	1.429
40	808.79	4.137	0.29	0.010	287.66	1.400
41	789.98	2.987	0.27	0.012	288.49	1.403
42	803.44	4.121	0.35	0.009	285.65	1.401
43	802.00	4.002	0.36	0.009	285.23	1.428
44	788.89	2.998	0.29	0.007	287.09	1.429
45	801.66	4.006	0.33	0.005	285.25	1.407
46	787.98	2.999	0.37	0.006	285.73	1.326
47	799.86	3.003	0.39	0.011	284.93	1.328
48	808.00	4.985	0,36	0.013	285.06	1.330
49	803.33	4.006	0.38	0.013	285.34	1.338
50	800.30	4.000	0.31	0.010	286.78	1.339
51	803.01	3.998	0.24	0.005	289.02	1.423
52	808.22	5.000	0.25	0.005	288.77	1.328
53	789.56	3.979	0.32	0.012	285.88	1.330
54	809.29	5.001	0.29	0.010	286.23	1.338
55	804.44	4.110	0.33	0.010	285.56	1.429
56	789.79	3.961	0.30	0.012	285.68	1.400
57	800.67	3.998	0.30	0.012	286.05	1.403
58	804.58	4.029	0.34	0.015	285.83	1.301
59	798.98	4.001	0.37	0.014	285.07	1.328
60	802.44	4.008	0.32	0.013	285.41	1.329
61	789.88	3.989	0.31	0.009	285.90	1.307
62	800.45	4.007	0.23	0.006	289.69	1.425

3.3.2 The influence of different polymeric composition on the equilibrium swelling ratio

3.3.2.1 The influence of carageenan, polyethylene oxide and ethylcellulose on the matrix stability

The formulations prepared with CG and PEO (F1- F12) (Table 3.2) presented with the highest level of matrix swelling as shown by their ESR values (0.55-0.76) (Table 3.18). The highest ESR was observed in F11 (ESR=0.76; Table 3.18). This was most likely due to the hydrophilic nature of CG and PEO. These polymers, being highly hydrophilic, rapidly absorbed water which led to a high degree of swelling. A similar trend was apparent in F3 (ESR=0.71) though to a lesser extent, due to relatively lower quantity of CG. The elimination of PEO and substitution of CG with EC resulted in a marked negative impact on matrix swelling in that a formulation with an ESR as low as 0.12 (F 19; Table 3.18) was obtained. This was due to the hydrophobic nature of EC.

3.3.2.2 The influence of guar gum, polyvinyl povidone, tragacanth and polyvinyl alcohol on matrix stability

GG produced matrix swelling to a large extent after 12 hours. PVP and TG were found to induce initial swelling and subsequent erosion of the formulation after 24 hours. PVA resulted in formulations with minimal matrix swelling (Table 3.18; F27; ESR=0.02) and therefore was considered to have the most favorable matrix stability among the entire group.

3.3.2.3 The influence of xanthan gum, beeswax and gelatin on the matrix stability

Xanthan gum provided substantially swollen formulations which remained intact (no evidence of erosion), in addition to being highly bioadhesive. Overall, XG, BWX and GL at low quantities (25mg each) (Table 3.18) resulted in formulations with minimal ESR (0.02-0.16). This may be due to the ability of XG, BWX and GL to form gel-like microstructure (Gimeno et al., 2003) which interspersed itself throughout.

3.3.2.4 The influence of the addition of methylcellulose, hydroxethylcellulose, hydroxypropylcellulose and hydroxylpropylmethylcellulose in the formulations, on matrix stability

The MC-containing formulations underwent initial massive swelling and thereafter erosion after 24 hours (formulations completely collapsed). HEC, HPC and HPMC-containing formulations remained stable for at least 72 hours. This finding may be attributed to the fact that these polymers are non-ionic and hydrophilic with water retention properties.

3.3.2.5 The influence of Eudragit® S100 and Eudragit® RS 100 on matrix stability

ED-S100 and ED-RS100 are random copolymers of methacrylic acid and ethyl acrylate, which are insoluble at acidic pH but become increasingly soluble in a neutral to weakly alkaline solution by forming salts (Guoqiang et al., 1995; Arasaratnam et al., 2000). Thus, the ED-S100- and ED-RS100-containing formulations presented with minimal swelling tendency (ESR=0.02 and 0.03) in simulated vaginal fluid (pH 4.5) (Table 3.18; F51 and F52).

Table 3.18: The equilibrium swelling ratios for the sixty two formulations that were subjected to screening using the OVAT approach

F#	ESR	±SD	F#	ESR	±SD
1	0.63	0.029	32	0.13	0.006
2	0.58	0.027	33	0.14	0.006
3	0.71	0.033	34	0.15	0.007
4	0.65	0.030	35	0.16	0.007
5	0.60	0.028	36	0.06	0.003
6	0.58	0.027	37	0.08	0.004
7	0.60	0.028	38	0.08	0.004
8	0.61	0.028	39	0.04	0.002
9	0.59	0.027	40	0.05	0.002
10	0.68	0.032	41	0.04	0.002
11	0.76	0.035	42	0.14	0.006
12	0.55	0.026	43	0.16	0.007
13	0.53	0.025	44	0.06	0.003
14	0.50	0.023	45	0.13	0.006
15	0.47	0.022	46	0.15	0.007
16	0.45	0.021	47	0.53	0.025
17	0.42	0.020	48	0.49	0.023
18	0.11	0.005	49	0.56	0.026
19	0.12	0.006	50	0.07	0.003
20	0.11	0.005	51	0.02	0.001
21	0.08	0.004	52	0.03	0.001
22	0.09	0.004	53	0.12	0.006
23	0.10	0.005	54	0.09	0.004
24	0.12	0.006	55	0.14	0.006
25	0.08	0.004	56	0.12	0.006
26	0.06	0.003	57	0.11	0.005
27	0.02	0.001	58	0.13	0.006
28	0.05	0.002	59	0.15	0.007
29	0.07	0.003	60	0.14	0.006
30	0.09	0.004	61	0.12	0.006
31	0.08	0.004	62	0.01	0.001

3.3.2.6 Equilibrium swelling ratios of the selected fifteen lead formulations screened through the OVAT approach

The ESRs for the selected 15 lead formulations are summarized in Table 3.19. Depending on the polymer combination and hydrophilic/hydrophobic proportions, all devices demonstrated a different swelling equilibrium as depicted by their ESR values (Table 3.19). Swelling ratio describes the amount of water that was contained within the device at equilibrium and is a function of the proportion between hydrophilicity and hydrophobicity in the device network structure. Ionization of the polymer functional groups, crosslinking density, charge density, and simulated vaginal fluid ionic strength,

may have played a role as well in this regard. The higher the hydrophobicity the lower the ESR and the higher the hydrophilicity the higher the ESR. The opposite holds true in both cases. Low ESR is an indication of low swelling rate and therefore high matrix stability and vice versa (Baumgartner et al., 2000; Girish et al, 2008; Wen et al., 2008). Among the 15 lead formulations, F62 presented with the lowest ESR (0.011). These findings may be associated with the presence of a high quantity of PLGA (400mg) in the formulation which prevented the influx of water into the IBPD matrix due to its high hydrophobicity. The presence of EC in the formulation at a relatively high quantity (200mg) may have as well attributed to the obtained results since EC is also a polymer with high degree of hydrophobicity. Most certainly, minimal quantities of the hydrophilic polymers PAA and PVA coupled the superior matrix resilience of $_{m}$PA 6,10 contributed as well to the small ESR value of F62. ED-S100 and ED-RS100 are both pH-dependent polymer materials that are only soluble at pH above 6.0 (Ohmura et al., 1991). Thus, at the simulated vaginal fluid pH (4.5), these polymers were insoluble indicating that they may have played a role in preventing the entrance of water molecules to the IBPD matrix, therefore leading to the low ESR values obtained in F51 (ESR=0.023) and F52 (ESR=0.033) respectively (Table 3.19).

The high tendency GL, BWX and XG to form a non-collapsible networked-structure may have improved the veracity of the IBPD matrix and therefore the relatively low values of ESR values obtained in the formulations containing these polymers (Table 3.19).The ESR for the best 15 formulations was in the following order F62>F27>F51>F52>F41>F39> F28 >F40>F44> F36>F29>F50> F37> F38>F31 (i.e. lowest to the highest) (Table 3.19). Overall, the high degree of hydrophobicity in the polymers constituting these formulations, prevented rapid penetration of water molecules into the IBPD matrix resulting in a low swelling tendency (Table 3.19) and therefore lower ESR values when compared to the rest of the tested formulations (Table 3.18).

Table 3.19: The selected fifteen lead formulations screened through the OVAT approach

Polymeric Composition (mg)											
F#	**P6**	**PL**	**PA**	**EC**	**PV**	**XG**	**GL**	**BW**	**ES**	**ER**	**ESR**
62	150	400	25	200	25	-	-	-	-	-	0.011
27	200	250	25	300	25	-	-	-	-	-	0.020
51	175	300	25	250	25	-	-	-	25	-	0.023
52	175	300	25	250	25	-	-	-	-	25	0.033
41	175	300	25	250	25	-	25	-	-	-	0.041
39	185	275	25	250	25	40	-	-	-	-	0.044
28	175	250	25	300	50	-	-	-	-	-	0.050
40	180	275	25	250	25	45	-	-	-	-	0.053
44	175	275	25	250	25	50	-	-	-	-	0.060
36	200	275	25	250	25	25	-	-	-	-	0.064
29	150	250	25	300	100	-	-	-	-	-	0.071
50	175	300	25	250	25	-	-	25	-	-	0.073
37	195	275	25	250	25	30	-	-	-	-	0.080
38	190	275	25	250	25	35	-	-	-	-	0.082
31	100	250	25	300	125	-	-	-	-	-	0.084

P6: *Modified polyamide 6,10;* ***PL:*** *Poly(lactic-co-glycolic acid);* ***PE:*** *Polyethylene oxide* ***PA:*** *Polyacrylic acid;* ***CG:*** *Carrageenan;* ***EC:*** *Ethycellulose;* ***PV:*** *Polyvinylalcohol;* ***XG:*** *Xanthan gum;* ***GL:*** *gelatin;* ***BW:*** *beeswax;* ***ES:*** *Eudragit S100;* ***ER:*** *Eudragit RS 100;* ***ESR:*** *Equilibrium swelling ratio*

3.4 Concluding Remarks

Through extensive screening of the developmental formulations using an OVAT approach, robust caplets with substantial matrix integrity were produced, as evidenced by the Equilibrium Swelling Ratios. Fifteen lead formulations with minimal swelling tendencies were obtained. F62 which is composed of $_{m}$PA 6,10 (150mg), PLGA (400mg), EC (200mg), PVA (25mg) and PAA (25mg) appeared to be the most suited in terms of minimal swelling capacity and highest matrix stability. Optimization was further conducted on the fifteen lead formulation employing ANN in Chapter 4.

CHAPTER FOUR

APPLICATION OF ARTIFICIAL NEURAL NETWORKS FOR THE ELUCIDATION OF AN OPTIMIZED SYNERGISTIC POLYMER COMBINATION FOR EFFECTIVE PERFORMANCE OF THE INTRAVAGINAL BIOADHESIVE POLYMERIC DEVICE

4.1 Introduction

Successful delivery of drugs to the human vagina is mainly dependent on the residence time as well as the ability of the delivery system to contain and release the drug in a controlled manner (Justin-Temu et a., 2004; Grassi et al., 2005; Andrews et al., 2009; Rohan and Sassi, 2009; Serra et al., 2009). Therefore, the selection of polymers to achieve good matrix integrity that will subsequently lead to the control of drug release is a vital goal in formulation optimization of an intravaginal drug delivery system. Conducting a comparative analysis of polymers for developing a specific drug delivery system is of paramount importance if the appropriate matrix integrity and controlled release effect is to be achieved. Comparative studies of polymers would also facilitate the process of formulation optimization. In addition, matrix integrity evaluation may enable identification of the physicochemical and physicomechanical properties of a polymeric delivery system which may be used to predict the *in vivo* behavior and therefore its suitability for intravaginal drug delivery.

Reproducibility of experimental procedures during formulation development is frequently hindered by numerous factors. In solving such problems, several techniques have been employed in attempting to discover the rules, patterns and relationships in a given data set. Software-based computational techniques namely Artificial Neural Networks (ANNs) are able to simulate the neurological processing ability of the human brain through mathematical modeling of its functional unit (Hopfied and Tank 1985; Achanta et al.,

1995; Arulsudar et al., 2005; Parojčić et al., 2007; Patel et al., 2007; Hassanien et al., 2008; Lee et al., 2008; Munasur et al., 2008; Lancashire et al., 2009). Essentially ANNs are networks of adaptable nodes, which store experimental knowledge through the learning process from the given samples (Yang et al., 2005; Lancashire et al., 2009). They can also be applied in establishing a non-linear relationship between causal factors and the required efficacy (Chen et al., 2002; Sun et al., 2003; Rizkalla and Hildgen 2005; Agatonovic-Kustrin et al., 2008; Najah et al., 2009). Thus, an ANN may be considered as an advanced non-linear regression model that can be used to assess the association of variables by means of iterative training of data obtained from a designed experiment (Takayama et al., 1999). Thus far, ANNs have successfully been used to solve various problems in pharmaceutical research, including pharmaceutical process optimization (Takahara et al., 1997; Peh et al., 2000), product development (Leane et al., 2003; Parojčić et al., 2007), predicting drug release patterns (Brier et al., 1995; Peh et a., 2000; Sun et al., 2003; Reis et al., 2004; Mendyk and Jachowicz, 2005; Fogel, 2008), predicting diffusion dynamics (Jha et al., 1995; Yuzgec et al., 2008; Lee et al., 2008), predicting the mechanism of action of drugs (Nair et al., 1994; Leane et al., 2003; Gunther et al. 2003; Ghaffari et al., 2006), predicting pharmacokinetic parameters, and pharmaceutical product coefficients (Weinstein et al., 1992; Hussain et al., 1993; Brier et al., 1995; Smith and Brier, 1996; Peh et al., 2000; Fu et al., 2003; Leane et al., 2003). Furthermore, ANNs are more accurate than conventional linear correlation techniques in identifying specific input variables such as the type of polymers that may substantially affect the performance of a formulation (Bourquin et al., 1998; Chen et al, 2002; Caputo and Pelagagge 2008). ANNs are also capable of generating non-linear input-output mappings for use in producing accurate confirmation or predictions of previous results within shortest periods and with highest computational precision (Agatonovic-Kustrin and Beresford 2000; Lee et al., 2008).

The aim of this Chapter was therefore to assess the matrix integrity of the initial selected 15 lead formulations derived from the OVAT-based developmental screening approach (Chapter 3), in terms of matrix erosion. ANN was used to optimize the erosion kinetics of the 15 lead formulations to generate an optimized formulation with the best erosion properties. Furthermore, to achieve ideal matrix erosion, ANN was employed to undertake sensitivity testing on the polymers that constituted the 15 lead formulations (Chapter 3; Section 3.3.2.6; Table 3.19) so as to optimize a selection of polymers that would ultimately lead to the achievement of this objective.

4.2. Materials and Methods

4.2.1 Materials

Materials employed are the same as in Chapter 3; Section 3.2.1.

4.2.2 Methods

4.2.2.1 Preparation of the intravaginal bioadhesive polymeric device

The polymers employed in this study were modified polyamide 6,10 ($_m$PA 6,10), poly(lactic-co-glycolic acid) (PLGA), polyacrylic acid (PAA 974), polyvinly alcohol (PVA), ethylcellulose (EC), polyethlene oxide (PEO), carageenan (CG) gelatin (GL), xanthan gum (XG), beeswax (BWX), Eudragit S-100 (ES100) and Eudragit RS-100 (ERS100). Different combinations and quantities of these polymers (Table 4.1), derived from the preliminary screening experiments that were conducted in Chapter 3, were blended by using a cube blender (Erweka® GmbH, Heusenstamm, Germany). The powder blends were then compressed at a pressure of 5 tons into caplet-shaped intravaginal bioadhesive polymeric devices (IBPDs) on a Manesty D3B 16 station tableting press equipped with D3B oblong tooling of 22×9mm in dimension (Manesty D3B L249Q, Liverpool, England).

Table 4.1: The Polymer combinations employed in the production of the intravaginal bioadhesive polymeric devices

Polymeric Composition (mg)											
F#	**P6**	**PG**	**PA**	**EC**	**PV**	**XG**	**GL**	**BW**	**ES**	**ER**	**WT**
62	150	400	25	200	25	-	-	-	-	-	800
27	200	250	25	300	25	-	-	-	-	-	800
51	175	300	25	250	25	-	-	-	25	-	800
52	175	300	25	250	25	-	-	-	-	25	800
41	175	300	25	250	25	-	25	-	-	-	800
39	185	275	25	250	25	40	-	-	-	-	800
28	175	250	25	300	50	-	-	-	-	-	800
40	180	275	25	250	25	45	-	-	-	-	800
44	175	275	25	250	25	50	-	-	-	-	800
36	200	275	25	250	25	25	-	-	-	-	800
29	150	250	25	300	100	-	-	-	-	-	800
50	175	300	25	250	25	-	-	25	-	-	800
37	195	275	25	250	25	30	-	-	-	-	800
38	190	275	25	250	25	35	-	-	-	-	800
31	100	250	25	300	125	-	-	-	-	-	800

P6: *Modified polyamide 6,10;* ***PL:*** *Poly(lactic-co-glycolic acid);* ***PE:*** *Polyethylene oxide* ***PA:*** *Polyacrylic acid;* ***CG:*** *Carrageenan;* ***EC:*** *Ethycellulose;* ***PV:*** *Polyvinylalcohol;* ***XG:*** *Xanthan gum;* ***GL:*** *gelatin;* ***BW:*** *beeswax;* ***ES:*** *Eudragit S100;* ***ER:*** *Eudragit RS 100;* ***WT:*** *Weight*

4.2.3. In-process validation tests

In-process validation tests involving indentation hardness, mass uniformity and friability analysis were performed to ensure the reproducibility of the matrices. Indentation hardness was represented by the Brinell Hardness Number (BHN) derived through the textural profiling analysis. A sample of 10 caplets from each formulation batch was tested for mass uniformity and friability using the same instruments, parameters and limits as described in Chapter 3, Section 3.2.2.2.

4.2.3.1 Determination of the Brinell hardness number

The BHN (N/mm^2) was computed for each set of devices using the ball probe approach. A calibrated Texture Analyzer (TA.*XTplus*, Stable Micro Systems, Surrey, UK) fixed with a ball probe indenter of diameter 3.125mm, indentation depth set at 1.5625mm and 5kg load cell for all readings was employed. The textural settings used to calculate the BHN values are depicted in Table 4.2. The typical force-distance profiles generated for the

calculation of the BHN value are illustrated in Figure 4.1. Each analysis was conducted in triplicate. The indentation hardness was represented by a conversion to the Brinell Hardness Number (BHN) (Equation 4.1).

$$BHN = \frac{2F}{\pi D\left(D - \sqrt{D^2 - d^2}\right)}$$

Equation 4.1

where F= force generated from indentation (N), D= diameter of ball probe indenter (3.125mm) and d= indentation depth (1.5625mm).

Table 4.2: Textural settings employed for BHN value calculations

Parameters	**Settings**
Pre-test speed	1.000 mm/sec
Test speed	0.500 mm/sec
Post-test speed	1.000 mm/sec
Compressive distance	1.563 mm
Trigger force	0.050 N

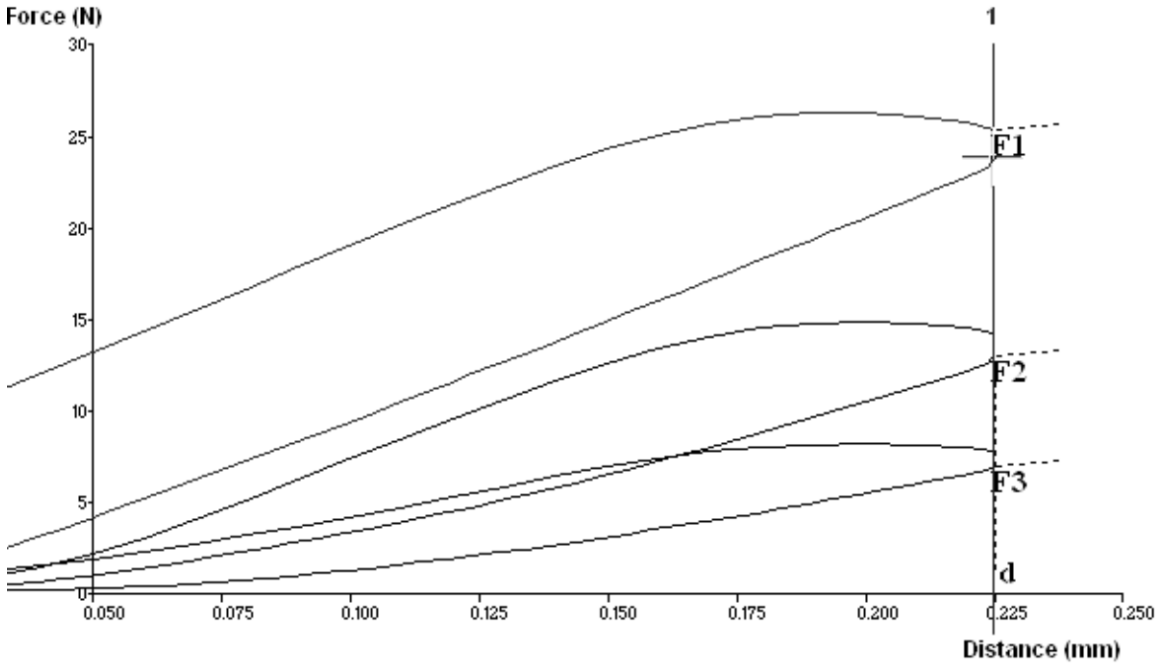


Figure 4.1: Typical force-distance profiles employed in the determination of matrix indentation hardness of the IBPD.

4.2.4 *In vitro* matrix erosion of the intravaginal bioadhesive polymeric device

Each formulation was subjected to *in vitro* matrix erosion studies. Formulations were immersed in 100mL of simulated vaginal fluid (pH 4.5; 37°C) using a sealable glass vessel (150mL) and then placed in an orbital shaking incubator (LM-530-2, MRC Laboratory Instruments Ltd., Hahistadrut, Holon, Israel) maintained at 20rpm. After 24 hours, each formulation was removed from the medium, blotted on filter paper (diameter 110mm and pore size 20µm) and dried to constant weight at 40±0.5°C in an oven. All determinations were conducted in triplicate. The mathematical expression stated in Equation 4.2 was employed to determine the percentage matrix erosion (ME) (i.e. $\%^{w}/_{w}$) of each formulation.

$$ME\ (\%) = \frac{OM - RM}{OM} \times 100 \qquad \textbf{Equation 4.2}$$

where ME is the matrix erosion, OM original mass, and RM the residual mass.

4.2.5 Optimization by ANN for the best polymer combination selection

Optimization was conducted by employing the feedback Multilayer Perceptron (MLP) neural network to train the empirical input matrix erosion data with static back propagation. Figure 4.2 illustrates the typical construction of the MLP network while Table 4.3 shows the input matrix erosion data (obtained from the matrix erosion studies) that was trained. The main advantage of these networks is that they can approximate any input/output map.

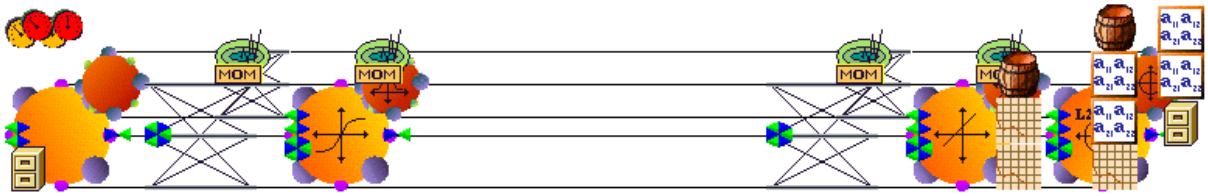


Figure 4.2: Constructed multilayer perceptron network.

A genetic algorithm with a Sigmoid Axon transfer function and Conjugated Gradient learning rule was employed for the hidden input and output layers. Figure 4.4 shows the network topology for the hidden input and output layers.

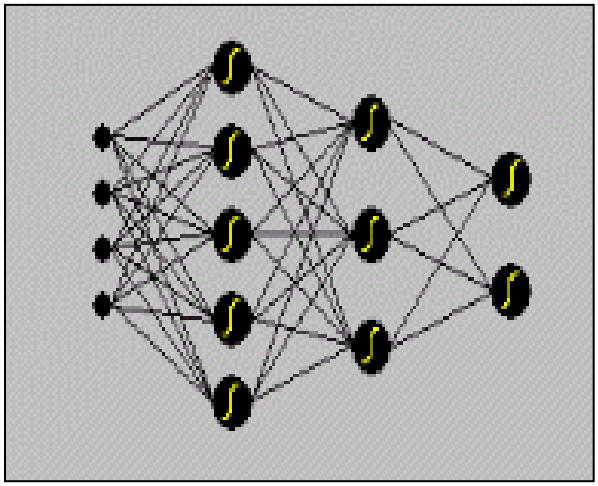


Figure 4.3: Network topology depicting the hidden input and output layers.

Table 4.3: The input matrix erosion data that was trained by the Multilayer Perception Network

Polymeric Composition											
F#	**P6**	**PG**	**PA**	**EC**	**PV**	**XG**	**GL**	**BW**	**ES**	**ER**	**ME%**
62	150	400	25	200	25	-	-	-	-	-	1.21
27	200	250	25	300	25	-	-	-	-	-	1.43
51	175	300	25	250	25	-	-	-	25	-	2.89
52	175	300	25	250	25	-	-	-	-	25	3.26
41	175	300	25	250	25	-	25	-	-	-	3.58
39	185	275	25	250	25	40	-	-	-	-	3.81
28	175	250	25	300	50	-	-	-	-	-	3.83
40	180	275	25	250	25	45	-	-	-	-	4.37
44	175	275	25	250	25	50	-	-	-	-	4.45
36	200	275	25	250	25	25	-	-	-	-	5.57
29	150	250	25	300	100	-	-	-	-	-	6.31
50	175	300	25	250	25	-	-	25	-	-	6.48
37	195	275	25	250	25	30	-	-	-	-	7.20
38	190	275	25	250	25	35	-	-	-	-	7.53
31	100	250	25	300	125	-	-	-	-	-	7.68

P6: *Modified polyamide 6,10;* ***PL:*** *Poly(lactic-co-glycolic acid);* ***PE:*** *Polyethylene oxide* ***PA:*** *Polyacrylic acid;* ***CG:*** *Carrageenan;* ***EC:*** *Ethycellulose;* ***PV:*** *Polyvinylalcohol;* ***XG:*** *Xanthan gum;* ***GL:*** *gelatin;* ***BW:*** *beeswax;* ***ES:*** *Eudragit S100;* ***ER:*** *Eudragit RS 100;* ***ME%:*** *Matrix erosion percentage*

A maximum of 10,000 epochs were run on NeuroSolutions Version 5.0 (NeuroDimension Inc., Gainsville, Florida) for ensuring optimal training of data. Sensitivity analysis was used for extracting the cause and effect relationship between the inputs and the outputs of the network. This could provide feedback pertaining to the input variable that was the most significant by testing the network with regard to its sensitivity about the matrix erosion for each formulation, thus elucidating the polymer combination type that was most significant. The parameters used to construct the neural network are as shown in Table 4.4

Table 4.4: Artificial neural network construction parameters employing a neural builder

Parameter	Setting
Hidden layer	2
Exemplars	17
Output Processing element	1
Transfer function	SigmoidAxon:sigmoid (0/1)
Learning rule	ConjugateGradient: second order method for gradient
Maximum Epochs	10,000: Supervised Learning Control Termination at Mean Square Error; Load Best Weights Approach
Probe Configuration	Quantitative-MatrixViewer, MatrixEditor; Qualitative-MegaScope, Hinton

4.3. Results and Discussion

4.3.1 In-process validation tests

The devices were uniform in mass (800±0.48mg). Textural analysis confirmed BHN values ranging from 2.687-4.981N/mm^2 while friability was at an average of 0.21% (Table 4.5), demonstrating desirable matrix compatibility and compressibility. F15 presented with the highest BHN value (4.981N/mm^2) while F1 presented with the lowest BHN value (2.687N/mm^2). The high compressibility of PLGA and EC coupled with the ability of GL, BWX and XG to form a gel-like macrostructure may have contributed to the good friability and BHN values obtained. In addition, GL, BWX and XG resulted in non-collapsible formulations with a swelling tendency which improved the veracity of the matrix networked-structure, which contributed to the highest matrix integrity and thus the high BHN values obtained.

Table 4.5: Brinell hardness number, mass, and friability of the intravaginal bioadhesive polymeric device

F#	BHN (N/mm^2)	±SD	Mass (mg)	±SD	%Friability	±SD
1	2.687	0.109	801.23	4.459	0.369	0.015
2	2.745	0.110	800.22	4.013	0.350	0.024
3	2.778	0.100	799.45	4.127	0.312	0.013
4	2.884	0.115	802.02	4.018	0.298	0.011
5	2.922	0.121	798.79	3.933	0.274	0.013
6	2.989	0.120	800.98	4.003	0.231	0.012
7	3.063	0.132	802.00	4.012	0.227	0.012
8	3.340	0.138	799.32	3.987	0.217	0.010
9	3.521	0.139	801.79	3.967	0.201	0.009
10	3.894	0.141	800.38	4.134	0.131	0.014
11	4.367	0.140	797.97	3.556	0.108	0.007
12	3.708	0.129	800.33	4.009	0.138	0.017
13	4.703	0.148	800.58	4.013	0.103	0.006
14	3.988	0.140	797.98	4.018	0.123	0.011
15	3.897	0.138	800.23	4.127	0.326	0.017

4.3.2 Matrix erosion evaluation

Matrix erosion, polymer swelling and drug dissolution are the important factors that determine drug release from polymer matrices (Colombo et al., 1996; Gao and Meury, 1996). Disolution systems can provide a means of overcoming the well-known advantage of a purely diffusion control system (Kim and Fassihi, 2000; Dürig and Fassihi, 2002; Al-Taani and Tashtoush, 2003; Lopes et al., 2007; Sriamornsak et al., 2007; Efentakis and Peponaki, 2008; Malik et al., 2008). Furthermore, synchronization between erosion and diffusion fronts may produce zero-order drug release kinetics (Lee, 1980; Pillay and Fassihi, 2000; Pillay and Danckwerts, 2002; Pillay et al., 2005; Al-Taani and Tashtoush, 2003). Normally in polymer matrices, polymer relaxation and erosion mechanisms may coexist (Christ et al., 1998; Bettini et al., 2001). The results of the matrix integrity as determined in terms of the matrix erosion percentage for the fifteen lead formulations are as shown in Table 4.3. Depending on the polymer combination and proportions of hydrophilic/hydrophobic polymer, all devices demonstrated different degrees of erosion as depicted in Table 4.3. F1 presented with the least matrix erosion (1.21%) while Formulation F31 presented with the highest matrix erosion (7.68%) (Table 4.3). Generally, the rate of matrix erosion from the IBPDs appeared to depend mainly on the networking

level of their hydrophilic and hydrophobic polymer-based microstructure. Matrix erosion in a polymer matrix is usually determined by the rate at which the polymer undergoes hydration and swelling (Roy et al., 2002; Khamanga and Walker, 2006; Sriamornsak et al., 2007; Choonara et al., 2008). Therefore, matrix erosion from the IBPDs depended on the relative magnitude of polymer hydration at the moving rubbery/glassy front within the IBPDs. F1 contained the highest content of PLGA (400mg) and a relativey high quantity of EC (200mg) (Table 4.3). These polymers are highly hydrophobic with high compressibility properties. The presence of relatively low quantities of the hydrophilic polymers in this formulation ($_m$PA 6,10: 150mg; PAA: 25mg; PVA 25mg) coupled with the hydrophobic nature of PLGA and EC are certainly the reasons for the minimal matrix erosion observed. The same reason holds for the rest of the formulations which apparently were the best formulations from the OVAT-based screening that was undertaken in Chapter 3. Thus, the high degree of hydrophobicity prevented rapid penetration of water molecules into the polymer matrices, leading to less hydration, swelling and erosion and therefore the lowest degree of matrix erosion.

PAA has played the leading role in fostering the bioadhesivity of the IBPD (see Chapter 5 later), but it had to be applied in minimal quantities due its high swelling properties. PAA in water swells up to 1000 times its original volume (Bonacucina et al., 2004), which permits the ionization of its carboxylic groups, that facilitate the formation of adhesive bonds with either the protein or glycoprotein of the vaginal epithelium (Figure 4.4). A chemometric structural model developed in our laboratories showed that the protein and polymer bonding at the cell surface of the vaginal epithelium, via the embedded glyco-protein and other surface proteins plays a key role in linking the incoming polymer or other smaller units. The saccharide part in the glycol-protein binds to the inherent surface protein and available functional groups in the protein and to some extent the glyco-proteins saccharide units are extended in binding to the polymer (i.e. PAA) (Figure 4.4).

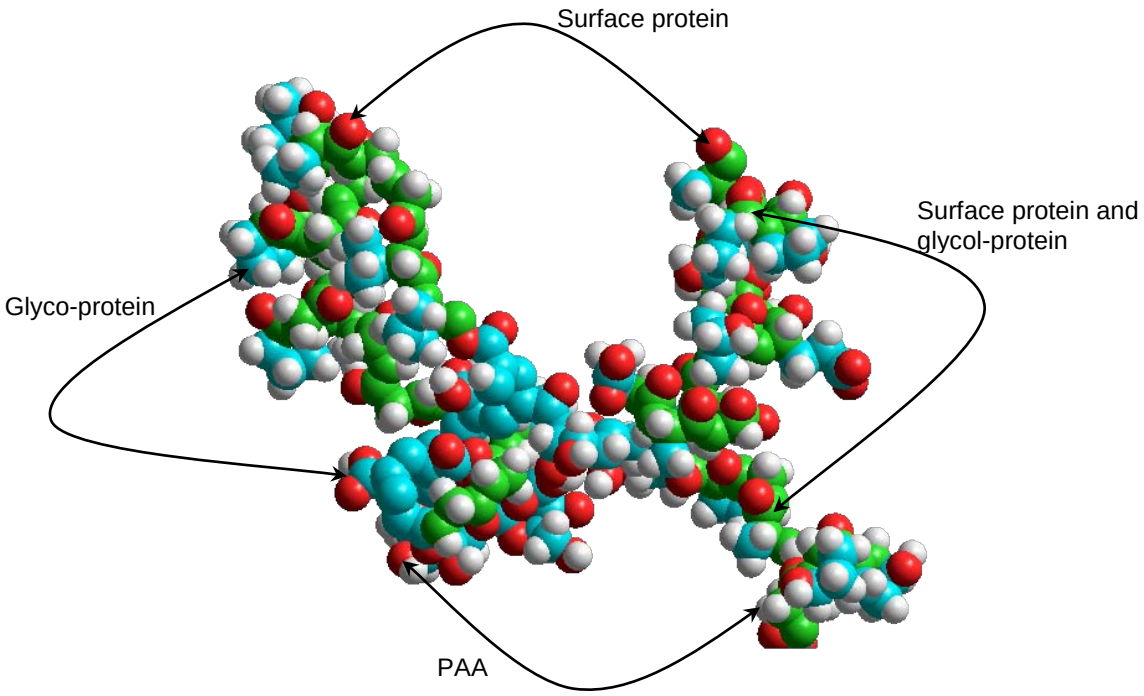


Figure 4.4: A chemometric structural model developed in our laboratories for a glyco-protein structure showing attachment of cellular surface protein (red) bound to the saccharide part of the glyco-protein (blue) and both (surface) protein and glyco-protein (green) bound to the polymer (here PAA) (white) for non-evenly distributed applied force during adhesion, with non-smooth and unevenly contacted two interacting surfaces with non-evenly distributed and non-constant, non-steadily (applied) force for the adhesion shown in a 3D optimized state with ball model. The coiling loop distortions for both protein and glycoprotein are also shown.

4.3.3 Selection of polymer combinations as per ANN optimization

For the 12 polymers that were employed in the study, the MLP network was able to accurately confirm that $_{m}$PA 6,10, PLGA, EC, PVA and PAA were the most significant input variables in terms of matrix integrity of the IBPD based on the empirical data. The approach followed in this work required prior assumption for the selection of a mathematical model before applying the ANN models so as to be able to confirm the sensitivity coefficients of the various polymer types as input variables that significantly contributed to characterizing the matrix integrity. In order to obtain accuracy and maximum degree of precision, the training was done twice (i.e. primary and secondary training). For the primary training, the three run outcomes are as shown in Figure 4.5a. Run # 2 gave the lowest level of the mean square error (MSE). The gradual leveling of the MSE with standard deviation (SD) boundaries for the training runs indicated a sequential

improvement of data modeling as illustrated in Figure 4.5b. Table 4.6 depicts the average of the MSE values for the three runs of the primary training, the best network run out 10,000 epochs, and the overall efficiency and performance of the neural network during the data training.

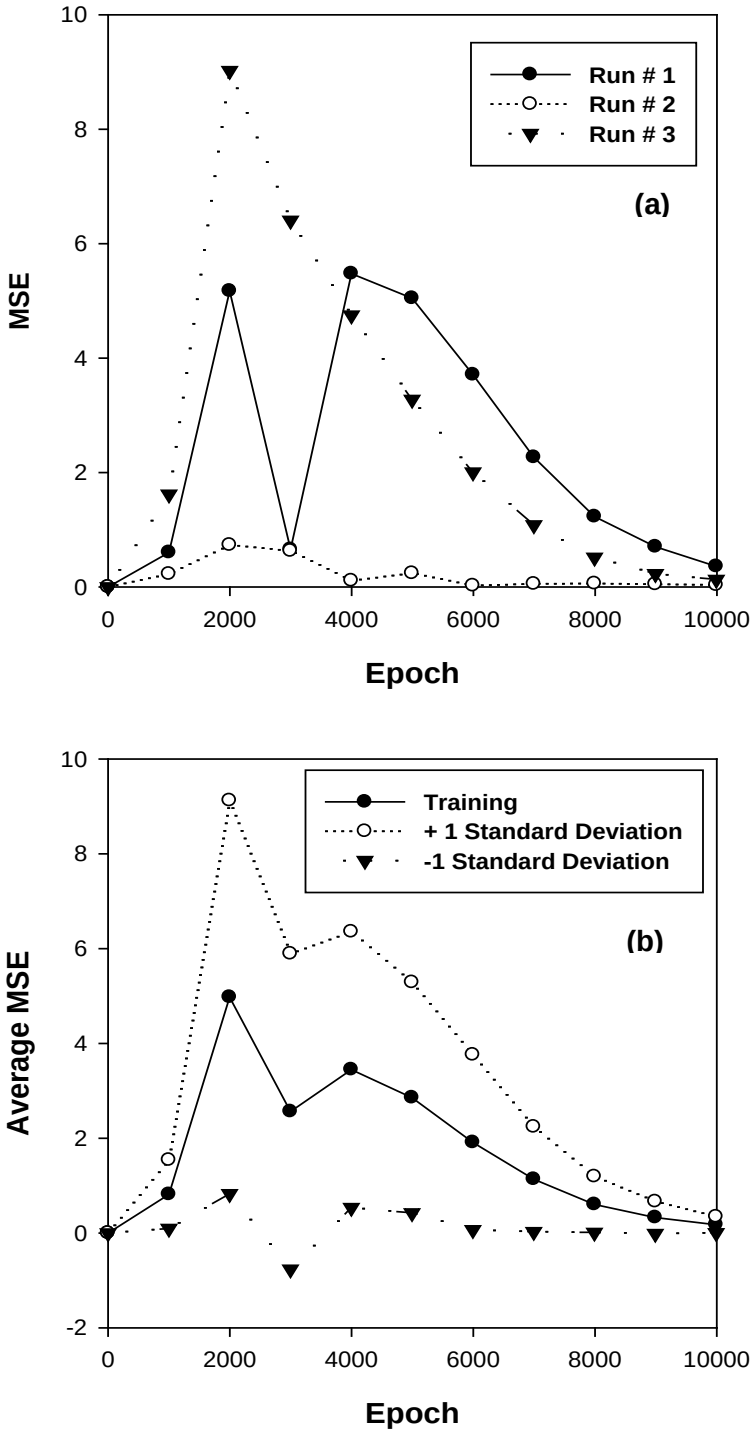


Figure 4.5: a) MSE for the primary ANN training, b) Average MSE for the primary ANN trains with standard deviations for 10,000 epochs.

Table 4.6: Neural network indicators characterizing the efficiency and performance of data in the primary training as per ANN

Best Network	Training	Performance	ME (%)
Epoch #	10,000	MSE	0.768
Minimum MSE	0.001	NMSE	0.010
Final MSE	0.002	MAE	0.519
-	-	Min Abs Error	0.002
-	-	Max Abs Error	2.512
R^2	-	-	0.99

MSE: Mean square error
NMSE: Normalized mean square error
MAE: Mean absolute error
Min Abs Error: Minimum absolute error
Max Abs Error: Maximum absolute error

Basing on the obtained results, it is evident that the employed training model was highly efficient (MSE=0.001). Results revealed a highly satisfactory fit for the input variables (R^2 = 0.99). The performance criterion employed to assess the closeness and correlation between the desired and the actual network output for matrix erosion of each formulation is listed in Table 4.7 and illustrated in Figure 4.6. The sensitivity coefficient of each polymer type (input variables) is depicted in Figure 4.7.

Table 4.7: Actual and desired network performance criterion data from the primary training

Desired output: ME (%)	Actual network output (%)
1.21	1.19905708
1.43	1.73679249
2.89	3.27070479
3.26	3.27070479
3.58	3.27070479
3.81	4.75966199
3.83	3.48203039
4.37	4.02684657
4.45	4.48380486
5.57	5.98735234
6.31	6.08292899
6.48	6.48950281
7.2	7.01026921
7.53	6.66716927
7.68	7.91356107

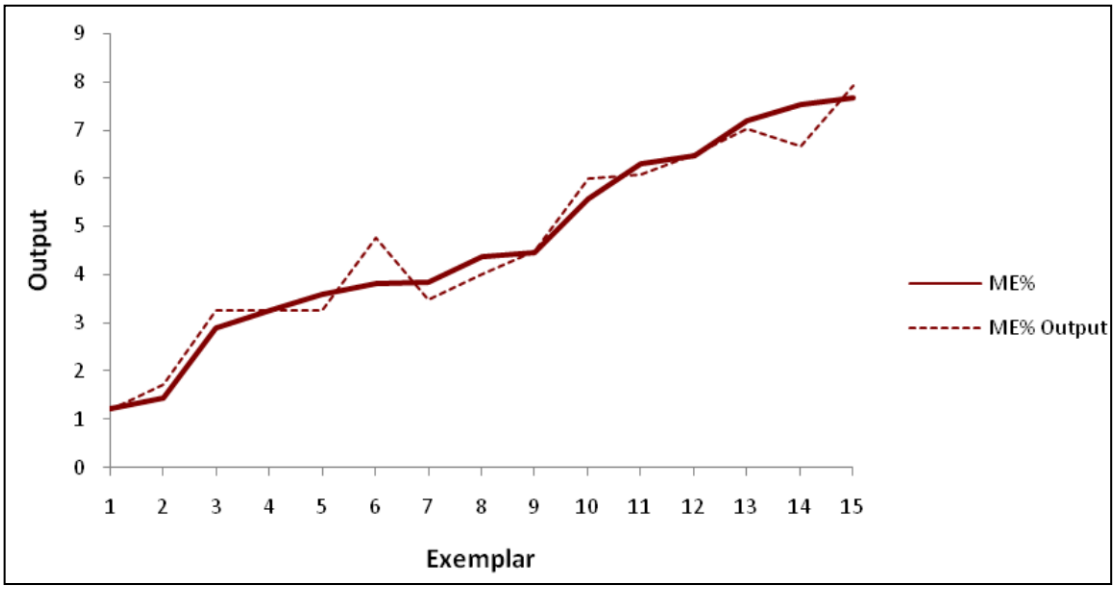


Figure 4.6: A profile depicting the a) desired and b) actual network output.

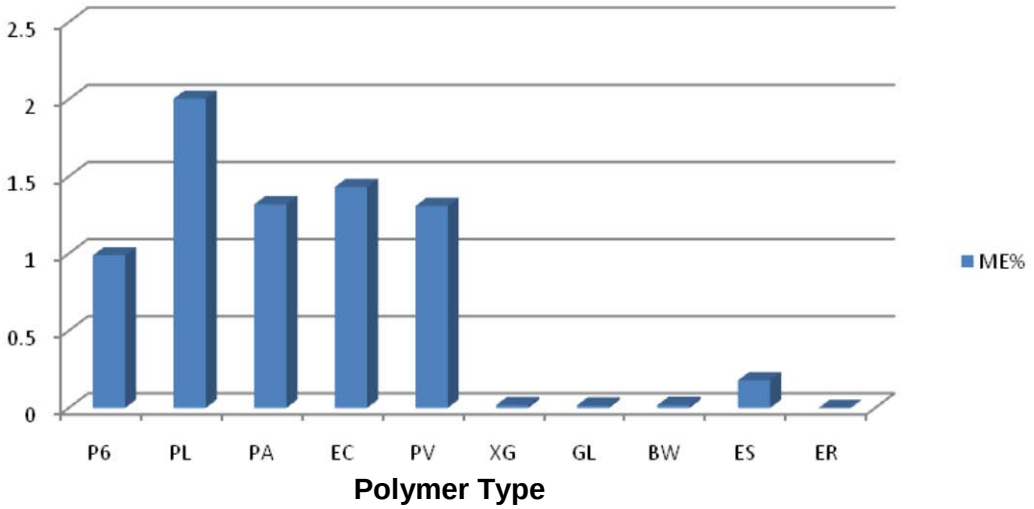


Figure 4.7: A typical bar chart graph depicting the sensitivity coefficients of each polymer type against the matrix erosion following the primary training.

From the primary ANN training it was apparent that 6 among 10 polymer types had the highest sensitivity against matrix erosion. These were $_{m}$PA 6,10, PLGA, EC, PEO PVA and PAA (Figure 4.7). Thus, a second (secondary) ANN training was run to explore the potential of these polymers to constitute an optimal formulation for developing the IBPD. The MSE for the secondary training is shown in Figure 4.8a. As can be noted, the secondary training resulted in highly reduced MSE values. For the primary training the

MSE ranged between 0.02-9.02 (Figure 4.5a) while for the secondary training the MSE ranged between 0.01-0.92 (i.e. less than 1) (Figure 4.8a). The gradual leveling of the MSE with standard deviation (SD) boundaries for the secondary training exhibited a sequential improvement of data modeling in that the average MSE decreased from values that were almost close to 10 (for primary training) (Figure 4.5) to values less than 1 as shown in Figure 4.8b. Overall these results depict a high degree of sensitivity for this set of polymers when compared to the rest.

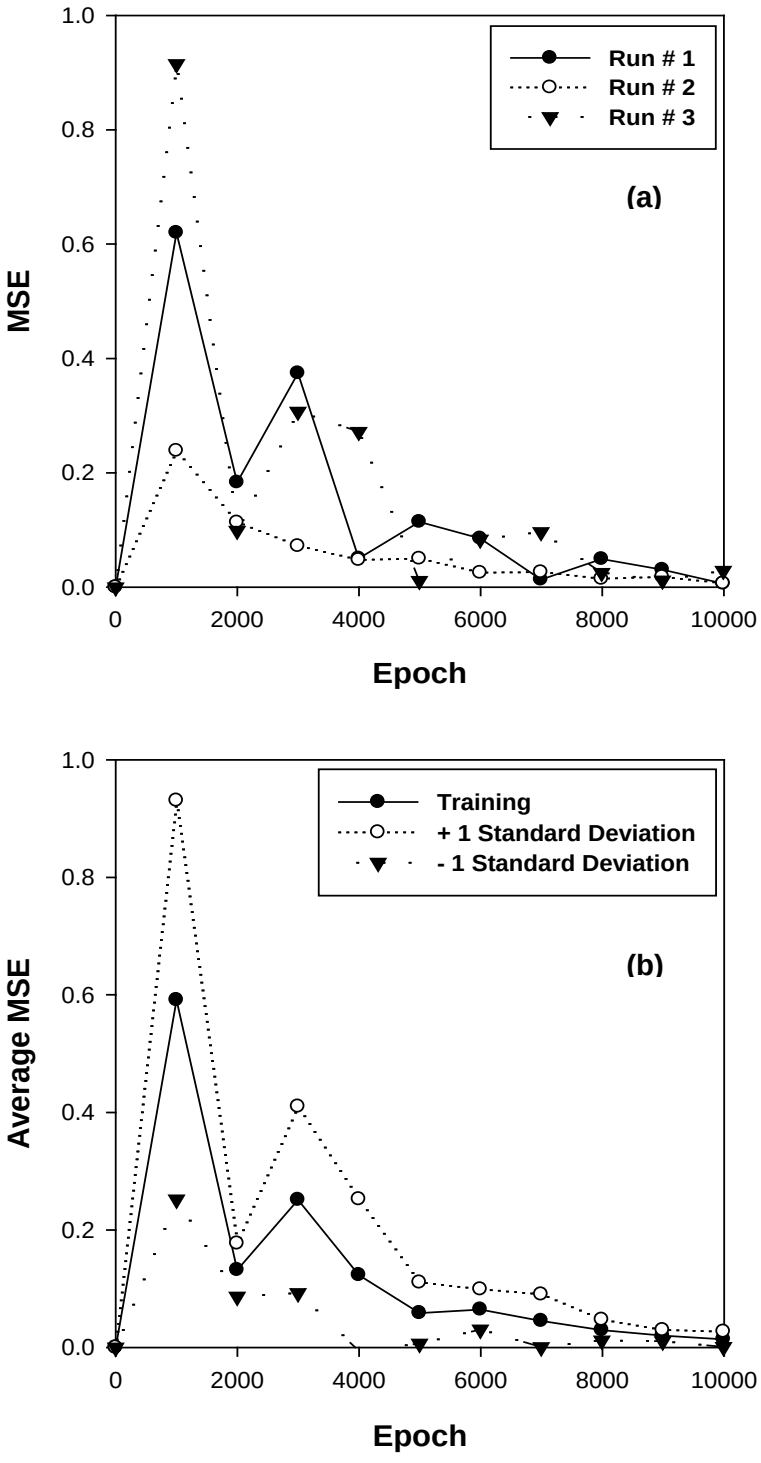


Figure 4.8: a) MSE for the secondary ANN training, b) Average MSE for the secondary training with standard deviations for 10,000 epochs.

As was the case with the primary training, the MSE for the secondary training was 0.001 with an excellent fit for the input variables as well (i.e. R^2 = 0.99). The sensitivity

coefficient of each polymer type against matrix erosion is as shown in Figure 4.9. EC presented with the highest sensitivity against matrix erosion followed by PLGA (Figure 4.9). This depicts their high capacity in controlling matrix erosion. This behavior may be attributed to their high degree of hydrophobicity. The sequence of the other remainng polymers in terms of sensivity against matrix erosion was PVA, PAA and $_{m}$PA 6,10. The results observed with PVA is a reflection of the results obtained earlier in matrix swelling tests where it had presented with minimal swelling (Chapter 3; Section 3.3.2.2). The low sensitivity against matrix erosion shown by PAA and PA 6,10 (Figure 4.9), may be associated with their hydrophilic nature.

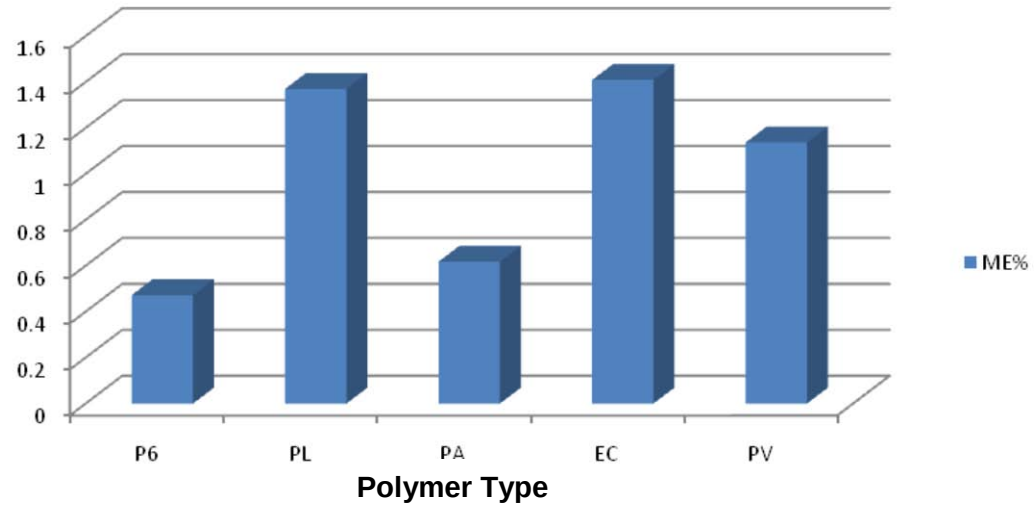


Figure 4.9: A typical bar chart graph showing the sensitivity coefficients of each polymer type against the matrix erosion following the secondary training.

The desirable matrix integrity exhibited by formulations with markedly reduced quantities of PAA is reflected in the formulation containing $_{m}$PA 6,10 (150mg), PLGA (400mg), EC (200mg), PVA (25mg) and PAA (25mg). This formulation was able to provide a matrix erosion value which was as low as 1.21% (Section 4.2.5; Table 4.3). These results are supported by the relationship between the matrix erosion and the varied input polymer quantity (Figure 4.10). PAA, which was mainly employed due to its bioadhesivity potential, showed a markedly high influence on matrix erosion in that even at quantities as low as 25mg, it could result in a static impact on matrix erosion (Figure 10). This is certainly due

to its high degree of hydrophilicity. PVA which was mainly used for the purpose of enhancing bioadhesivity and controlled release to the IBPD, portrayed a substantially high positive sensitivity for matrix erosion (Figure 10) and therefore the reason why it was also used in minimal quantities (25mg). $_m$PA 6,10 which was employed for the purpose of achieving desirable controlled drug release, presented with a relatively low sensivity to matrix erosion compared to the other hydrophilic polymers i.e PAA and PVA. PLGA and EC which are highly hydrophobic polymers, presented with a markedly high degree of negative sensitivity for matrix erosion (Figure 4.10). The substantially high matrix integrity of this formulation, may therefore be attributed to the high degree of hydrophobicity in PLGA and EC, good matrix resilience of $_m$PA 6,10, and the hydrophilic nature of PAA and PVA which allowed formation of the networked microstructure in the IBPD polymer matrix. The bioadhesivity capacity of the optimized formulation is assessed later in Chapter 5.

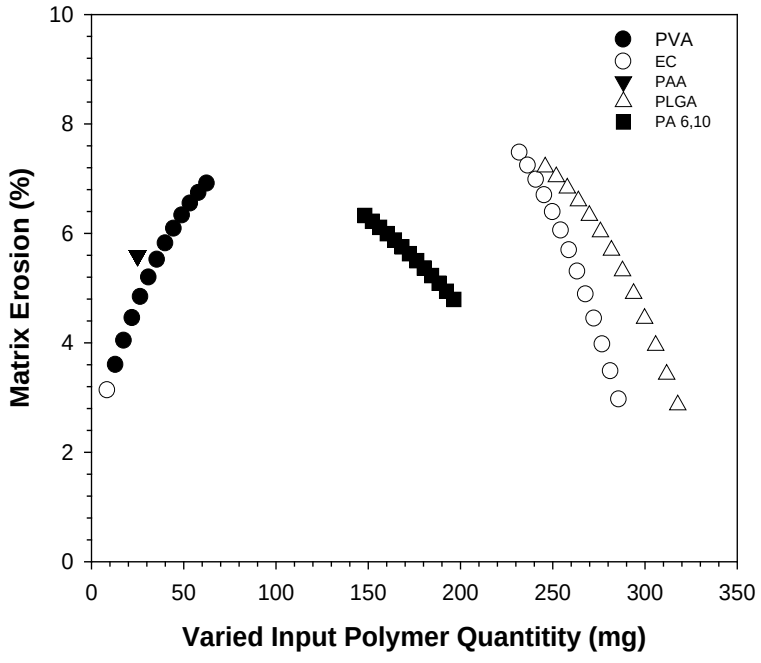


Figure 4.10: Correlation between matrix erosion and polymer quantity.

4.4. Concluding Remarks

A comprehensive analysis of several polymers for the development of the IBPD was conducted in terms of matrix erosion. The results of this study have highlighted polymeric effects on the matrix erosion of the IBPD. The high sensitivity against matrix erosion shown by the best 5 polymers (Figure 4.7 and 4.9) in both primary and seconadary ANN training was an indication of their suitability in formulating a stable IBPD. The ANN approach confirmed that $_{m}$PA 6,10 (150mg), PLGA (400mg), EC (200mg), PVA (25mg) and PAA (25mg) could lead to the development of an optimized intravaginal bioadhesive polymeric drug delivery device.

CHAPTER FIVE

IN VITRO AND *EX VIVO* BIOADHESIVITY ANALYSIS ON THE OPTIMIZED INTRAVAGINAL BIOADHESIVE POLYMERIC DEVICE COMPOSED OF DIFFERENT CROSSLINKED POLYACRYLIC ACIDS

5.1 Introduction

The development of bioadhesive intravaginal drug delivery systems allows for therapeutic levels of bioactive to be maintained locally, extends the bioactive residence time within the vagina and reduces the dosing frequency and quantity of bioactive administered (Mathowitz and Jacob, 1999; Ugwoke et al., 1999; Ceschel et al., 2001). Thus, the therapeutic efficacy of locally-acting bioactives may be improved due to the increased availability at the vaginal epithelium afforded through bioadhesion (Elson et al., 2000; Genc et al., 2000; Mandal, 2000). Currently, the majority of bioadhesive polymers include polyacrylic acid and cellulose derivatives (Repka and McGinity 2001), poly(vinylpyrrolidone) (PVP), poly(vinylalcohol) (PVA), chitosan and various gum-based polymers such as xanthan gum (XG) (Tambwekar et al., 2002). Multifunctional polymers exhibiting bioadhesive and/or gelling properties such as the thiolated polyacrylates and poloxamers represent a useful framework for the design of intravaginal drug delivery systems (Bogentoft et al., 1996; Remuñán-López et al., 1998; Lee et al., 2000; Lehr, 2000;Valenta et al., 2001; Kast et al., 2002; Robinson and Bologna, 2002; Valenta et al., 2002; Tang et al., 2005 ; Bonferoni et al., 2006; Al-Tahami and Singh, 2007; Trapani et al., 2009).

Bioadhesivity is essentially defined as the interfacial force between the polymeric drug delivery system and the mucus layer coating an epithelium (Peppas and Buri, 1985). Bioadhesion may occur in three distinct phases namely wetting, interpenetration and

subsequent mechanical interlocking between mucin and the polymer (Longer and Robinson, 1986; Jast et al., 2003). Bioadhesion is thus thought to be a result of the presence of hydrogen bonding groups, strong anionic/cationic charges, high molecular mass, chain flexibility and surface energy interactions which favor spreading onto the vaginal tissue. Basic theories such as electronic, adsorption, wetting and diffusion phenomena have been described and associated with the mechanisms by which bioadhesion occurs (Helfand and Tagami, 1972; Kaelble, 1977; Deryaguin et al., 1997). Each of the steps involved in bioadhesion may be facilitated by the polymeric composition of the drug delivery system and the mode of application (Hussain, 2000). Several techniques for the *in vitro* assessment of bioadhesion have been explored and include adhesion mass methods (Smart and Kellaway, 1982), fluorescent probe methods (Park and Robinson, 1984), shear stress testing (Smart et al., 1984), flow channel techniques (Mikos and Peppas, 1986), tensile testing (Park and Robinson, 1987), colloidal gold staining methods (Park, 1989), averted sac technique and the CAHN microbalance (Santos, 1999). However, these techniques lack sufficient sensitivity.

Therefore the aim of this Chapter was to investigate the use of a comprehensive multi-dimensional approach including chemometrical molecular modeling and pertinent mathematical descriptors to assess the polymeric bioadhesivity to vaginal tissue. The Area Under the Force-Distance Curve (AUC_{FD}) under a global locale was computed for all data points iterated during bioadhesion testing of optimized polyacrylic acid (PAA)-based intravaginal matrices generated through an Extreme Vertices Mixture Experimental Design template. Bioadhesivity testing was conducted on both simulated vaginal membrane and freshly excised pig vaginal tissue. The ideal polymers obtained through the optmization by ANN approach i.e. modified polyamide 6,10, ($_m$PA 6,10), poly (lactic co-glycolic acid (PLGA), ethylcelloluse (EC), polyvinyl alcohol (PVA) and polyacrylic acid (PAA) were employed for the study. Apart from the ensured matrix integrity, these polymers also have the potential for controlling drug release, ensuring desirable

erodability, homeostatic control of the vaginal environment and bioadhesive potential. Studies have shown that PAA is a highly bioadhesive polymer (Semalty et al., 2006; Semalty and Semalty, 2008). Therefore, for the purpose of achieving optimal bioadhesivity, this study explored two combinatory matrices comprising PAA, namely allyl sucrose-crosslinked PAA (AS-PAA) and allyl penta erythritol-crosslinked PAA (APE-PAA).

5.2 Materials and Methods

5.2.1 Materials

The simulated vaginal membranes employed were dialysis flat sheet membranes of M_w=12,000-14,000g/mol purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA). Allyl sucrose-crosslinked PAA (AS-PAA) and allyl penta erythritol-crosslinked PAA (APE-PAA) were purchased from Noveon Inc Cleveland, OH, USA. Pig vaginal tissue was excised from euthanized pigs obtained from the University of the Witwatersrand Central Animal Services Unit. The modified PA 6,10 was synthesized as per the method described in Chapter 3, Section 3.2.1.1. The rest of the materials used were as described in Chapter 3.

5.2.2 Methods

5.2.2.1 Preparation of the intravaginal bioadhesive polymeric device caplets composed of allyl sucrose and allyl penta erythritol-crosslinked PAA

An Extreme Vertices Mixture Design template was generated employing Minitab® V15 (Minitab® Inc., PA, USA) statistical software to produce various caplet formulations comprising 11 polymer combinations (Table 5.1). Each formulation had an equivalent mass of 800mg. Formulation response optimization was performed using an inherent D-optimal technique by combining mixture components and processing factors to converge to pre-optimal settings prior to achieving a global optimized solution with the desirable polymeric proportions for the AS-PAA and APE-PAA caplets that were subsequently prepared and further tested.

Table 5.1: Extreme Vertices Mixture Formulation Template for caplet preparation

F #	$_m$PA 6,10 (mg)	PLGA(mg)	EC (mg)	PVA (mg)	PAA[a] (mg)	PAA[b] (mg)
1	150	50	100	100	200	200
2	100	50	100	150	250	200
3	100	50	100	100	205	250
4	130	55	105	105	205	205
5	105	80	105	105	200	205
6	100	100	100	100	205	200
7	105	55	130	105	230	205
8	105	55	105	105	210	230
9	110	60	110	110	200	210
10	100	50	150	100	205	200
11	105	55	105	130	200	205

[a]*AS-PAA: allyl sucrose-crosslinked PAA*
[b]*APE-PAA: allyl penta erythritol-crosslinked PAA*

PLGA, $_m$PA 6,10, EC, PVA and the two crosslinked PAA variants were weighed in triplicate and subsequently blended using a cube blender (Erweka Apparatebau, Heusenstamm, Germany). Two sets of blends were prepared in each case, one containing AS-PAA and the other APE-PAA. The polymer blends were then compressed into IBPD caplets using a Manesty D3B 16 station tableting press (Manesty D3B L249LQ, Liverpool, England) equipped with D3B oblong tooling of 22×9mm in dimension at a compression pressure of 5 tons.

5.2.2.2 In-process validation tests

Diametric hardness, mass uniformity and friability analysis were conducted on the IBPD caplets to ensure adequate manufacturing reproducibility. A sample of 10 caplets from each formulation batch was tested for diametric hardness, mass uniformity and friability using the same instruments, parameters and limits as described in Chapter 3, Section 3.2.2.

5.2.2.3 *In vitro* and *ex vivo* bioadhesivity analysis

Bioadhesivity testing was performed on all experimental formulation batches and both sets of optimized caplets (i.e. the AS-PAA and APE-PAA caplet formulations) using a highly sensitive Texture Analyzer (TA.XT*plus*, Stable Microsystems, UK). This approach was employed to principally measure the bioadhesiveness using a cylindrical probe that was robotically guided to make contact and induce a test force onto the non-deformed substrate surface comprising either a simulated vaginal membrane or freshly excised pig vaginal tissue and thereafter disengage in order to simultaneously measure the force required to detach the probe containing the vaginal tissue from the hydrated caplet. The maximum force required to detach the simulated vaginal tissue attached to the cylindrical probe from the secured caplet on the platen was obtained from the Peak Adhesive Force (PAF) and also computed from the Area Under Force-Distance Curves (AUC_{FD}.) representing the Work of Adhesion. In each case, the simulated vaginal membrane and the caplets were hydrated for 30 minutes in simulated vaginal fluid (pH 4.5; 37°C) (Chapter 3, Section 3.2.2.3, Table 3.3) prior to testing (N=3). For further method and formulation validation bioadhesivity testing was also conducted using freshly excised vaginal tissue obtained from a pig model on the APE-PAA and AS-PAA caplets. The textural analysis parameter settings employed were pre-test and test speeds = 2.0mms^{-1}, a post test speed = 10mms^{-1}, a trigger force = 0.5N and a contact time of 5s.

5.2.2.4 Removal of vaginal tissue from the pig model for bioadhesivity testing

A Large White female pig (84kg) was euthanized with 40mL of sodium pentobarbitone (200mg/mL) administered intravenously. The pelvic canal of the pig was opened by dissecting through the symphysis pubis and then exposing the intra-abdominal vaginal tract. The external vaginal tract was carefully dissected from the surrounding tissues before removing the vaginal tissue (Figure 5.1). An incision was made through the vaginal canal to expose the inner lining of the tissue, which was then placed in an airtight specimen jar and immediately subjected to bioadhesivity testing.



Figure 5.1: The dissection process to remove the vaginal tissue from the pig.

The freshly excised vaginal tissue was secured onto a cylindrical textural probe of a Texture Analyzer (TA.XT*plus*, Stable Microsystems, UK). An optimized APE-PAA caplet was fixed onto the textural platen after exposure to simulated vaginal fluid (pH 4.5; 37°C) for 30 minutes. Bioadhesive testing was then conducted immediately by measuring the maximum force (N) required to detach the vaginal tissue from the hydrated APE-PAA caplet matrix. Data was represented as the Peak Adhesion Force (PAF) and the work of adhesion (AUC_{FD}.) (Figure 5.2). The work of adhesion per unit area ($wA\ \alpha\beta\delta$), was characterized by the work executed on the matrices when the two contact phases i.e. the vaginal tissue (α) and the caplet matrix (β), formed an interface of unit area which were subsequently separated reversibly to form unit areas of each of the $\alpha\delta$- and $\beta\delta$- interfaces. This relationship is mathematically described by Equation 1.

$$wA\alpha\beta\delta = \gamma\,\alpha\,\delta + \gamma\,\beta\,\delta - \gamma\,\alpha\beta \qquad \textbf{\textit{Equation 5.1}}$$

Where, $\gamma\,\alpha\beta$, $\gamma\,\alpha\delta$ and $\gamma\,\beta\delta$ are the surface tensions between the two bulk phases comprising the vaginal tissue and the APE-PAA IBPD caplets , α, β; α, δ and β, δ respectively.

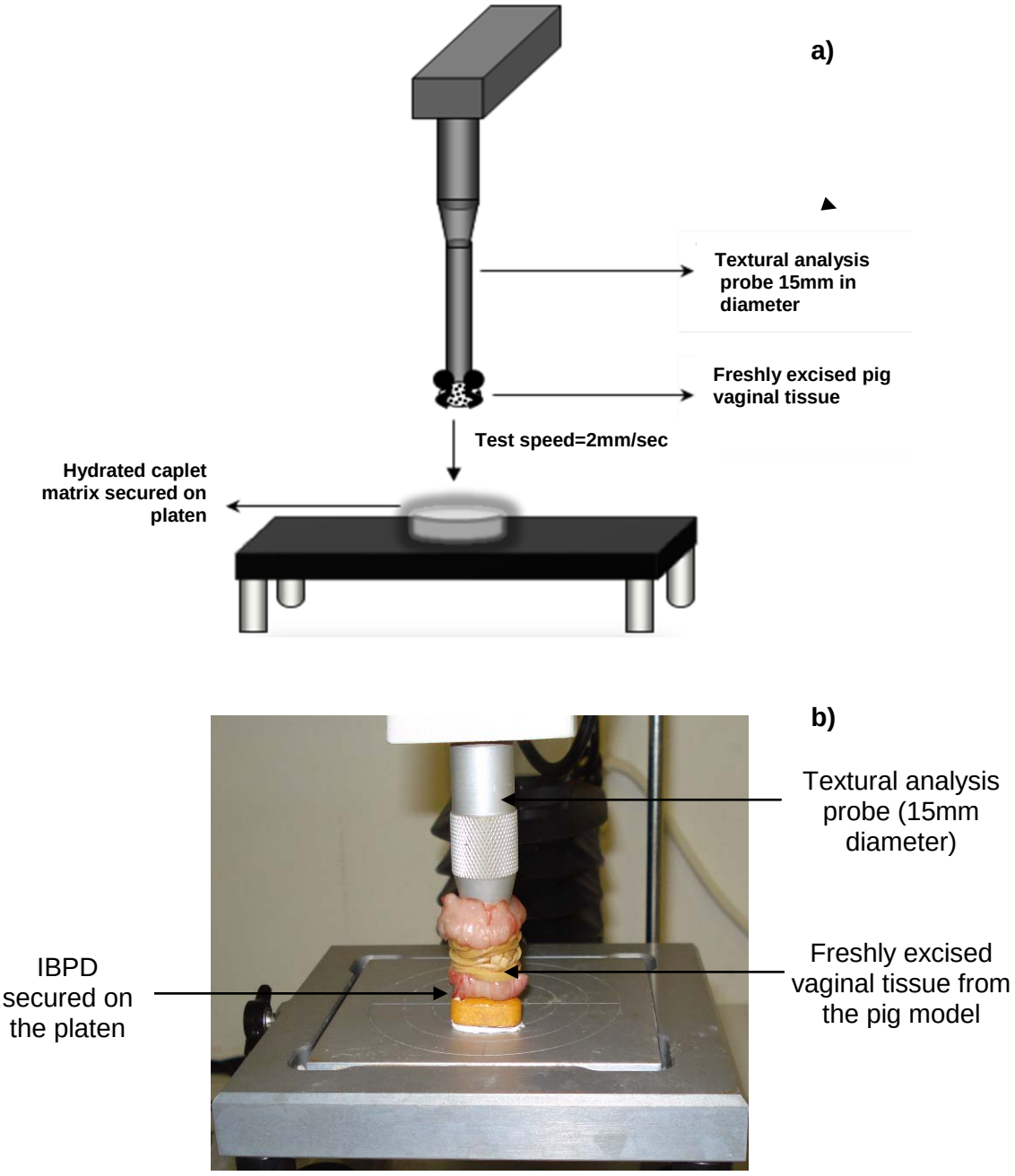


Figure 5.2: Textural analysis method employed to generate Force-Distance profiles for assessing the caplet bioadhesivity to freshly excised pig vaginal tissue indicating a) blue paint design, b) actual process.

5.2.2.5 Rheological analysis of hydrated AS-PAA and APE-PAA polymers

The ability of PAA to partially hydrate within the IBPD matrix ultimately results in wetting and volumetric distension of the system which facilitates the intimate contact and interaction with the simulated vaginal membrane and freshly excised pig vaginal tissue. Rotational rheological analysis was therefore performed to generate flow curves for

analyzing relationships between the viscosity and shear stress as a function of shear rate using a Modular Advanced Rheometer system (ThermoHaake MARS Rheometer, Thermo Fischer Scientific, Karlsuhe, Germany) equipped with a T-type spindle sensor (C35/1^{o}Ti) set at a sample gap of 1mm and a Thermocontroller (UTC-MARS II). The AS-PAA and APE-PAA polymers were dissolved in simulated vaginal fluid at a concentration of 2%$^{w}/_{v}$, vortexed for 2 minutes, and then assessed for their rheological behavior at 25±1.0^{o}C with a test time of 200s and a controlled rate starting at 5.00s^{-1} decreasing to 0.001s^{-1} and a shear rate ranging from 0 to 500s^{-1}.

5.2.2.6 Mechanistic postulation of caplet bioadhesion employing chemometric and molecular modeling and associated interactive energy paradigms

Cellular adhesion will play a critical role toward the mechanisms of achieving superior bioadhesion. While a number of computational models and experimental studies have addressed the issue of cell adhesion to surfaces, no model or theory has adequately addressed cell adhesion at the molecular level employing freshly excised pig vaginal tissue as the bioadhesive substrate (King, 2001; Yang and Zaman, 2007). Computational and molecular structural modeling was performed in our laboratories to deduce the transient mechanisms of bioadhesivity, chemical interactions and inter-polymeric interfacing during caplet adhesion to the freshly excised pig vaginal tissue as the adhesive substrate. This approach led to predictive findings solely based on the chemical interactions underlying the bioadhesion of the caplet to the vaginal tissue substrate. Semi-empirical quantum mechanics was used to generate molecular interactions and computational energy paradigms of the caplet components based on inherent interfacial phenomena underlying the mechanisms of bioadhesion provided by the inter-polymeric blended caplet. Models and graphics supported on the step-wise molecular caplet-tissue interaction, polymeric interconversion and bioadhesion as envisioned by the molecular behavior and stability of the hydrated caplet network were generated on ACD/I-Lab, V5.11 (Add-on) software (Advanced Chemistry Development Inc., Toronto, Canada,

2000). The energy distribution curves assumed a componential progression of bioadhesion stability, a spatially uniform distribution of polymers within the caplet, and an irreversible gelation process (Schroeder et al., 1998; Yu et al., 1998; Picout et al., 2000a and b; Kalugin et al., 2001; Gilsenan et al., 2003a, b, c).

A comprehensive chemometric molecular understanding of the mechanisms by which certain macromolecules adhere to the vaginal tissue epithelium is not yet available (Mikos and Peppas, 1990; Valenta, 2005). Therefore a thermodynamic model that addresses receptor-mediated cell adhesion at the molecular level was explored. By incorporating the entropic, conformational, solvation, and long- and short-range interactive components of receptors and the extracellular matrix molecules, adhesive free energy as a function of a number of key variables such as surface coverage, interaction distance, molecule size, and solvent conditions were predicted. This allows the computation of the free energy of adhesion in a multi-component system in order to simultaneously elucidate adhesion receptors and ligands of different sizes, chemical identities, and conformational properties. This approach not only provides a fundamental understanding of adhesion at the molecular level but may also aid in identifying possible strategies for designing novel biomaterials to improve bioadhesion of the caplet to the pig vaginal tissue substrate.

5.3 Results and Discussion

5.3.1 Assessment of caplet bioadhesivity

Work of adhesion values for the APE-PAA caplets were significantly higher ($P<0.05$) than those of AS-PAA caplets. The difference in work of adhesion was 94.1% (Figure 5.3). This clearly indicated that the APE-PAA caplets had superior bioadhesivity than the AS-PAA caplets. The difference in bioadhesivity may be due to the difference in percentage crosslinking of allyl sucrose and allyl penta erythritol to PAA. Allyl sucrose is 1.5% crosslinked (Corring, 1992) while penta erythritol is 2.0% crosslinked (Wilber et al., 2003).

This slightly higher density of crosslinking in APE-PAA may therefore have translated into an intense hydrated network capable of facilitating superior bioadhesion.

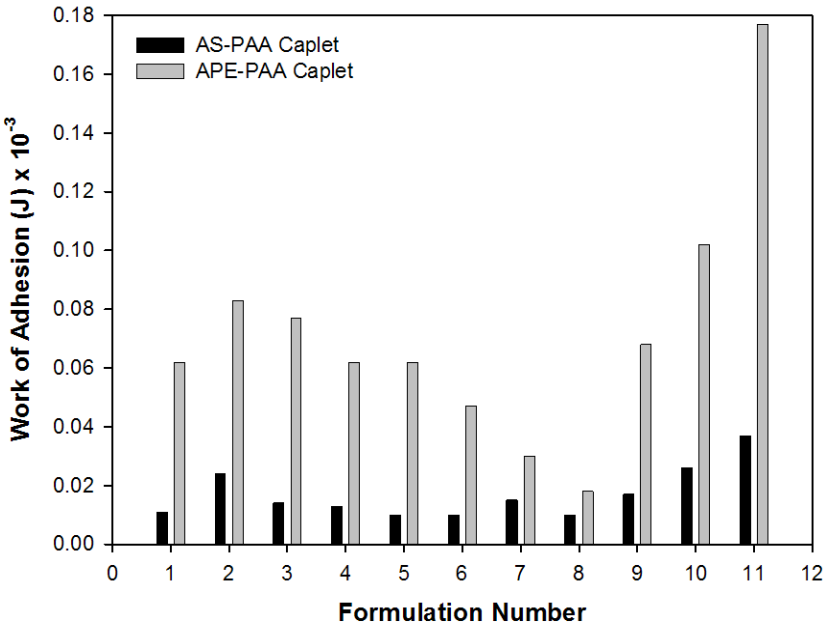


Figure 5.3: A comparison of work of adhesion (AUC_{FD}) (J) between the AS-PAA and APE-PAA caplets (N=3; SD<0.03).

5.3.2 Response optimization via textural analysis outputs from the optimized AS-PAA and APE-PAA caplets

Response optimization of the AS-PAA and APE-PAA caplets using Minitab® V15 (Minitab® Inc., PA, USA) software revealed the optimum level for each polymer ($_m$PA 6,10, PEO, PLGA, CG and PAA) within the caplets in order to provide the most desirable relative bioadhesivity (Figure 5.4). The bioadhesivity values for the *in vitro* test were significantly higher compared to the *in vivo* test (P<0.01) (Table 5.2). The reason is mainly based on the nature of the surfaces with which the caplets made contact with to establish the bioadhesive force. The *in vitro* test employed artificial simulated vaginal membrane whereas the *in vivo* test employed freshly excised vaginal membrane. The mucus present on the surface of the freshly excised vaginal membrane underwent a constant turn-over with the old layer being replaced by a new one. This process interrupted the establishment of hydrogen bonds and van der Waal forces between the caplet and the

vaginal membrane thereby resulting in low bioadhesion. With articial membrane, there was no turn-over effect and therefore once a bond formed, it remained intact (i.e. the interaction holds) which may thus be the reason for the higher bioadhesivity observed. Furthermore, mucus degrades while artificial membranes do not and therefore the biodhesive forces established in the later, remained intact and undisturbed. The difference in bioadhesivity between the AS-PAA and APE-PAA caplets was 46.7% (Figure 5.5). The desirability (D) values were 0.98 and 0.92 respectively indicating the sufficient convergence of data toward an optimized global solution.

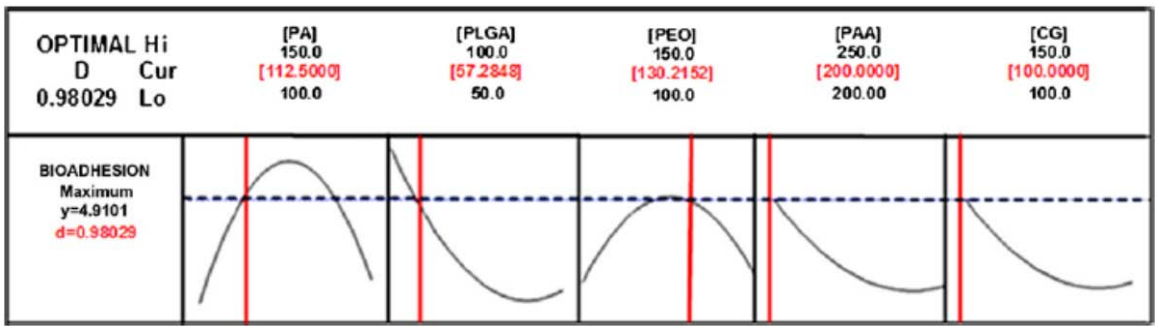


Figure 5.4: A typical response optimization plot for the AS-PAA and APE-PAA caplets.

Table 5.2: Comparative *in vitro* and *ex vivo* bioadhesivity results for the optimized caplets

Textural parameters	Predicted ($x10^{-3}$)	Experimental ($x10^{-3}$)
***In vitro* analysis of optimized AS-PAA and APE-PAA caplets**		
	Force of adhesion (N)	
AS-PAA caplet	1300.00	1168.10 ± 93.270
APE-PAA caplet	2000.00	1671.60 ± 23.221
	Work of adhesion (J)	
AS-PAA caplet	0.50	0.32 ± 0.13
APE-PAA caplet	0.90	0.60 ± 0.21
***Ex vivo* analysis of the optimized APE-PAA caplet**		
Force of adhesion (N)	1000.00	883.00 ± 82.73
Work of adhesion (J)	0.35	0.30 ± 0.035

5.3.3 *Ex vivo* bioadhesivity analysis of the optimized APE-PAA caplets on freshly excised vaginal tissue

The PAF value obtained was 0.883±0.08273N while the work of adhesion (AUC_{FD}) was 0.0003±3.53553 $X10^{-5}$J. The results portrayed a direct correlation between the PAF and the work of adhesion (Table 5.2 and Figure 5.6). The optimized caplet matrices with desirability (D) values close to 1 depicted excellent correlation (R^2=0.99) between the predicted and the experimental results (Table 5.2).

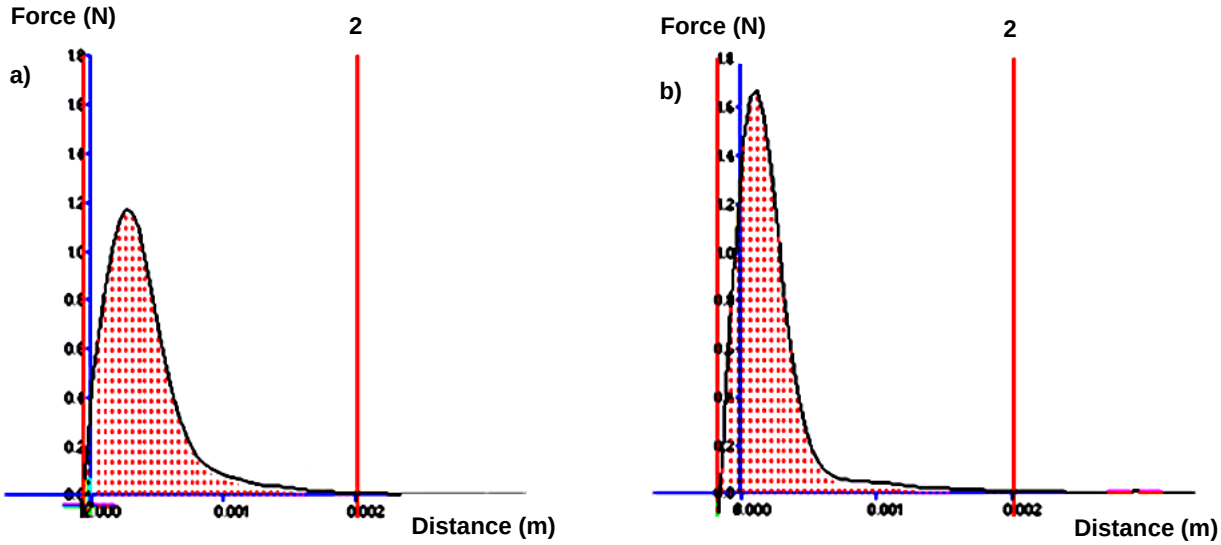


Figure 5.5: Typical textural profiles elucidating the peak adhesive force (PAF) (N) and work of adhesion (AUC_{FD}) (J) for the optimized composed of: a) AS-PAA, and b) APE-PAA.



Figure 5.6: Typical textural profile elucidating the force (N) and work of adhesion (AUC_{FD}) (J) for the optimized APE-PAA caplets on freshly excised vaginal tissue.

5.3.4 Rheological analysis of the hydrated AS-PAA and APE-PAA polymer solutions

AS-PAA and APE-PAA are polymers that are able to produce mucilages with short rheological flow properties that can be associated with the high degree of crosslinking in both polymers. In terms of rheological properties, APE-PAA was found to be highly viscous ($900x10^{-3}$mPas) in comparison to AS-PAA ($80x10^{-3}$mPas) with a subsequent higher shear stress (180Pa vs. 58Pa) (Figure 5.6). Justifiably, this rheological observation may explain the superior bioadhesivity of the APE-PAA caplets over the AS-PAA caplets. In both cases the viscosity decreased as the shear rate increased (Figure 5.7).

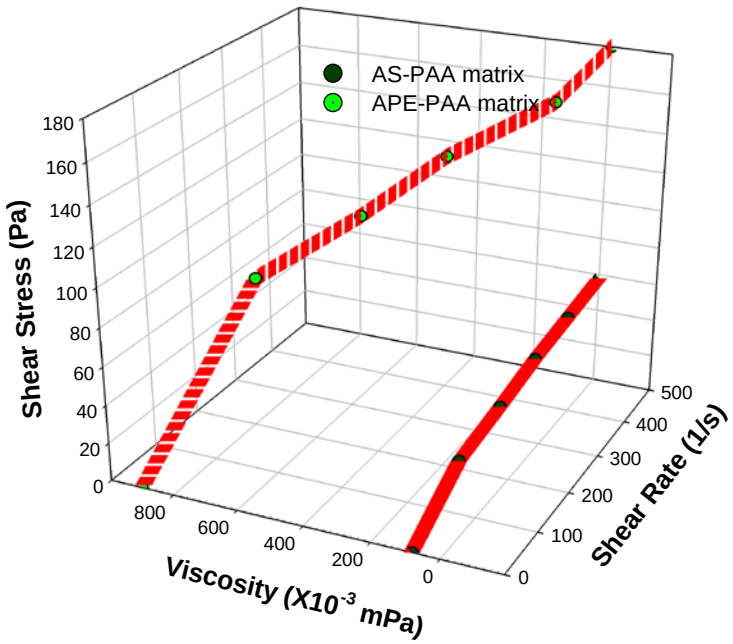


Figure 5.7: Rheological behavior of 2%$^{w}/_{v}$ AS-PAA and APE-PAA solutions at a shear rate between 0-500s^{-1}.

The bioadhesivity behavior of the caplets to the freshly excised pig vaginal tissue is a typical reflection of the fact that the polymer combination employed for matrix formation typically possesses different structural deformation mechanisms due to their backbone, molecular mass, polymeric concentration, chain flexibility, spacial conformation, pH of volumetric distention at the polymer-substrate interface, the associated forces, the initial contact time and the extent of mucin turn-over. This may be a result of their variation in moduli and rheological deformation energies. Hence deformation or flow at the polymer-vaginal tissue substrate interface occurred within the polymeric matrix with subsequent failure at the interface to induce and control the degree of bioadhesion between the caplet and the freshly excised vaginal tissue.

5.3.5 Postulative chemometric and molecular modeling of the mechanisms of caplet bioadhesion and the associated interactive energy paradigms

Results obtained from chemometric and computational analysis of the associated energy paradigms have revealed that the caplet bioadhesivity to the freshly excised pig vaginal tissue substrate may be explained in terms of the Differential Adhesion Hypothesis (DAH)

theory of cell adhesion (Steinberg, 1996; Brodland, 2003; Foty and Steinberg, 2005; Steinberg, 2007; Katsamba et al., 2009). The pig vaginal tissue substrate acted as a viscoelastic flexible semi-solid with a tissue surface tension corresponding to a mutual classification behavior occupying an internal position relative to the gelled caplet with a lower surface tension during interaction. Quantitative energy variations in homo-and hetero-typic bioadhesion were sufficient to account for the phenomenon without the need to postulate cell-type specific bioadhesion. The caplet bioadhesion occurred in a stepwise mechanism involving weak inter-atomic and inter-molecular physical forces that interplay at the outset (Figure 5.8).

During initial contact of the caplet with the freshly excised pig vaginal tissue substrate mechanical bioadhesion occurred with all surface voids between the caplet and tissue substrate filled and therefore resulted in surface interlocking. As the caplet hydrated a more unyielding chemically adhesive interaction progressed between the hydrated inter-polymeric blend of the caplet to form a bioadhesive compound at the interface either through ionic or covalent bonding prior to caplet dispersion. In the dispersive phase of bioadhesion the hydrated caplet and vaginal tissue substrate were bound by van der Waals forces as a result of polymeric ionic species generated due to disentanglement and volumetric distention of the constituent polymers. In the simplest case, molecules during this phase of bioadhesion were polar with respect to the average charge density and may be due to Keesom forces or a transient effect of London forces. Once the caplet and vaginal tissue substrate diffusively merged, both interfaces were miscible through a sintering-like mechanism. The strength of bioadhesion depended on a hybrid between the dispersive and diffusive phases as well as the contact surface area. Polymers such as the AS-PAA and APE-PAA were able to significantly wet during sol-gel interconversion and therefore provided a reduced contact angle facilitating bioadhesion.

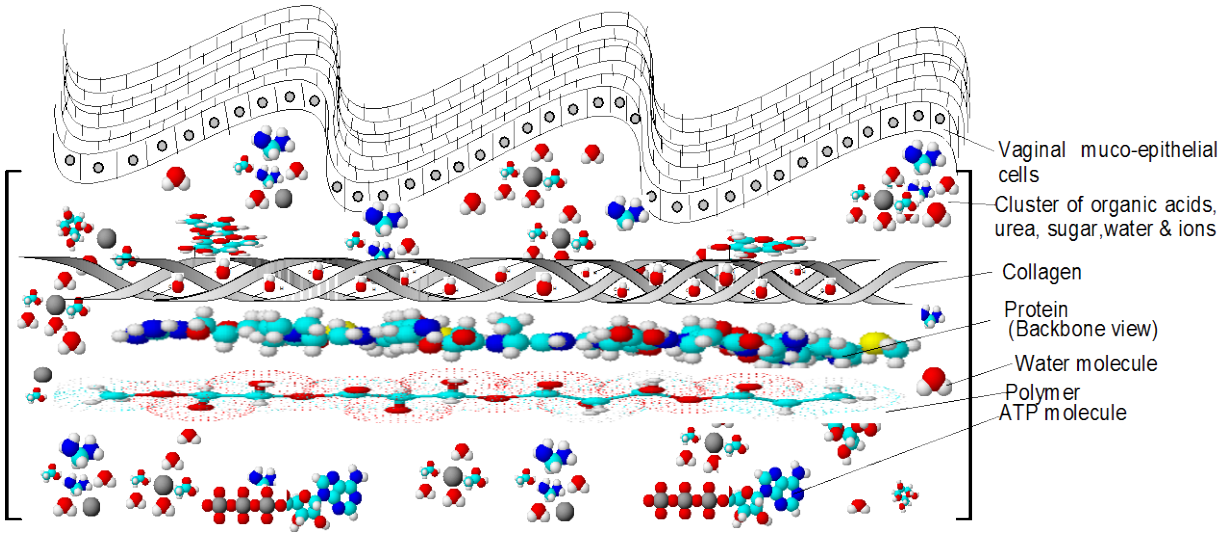


Figure 5.8: A chemometric structural model developed in our laboratories depicting caplet bioadhesion to freshly excised pig vaginal tissue with muco-epithelial cell secretions and surface bio-molecule interactions.

Figure 5.9a-d depicts the energy estimates for bioadhesion representing the physical forces, ionic and chemical interactions as well as ion-exchanges during bioadhesion. This involved electron donations and sharing of covalent bonds between the xenobiotic polymers of the caplet and cellular proteins of the vaginal tissue substrate. Interactions with collagen in the substrate surface with polymer-protein interactions were also present. The extent of hydration also played a significant role in hydration of the caplet resulting in ionic interaction pockets that contributed towards bioadhesion. Structural deformities of the caplet and proteins occurred with proteins transitioning into coiling at primary, secondary, tertiary and quaternary structure levels that participated in bonding to the polymers and facilitated bioadhesion.

The energy barrier between the compartmental interactions determined the extent of bioadhesion as a factor of energy (Figure 5.9a-d). The protein and polymer bonding at the cell surface via the embedded glyco-protein and other surface proteins played a role in linking the polymer. The saccharide component of the glyco-proteins bonded to the inherent surface protein and available functional groups and to some extent also bonded to the AS-PAA and APE-PAA polymers within the caplet. The energy differences and

level played a rate-limiting factor in the proposed bioadhesion phenomenon. Helical structural transitions and distortions within the polymer were predicted by molecular modeling and also affected by stereo-orientation though no effects on the coiling or helical turns of the protein were observed. The embedded glyco-proteins were least affected and provided the anchoring groups for polymer-substrate bioadhesion.

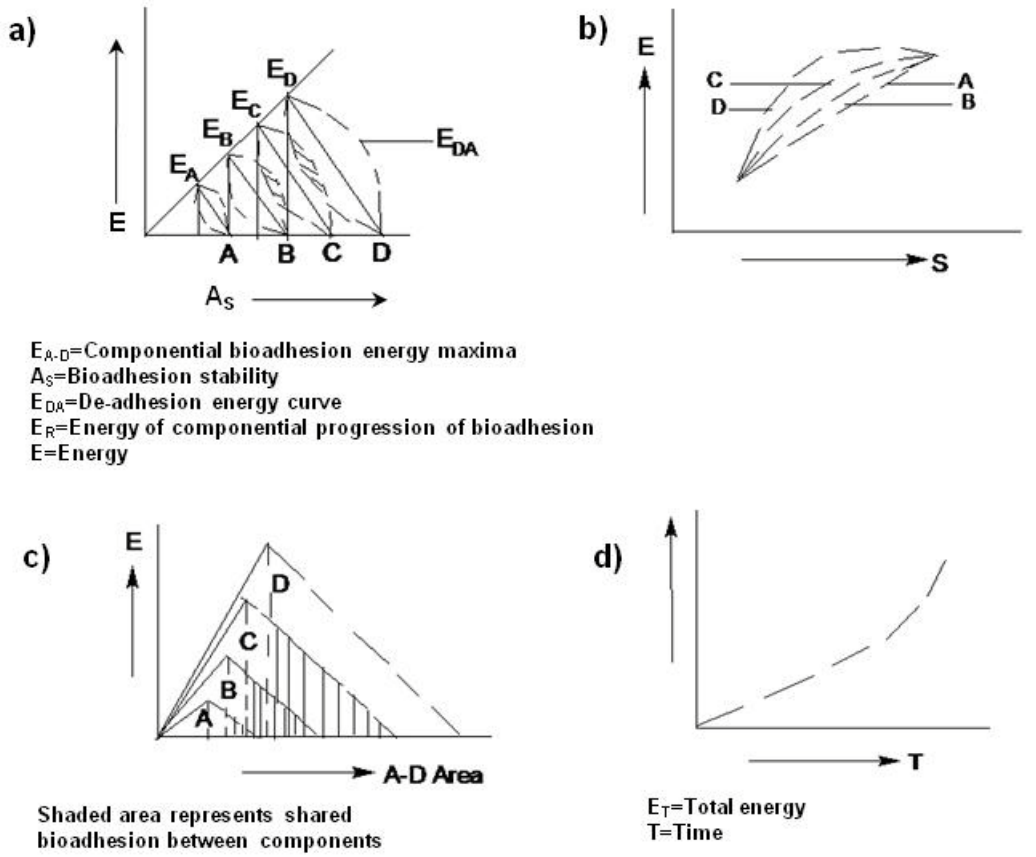


Figure 5.9: Energy estimates for the mechanism of bioadhesion developed in our laboratories: a) compartment A=interactions at physical forces levels, B=ionic interactions and ion-exchanges producing bioadhesion, C= chemical interaction involving covalent bonding between the xenobiotic polymer and cellular protein, D=interaction with surface collagen in the tissue substrate includes polymer-protein-collagen interactions.

Hydration also played a role and facilitated polymeric gelling and induction of ionic interaction pockets that contributed towards bioadhesion. Furthermore, collagen was dissolved from the fibrils by proteases and structurally adjusted to the requirements of bioadhesion as a result of polymer-protein and polymer-collagen interactions. Generally, the barriers to break weak physical bonds do not usually exceed 10–20 times the thermal

energy, *kT* (where *k* is the Boltzmann constant and *T* is the absolute temperature). The lifetime of these bonds can vary from microseconds to seconds. Hence, polymers form and break these weak physical bonds numerous times during the course of bioadhesion.

The caplets comprised two types of polymeric chain molecules arising from the AS-PAA and APE-PAA within the matrices. In order to predict the mechanisms of cell-matrix bioadhesion it was assumed that both surfaces (i.e. the caplet and vaginal tissue substrate) were two dimensional parallel planar impenetrable surfaces. In addition to polymeric chain molecules, the residual voids between the two surfaces were assumed to be filled with simulated vaginal fluid. To simulate protein interactions with the polymers, “generic” amino acids were employed and conformations chosen randomly from sterically allowable regions using dihedral angles. The polymer molecules were simulated with a similar strategy. Bioadhesive mechanisms were modeled to capture the essential features of polymer-protein interactions. Increasing the number of sampled conformations did not significantly alter the results and for simplicity only interactions between polymer-protein, collagen-polymer and polymer-protein-collagen were considered with self-interaction terms ignored. All interaction terms contained attractive and repulsive phases with an incompressibility condition or volume-filling constraint introduced to account for repulsive interactions. Under this assumption, no two segments could occupy the same volume which was the result of repulsive interactions.

5.4 Concluding Remarks

The polymer-based caplets adhered to the simulated vaginal membranes and the freshly excised pig vaginal tissue revealing the potential for application in intravaginal drug delivery. Chemometrical and molecular structural modeling deduced that the potential mechanisms of bioadhesivity are a combination of mechanical, chemical, dispersive and diffusive bioadhesive phenomena. Rheological analysis revealed that AS-PAA and APE-PAA displayed varying rheological behavior contributing to bioadhesion to the *in vitro* and

ex vivo substrates evaluated. Furthermore, the results demonstrated that APE-PAA caplets were more bioadhesive than the AS-PAA caplets and therefore capable of providing an optimized IBPD formulation. The bioadhesivity testing approach employed using freshly excised pig vaginal tissue suggests that the method developed may be useful for measuring the bioadhesivity of other intravaginal drug delivery systems on vaginal tissue substrates. Rational design of future bioadhesive intravaginal drug delivery systems should focus on the polymer properties that conform to vaginal retention to target bioactives more appropriately.

CHAPTER SIX

INVESTIGATION OF THE PHYSICOCHEMICAL AND PHYSICOMECHANICAL PROPERTIES OF THE OPTIMIZED INTRAVAGINAL BOADHESIVE POLYMERIC DEVICE

6.1 Introduction

The thermodynamic behavior of polymers is known to have a significant impact on the physicomechanical properties and therefore their final performance (Pillay and Fassihi, 1999; Liu et al., 2003; Parija, et al., 2004). Therefore, accurate thermal analysis of polymer blends employing temperature modulated differential scanning calorimetry (TMDSC) is critical during formulation design (Ribeiro and Grolier, 1999). TMDSC provides a clearer interpretation of thermal transitions on analyzed samples due to the improved sensitivity and resolution, coupled with the ability to separate reversible glass transitions that have diminutive transitions in heat capacity from overlapping non-reversible relaxation endotherms. TMDSC is also used to reveal thermal transitions at sub-zero temperatures that cannot be detected by conventional DSC due to poor equilibrium of the heat flow baseline below 0°C (Pijpers et al., 2000). Thus, the development of multi-polymeric intravaginal drug delivery systems requires a comprehensive understanding of the thermodynamic stability of the polymeric constituents used in designing the system.

The natural and most important protection against potential infections in the vagina is the presence of a normal, healthy vaginal flora (Vitali et al., 2007; Obiero et al., 2008; Mastromarino et al., 2009). An acidic vaginal pH value ranging from 4-5 is a crucial requirement for the optimal functioning of the body's natural protective system in the vagina. Thus, maintenance of this pH range in the vagina is important as it can effectively

avoid colonization by pathogens which may lead to gynecological problems since most of the pathogens cannot thrive under acidic conditions (Boskey et al., 2001; Ramsey 2002; Mastromarino et al., 2009). There are numerous factors that regulate the composition and dynamics of the vaginal microbial ecosystem and that maintain the natural protective functions of the vagina. The most important one, however, is the breakdown of glycogen (released from the upper vaginal epithelium), which is then metabolised to glucose, and finally lactic acid by *Lactobacilli* (Boskey et al., 2001). This results in an ideal acidic environment in the vagina, thus providing effective protection against infection. Generally, drug permeation rates correlate with partition coefficients and are inversely proportional to the molecular mass of the drug (Iyer et al., 2008). Thus, successful development of an intravaginal drug delivery system should consider permeation characteristics as a result of the formulation design, the therapeutic agent and vaginal physiology (Verman and Garg, 2000; Iyer et al., 2008; Ndesendo et al., 2008).

Therefore the aim of this Chapter was to design, develop and evaluate the bioadhesivity, permeability, thermal stability and the *in vitro* drug release of a novel drug-loaded intravaginal bioadhesive polymeric device (IBPD) for the prevention of HIV and other STIs employing model drugs AZT and PSS. The device should provide controlled drug release over an experimental period of at least one month when inserted into the posterior fornix of the vaginal cavity. Pig vaginal tissue was employed for permeation analysis due to the fact that the pig vaginal mucosa is very similar in many respects to human vaginal mucosa (Kremer et al., 2001; Thomson et al., 2001; Davis et al., 2003; Squier et al., 2008). Both types of mucosa consist of stratified squamous epithelium that is supported by connective tissue and lamina propria with lipid compositions that are comparable (van Kremer et al., 2001; Thomson et al., 2001). The specific polymers expressly selected in formulating the device included combinations of modified polyamide 6,10 ($_{m}$PA 6,10), poly(lactide-co-glycolide) (PLGA), allyl penta erythritol-crosslinked PAA (APE-PAA), polyvinyl alcohol (PVA) and ethylcellulose (EC) that were primarily employed for

controlling the release of AZT and PSS from the IBPD. PSS played a dual role as a polymer matrix and a bioactive (Garg et al., 2004a; Chu et al., 2007) (Chapter 3, Section 3.2.1.1). PAA was used as both polymer matrix constituent as well as a coating agent for the purpose of achieving extended bioadhesivity within the posterior fornix of the vagina (Bonacucina et al., 2006; Ndesendo et al., 2009).

The acidic environment in the vagina may be influenced by the changes in pH and ionic concentrations as a result of PLGA degradation into lactic and glycolic acid through cleavage by enzymatic or non-enzymatic hydrolysis (Park et al., 2005; Yasukawa et al., 2006; Kulkarni et al., 2007). Thus, since PLGA was one of the polymers selected for formulating the IBPD, it was important to determine the changes in micro-environmental pH the simulated vaginal fluid when exposed to the degradating constituents of the IBPD. A specific X-ray imaging approach employing radiopaque barium sulfate ($BaSO_4$) was developed for detecting and determining the retention and sequential biodegradation pattern of the IBPD device within the vagina of the Large White pig. In addition to having radiopaque properties, $BaSO_4$ (which is biocompatible) was employed as a polymer matrix stabilizer for the purpose of enhancing matrix integrity (Bailey and Swett, 2007). Furthemore, since chemometric and molecular modeling approaches can precisely explicate various interactive mechanisms of drug release and permeation dynamics that occur from a drug delivery system, a model was developed in our laboratories to elucidate these mechanisms at a molecular level.

6.2 Materials and Methods

6.2.1 Materials

The two model drugs were AZT (Evershine Ind., Naejar Malad, Mumbai, India) and PSS (Omega (Pty) Ltd., Montreal, Canada). Methylparaben (Merck (Pty) Ltd., Darmstadt, Germany), shellac (Roeper GmbH, Hamburg, Germany), castor oil (Jayant Oils and Derivatives Ltd., Mumbai, India), barium sulphate ($BaSO_4$) (Merck-Schuchardt,

Hohenbrunn, Germany), pentobarbitone, ketamine (Bayer (Pty) Ltd, Wrenchwerg, Isando, South Africa), midazolam (Roche Products (Pty) Ltd, Isando, Gauteng, South Africa) and isoflurane (Safe Line Pharmaceuticals (Pty) Ltd, Florida, South Africa) were utilized to facilitate the *in vivo* studies. The mobile phase solvents comprising acetonitrile (99.9%) and methanol (99.9%) were purchased from Romil-SpS™ (Cambridge, UK). All other materials employed were the same as in Chapter 3.

6.2.2 Methods

6.2.2.1 Preparation of the intravaginal bioadhesive polymeric device

Biodegradable and biocompatible polymers namely $_m$PA 6,10 (150mg), PLGA (400mg), APE-PAA (25mg), PVA (25mg) and EC (200mg) were blended with model drugs AZT and PSS (separately and in combination) as well as radio-opaque barium sulfate ($BaSO_4$), using a cube blender (Erweka® GmbH, Heusenstamm, Germany), and then compressed into robust devices on a tableting press. One set of devices was coated with 2%$^w/_v$ APE-PAA while another set remained uncoated. In process validation tests were performed to ensure that the IBPD device had desirable quality attributes in terms of diametric hardness, uniformity in mass and friability.

6.2.2.2 Pan coating of the intravaginal bioadhesive polymeric device

A dual coating process using the Thai Coater® (Pharmaceutical and Medical Supply Limited Partinership, Yannawa, Bankok, Thailand) was employed with a protective undercoat comprising shellac and thereafter a mixture of XG and APE-PAA as an overcoat in order to prevent any irritation to the vaginal tissue during device insertion. The addition of APE-PAA was to facilitate bioadhesion of the IBPD to the posterior fornix of the vagina. The process involved firstly undercoating the IBPD with a combination of shellac (4mg/device), cold pressed castor oil (3mg/device) and ethanol (96%). This was followed by an overcoat of XG (2%$^w/_v$) and APE-PAA (2%$^w/_v$). XG was used for its viscoelastic non-collapsible swellability in order to facilitate bioadhesion of the IBPD in

conjunction with APE-PAA that was employed as a biodhesive polymer (Iseki et al., 2001; Gimeno et al., 20003; Verhoeven et al., 2006). The processing conditions utilized for effective coating of the IBPDs are listed in Table 6.1. A non-coating period of 30 minutes was allowed after each coating phase to effect a reduction in pan temperature and avoid sticking or fracture of the undercoat or overcoat seal. The increase in weight after coating the IBPD was determined using an electronic balance (Mettler, Model AE 240, Griefensee, Switzerland) while the increase in thickness was determined using a digital vernier caliper (Taizhou hangyu tools gauge and blades Co., Ltd, Wenqiao, Zhejiang, China) with a sensitivity of 0.01.

Table 6.1: Parameters and settings employed for coating the intravaginal bioadhesive polymeric device

Parameter	Settings
Temperature	50-56°C
Relative humidity	23-28%
Warming-up period	10min
Pan rotation	2-3rpm
Spray rate	4g/min
Volume of coating	2L
Number of caplets coated	2000
Undercoating duration	30min
Over-coating duration	60min

6.2.2.3 Influence of the intravaginal bioadhesive polymeric device on the micro-environmental pH of the vagina

The changes in micro-environmental pH within simulated vaginal fluid due to the presence of the IBPD were assessed by incubation of 3mL simulated vaginal fluid (containing the IBPD) in a Multi-Purpose Titrator (MPT-2) equipped with a rapid response, liquid filled glass pH micro-electrode supported on a vertical puller (Malvern Instruments Ltd., Worcestershire, UK). The changes in pH were evaluated from a pH-time profile over 30 days. The electrode calibration standards were adjusted to cover the buffer range from pH 3.5-5.5 with a linear Nernstian response maintained.

6.2.2.4 Thermal analysis of the polymeric composition and the coated intravaginal bioadhesive polymeric device

TMDSC was performed on the constituent polymers ($_m$PA 6,10, PLGA, APE-PAA, PVA, and EC) as well as the unhydrated and hydrated physical mixtures of the polymers and the IBPD (Mettler Toledo, DSC1, STAR^e System, Schwerzenback, Switzerland). The thermal events were explicated in terms of the glass transition (T_g) measured as the reversible heat flow (ΔH) due to changes in the magnitude of the C_p-complex values (ΔC_p), melting (T_m) and crystallization (T_c) temperature peaks which are consequences of irreversible and reversible ΔH values corresponding to the total heat flow. The temperature calibration was accomplished with the melting transition of indium. The transitions of the individual polymers and their physical mixtures were compared with the transition of the composite IBPD matrix. Samples were weighed (5mg) on perforated 40µL aluminum pans, crimped and then ramped from -35-230°C under a nitrogen (Afrox, Germiston, Gauteng, South Africa) atmosphere in order to diminish oxidation at a rate of 1°C/min. The instrument parameters and settings employed are listed in Table 6.2.

Table 6.2: Temperature modulated differential scanning calorimetry settings employed for thermal analysis of the intravaginal bioadhesive polymeric device and its formulation components

Segment Type	Parameter Setting
SINE PHASE[a]	
Start	-35°C
Heating rate	1°C/min
Amplitude	0.8°C
Period	0.8°C
LOOP PHASE[b]	
To segment	1
Increment	0.8°C
End	230°C
Count	436

[a] *Sinusoidal oscillations*
[b] *Oscillation period*

6.2.2.5 *Ex vivo* bioadhesivity testing of the coated drug-loaded intravaginal bioadhesive polymeric device

The excision of vaginal tissue from the pig model for bioadhesivity testing was undertaken following the method described in (Chapter 5, Section 5.2.2.4).

6.2.2.6 Textural profiling analysis to determine the bioadhesivity of the intravaginal bioadhesive polymeric device

Bioadhesivity of the IBPD was determined using a method described in one of our recent publications (Ndesendo et al., 2009). Briefly, the freshly excised pig vaginal tissue was secured on the textural probe and the IBPD was fixed onto the heated textural platen after exposure to simulated vaginal fluid (pH 4.5, 37°C) (Chapter 3, Section 3.2.2.3, Table 3.3) for 30 minutes. Testing was then conducted by measuring the maximum force (N) required to detach the vaginal tissue from the fixed device. This was determined by measuring the Peak Adhesive Force (PAF) or the Work of Adhesion that was computed as the area under the curve of a Force-Distance textural profile (AUC_{FD}).

6.2.2.7 Insertion of the intravaginal bioadhesive polymeric device into the vagina of the pig

Three Large White pigs each weighing 35kg were anaesthetized with midazolam (0.3mg/kg I.M.) and ketamine (11mg/kg I.M.). 2% isoflurane in 100% oxygen was administered via a face mask to maintain anesthesia. The IBPD was then deeply inserted into the posterior fornix of the vagina of each pig with the aid of an applicator and a speculum as shown in Figure 6.1a and b.

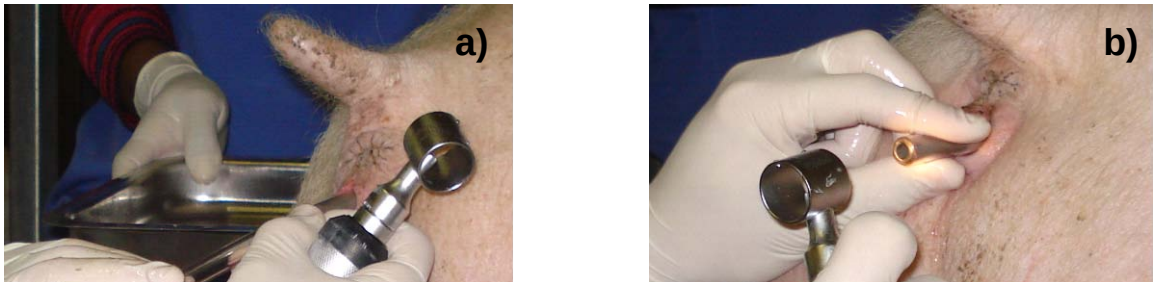


Figure 6.1: Digital images depicting a) insertion of the IBPD into the vagina of the pig and b) tracking the location of the IBPD in the vagina using a speculum.

6.2.2.7.1 X-ray imaging of the pig for detection of the intravaginal bioadhesive polymeric device

To detect the presence and position of the IBPD in the pig vagina after insertion, animals were X-rayed (Siemens AG, Medical Engineering Group, Erlangen, Germany) directly after device insertion and thereafter three times weekly for 2 weeks, then twice weekly for a further 2 weeks to confirm the retention of the IBPD in the vagina and to qualitatively analyze its swellability and bioerosion dynamics.

6.2.2.8 *In vitro* drug release from the coated and uncoated intravaginal bioadhesive polymeric device

In vitro drug release evaluation was conducted on the APE-PAA coated and uncoated AZT-loaded IBPDs and coated AZT-and PSS-loaded IBPDs

6.2.2.8.1 Analysis of the effect of device coating on the drug release

To assess the effect of coating on drug release, analysis was conducted on IBPDs (coated and uncoated) containing AZT as a representative drug model due to its hydrophilicity. An IBPD was immersed in a 100mL (Umamaheshwari et al., 2004; Charde et al., 2008) simulated vaginal fluid (pH 4.5; 37°C) (Chapter 3, Section 3.2.2.3, Table 3.3) using a sealable glass vessel (150mL) and placed in an orbital shaking incubator (LM-530-2, MRC Laboratory Instruments Ltd., Hahistadrut, Holon, Israel) maintained at 20rpm and a temperature of 37°C. For the determination of AZTconcentration, 3mL samples

were withdrawn at predetermined time intervals over a period of 30 days and subjected to Ultra Performance Liquid Chromatography (UPLC) analysis. An equivalent volume of drug-free simulated vaginal fluid was replaced into the release medium to maintain sink conditions. The analysis was conducted in triplicate. A correction factor was appropriately applied in all cases where dilution of samples was required.

6.2.2.8.2 Analysis of the drug release from the coated devices containing AZT and PSS separately and in combination

For the analysis of the drug release from the coated devices containing AZT and PSS separately and in combination, the same procedure as in Section 6.2.2.8.1 was employed the only difference being that in this case samples for analysis were withdrawn over a period of 72 days.

6.2.2.8.3 Chromatographic conditions for the analysis of AZT and PSS concentration

Quantitative analysis was performed using a Waters® Acquity Ultra Performance Liquid Chromatographic (UPLC) system (Waters Corp., Milford, MA, USA), equipped with a photodiode array (PDA) detector and interchangeable columns, namely, a UPLC® BEH phenyl column (1.7µm; 2.1×50mm) for AZT separation, and a UPLC® BEH C_{18} column (1.7µm; 2.1×100mm) for PSS separation. The binary mobile phases were composed of water/acetonitrile (60:40$^{v}/_{v}$) and methanol/water (50:50$^{v}/_{v}$) for AZT and PSS respectively. All solutions were filtered using a 0.22µm membrane filter (Millipore Corp., Bedford, Massachusetts, USA) prior to injection onto the UPLC column. A gradient assay method was used for AZT separation with a column temperature set at 25°C, injection volume of 2µL and a UV detection wavelength of 267nm. The gradient settings for the assay method are shown in Table 6.3. An isocratic assay method was used for PSS separation employing methanol/water (50:50$^{v}/_{v}$) as the mobile phase, a flow rate of 0.2mL/min, a

column temperature of 25°C, an injection volume of 1.7µL and a UV detection wavelength of 244nm.

Table 6.3: Chromatographic mobile phase gradient settings used for the separation of AZT

Separation events	Time (min)	Flow Rate (mL/min)	% A (Water)	% B (Acetonitrile)
1	0.00	0.500	60.0	40.0
2	1.00	0.500	5.0	95.0
3	2.60	0.500	5.0	95.0
4	3.50	0.500	60.0	40.0

6.2.2.8.4 Preparation of standard solutions and calibration curves

The internal standard employed for both model drugs was methylparaben (MP). Standard solutions of AZT, PSS and MP (internal standard) were separately prepared by mixing specific quantities in water/acetonitrile (60:40$^{v}/_{v}$) for AZT and methanol/water (50:50$^{v}/_{v}$) for PSS to yield a concentration of 0.1mg/mL in each case. The standard solutions employed in preparing the calibration curve of the test drug and internal standard were obtained by further serial dilutions with a final concentration range of 25-10,000ng/mL. The internal standard solution was prepared at a concentration of 5000ng/mL and was added to all samples prepared for UPLC analysis. Calibration curves were developed using blank simulated vaginal fluid (pH 4.5) and computed as a ratio of the Area Under the Curve (AUC) of AZT and PSS chromatographic peaks to that of the internal standard MP against the corresponding standard concentrations of AZT and PSS (Figure 6.2a and b).

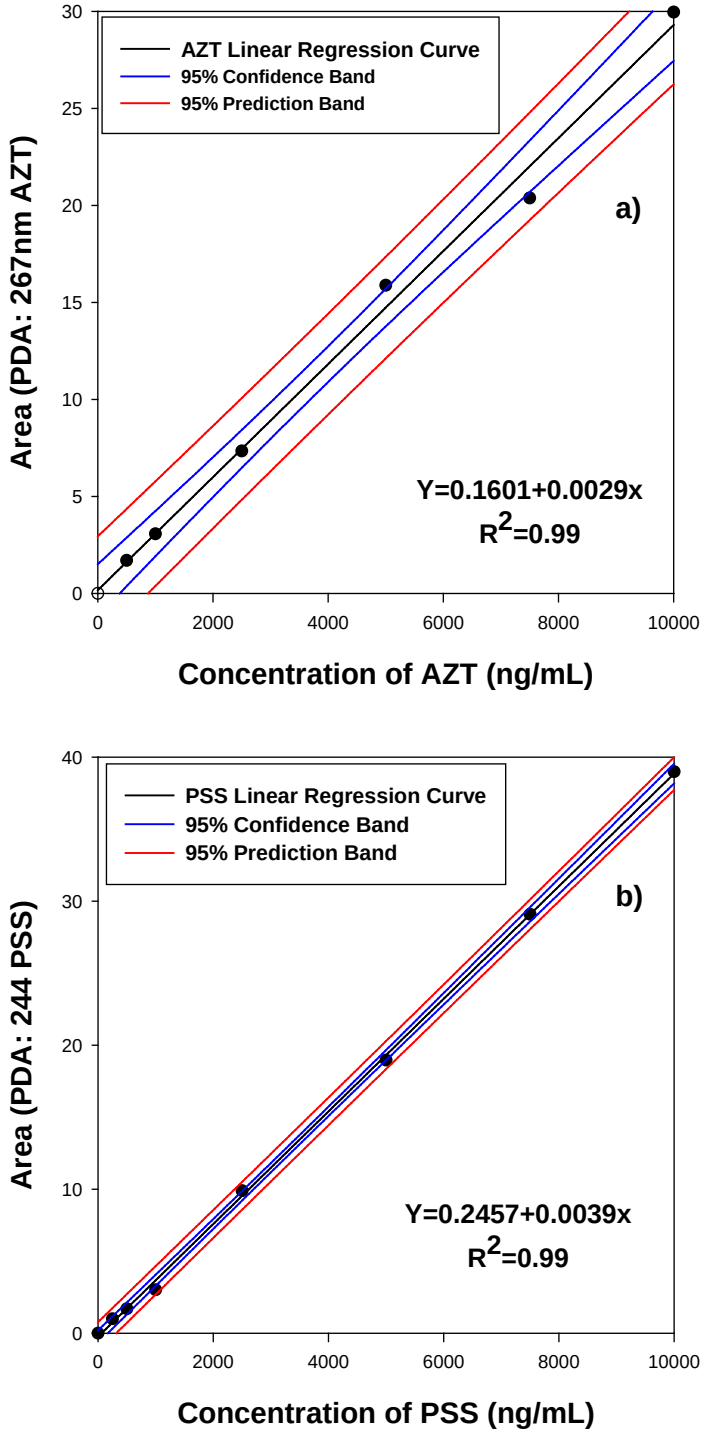


Figure 6.2: a) Calibration curves for: a) AZT in simulated vaginal fluid 267nm (N=3, SD<0.001 in all cases); b) PSS in simulated vaginal fluid 244nm (N=3, SD<0.003 in all cases).

6.2.2.8.5 The solid phase extraction procedure employed in the extraction of the drugs from simulated vaginal fluid samples for UPLC analysis

This was carried out by using single use Water Oasis® HLB 3cc (60mg) extraction cartridge (Waters Corporation, Milford, Massachusetts, USA) adapting a method developed by Notari and co-workers (2006). The Solid Phase Extraction (SPE) cartridge was conditioned with 1.0mL methanol followed by 1.0mL water Milli-Q. For AZT sample preparation, 1mL of sample was mixed with 1mL of acetonitrile vortexed for 1 minute and centrifuged (Nison Instrument (Shangai) Limited, Shangai, China) at 13,000rpm for 6 minutes at 24^{o}C. 650µL of the supernatant was diluted by adding water Milli-Q (1mL) and loaded in the cartridge. Thereafter, the cartridges were washed with 1.0mL of 5%$^{v}/_{v}$ methanol in water Milli-Q. Analytes were eluted by washing cartridges with 550µL 0.01M KH_2PO_4 followed by 2mL methanol. The eluate was evaporated to dryness in a slow stream of high purity nitrogen gas (Afrox, Germiston, Gauteng, South Africa). The extracted sample was re-constituted with 100µL absolute methanol, mixed with 400µL of MP, and then filtered into the injection vials using 0.22µm syringe-driven filter units (Millipore Corporation, Bedford, Massachusetts, USA) for UPLC analysis. The same procedure was followed for PSS samples. However, methanol was used as the mobile phase.

6.2.2.9 *Ex vivo* drug permeation studies through pig vaginal tissue from the intravaginal bioadhesive polymeric device using the Franz Diffusion Cell Apparatus

The permeation studies to assess the extent of drug permeation across pig vaginal tissue were performed using a Franz Diffusion Cell (FDC) apparatus (PermeGear Inc. Bethlehem, PA, USA) equipped with a 12mL receptor compartment, clamp and stirrer-bar. Freshly excised pig vaginal tissue obtained from the euthanized Large White pig described above was placed between the donor and receptor compartments of the FDC apparatus (Figure 6.3). 10mL simulated plasma (pH 7.4; 37°C) (Giannola et al., 2007) (Table 6.4) was used in the receptor compartment and continuously agitated. IBPD device

containing AZT and PSS was dissolved in 50mL simulated vaginal fluid (pH 4.5; 37°C) (Chapter 3; Section 3.2.2.3; Table 3.3), by crashing and triturating it into a powder form and then solubilising the powder by the aid of a sonicator (SB 5200D, Ultrasonic, Ningbo Scientists Biotechnology Co Ltd, Ningbo, China). The solutions were then assessed for the permeation of the drug across the pig vaginal tissue (thickness=1.5±0.06mm; permeation area=2.0±0.01cm^2) and into the simulated plasma in the receptor compartment. Samples (2mL) were withdrawn from the receptor compartment, at predetermined intervals over a period of 24 hours, and subjected to quantitative drug analysis using UPLC. An equivalent volume of drug-free simulated plasma was replaced into the receptor compartment to maintain sink conditions throughout the permeation study. The analyses were conducted in triplicate. A correction factor was appropriately applied in all cases where dilution of samples was required.

The extent of permeation of AZT and PSS across the pig vaginal tissue was determined in terms of drug flux. The flux (mg.cm^{-2}.hr^{-1}) of drug across the vaginal tissue was calculated at steady-state per unit area by linear regression analysis of permeation data using Equation 6.1.

$$J_S = \frac{Q_r}{A \times t} \qquad \textbf{\textit{Equation 6.1}}$$

where, *Js* is the flux (mg.cm^{-2}.hrg^{-1}), Q_r (mg) is the quantity of AZT or PSS that diffused through the pig vaginal tissue into the receptor compartment, *A* (cm^{-2}) is the effective cross-sectional area available for drug permeationand *t* (h) is the time of drug exposure to the vaginal tissue.

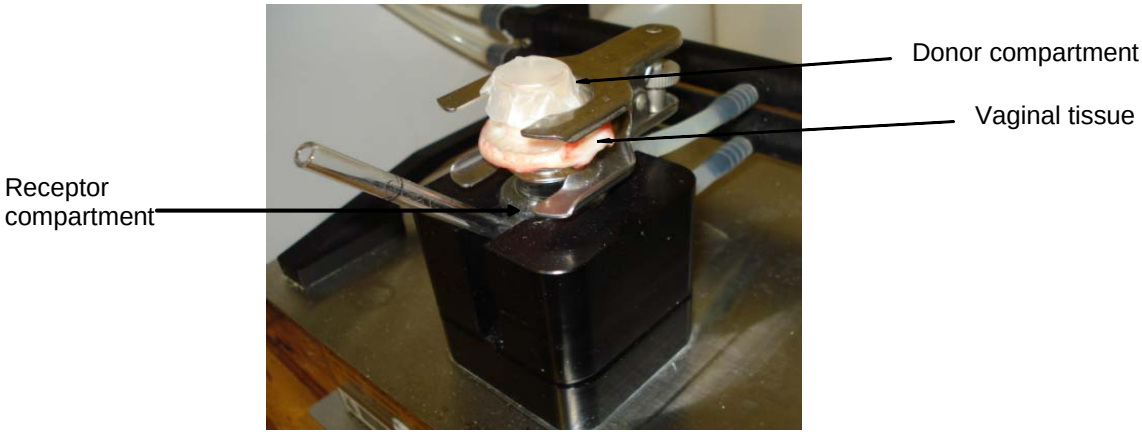


Figure 6.3: Permeation studies of AZT and PSS across pig vaginal tissue using a Franz Diffusion Cell apparatus.

Table 6.4: Constituents used to prepare the simulated plasma fluid

Simulated Plasma	
Component	**Quantity (g/L)**
KH_2PO_4	0.144
Na_2HPO_4	0.795
NaCl	9.000

(Giannola et al., 2007)

6.2.2.9.1 Chromatographic conditions for the analysis of AZT and PSS concentration

The chromatographic conditions for the analysis of AZT and PSS concentration in the simulated plasma fluid were the same as described in Section 6.2.2.8.3.

6.2.2.9.2 Preparation of standard solutions and calibration curves

This was conducted in the same way as described in Section 6.2.2.8.4, the only difference being that calibration curves were developed using blank simulated plasma fluid (pH 4.5) (Section 6.2.2.9, Table 6.4) instead of simulated vaginal fluid. The constructed calibration curves are as shown in Figure 6.4a and b.

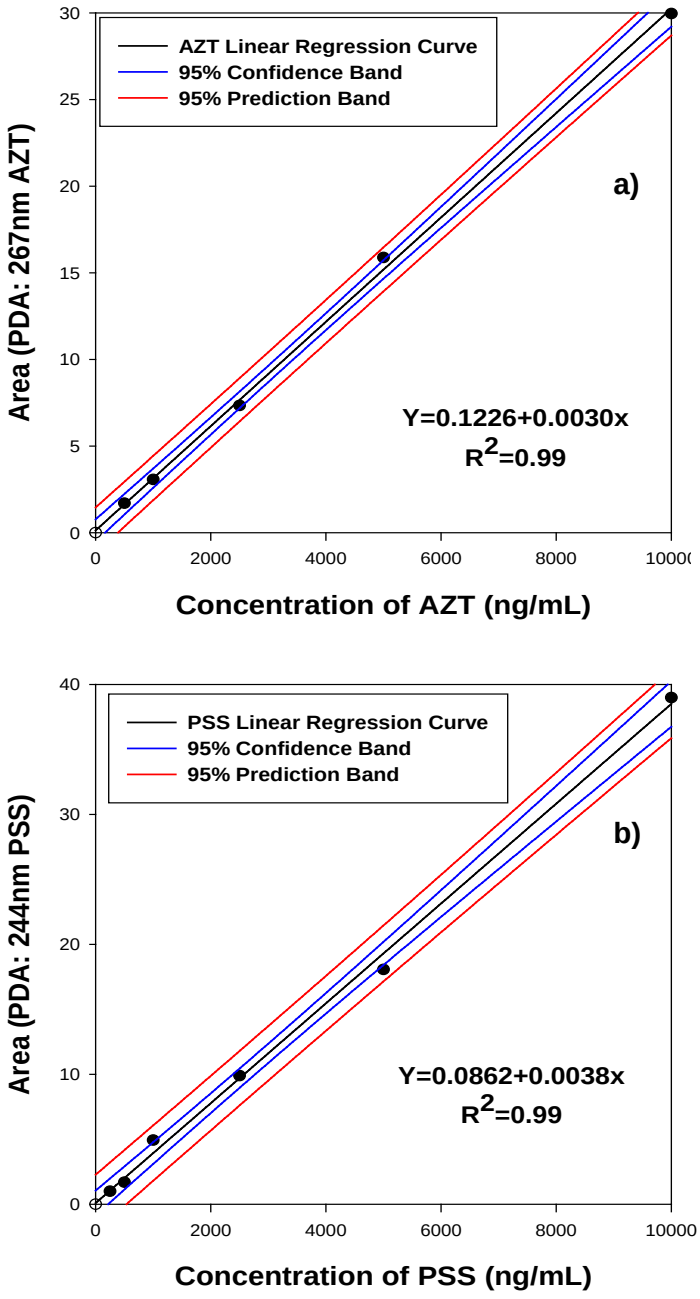


Figure 6.4: a) Calibration curves for: a) AZT in simulated plasma fluid 267nm (N=3, SD<0.001 in all cases); b) PSS in simulated plasma fluid 244nm (N=3, SD<0.003 in all cases).

6.2.2.9.3 The solid phase extraction procedure employed in the extraction of the drugs from simulated plasma fluid samples for UPLC analysis

The solid phase extraction procedure employed in the extraction of the drugs from simulated plasma fluid samples was the same as described in Section 6.2.2.8.5.

6.2.2.10 Postulated mechanism of the drug permeation and dissolution dynamics from the intravaginal bioadhesive polymeric device employing chemometric and molecular modeling

Chemometric and molecular structural modeling was used to deduce the transient mechanisms of diffusion and dissolution, chemical interactions and inter-polymeric interfacing during the dissolution of the IBPD device and the permeation of AZT and PSS across the vaginal tissue. This approach allowed us to make predictive findings based on the chemical and physical interactions underlying the dissolution of the IBPD and the diffusion of AZT/PSS from the IBPD (contained in the simulated vaginal fluid) and finally the permeation of these drugs to simulated plasma fluid across the pig vaginal tissue. In addition, semi-empirical quantum mechanics were employed to generate molecular interactions and computational energy paradigms of the IBPD components based on inherent interfacial phenomena underlying the mechanisms of dissolution and diffusion as provided by the inter-polymeric blended IBPD. Models and graphics supported on the step-wise molecular IBPD-simulated fluids and IBPD-tissue interactions, polymeric interconversion, dissolution and diffusion as envisioned by the molecular behavior and stability of the gelled IBPD network were generated on ACD/I-Lab, V5.11 (Add-on) software (Advanced Chemistry Development Inc., Toronto, Canada, 2000).

6.3 Results and Discussion

6.3.1 Coating of the intravaginal bioadhesive polymeric device

Uniformly coated IBPDs were produced. The final mass for the coated caplets was 1278±0.04mg while the uncoated caplets weighed 1200±0.01mg. Thus, the increase in weight was 6.5±0.02%$^{w}/_{w}$ for which the thickness of the coat was 0.520±0.005mm.

6.3.2 Micro-environmental pH variation analysis within the simulated vaginal fluid

It was generally observed that the superficial simulated vaginal fluid immediately adjacent to the immersed IBPD exhibited higher pH values than the simulated vaginal fluid

immediately surrounding the device. The initial pH, measured as close as possible to the device, upon insertion of the IBPD into the titration system (MPT-2) was 4.5±0.01 (N=3). The pH electrode was inserted using a Narashige micro-manipulator and was submerged towards the IBPD by careful hydraulic micro-movements to avoid creating any unnecessary turbulent hydrodynamic flow. A slight drop in pH was routinely recorded as the electrode passed in proximity to the IBPD with a slightly more acid pH than the entire simulated vaginal fluid (pH 4.48±0.02) around a superficial surface diameter of 5mm. The pH, measured away from the superficial layer was 4.58±0.03 (N=3). The relatively higher pH at the superficial layer may have been due to the extruding OH^- ions from $_m$PA 6,10, EC, PAA or PVA. The relatively lower pH observed at the IBPD proximity (micro-environmental pH) could most certainly have been due to the break down of PLGA into lactic and glycolic acids. This biphasic response in pH was observed over an experimental period of 30 days. A profile depicting this sequence is shown in Figure 6.5.

Attempts were made to perturb the inherent pH of the simulated vaginal fluid by adding incremental volumes of simulated seminal fluid of pH 7.4 (a significantly > volume than would be produced on degradation of the PLGA-based IBPD in the simulated vaginal fluid). After the addition of simulated seminal fluid, the pH recorded within the simulated vaginal fluid was 4.58+0.02 (N=3) and not significantly different from the resting pH of 4.50 ($P<0.01$). Upon further addition of simulated seminal fluid into the MPT-2, the inherent pH was reduced to 4.52±0.03 (N=3) indicating that the simulated vaginal fluid was able to maintain its internal pH close to a value of 4.50 in the presence of a more alkaline medium (e.g. seminal fluid). Overall the results portrayed the potential of the IBPD to control and buffer the pH range of 3.5-5.5 in the simulated vaginal fluid by the virtue of the degradation of PLGA to lactic and glycolic acids. This pH range is the same as that produced by *Lactobacilli* species (Boskey et al., 2001) which plays a vital role in keeping the vagina healthy. It also covers the average pH range of a normal healthy

human vagina which is 4-5 (Smith, 1993; Ferris et al., 2006; WebMD, 2008) indicating the suitability of the IBPD for the intended purpose.

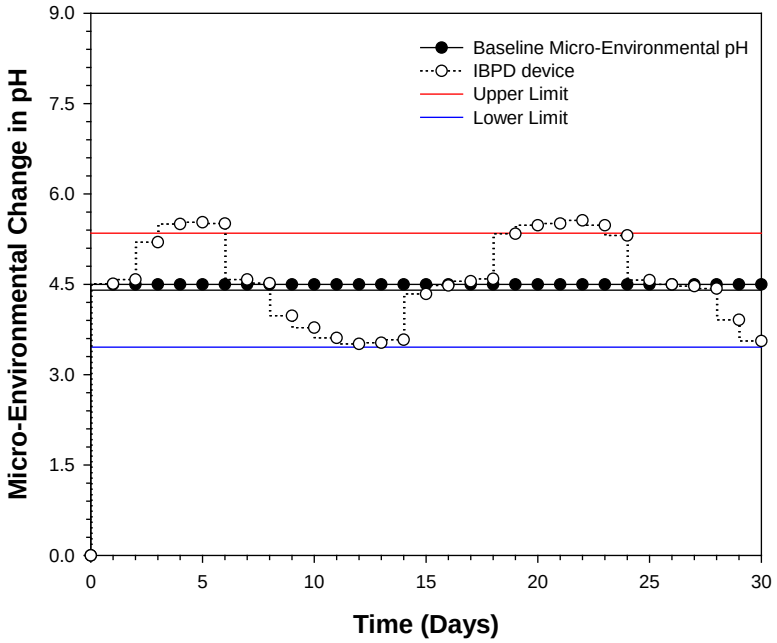


Figure 6.5: Micro-environmental pH variation in the simulated vaginal fluid containing the IBPD (N=3).

6.3.3 Thermal analysis of the polymer constituents of the intravaginal bioadhesive polymeric device

The thermal stability of the constituent polymers as well as the composite unhydrated IBPD was investigated by TMDSC at a temperature range from -35-230°C. The corresponding TMDSC thermograms are shown in Figures 6.6 to 6.13. The polymers displayed multi-transitional thermal behaviors with multiple T_g, T_m and T_c values (Table 6.5) that were attributed to the existence of reversing and non-reversing endothermic signals arising from the transient melting of molecules within each polymer.

Table 6.5: Critical thermal events evidenced by diverse temperature inflection peaks for the polymer constituents of the intravaginal bioadhesive polymeric device

	Critical temperature transition points (°C)		
Sample Analyzed	**T_g**	**T_c**	**T_m**
$_m$PA 6,10	163	120; 200	65; 140
PLGA	45-55; 210	230	22; 220
APE-PAA	90	60	30; 130-170
PVA	22; 115	163; 215	30; 180
EC	100	130	170
Hydrated polymer blend	70; 160	210	22; 163; 200
Unhydrated polymer blend	170	220	200
Hydrated IBPD	83; 163	160	-10; 38; 85
Unhydrated IBPD	150	140; 220	222

T_g=Glass transition temperature
T_c=Crystallization temperature
T_m=Melting temperature

6.3.3.1 Thermal characterization of the native unhydrated polymer constituents of the intravaginal bioadhesive polymeric device

6.3.3.1.1 Ethylcellulose (EC)

EC was characterized by a T_g at 100°C and an exothermic T_c at 130°C (Figure 6.6). The T_m of EC occurred at 170°C which was relatively high compared to the other polymers employed. This revealed the superior thermodynamic stability of the polysaccharide subunits of EC.

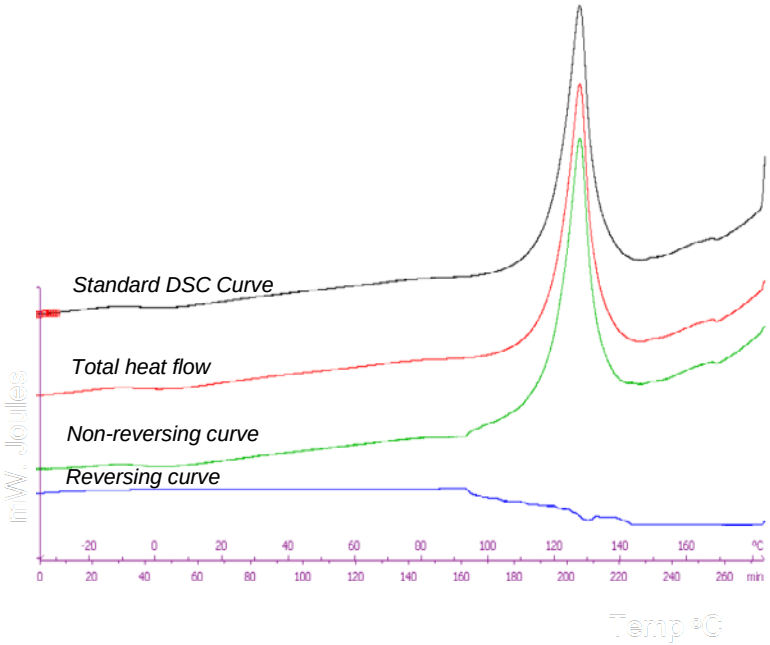


Figure 6.6: TMDSC thermogram for the unhydrated ethylcellulose.

6.3.3.1.2 Modified polyamide 6,10 ($_m$PA 6,10)

The $_m$PA 6,10 had a distinct T_g at 163^{o}C and two endothermic T_m values at 65°C and 140°C. In addition, the exothermic T_c peaks observed at 120°C and 200°C followed this melting phases. This suggested partial decomposition of $_m$PA 6,10 that may be related to matrix erosion (Figure 6.7) and the fact that the aliphatic polyamides inherently have a high heat of fusion and low entropy of fusion dependent on the collective dissociation energy of intramolecular H-bonds before any macroscopic dimensional changes can be realized (de Candia et al., 1998; Murthy, 2006).

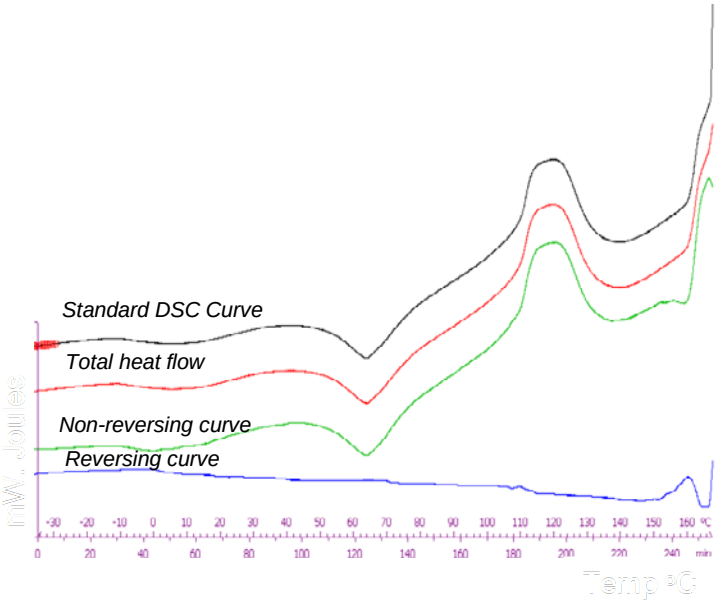


Figure 6.7: TMDSC thermogram for the unhydrated polyamide 6,10 (PA 6,10).

6.3.3.1.3 Poly(lactic-co-glycolic acid)

PLGA was characterized by two T_g values (Figure 6.18). The first T_g peak appeared during the initial heating scan in which an enthalpy of relaxation peak superimposed the T_g between 20-30°C. The second T_g value at 210°C was also noted as PLGA is a quench cooled amorphous material (Teixeira et al., 2005; Mainardes et al., 2006; Rouse et al., 2007). Furthermore, thermograms representing the total heat flow depicted a transient endothermic T_m peak at 22°C which was a recovery of heat loss. As heating proceeded, the T_g and the temperature at the apex of the overheating peak shifted to higher temperatures (T_g=210°C; T_m=220°C) due to the formation of a percolated network structure resulting from the quench cooling properties of PLGA.

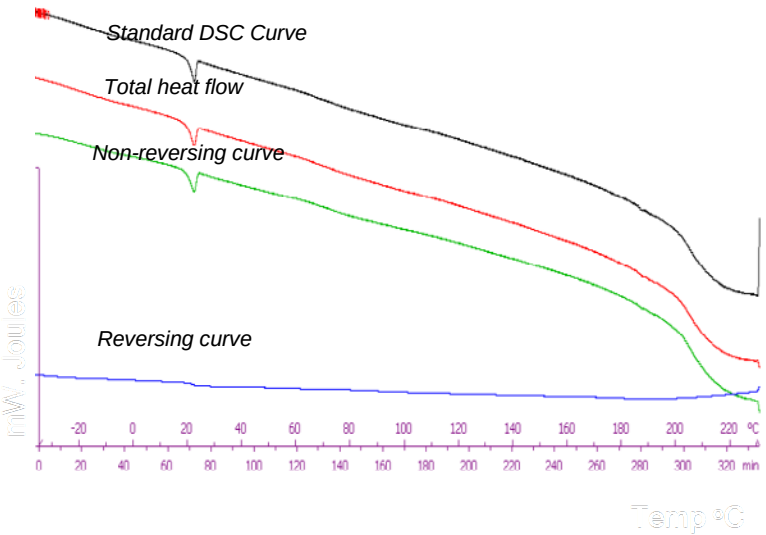


Figure 6.8: TMDSC thermogram for the unhydrated poly (lactic-co-glycolic acid).

6.3.3.1.4 Polyvinyl alcohol

PVA is a semi-crystalline polymer in which high physical polymeric chain interactions exist due to H-bonding between the OH^- groups. Thermograms for PVA revealed two T_g values at 22°C and 115°C and two endothermic T_m peaks at 30°C and 180°C (Figure 6.9) due to the thermal decomposition of the ordered PVA elements. Physical interactions between the polymer chains such as H-bonding contributed to the high enthalpy of fusion and T_m values observed for PVA. Thermodynamic analysis of PVA isotherms and crystallite growth rates has shown that crystallization of PVA (163°C; 215°C) is one-dimensional (Peppas and Hansen, 2003). Thus, due to the absence of water or other swelling agents, kinetic hindrances predominated as a result of interactions between the OH^- groups of PVA.

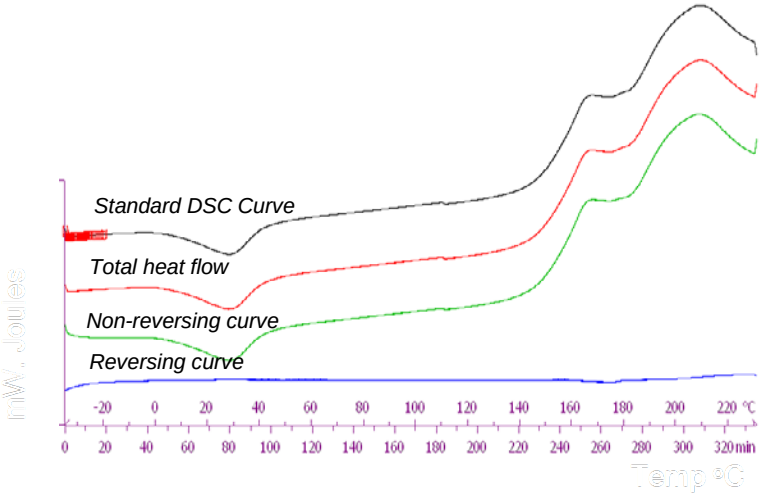


Figure 6.9: TMDSC thermogram for the unhydrated polyvinyl alcohol.

6.3.3.1.5 Allyl penta erythritol-crosslinked poly(acrylic acid)

The salient thermal events for APE-PAA are shown in Figure 6.10 and Table 6.5. The broad endothermic peak (130-170°C) resulted from conformational changes in the macromolecules of APE-PAA related to the magnitude of crosslinking interactions between the proton-donating pendent $COOH^-$ groups and polar OH^- groups of APE-PAA during synthesis (Sugama et al., 2007). The presence of $COOH^-$ groups favored the bioadhesiveness of APE-PAA within the pig vaginal tissue when used as a coating agent for the IBPD device.

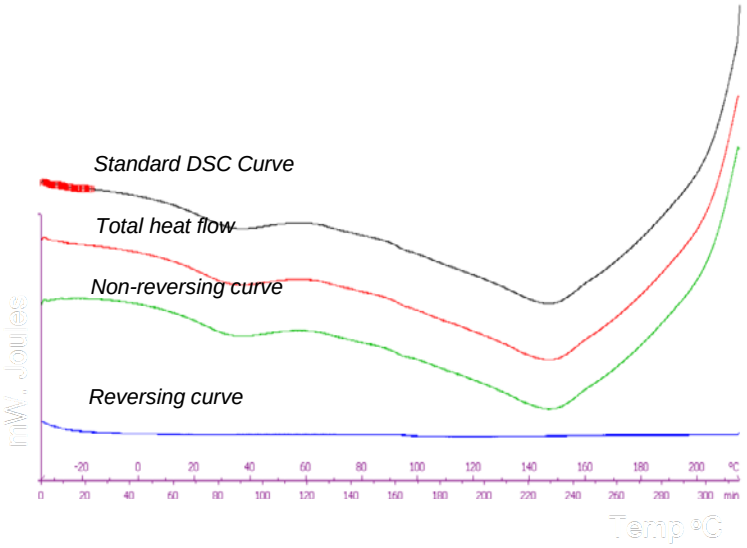


Figure 6.10: TMDSC thermogram for the unhydrated allyl penta erythritol-crosslinked poly(acrylic acid).

6.3.3.2 Thermal characterization of the unhydrated intravaginal bioadhesive polymeric device

Thermal analysis of the unhydrated IBPD revealed a T_g at 150°C, two T_c peaks at 140°C and 220°C and a T_m peak at 220°C (Figure 6.11). The presence of transient T_m peaks in the total TMDSC signals for the unhydrated IBPD indicated that the polymers were dispersed within the device matrix. In addition, diminutive exothermic events were observed at the corresponding T_c ranges for the constituent polymers indicating a high degree of crystallinity within the matrix structure. The deconvolution of the total TMDSC signals for the unhydrated IBPD in terms of reversing and non-reversing events reflected the average of the equivalent signals for each polymer. However, for the unhydrated IBPD the T_m appeared to be predominantly reversing due to the concurrent re-crystallization and melting phenomena that offset one another. This indicated that solid-solid phase transitions may have occurred within the unhydrated IBPD due to polymeric compression, and subsequently contributed to the prolongation and control of drug from the device. Thus, the thermodynamic stability of polymer blends may affect the drug release process and can therefore be used to predict the drug release behavior based on unequivocally defined thermodynamic events.

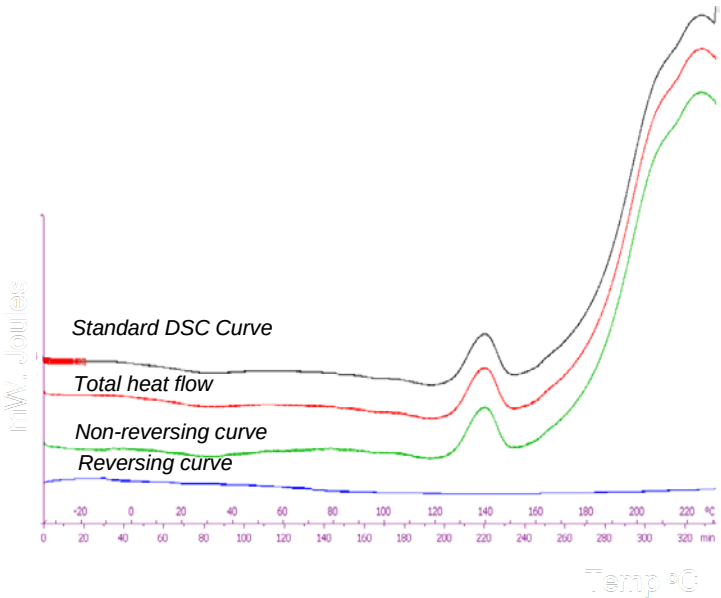


Figure 6.11: TMDSC thermogram for the unhydrated IBPD.

6.3.3.3 Thermal characterization of unhydrated and hydrated physical polymer blends as well as hydrated intravaginal bioadhesive polymeric device

TMDSC analysis was also performed on hydrated and unhydrated physical blends of the constituent polymers of the IBPD as well as the hydrated IBPD in order to determine the effect of compression on the polymer blend. Thermograms obtained on the hydrated and unhydrated physical polymer blends as well as the hydrated and unhydrated IBPD are depicted in Figures 6.12 and 6.13 and in Table 6.5. Overall, there was a distinct similarity between thermal events of the hydrated physical polymer blend and the hydrated IBPD (Figures 6.16a and b). Thermograms presented with regions associated with very low temperatures (-10°C and -15°C) for the hydrated samples of the physical polymer blend and the IBPD while dehydration was complete at 200°C (Figure 6.16a and b). For the hydrated physical polymer blend (Figure 6.15a), the onset of the low-temperature endothermic T_m peak (22°C) was attributed to the high moisture content in the physical polymer blend while the apparent T_m peaks (163°C and 200°C) resulted from the loss of residual water as heating proceeded. The T_m endotherms were distinctly separated from the total heat flow in the non-reversing signal.

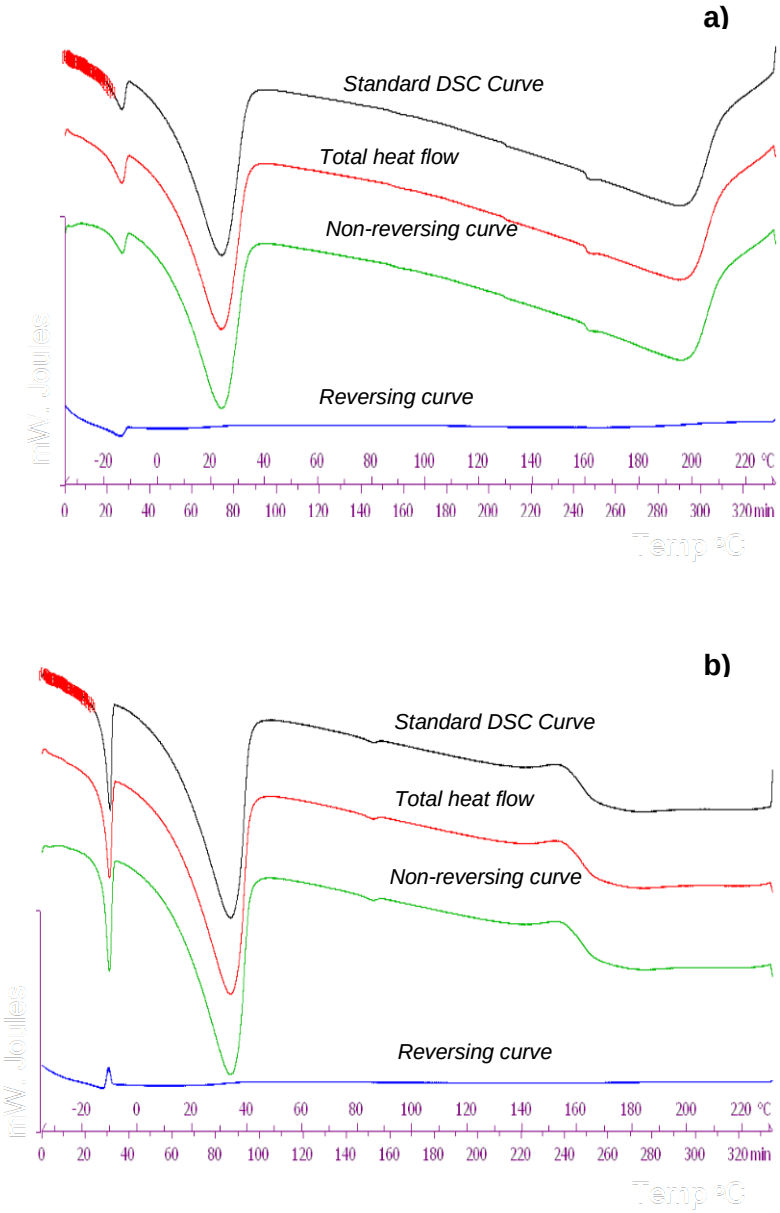


Figure 6.12: TMDSC thermogram for a) the hydrated physical polymer blend, b) the hydrated IBPD.

The thermal behavior for the unhydrated physical polymer blend was markedly different from that of the unhydrated IBPD (Figure 6.13a and b). This was attributed primarily to the effect of polymer compression on the physical polymer blend to produce the device. Contrary to the hydrated physical polymer blend, the unhydrated polymer blend showed fewer thermal events (Figure 6.13a). A single T_g at 170°C and a T_m peak at 200°C were observed (Figure 6.13a). Furthermore, the T_g and T_m peaks that appeared for the

hydrated physical polymer blend prior to 170°C, were absent in the unhydrated polymer blend (Figure 6.13a). This may be associated with a baseline transition at ±170°C in the reversing heat flow signal. Overall, the hydrated physical polymer blend presented with lower T_m peaks (22°C and 163°C). These observations were consistent with previous results reported by Frushour (2004), where upon hydrating a physical polymer blend, the T_m peak was reduced well below the onset temperature.

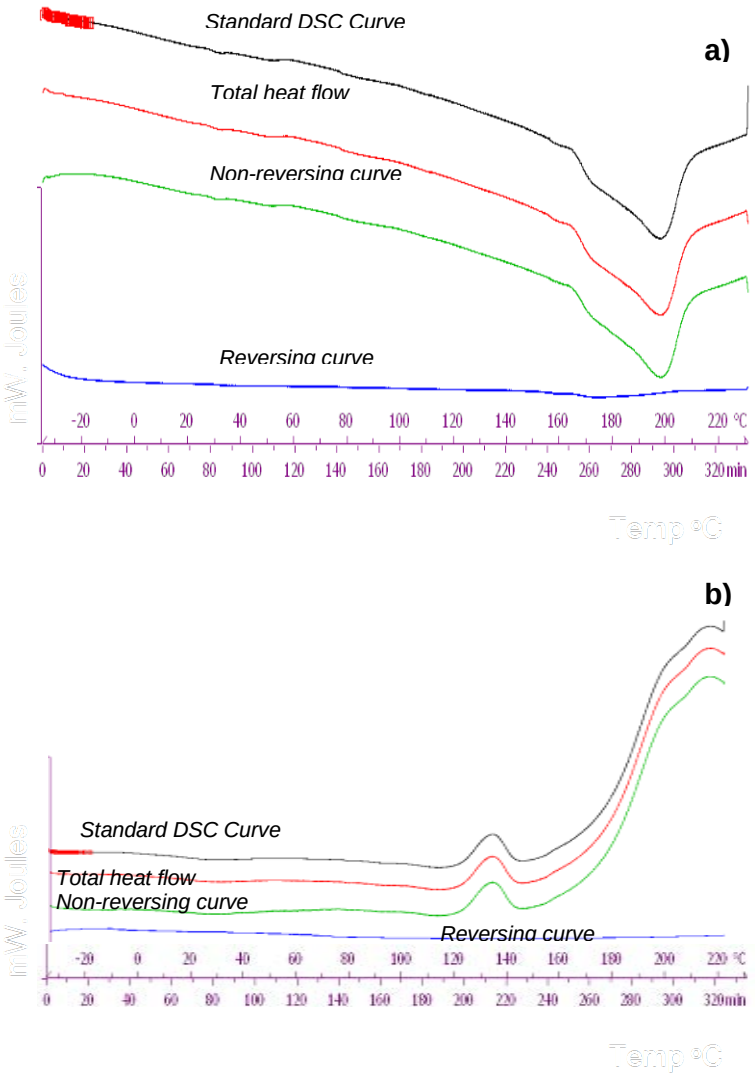


Figure 6.13: TMDSC thermogram for a) the unhydrated physical polymer blend, b) the unhydrated IBPD.

6.3.4 Analysis of the drug release behavior from the coated and uncoated optimized drug-loaded intravaginal bioadhesive polymeric device

6.3.4.1 Chromatographic separation of 3'-azido-3'-deoxythymidine and polystyrene sulphonate with methyparaben as an internal standard

A UPLC assay method was used for quantifying the concentration of AZT and PSS released from the IBPD. The 3 dimensional chromatographic analysis of blank simulated vaginal fluid revealed complete separation with no interfering peaks at the retention times within the UV wavelength range of 200-400 (AZT/PSS and MP) as shown by the typical representative 3D UPLC profile in Figure 6.14. Chromatograms depicting the retention times for MP (internal standard), AZT and PSS in simulated vaginal fluid are as shown in Figure 6.15a and b.

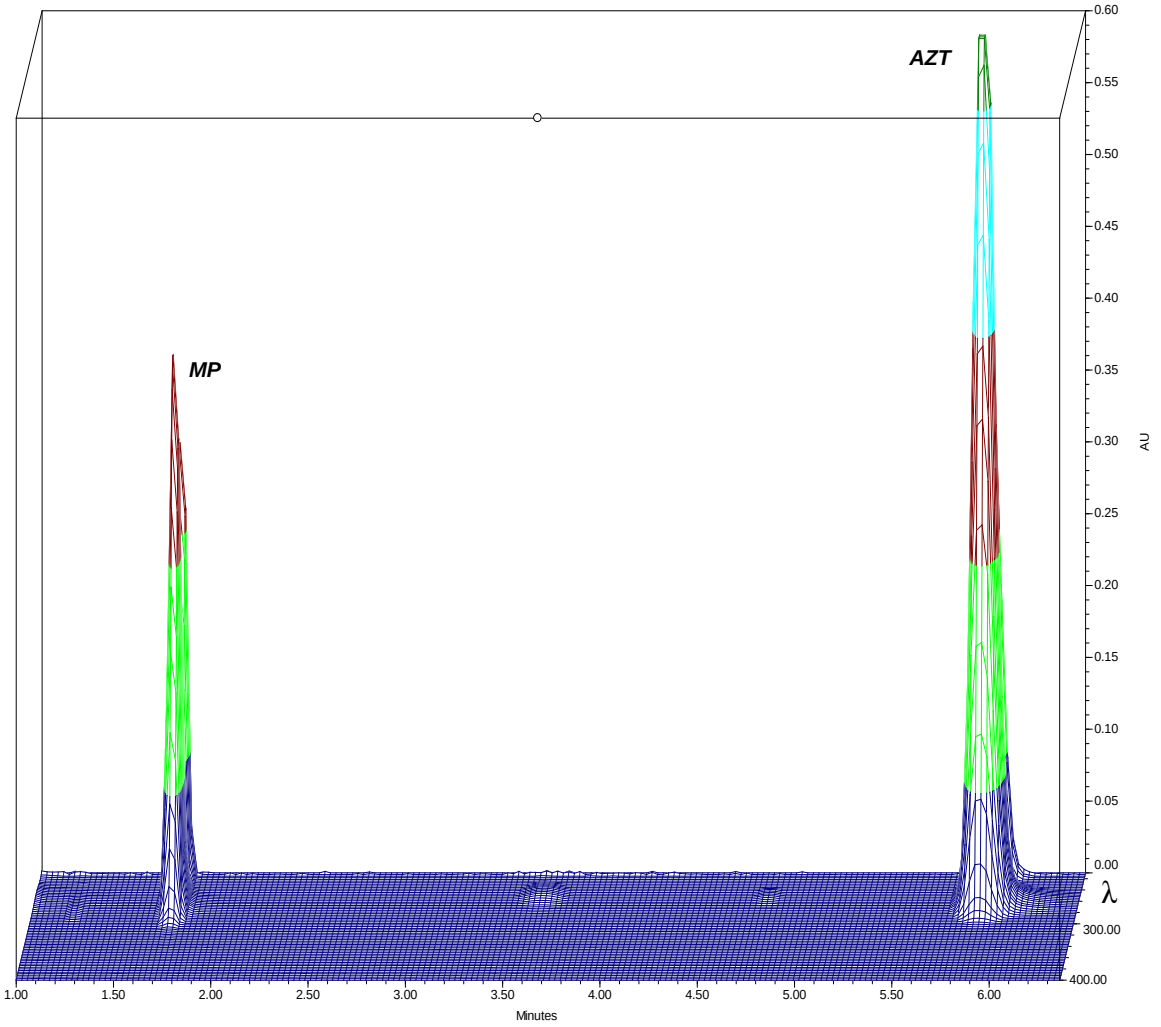


Figure 6.14: A typical 3D UPLC profile showing a complete separation between AZT and MP (internal standard).

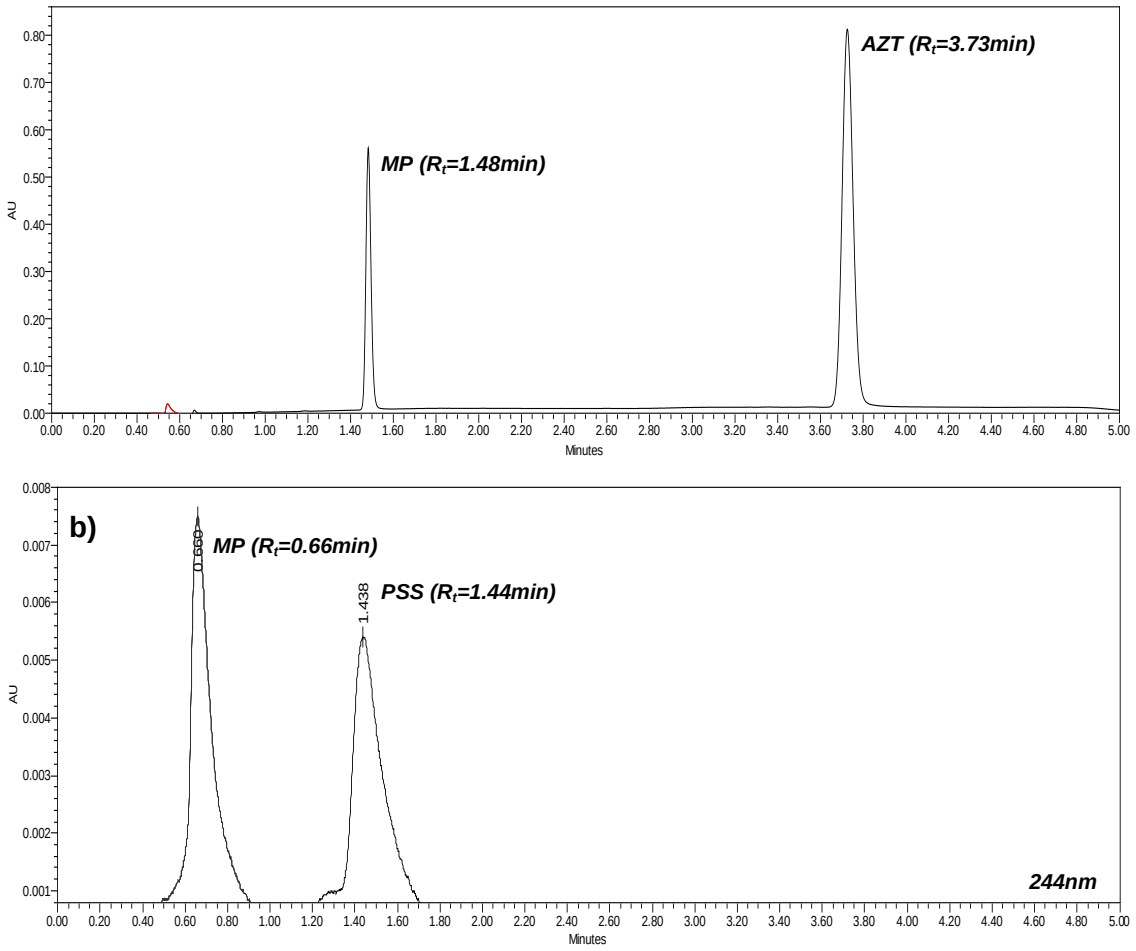


Figure 6.15: UPLC chromatograms depicting the separation of a) AZT and MP (internal standard) and b) PSS and MP (internal standard) in simulated vaginal fluid (pH 4.5; 37°C).

6.3.4.2 Assessment of the effect of coating on the drug release from the intravaginal bioadhesive polymeric device

The shellac/APE-PAA-coated IBPD containing only one of the model hydrophilic drugs, AZT, demonstrated extended drug release when compared to the uncoated devices (28 vs 20 days) (Figure 6.16a and b). This was due to the shielding effect of the initial shellac undercoat and APE-PAA overcoat applied. Once the APE-PAA coating was hydrated the shellac gradually solubilised in a manner that diffusion channels formed within the coating layer. This facilitated the drug diffusion from the IBPD. Furthermore, shellac (used as an undercoat) shielded the device against the ingress of release medium due to its wax-like

properties (Sinha, 2003). This may be attributed to its inherent moisture protecting properties, as well as its ability to act as a plasticizer.

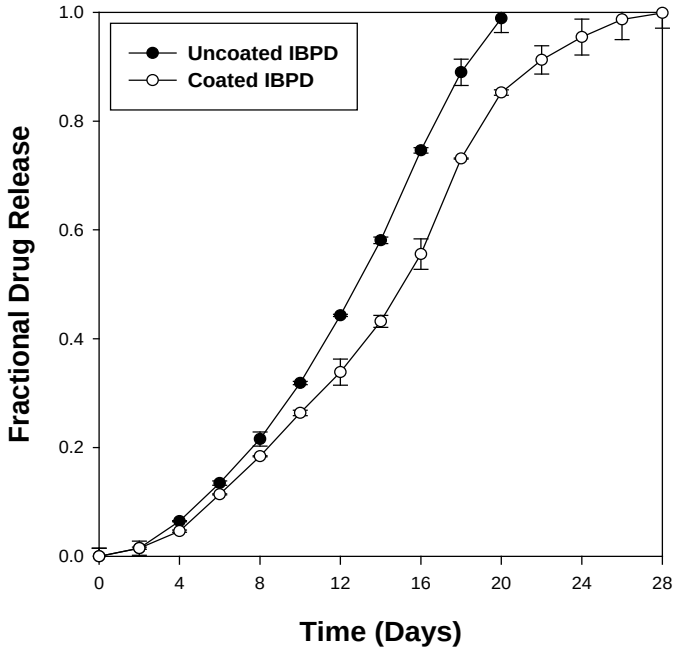


Figure 6.16: A typical profile showing the effect of coating on the model hydrophilic drug AZT from a) uncoated IBPD and b) shellac/APE-PAA-coated IBPD in simulated vaginal fluid (pH 4.5; 37°C) (N=3; SD<0.18 in all cases).

6.3.4.3 Analysis of the drug release behavior from the optimized coated intravaginal bioadhesive polymeric device containg AZT and PSS separately and in combination

The substantial matrix integrity imparted by the polymers used to formulate the IBPD resulted in the minimization of the rate of matrix disentanglement and consequently prolonged and controlled the release of AZT and PSS from the IBPD. Controlled drug release representing zero-order was realized consistently over 40 days for AZT and 72 days for PSS (Figure 6.17). These results can be attributed to the hydrophobic nature and high compressibility of EC, PLGA and PSS, coupled with the superior matrix resilience of $_{m}$PA 6,10. The electrolytic nature of $BaSO_4$ may have also contributed to the prolongation and control of drug release. The PSS-loaded IBPD achieved superior drug release behavior with consistent and controlled release over a period of 72 days (Figure 6.17d). For the IBPD loaded with both PSS and AZT, the release of PSS occurred over 56 days

compared to 40 days for AZT (Figure 6.17b and c). However this was still diminutive in comparison to the 72 days achieved from the PSS-only loaded IBPD (Figure 6.17d). Conversely, the period of AZT release from the IBPD was more prolonged than from the AZT-only loaded IBPD device (i.e. 40 vs 28 days) (Figure 6.17a and b). This was clear that the inclusion of PSS had a significant role in controlling the release of AZT from the IBPD due to the hydrophobicity of PSS and electrostatic properties (as a sodium salt) arising from its polymeric segmental charge density (Singh et al., 1995). Studies have shown that charge density of polyelectrolytes such as PSS enhance binding interactions and favors oppositely charged compounds, culminating in the lowering of the rate of desorption and diffusion thereby slowing the drug release rate from the polyelectrolyte compound (Singh et al., 1995; Vishalakshi, 1995, Pu et al., 2004). The hydrophobicity of PSS results from the presence of strong electrostatic charges and internal linkages (H- and S-bonds) in the residual un-sulfonated aromatic moieties of the PSS molecule (Jiang et al., 2003; Bonifazi et al., 2007). In addition, previous studies conducted on polyelectrolytes such as PSS have shown that the higher the osmotic coefficient and radius of gyration, the greater the ability to control the rate of drug release (Alvarez-Lorenzo et al., 1999; Griffiths et al., 2004; Le Cer et al., 2004; Sen et al., 2007; Thapa et al., 2009; Viridén et al., 2009).

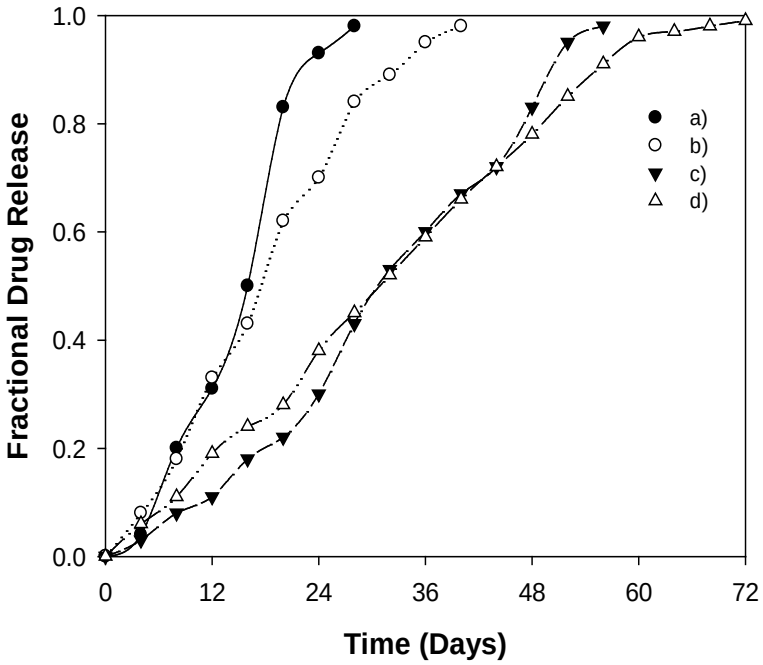


Figure 6.17: Drug release profiles of a) AZT (AZT-loaded IBPD), b) AZT (AZT/PSS-loaded IBPD), c) PSS (AZT/PSS-loaded IBPD) and d) PSS (PSS-loaded IBPD), in simulated vagina fluid (pH 4.5; 37°C) (N=3; SD<0.38 in all cases).

6.3.5 Assessment of drug permeation across the pig vaginal tissue

The flux of AZT and PSS across the pig vaginal tissue over time is shown in Figure 6.18. A relatively constant increase in the rate of flux occurred over the initial 12 hours and thereafter a steady-state was achieved up to 24 hours (Figure 6.18). This suggests that the mechanism of passive drug transport across the pig vaginal tissue became saturated with drug. The lower flux of PSS reflects its high degree of hydrophobicity coupled with the presence of strong intermolecular charges in the polymer (Knudsen et al., 2004; Pu et al., 2004; Chu et al., 2007). This may therefore have contributed to controlling the permeation of the drug across the vaginal tissue. Overall, it can therefore be proposed that most of the drug was retained within the vaginal tissue.

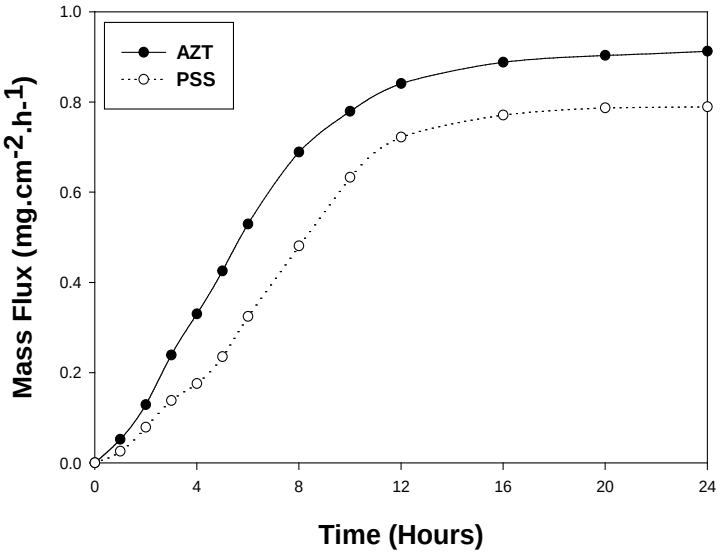


Figure 6.18: Profiles showing the flux of AZT and PSS across pig vaginal tissue over a period of 24 hours (N=3, SD<0.23 in all cases).

6.3.6 *Ex vivo* bioadhesivity analysis of the intravaginal bioadhesive polymeric device

The devices that were produced were strongly bioadhesive. Textural profile analysis indicated that the uncoated devices had the lowest bioadhesivity (PAF=1.1976±0.150N; AUC_{FD}=0.0019±0.0001J) compared to the PAA-coated devices (PAF=3.699±0.0.464N; AUC_{FD}=0.0098±0.0004J) (Figure 6.19a and b). This indicates the superiority of APE-PAA as a bioadhesive coating as may be attributed to its hydrophilicity, H-bonding capacity, the high molecular mass and the surface tension properties of the polymer. APE-PAA controlled the extent of interpenetration between the polymer and the vaginal mucosal/epithelial surface. The high hydrophilicity of APE-PAA enabled the formation of strong bioadhesive bonds due to the high water content within the mucosal layer of the pig vaginal tissue. The presence of OH^- and $COOH^-$ groups in APE-PAA may have favored the formation of H-bonds between the entangled APE-PAA chains and the pig vaginal tissue that ultimately resulted in bioadhesion (Chapter 4; Section 4.3.2; Figure 4.4). In addition, the desirable surface tension of PAA facilitated spreading over the epithelial surface of the vaginal mucosal layer thereby enhancing bioadhesion.

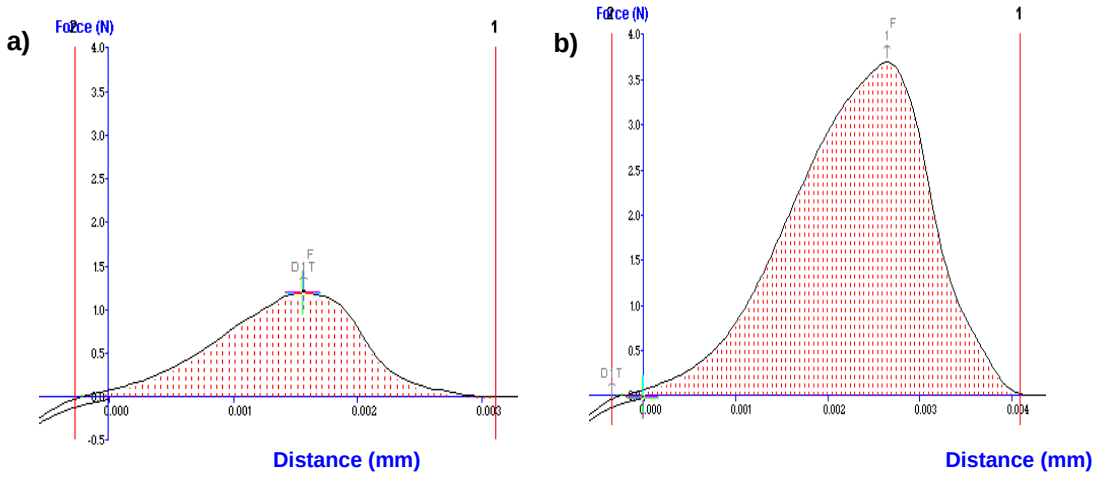


Figure 6.19: Typical Force-Distance textural profiles used for computing the Peak Adhesion Force (PAF) and Work of Adhesion (AUC_{FD}) for a) uncoated devices and b) PAA-coated devices on freshly excised pig vaginal tissue.

6.3.7 Retention of the intravaginal bioadhesive polymeric device within the pig vagina

Analysis of X-ray images (Figure 6.20) revealed that the devices were maintained in the posterior fornix of the pig vagina for the experimental period up to 30 days. The devices underwent swelling and gradually eroded over time as shown in Figures 6.20a, b and c which is in accordance with the stipulated design in which the formulation is expected to initially swell in order to facilitate bioadhesion and thereafter gradually erode and release the drug over the vaginal tissue for the required clinical preventative effect.

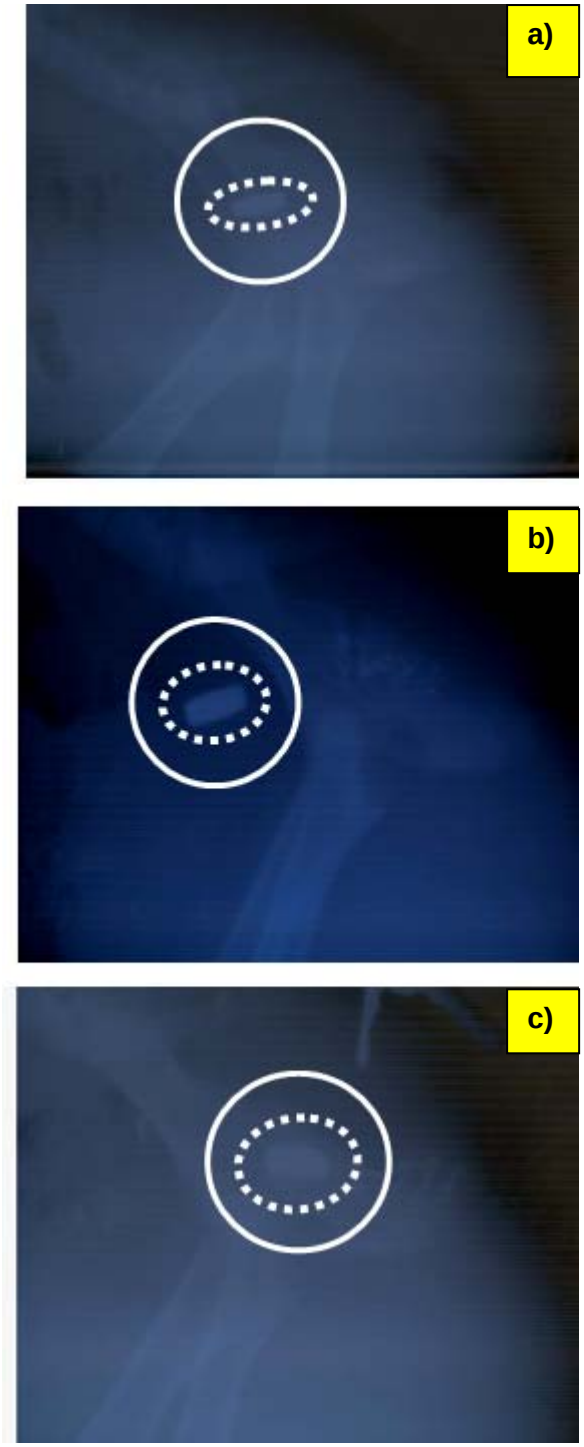


Figure 6.20: X-ray images depicting the presence of the intravaginal bioadhesive polymeric device at a) day 1, b) day 14, and c) day 30 after insertion into the posterior fornix of the pig vagina.

6.3.8 Chemometric and molecular modeling of the intravaginal bioadhesive polymeric device drug dissolution and diffusion

6.3.8.1 Postulation of dissolution dynamics and subsequent effect on drug release

Chemometric and computational analysis conducted in our laboratories revealed that polymer-polymer and polymer-drug ratios, as well as the ratio between the coating polymers and components of the dissolution medium contributed substantially to drug dissolution kinetics obtained. Figure 6.21 depicts a step-wise model of the IBPD undergoing dissolution.

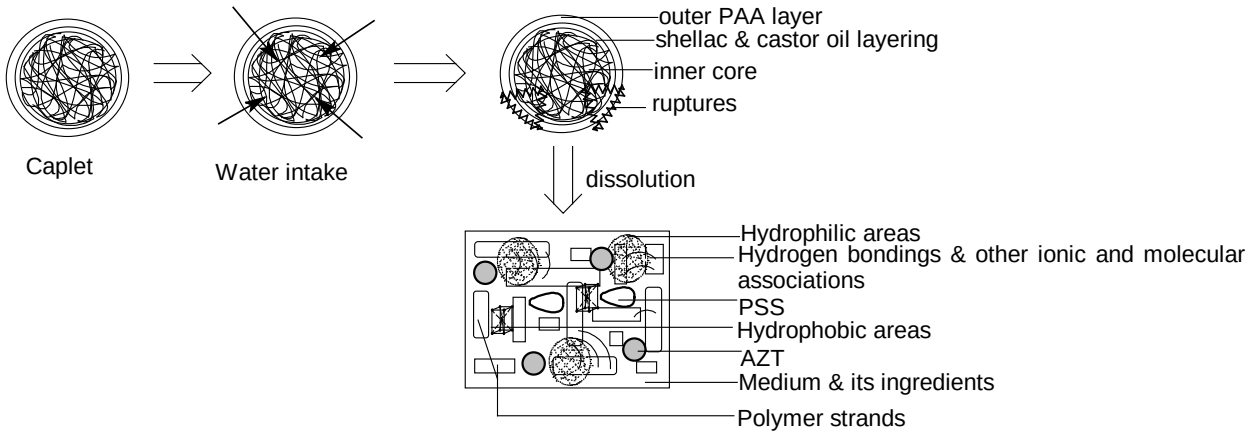


Figure 6.21: Molecular model mechanistically depicting the IBPD dissolution process with lesser H-bond formation due to the excessive of simulated vaginal fluid providing more freedom to polymeric strands to disentangle.

Regarding the physicochemical associations of the polymers, AZT and PSS, all components were homogenized to produce a characteristic even distribution within the IBPD matrix. The hydrophile-hydrophobe properties, molecular interactive sites and functional groups in the mass and stoichiometry of the matrix was in a 2:3 ratio as depicted in Figures 6.22a-c for a typical IBPD matrix. The core of the IBPD was a heterogenous matrix medium in the active solid-state and molecular properties of the polymers and excipients were in a dormant solid-state. The dissolution medium provided the induction phase for the dormant properties to become activated. With the presence of excess dissolution medium and subsequent complete dissolution of the IBPD, this

weakened the interactions and physicochemical associations with no further channel formations that may not be conclusive for channelized diffusion of drug. The hydrophilic and hydrophobic areas of certain polymeric components within the matrix and their association provided the flexible hydration sites. The hydrophilic sites were located within the outer regions of the matrix and the hydrophobic sites were confined to fewer interactive regions at the center of the IBPD matrix. AZT was confined near hydrophilic regions of the matrix while PSS consolidated the inner core as well as areas associated with other hydrophobic polymeric interactions such as PLGA and EC. This segregated hydrophile-hydrophobe clusters within the IBPD matrix was primarily responsible for modulating the diffusion path of drug molecules through the matrix and subsequently controlling drug release. Figure 6.22 depicts the IBPD matrix in a solid and heterogeneous form as well as the inter-polymeric entanglements that involved the polymer-polymer, polymer-drug associations and other componential interactions.

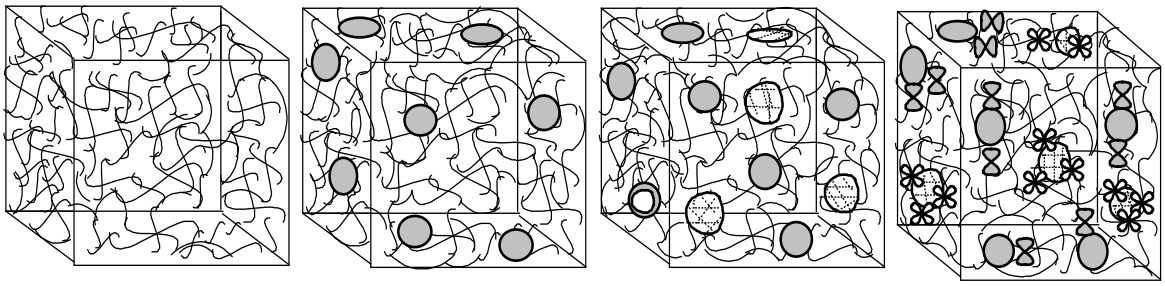


Figure 6.22: A 3D model representing the IBPD matrix under dissolution along with drug associations: a) the polymer matrix, b) fluid interactions producing hydrophilic pockets and swelling of the device, c) generation of hydrophobic areas in the matrix due to critical formulation excipients and d) the drug molecule (AZT) in association at hydrophilic sites prior to release.

The chemometric and molecular modeling revealed that, remarkably, the rate of transport for the hydrophilic drug AZT compared to the hydrophobic PSS was found to be in a ratio of 3:2 indicating that AZT diffused at a rate that was 1.5 times faster than PSS. This explains the longer controlled release effect obtained when PSS was incorporated into the IBPD device. Through chemometric modeling, it was also ascertained that the difference in molecular mass between AZT and PSS was not solely responsible for this behavior and

that the high degree of charge density in PSS, the ionic interactions between the cationic PSS and the polymer constituents as well as the high osmotic coefficient and radius of gyration of PSS, were the primary contributing factors. Figure 6.23 depicts the polymeric strands in the dissolution medium, the free floating AZT in the simulated vaginal fluid and PSS occupying the inner core regions of the matrix, in comparison to the interactions/entanglement in the solid/semi-solid/gel state at the onset of the dissolution process when the IBPD device was not fully dissolved.

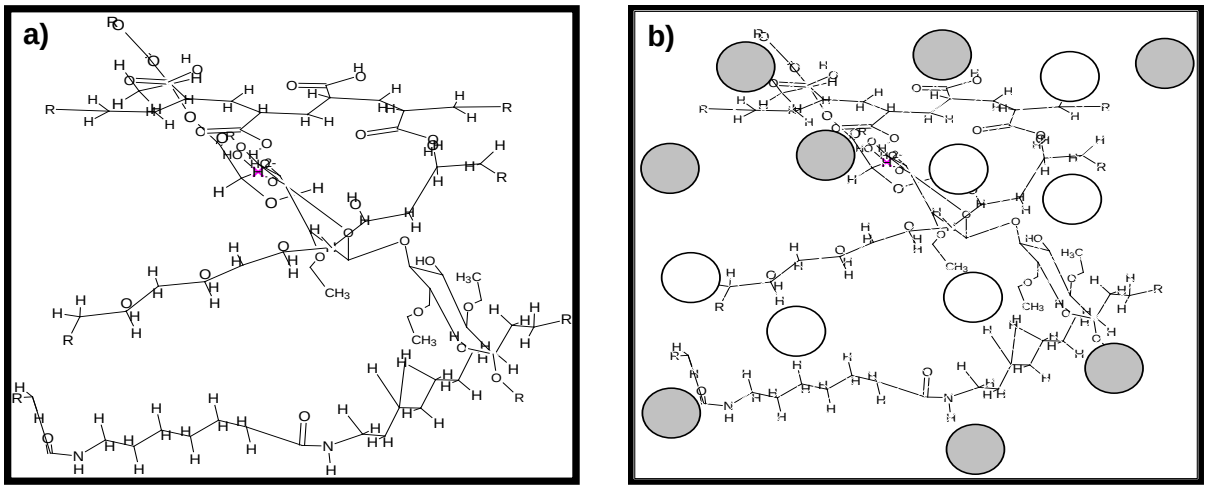
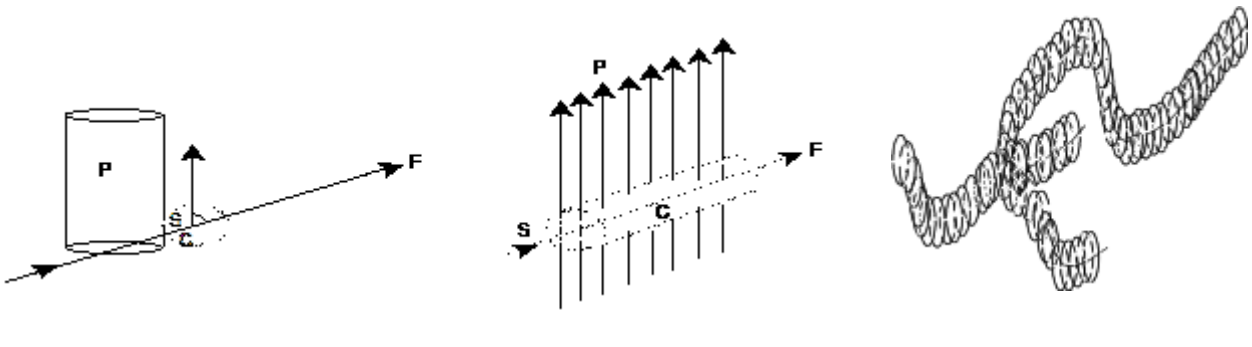


Figure 6.23: Chemometric models depicting a) dissoluted polymeric strands and b) AZT molecules (grey-colored circles) and PSS (white-colored circles) in the free float and associated forms with the loosely interacting polymeric strands in the dissolution medium.

6.3.8.2 Diffusion kinetics depicting the drug flux mechanism during *ex vivo* studies

The presence of excess simulated vaginal fluid, led to complete dissolution of the caplet in the donor compartment of the Franz Diffusion Cell apparatus during the *ex vivo* vaginal tissue permeation studies weakening the interactions and physicochemical associations. It was observed that approximately 21% of AZT and 14% of PSS permeated across the pig vaginal tissue in 24 hours. Thus, the actual transport of the drugs (considering that 16.67% of both drugs i.e. 200mg each in a 1200mg IBPD matrix) from the donor compartment to the receptor compartment was 42mg and 28mg for AZT and PSS respectively. This finding substantiates the results obtained in Section 6.3.5 for which it

was proposed that most of the drug remained in the vaginal tissue. A chemometric model depicting the step-wise process of generating diffusion/transport channels perpendicularly to a polymer-strand localized in the IBPD is depicted in Figure 6.24.



A **B** **C**

Figure 6.24: Chemometric model depicting the development of a diffusion channel with a) a single polymer strand P, situated perpendicularly to a forming pore C, b) a group of strands also denoted collectively as P giving rise to the channel C which is formed perpendicular to the polymer strands backbone with F=the direction of flow from the starting point S to form a a networked 3D channel and c) a polymeric strand with the generated diffusion channel.

With regard to the diffusion kinetics of the IBPD device, the pH of the simulated vaginal fluid had a contributory effect on the transport of drug across the pig vaginal tissue. An osmotic gradient mediated transport across the vaginal tissue based on the higher concentration of drugs, ions and other molecular entities in the 2mL donor compartment of the Franz Diffusion Cells. The permeation of drug was also inversely proportional to the molecular mass and almost all components i.e. ions, drugs, polymeric strands, protein, salts and acids as well as urea from the donor cell permeated though in varying concentrations. Figure 6.25 shows the actual events that occurred during the generation of diffusion/transport channels within the IBPD matrix.

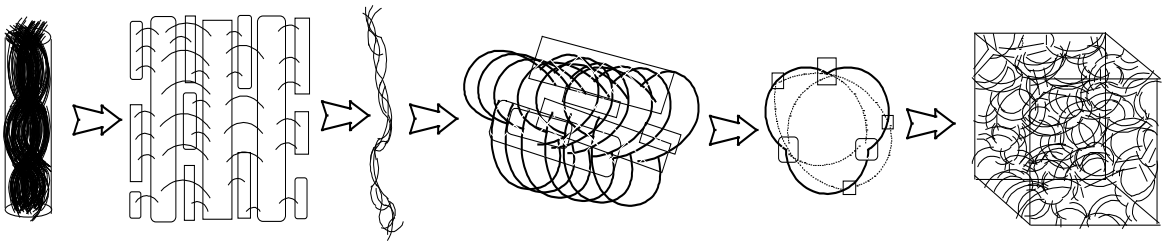
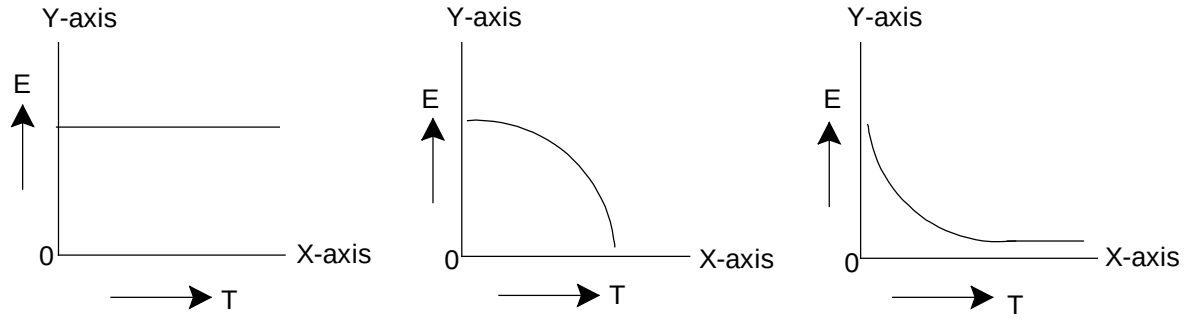


Figure 6.25: 3D models depicting the generation of diffusion channels with the IBPD matrix with a) polymeric strands, b) inter-strand physicochemical interactions, c) stereo-orientation within a group of associated polymeric strands generating an inter-polymeric cavity d) the cavity formed, e) associations between different polymeric strands and f) the blocked cavity due to excessive associative networking at various localized sites incorporating other components such as drug in different associative settings.

The considerable energy paradigms were governed by the solvation of the dissolution medium and osmotic flux. The qualitative status of energy paradigms and energy-time relationships for the IBPD matrix is as shown in Figure 6.26a-c. A simple energy status is depicted as a qualitative energy-time relationship for the IBPD matrix as clearly portrayed in Figure 6.26b.



a) **b)** **c)**

Figure 6.26: Qualitative status of energy paradigms and energy-time relationship for the IBPD matrix with a) stable energy status as a solid matrix, b) energy change following dissolution and c) energy transaction and changes for the drug permeation across the pig vaginal tissue with the static energy status at equilibrium without flux also shown.

6.4 Concluding remarks

Robust polymeric devices were produced that confirmed the ability of the polymer blend selected (PA 6,10, PLGA, PVA, PAA and EC) to control the release of the model drugs AZT and PSS over a period of 72 days (PSS). The thermodynamic stability of the native

polymers and device was substantiated by TMDSC thermograms. *Ex vivo* bioadhesion and permeation studies revealed that the APE-PAA-coated devices were desirably bioadhesive and a relatively substantial fraction of the drug-load was confined within the vaginal tissue. The produced IBPDs showed the potential of maintaining the acidic microenvironmental pH of the simulated vaginal fluid upon degrading which is a desirable feature in the vagina. The chemometric and molecular structural modeling approach qualitatively supported the deduction of the IBPD rate of dissolution and has shown that the drug release rate was dependant on the stoichiometric parameters between the polymers, drugs and the simulated vaginal fluid. Furthermore, it was mechanistically deduced that the permeation of drug across the pig vaginal tissue during *ex vivo* studies was based on an osmotic gradient and depended on the degree of ionization as well as the size and molecular mass of the drug molecules. Thus, the developed IBPD may be suitable for use as a localized intravaginal drug delivery system for the potential treatment and prevention of HIV infection and STIs.

CHAPTER SEVEN

IN VIVO DRUG CONTENT ANALYSIS AND HISTOPATHOLOGICAL EVALUATION OF THE INTRAVAGINAL BIOADHESIVE POLYMERIC DEVICE

7.1 Introduction

Prevention of sexual transmission of HIV at the vaginal mucosal epithelial level is a critical global health priority because of the devastating impacts of HIV/AIDS on women, young adults and infants (Keller et al., 2005). Studies using cervical organ culture as a model of primary infection, have demonstrated that HIV-infected cells mostly reside within subepithelial mucosa (Miller et al., 1992; Nuovo et al., 1993; Padian et al., 1997). This indicates that the establishment of HIV infection requires transepithelial penetration and therefore blocking this step may assist to prevent the HIV infection. HIV pathogens cross the mucosal surface either by: a) direct HIV infection of epithelial cells; b) transcytosis of HIV through epithelial cells; c) epithelial transmigration of HIV-infected donor cells; d) uptake of HIV by intraepithelial dendritic cells (e.g. Langerhans cells) and e) circumvention of the epithelial barrier through physical breaches (Ibata et al., 1997; Shattock, 2001, Saïdi et al., 2009). Furthermore, it has been shown that penetration of the mucosal surface by HIV to reach the submucosal target cells (macrophages, lymphocytes and dendritic cells) may be modulated by: a) supraepithelial factors, such as seminal complement components (opsonized HIV); b) epithelial factors released in the submucosal microenvironment, such as antimicrobial soluble factors, cytokines and chemokines; and c) by the potent intraepithelial and submucosal innate immunity (Kozlowski and Neutra, 2003; Saïdi et al., 2009).

Following the translocation across the mucosal epithelium, HIV-1 finds potential target cells in the dense lymphocyte and macrophage populations of the lamina propria. Cervical

mucosa, lamina propria and $CD4^+$ T lymphocytes are early target cells for HIV-1 and Simian Immunodeficiency Virus (SIV) in humans and support viral replication (Spira et al., 1996; Zhang et al., 1999; Collins et al., 2000; Greenhead et al, 2000; Gupta et al., 2002; Moore et al., 2004; Shen et al., 2009). Thus, the trajectory of anti-HIV microbicide delivery system development approach should preferably be towards agents that can block specific virus–host cell interactions at the vaginal mucosal epithelial level. Targeting HIV entry has been a favored approach because it is the first step in the process of HIV infection (D'Cruz and Uckun, 2004; Cutler and Justman, 2008). An anti-HIV microbicide delivery system that can block transmission of HIV at the vaginal mucosal epithelial level may provide a realistic method of intervention worldwide.

The strong epidemiological association of inflammatory ulcerative venereal disease with HIV transmission and the observation that mucosal SIV transmission may be enhanced following thinning of vaginal epithelium by steroids such as progesterone implants suggests an important barrier role of the genital epithelium (Marx et al., 1996; Royce et al., 1997). Thus, following any study in the vagina that involves administration of a foreign body (tablet, caplet, ring etc), it is imperative to undertake histopathological and histomorphological evaluation on the vaginal epithelium since it plays a vital role, both as a physical and as an immunological barrier, in preventing entry of bacterial and viral pathogens into host tissues (Catalone et al., 2005; Hirbod and Broliden 2007; Rebbapragada and Kaul, 2007; Levinson et al., 2009). Disruption of this barrier and the presence of genital tract inflammation are risk factors for HIV infection as well as other STIs (Coombs et al., 2003; Catalone et al., 2005; Dhawan and Mayer, 2006; Sundstrom et al., 2007; Brenchley and Douek 2008; Kaul et al., 2008; Sheung et al., 2008; Broliden et al., 2009; Hendrix et al, 2009; Shafir et al., 2009). Therefore, it is important for a vaginal delivery system to not only target STI pathogens but also to have negligible or no toxic effects on the vaginal epithelium (Levine et al., 1998; Stafford et al., 1998; Greenhead et al, 2000; Miller and Shattock, 2003; Shen and Iwasaki, 2006; Iijima et al., 2008).

The aim of this Chapter therefore, was to report on the *in vivo* study that was conducted in a pig model administered with AZT and PSS-loaded IBPD with the following purposes: i) to analyze the drug content contained in plasma (if any) as well in the vaginal tissues after sacrificing the animals; and ii) to conduct a thorough toxicity study that involved histopathological evaluation on the vaginal tissues exposed to drug-containing IBPDs and placebo in comparison to a control. In each case, the IBPDs (properly coated to avoid irritation to the vaginal tissue as well as to facilitate easy insertion) were inserted into the vagina using a special applicator designed in our laboratories. A pig model was selected because of many similarities with the human vagina, particularly in genital tract physiology and histology. (Pond and Houpt, 1988; D'Cruz et al., 2005b; Wang et al., 2009).

7.2 Materials and Methods

7.2.1 Materials

For anesthesia, ketamine was purchased from Bayer (Pty) Ltd (Bayer Pty Ltd, Isando, Gauteng, South Africa., midazolam from Roche products (Pty) Ltd (Roche products (Pty) Ltd, Isando, Gauteng, South Africa) and isoflurane from Safeline Pharmaceuticals (Pty) Ltd (Safeline Pharmaceuticals (Pty) Ltd, Florida, South Africa). The digestive enzyme for the vaginal tissues was subtilisin which was purchased from Merck Co. Ltd (Merck-Schuchardt®, Hohenbrunn, Germany). The rest of the materials employed were the same as in Chapter 6.

7.2.2 Methods

7.2.2.1 *In vivo* studies in the pig model using the drug-loaded intravaginal bioadhesive polymeric device

The study involved 20 female, healthy Large White pigs (35kg) divided into 4 groups of 5 each. Group 1 was a control group in which no IBPDs were inserted. Groups 2 and 3 were used for testing the model drugs, and therefore IBPDs with drugs were inserted. Group 4 was used as a placebo group in which IBPDs with no drug (i.e. formulated with

native polymers) were inserted. The study period for each group was 1 month spanning across a total of 4 months in staggered manner. In Group 2 and Group 3, IBPDs containing AZT and PSS respectively were inserted after anaesthetizing the pigs. X-ray imaging was done three times a week (1^{st}, 3^{rd} and 5^{th} day) for two weeks and then twice a week (1^{st} and 5^{th} day) for another two weeks, each time under anesthesia. Furthermore, blood samples (10mL) were withdrawn from the jugular vein of each pig on days 1, 3, 5, 7, 14 and 28 while the pigs were still under anesthesia. In Group 4, placebo IBPDs were inserted and then subjected to the same procedure as above. Group 1 was subjected to blood sampling procedures on the scheduled days. On the 28^{th} day, each pig was euthanized, followed by dissection of the vaginal tissue for drug content analysis using UPLC, and toxicity studies. The toxicity studies involved histological examination for inflammation, exocytosis, hyperplasia, hypoplasia, epithelial exudate, ulceration, polymorphonuclear infiltration and any evidence of infection. A summary of the *in vivo* study is shown in Figure 7.1.

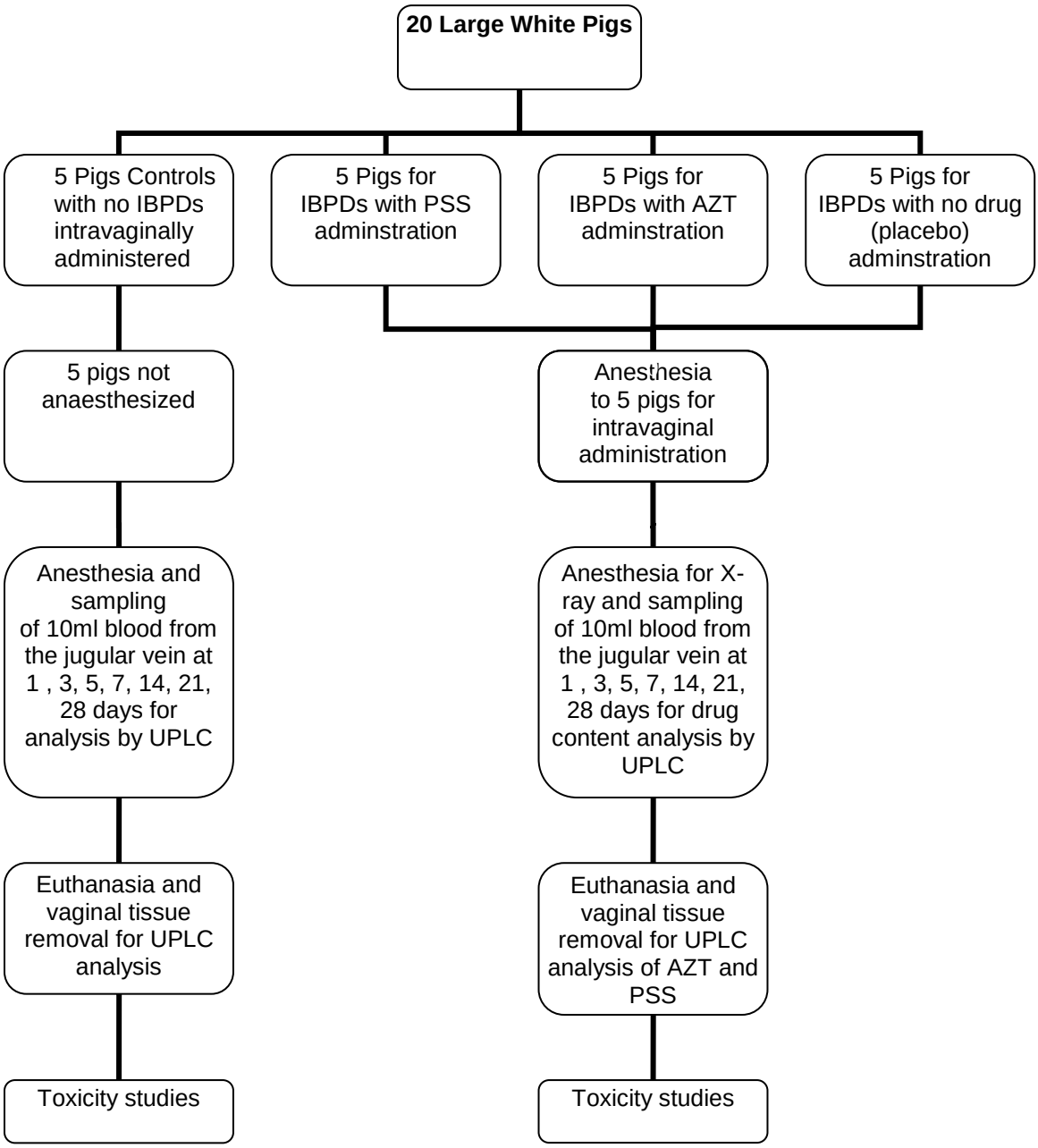


Figure 7.1: Summary of the *in vivo* study.

7.2.2.2 Anesthesia, X-ray imaging and blood sampling

Each group of pigs was anesthetized with ketamine (11mg/kg I.M.) and midazolam, (0.3mg/kg I.M.). The pigs were then intubated and anesthesia was maintained with 2% isoflurane in 100% oxygen. X-ray imaging of the pelvic region was performed (Figure 7.2a), and blood samples were taken following the procedure stipulated in Section 7.2.2.1 (Figure 7.2b). All blood samples were immediately transferred into heparinised vacutainers (BD Vacutainer®, Plymouth, BD Beliver Industrial State, UK) and then stored

in a refrigerator at 4^{o}C for one hour. The blood was then centrifuged (Nison Instrument (Shangai) Limited, Shangai, China) at 15,000rpm for 10 minutes and the supernatant was pipetted as blank plasma using an adjustable volume micropipette (Boeco Gmbh, Hamburg, Germany). The blank plasma was stored in a refrigerator at -70^{o}C for the quantitative drug analysis using UPLC.

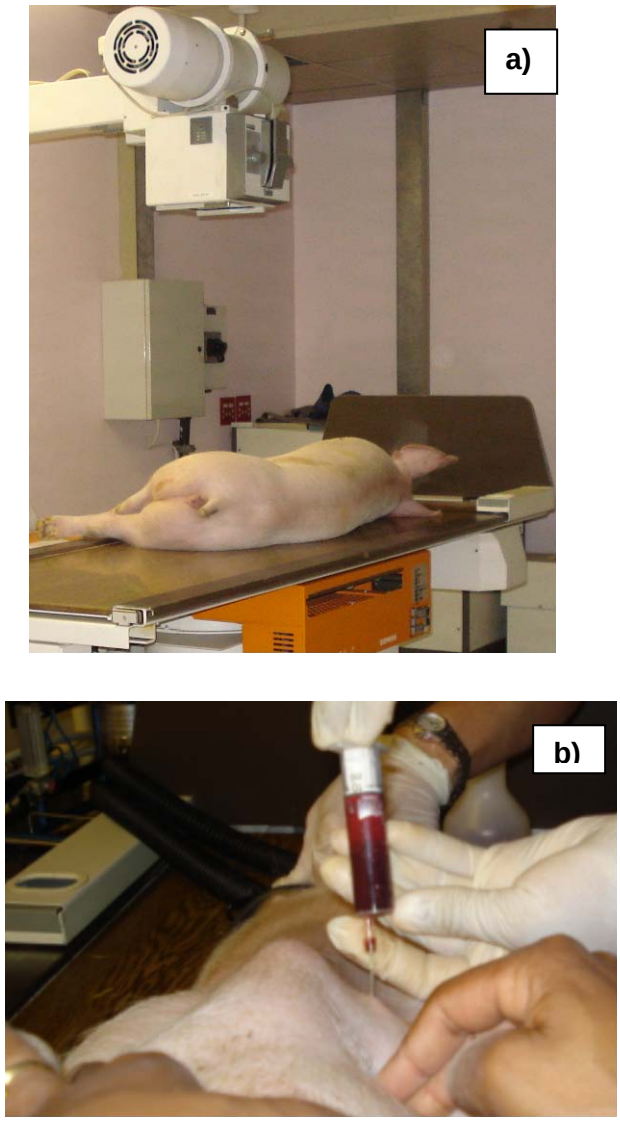


Figure 7.2: a) X-Ray imaging process and b) blood sampling procedure from the jugular vein of the pig.

7.2.2.3 Vaginal tissue removal for drug analysis and histopathological studies

On 28th day, each pig (weighing between 80-85kg) was euthanized and then dissecting out the vaginal tract following the procedure described in Chapter 5, Section 5.2.2.4, Figure 5.1. Transverse cuts of tissues (at the anterior, middle and posterior part of the vagina) with a cross-sectional size 1.5×3.0cm were made through the vaginal wall. Half of the tissues were kept in 100mL of 10% formalin and then subjected to histopathological studies (including toxicity evaluation). The remaining tissue was immediately frozen using liquid nitrogen, and then stored in a refrigerator at -70^{o}C for quantitative drug analysis.

7.2.2.4 Quantitative analysis of AZT and PSS in the blood and the vaginal tissue of the pig.

7.2.2.4.1 Preparation of standard solutions and calibration curves

The same procedure as previously described under Chapter 6, Section 6.2.2.8.4, was used except that in this case calibration curves were developed using blank pig plasma instead of simulated vaginal fluid. The constructed calibration curves are as shown in Figures 7.3a and b.

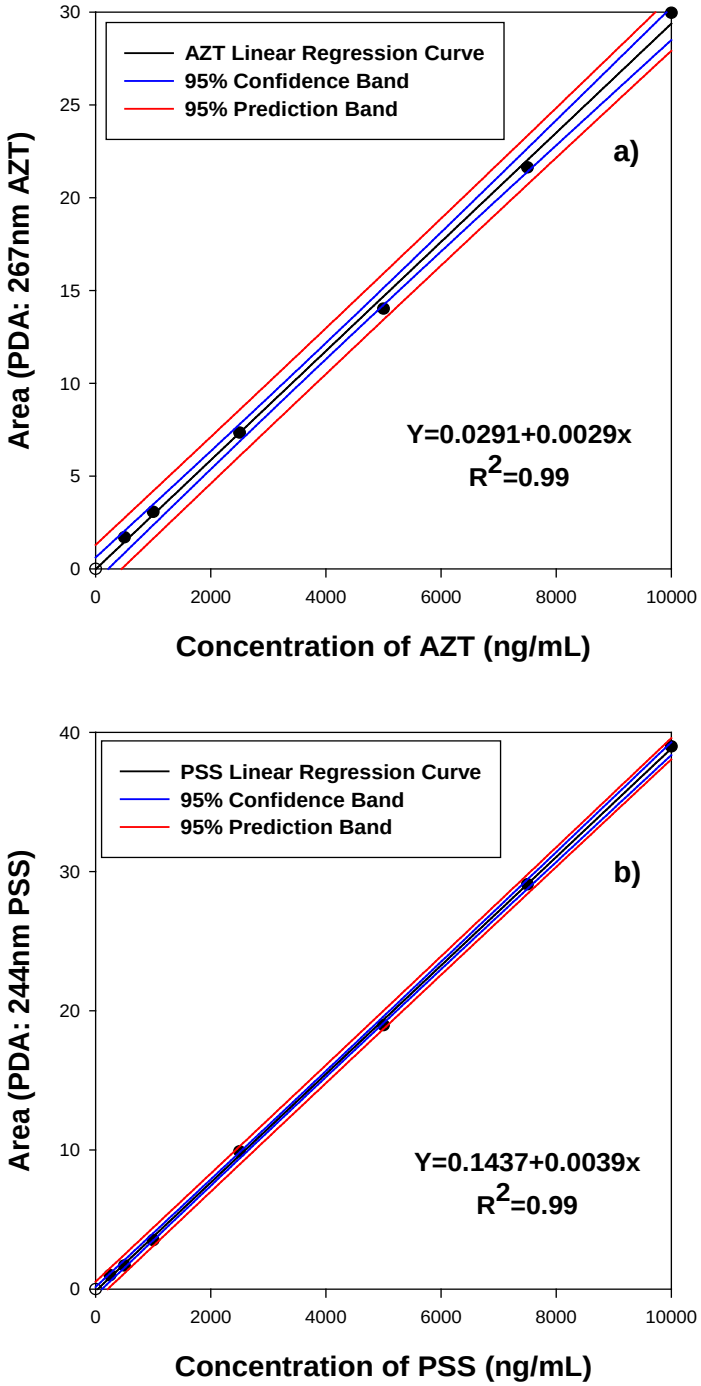


Figure 7.3: a) Calibration curves for: a) AZT in blank plasma 267nm (N=5, SD<0.001 in all cases); b) PSS in blank plasma 244nm (N=5, SD<0.009 in all cases).

7.2.2.4.2 The drug extraction method from the plasma samples for UPLC analysis

This was conducted employing a single use Water Oasis® HLB 3cc (60mg) extraction cartridge (Waters Corporation, Milford, Massachusetts, USA) adapting a method

developed by Notari and co-workers (2006). The Solid Phase Extraction (SPE) cartridge was conditioned with 1.0mL methanol followed by 1.0mL ultra pure water Milli-Q. 1mL of acetonitrile (for AZT) was added to 600µL of pig plasma. The solution was vortexed for 1 minute and then centrifuged (Nison Instrument (Shangai) Limited, Shangai, China) at 15,000 rpm for 6 minutes at 24^{o}C. 650µL of the supernatant was diluted by adding ultra pure water Milli-Q (1mL) and loaded in the cartridge. Thereafter, the cartridges were washed with 1.0mL of 5%$^{v}/_{v}$ methanol in ultra pure water Milli-Q. Analytes were eluted by washing cartridges with 550µL 0.01M KH_2PO_4 followed by 2mL methanol. The eluate was evaporated to dryness in a slow stream of high purity nitrogen gas (Afrox, Germiston, Gauteng, South Africa). The extracted sample was re-constituted with 100µL absolute methanol, mixed with 400µL of MP, and then filtered into the injection vials using 0.22µm syringe-driven filter units (Millipore Corporation, Bedford, Massachusetts, USA) for UPLC analysis. The same procedure was followed for PSS but substituting acetonitrile with methanol as a mobile phase.

7.2.2.4.3 Drug extraction from the vaginal tissue for UPLC analysis

To extract the drug from the tissues, a method developed by Wang and co-workers (2009) was adapted but with some modifications. Briefly, each tissue sample (8g each) was homogenized in 16mL of simulated plasma (pH 7.4). The homogenized sample was digested by incubating it with 10 mg of subtilisin (Merck-Schuchardt®, Hohenbrunn, Germany), vortexed for 1 minute, and then placed in a thermostatic bath for 1 hour at 56°C while mixing after every 10 minutes to ensure complete degradation of the tissue. After enzymatic digestion, the sample was centrifuged at 10,000rpm for 15 minutes. For the AZT sample preparation, 1mL of the supernatant was withdrawn, mixed with 1mL of acetonitrile and then pipetted into the centrifuge tubes into which 550µL of 0.01M KH_2PO_4 was added as an extracting medium. The tubes were vortexed for 1 minute and then centrifuged at 13,000 rpm for 6 minutes at 24^{o}C. 650µL of the supernatant was diluted by adding ultra pure water Milli-Q (1mL) followed by the addition of 2mL absolute methanol.

The supernatant was placed into sample vials and then evaporated to dryness in a slow stream of high purity nitrogen gas (Afrox, Germiston, Gauteng, South Africa). The samples were re-constituted with 100µL absolute methanol mixed with 400µL of MP, then filtered into the injection vials using 0.22µm syringe-driven filter units (Millipore Corporation, Bedford, Massachusetts, USA). The same procedure was followed for the PSS sample preparation but substituting acetonitrile with methanol as the mobile phase.

7.2.2.4.4 Instrumentation and chromatographic conditions for the quantitative analysis of AZT and PSS from plasma and vaginal tissue samples

Quantitative analysis was performed using the Waters Acquity Ultra Performance Liquid Chromatographic (UPLC) system (Waters Corporation, Milford, Massachusetts, USA), equipped with the Acquity Photodiode Array (PDA) and Evaporative Light Scattering (ELS) detectors. The columns employed were Acquity UPLC® BEH Phenyl 1.7µm, 2.1×50mm column and Acquity UPLC® BEH C_{18}, 1.7µm, 2.1×100mm for AZT and PSS analysis respectively. The mobile phases were composed of water/acetonitrile ($60/40^{v}/_{v}$) and methanol/water ($50/50^{v}/_{v}$) for AZT and PSS respectively. The wash solutions used, namely strong and weak washes were composed of 90/10 acetonitrile/water, 10/90 water/acetonitrile, and 100% ultra pure water Milli-Q, respectively. All prepared solutions were filtered using 0.22µm membrane filters (Millipore Corporation, Bedford, Massachusetts, USA) under vacuum and degassed before their use. For AZT analysis, a gradient method was used at a column temperature of 25°C, injection volume was 2µL and UV detection wavelength of 267nm. The employed gradient settings were as shown in Table 7.1. For PSS analysis, an isocratic method was employed using methanol/water ($50/50^{v}/_{v}$) as a mobile phase, flow rate 0.2mL/minute, column temperature 25°C, total run time of analysis was 3 minutes, injection volume was 1.7µL and UV detection wavelength of 244nm.

Table 7.1: Parameter settings for the AZT gradient method.

Separation events	Time (min)	Flow (mL/min)	% A (Water)	% B (Acetonitrile)
1	0.00	0.500	60.0	40.0
2	1.00	0.500	5.0	95.0
3	2.60	0.500	5.0	95.0
4	3.50	0.500	60.0	40.0

7.2.2.4.5 Histopathological evaluation of the vaginal mucosa

The vaginal tissue specimens obtained were cut into three tissue blocks containing the anterior, middle and posterior sections. These blocks were processed with routine histological methodology in an automated tissue processor. They were then sectioned at 5µm, placed on slides and stained with haematoxylin and eosin in an automated stainer (Rankin Biomedical Corporation, Michigan, USA) and finally subjected to thorough histological evaluation. The evaluation was divided into three parts. The first was an evaluation on epithelial histological lesions; the second one was an assessment of the lamina propria and the third was an evaluation on the subepithelial tissues and vaginal wall. The evaluation of epithelial histological lesions encompassed: i) hyperplasia which is an increase in epithelial cell layers which finally results in acanthosis and thickening of the epithelium; ii) exocytosis which refers to transepithelial leukocyte migration of inflammatory cells; iii) exudate on the epithelial surface; and iv) ulceration. In the lamina propria, mononuclear inflammation, polymorphonuclear infiltration and foreign body inflammation were assessed. The evaluation of subepethelial tissues and vaginal wall was mainly concerned with a search for perivascular inflammation.

7.3. Results and Discussion

7.3.1 Chromatographic separation and retention times of AZT and PSS from methylparaben (MP) (Internal Standard)

AZT/PSS and MP were eluted at 1.701±0.43 and 1.184±0.18 for AZT and 0.583±0.15 and 1.275±0.13 for PSS after extraction from pig plasma (Figures 7.4a and b). Assay method

validation analysis revealed that the intra-and inter-day precision and accuracy were satisfactory (R^2=0.9987 for AZT; R^2=0.9998 for PSS). The signal to noise ratio at the lower limit of quantification (25ng/mL) was >10.8 in both cases. For the validation testing of the linear regression model, the weighting factors were selected to be proportional to the reciprocal of the standard deviations which was optimal under the least squares estimation with R^2>0.99. The three dimensional chromatographic analysis of blank plasma revealed no interfering peaks at the retention times within the UV wavelength range of 200-400 (AZT/PSS and MP).

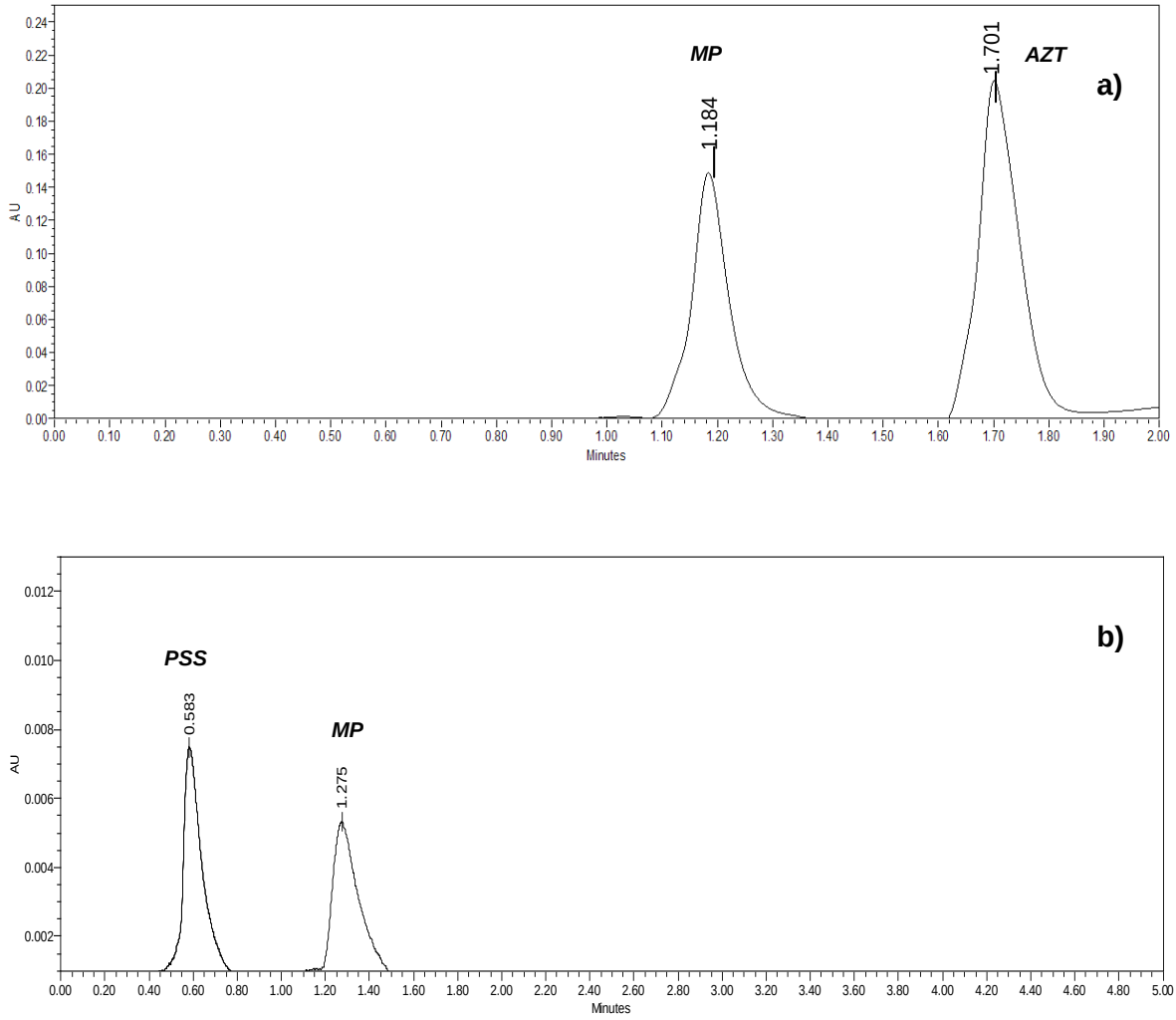
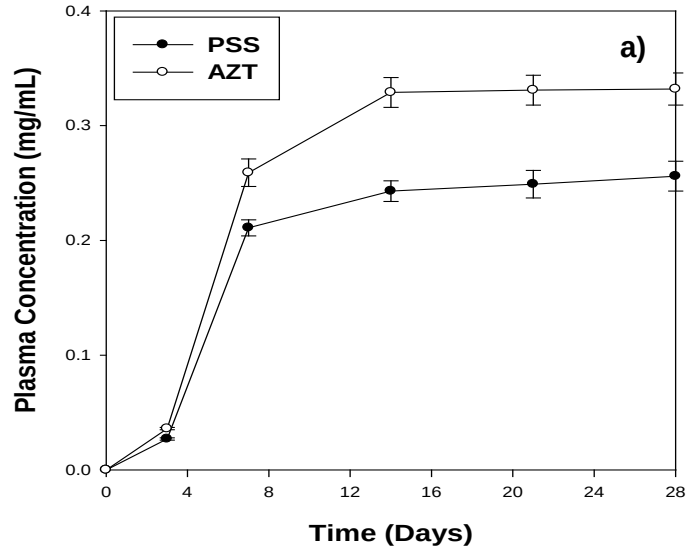


Figure 7.4: UPLC chromatograms depicting the retention times for the standard solutions of a) AZT, b) PSS and MP as an internal standard in blank plasma.

7.3.2 AZT and PSS concentrations in the blood and vaginal tissue of the pig model

The drug concentration in the plasma from day 1-28 ranged between 0.012-0.332mg/mL (AZT) and 0.009-0.256mg/mL (PSS) (N=5) (Figure 7.5a). This indicates that only a minimal quantity of the AZT and PSS crossed into the systemic circulation. This may be attributed to the hydrophobicity and high matrix resilience of the polymers employed. A steady-state concentration of AZT/PSS was observed from day 14 through day 28 indicating that no further drug crossed into systemic circulation. The lower concentration value of PSS may be due to the presence of strong intermolecular forces in the polymer that reduced the rate of permeation of the drug into blood compartment (Pu et al., 2004; Chu et al., 2007). The mean drug concentration in the vaginal tissue at day 28 was 1.2148±0.062mg/mL (N=5) for AZT and 1.4004±0.071mg/mL (N=5) for PSS, while the plasma concentration was 0.332±0.014mg/mL (N=5) for AZT and 0.256±0.013mg/mL (N=5) for PSS (7.5b). This is an indication that the majority of the drug was retained in the vaginal tissue. For each pig, the vaginal tissue drug concentration at three vaginal sites (namely anterior, middle and posterior) was approximately the same ($P>0.05$). On average, PSS presented with the highest vaginal tissue drug concentration at the 28^{th} day (Figure 7.5b). This indicates that PSS was better retained in the vaginal tissue than AZT. This may be attributed to the high degree of hydrophobicity coupled with the presence of strong intermolecular charges in PSS. Figure 7.6 depicts a typical profile for the PSS content in the vaginal tissue. The finding corresponds well with the earlier results of drug flux analysis (Chapter 6, Section 6.3.5) whereby PSS had lower flux values, indicating that its permeation rate across the vaginal tissue was slower compared to AZT. Likewise, the results of the analysis of drug concentration in the plasma revealed that a lower concentration of PSS crossed into the blood compared to AZT. Overall, the results portrayed a direct correlation ($R^2 = 0.99$) between the drug concentration in the blood and in the vaginal tissue (Figures 7.7a and b).



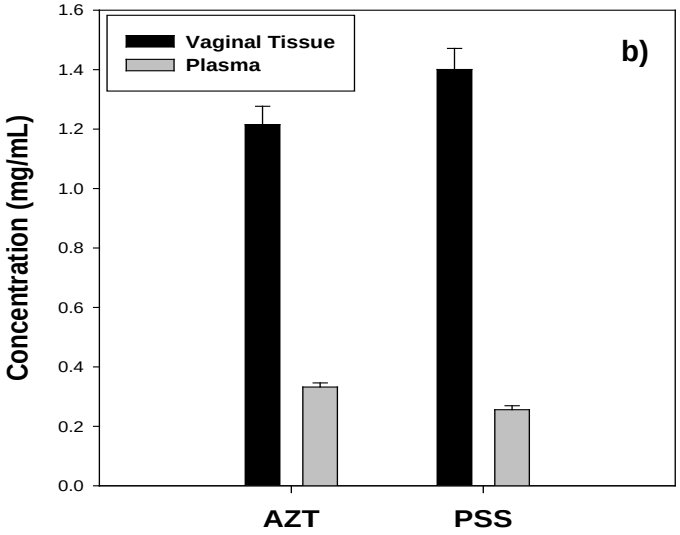


Figure 7.5: a) AZT and PSS plasma concentration profiles (N=5, SD<0.01), b) AZT and PSS mean vaginal tissue and plasma concentration values on day 28 (N=5, SD<0.014 in all cases).

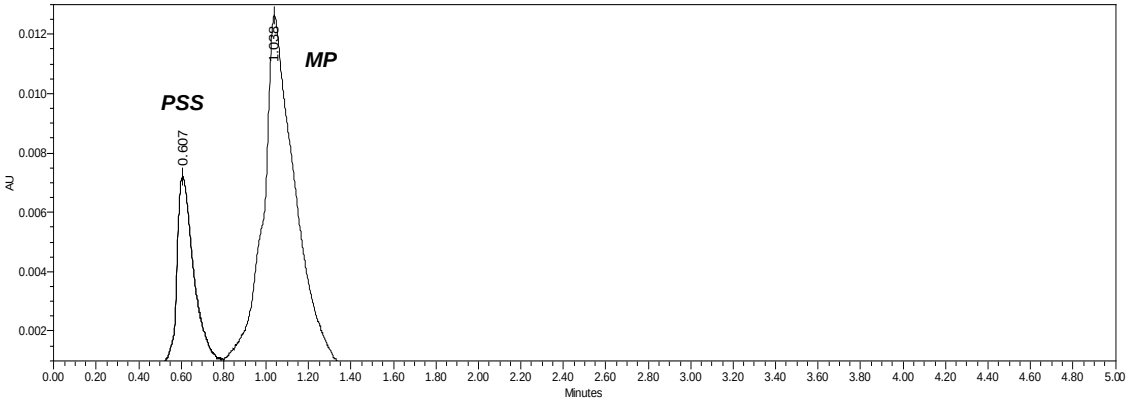


Figure 7.6: A typical profile depicting the content of PSS in the vaginal tissue.

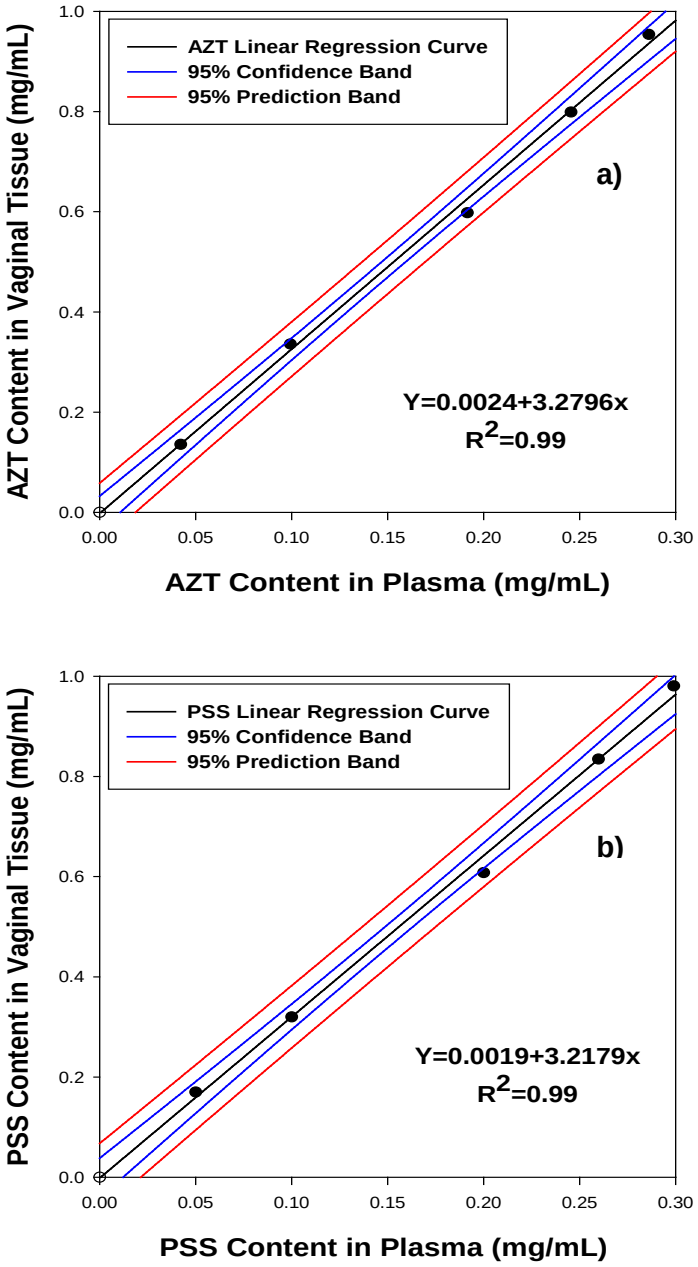


Figure 7.7: Correlation between a) AZT, b) PSS concentrations in the blood plasma and vaginal tissue for the 5 pigs tested at the 28th day.

On the 28th day, the IBPD was still found retained in the vagina. The adherence of the IBPD to the vagina was mechanistically deduced (in our laboratories) to be a result of the role played by the components forming the vaginal epithelium and the polymers of the IBPD as depicted in Figure 7.8. However, the IBPD was found to have broken into several parts (close to the cervix) and most of it had eroded (Figure 7.9). A gel-like soft matrix was also observed at the vaginal epithelial lining. This is an indication that the IBPD was

degrading slowly into a gel-like matrix that adhered to the vaginal walls. These findings correlate well with the objectives of the study whereby the IBPDs were supposed to degrade, adhere to the vaginal walls and release the ARV and microbicide simultaneously.

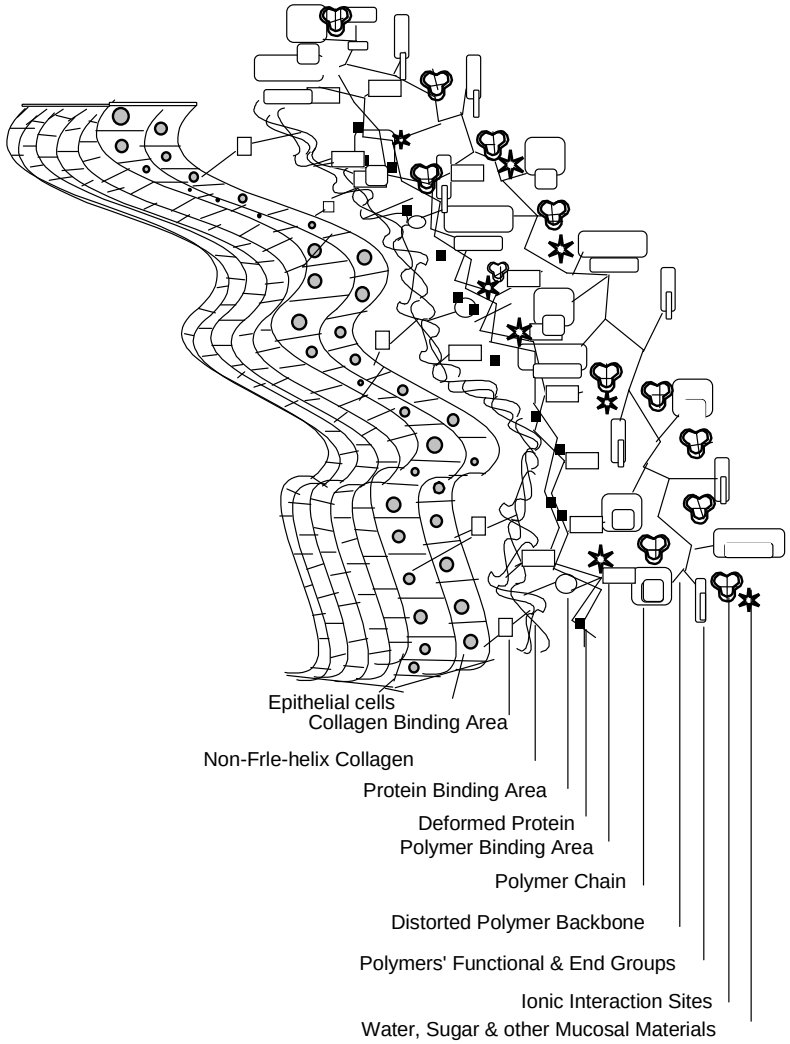


Figure 7.8: A schematic modeled in our laboratories depicting the mechanistic approach of adhesion between a polymer and the vaginal epithelium. Figure generated on ACD/I-Lab, V5.11 (Add-on) software (Advanced Chemistry Development Inc., Toronto, Canada, 2000).



Figure 7.9: Intravaginal Bioadhesive Polymeric Device (IBPD) as observed in the excised pig vagina on the 28th day.

7.3.3 Histopathological evaluation of the vaginal tissue

In this study, negative to mild epithelial hyperplasia was observed (Figure 7.10a) except for pig number 3, 9 and 14 which presented with moderate to severe hyperplasia (Table 7.2). This was noted from an increase in the epithelial rete ridges (Figure 7.10b). The controls showed no epithelial hyperplasia thus showing normal vaginal epithelium (Figure 7.10c) (Table 7.2). The reason for the moderate to severe hyperplasia observed in pig number 3, 9 and 14 may have been due to the small vaginas of these pigs. In these pigs, the insertion of the IBPD was achieved with some difficulty resulting in a relatively higher degree of inflammation. Some irritation from the polymeric device was most likely responsible for the hyperplastic epithelial response. Regarding exocytosis, neutrophils were detected in intercellular spaces in the vaginal epithelium, in the process of migrating to the vaginal lumen. The exocytosis varied among the different test groups as well as the placebo, but was not found in the control animals.

The exudate attached to the mucosal surface followed the transepithelial migration and exocytosis of neutrophils into the lumen. This was found in only one of the animals

receiving PSS and two that received AZT (Figure 7.10d). However it was not observed in the placebo and control pigs. Ulceration in the vagina refers to the complete loss of superficial epithelium due to an underlying submucosal inflammatory process and was confirmed only in a few animals from the AZT group (Table 7.2), and more so in pig number 9 which again may have been caused by the epithelial damage or necrosis during the insertion process due to the small size of the vagina. Mononuclear inflammatory cells (lymphocytes and plasma cells) were observed in the lamina propria and stromal tissue directly underneath the vaginal epithelium (Figure 7.10e). As shown in Table 7.2, (Figure 7.10e), moderate mononuclear inflammation was recorded in PSS (N=1) and AZT-containing IBPDs (N=2) as well as placebo animals (N=1), but was not visible in the controls. Polymorphonuclear cells (eosinophils and neutrophils), were found in the submucosa as well as in the superficial exudate. These cells originated from the circulation and migrated into the tissues from the blood vessels and also by exocytosis into the lumen of the vagina. The mononuclear and polymorphonuclear inflammation indicates that there was mild to moderate chemotaxis of polymorphonuclear cells as well as an antigenic stimulation of mononuclear leukocytes arising from the polymeric devices. This is normal for any foreign object introduced onto an epithelium.

In pig number 9, a foreign body granuloma and inflammation was observed in the focal area of the vaginal epithelium. The foreign material appeared as homogenous eosinophilic staining and was surrounded by numerous macrophages, epithelioid cells and multinucleated foreign body giant cells. This reaction was also associated with ulceration in the epithelium. Possibly the foreign body reaction was related to the traumatic insertion process in this pig. The perivascular inflammatory infiltrates consisted mainly of mononuclear cells with few polymorphonuclear infiltrates dispersed among the mononuclear cells in the submucosa and the wall of the vagina (Figure 7.10f) (Table 7.2). It was negative to mild and could be demonstrated in just few individual pigs (PSS: N=1; AZT: N=2; placebo: N=1).

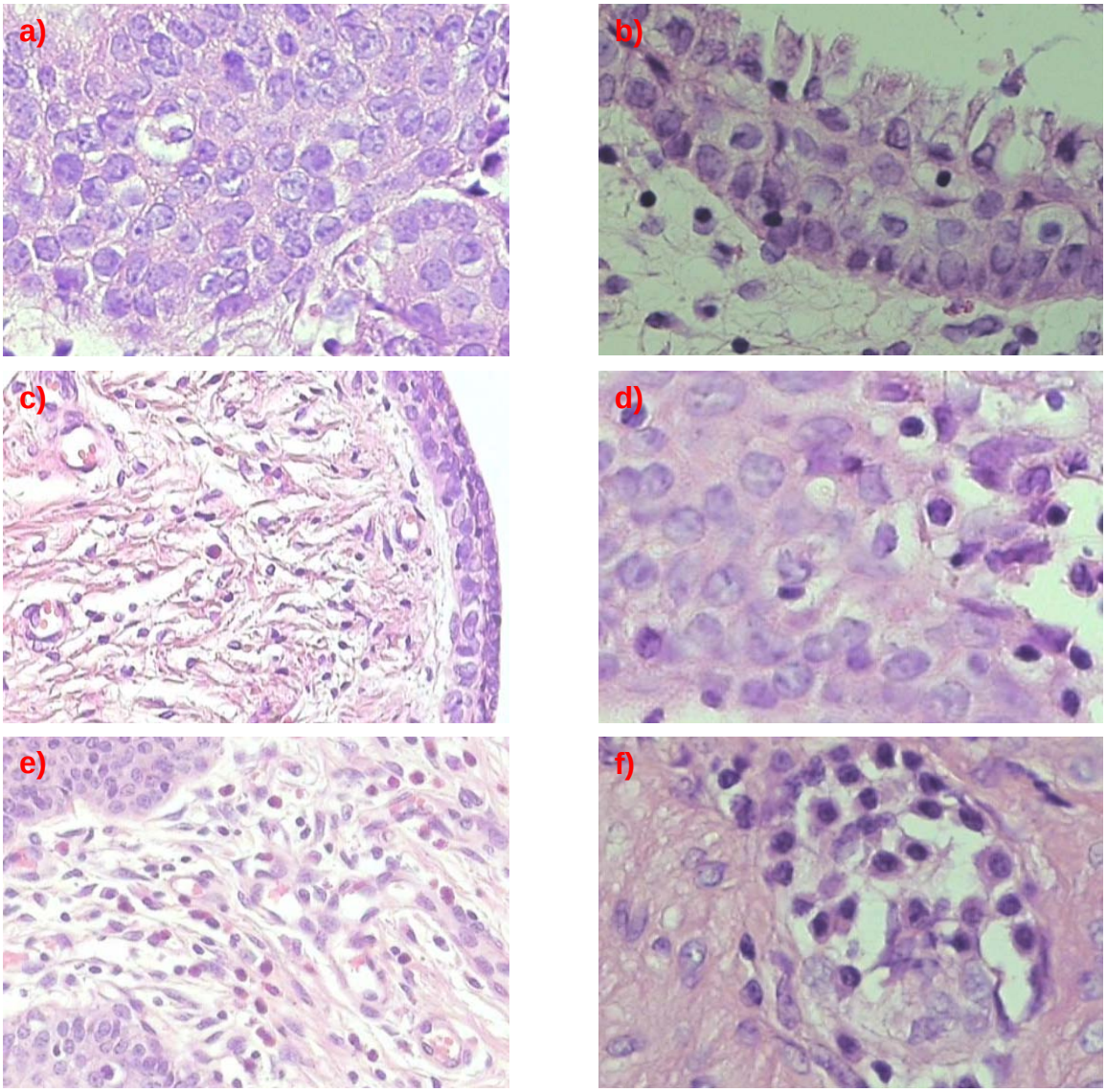


Figure 7.10: Histological images of haematoxylin and eosin stained pig vaginal tissue samples depicting a) Epithelial hyperplasia of the vagina x 40, b) Epithelial hyperplasia, exocytosis and superficial exudate x 40, c) Lamina propria with no inflammatory cells and normal vagial epithelium x 20, d) Hyperplastic epithelium with exocytosis x 40, e) Mononuclear cell infiltrates in the lamina propria and f) Perivascular inflammation in the submucosal wall x 40.

Table 7.2: Histopathological findings in the vaginal tissue

Tested item →	IBPD with PSS					IBPD with AZT					Placebo					Negative control				
Animal No. →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Histopathological findings ↓																				
Epithelium																				
Hyperplasia	1+	1+	2+	1+	1+	-	1+	1+	3+	1+	1+	1+	1+	2+	1+	-	-	-	-	-
Exocytosis	1+	-	2+	1+	1+	2+	1+	1+	2+	1+	1+	1+	2+	2+	1+	-	-	-	-	-
Exudate on epithelial surface	-	1+	2+	-	-	2+	1+	-	-	1+	-	-	-	-	-	-	-	-	-	-
Ulceration	-	-	-	-	-	1+	-	-	2+	-	-	-	-	-	-	-	-	-	-	-
Lamina propria																				
Mononuclear inflammation	-	1+	2+	_	1+	1+	1+	1+	2+	2+	1+	1+	2+	1+	1+	-	-	-	-	-
Polymorphonuclear infiltration	1+	-	2+	1+	1+	1+	2+	1+	1+	-	2+	1+	1+	1+	1+	1+				1+
Foreign body inflammation	-	-	-	-	-	-	-	-	3+	-	-	-	-	-	-	-	-	-	-	-
Submucosa and vaginal wall																				
Perivascular inflammation	-	-	1+	-	-	-	2+	_	1+	1+	-	1+	-	-	-	-	-	-	-	-

- *Negative*
1+ Mild
2+Moderate
3+ Severe

7.4 Concluding Remarks

The results of the drug content analysis in the blood and vaginal tissue revealed that only 0.332mg/mL (16.78%) of AZT and 0.256mg/mL (12.94%) of PSS crossed into the systemic circulation, indicating that the majority of the drugs was not absorbed and was thus in the vaginal lumen and possibly the tissues. This shows the superior ability of the employed polymers in retaining the drugs within the vaginal tissue. The observed gradual erosion of the IBPD and the subsequent adherence of its byproducts to the vagina is a clear indication that the developed IBPDs had the required matrix integrity attributes and were also substantially bioadhesive. The microscopical examination of the vaginal tissue following the termination of the pigs revealed that the histopathological changes (e.g.

inflammation, ulceration) were either negative, mild or moderate, which was pathologically or clinically a sign of non-toxicity. The developed microbicide-ARV delivery system meets most of the essential requirements of an ideal microbicidal delivery system with characteristics that are defined as desirable in that they have shown substantial retention to the vaginal epithelium with very low systemic absorption and high permeation of the drug within the vaginal tissue.

CHAPTER EIGHT

CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

An effective anti-HIV intravaginal microbicide delivery system represents a practical and accessible means of reducing male to female sexual transmission of HIV. However, successful design and development of such a system brings with it a multitude of challenges, evidenced by a need for efficacy, acceptability, accessibility and affordability. Thus, exhaustive efforts have been made during the design and development of an IBPD in this study to address these challenges, leading to the successful achievements of the aim and objectives. The current status of the treatment modalities of HIV/AIDS has been well described. Factors such as vaginal physiology, vaginal pH, the type and the intrinsic properties of the polymer vehicles as well as the drugs employed have been well addressed.

Numerous safe and effective HIV vaginal microbicide delivery systems are still being evaluated at various stages in clinical trials, meaning that this research has been conducted at a period when the successful development of an effective anti-HIV intravaginal microbicide delivery system is still an ongoing agenda. Polymeric biomaterials appear to have great potential for intravaginal drug delivery development because of their peculiar physicochemical and physicomechanical properties which allow delivery and maximization of the drug concentration at the required site, ultimately resulting in improved efficacy of drug therapy. Striking a balance between the hydrophobicity and hydrophilicity was an essential criterion upon which polymer selection was based using the equilibrium swelling ratio. The polymeric materials employed after undergoing a

thorough screening have resulted in the development of a delivery system that demonstrated the potential for providing controlled release over a period of 72 days. This is a significant achievement in the current research.

The major prerequisite for the developed IBPD was proper matrix integrity which would eventually ensure highly controlled drug release and substantial bioadhesion (retention) to the vaginal epithelium. To attain these goals, this research utilized multifunctional polymers with intrinsic properties that could lead to the development of a delivery system with a high degree of matrix integrity and subsequently controlled drug release. Successful optimization mainly based on polymer matrix erosion as a measure of matrix integrity, and was carried out using the most recent highly-enabled, mathematically-based technology of Artificial Neural Networks.

Extensive bioadhesivity testing on the employed polymers on both a simulated artificial membrane and on freshly exercised pig vaginal tissue under conditions simulating the vaginal fluid pH demonstrated the bioadhesivity capacity of the developed intravaginal drug delivery system. Deduction of the transient mechanisms of bioadhesivity and the associated interactive energy paradigms during caplet adhesion to the freshly excised pig vaginal tissue was successfully carried out using computational and structural modeling techniques.

Economic and social conditions as well as patient's preference are prime factors that needed to be considered during the design and development of the IBPD. Acceptability depends on affordability, ease of use and minimal side-effects. Failure in this regard would result in non-compliance and non-adherence, amounting to no progress with the intended mission. Thus, it was absolutely important to conduct a thorough scientific evaluation of the physicochemical and physicomechanical properties of the IBPDs. The benefit of this evaluation, attested to by the good matrix hardness, matrix integrity,

thermal stability and acceptable friability of the IBPD, also ensured safety, functionality and efficacy and therefore its suitability as a microbicide-ARV delivery system. Furthermore, the highly controlled release pattern shown by the IBPD, the permeation dynamics obtained and the fact that the IBPD could adhere to the vaginal epithelium, satisfied the formulation requirements. This entailed, among other things, the ability of the IBPD to maintain a steady-state concentration of the bioactives within the vaginal tissue.

Numerous challenges are currently faced by the pharmaceutical industry. Therefore, there is a pressing need for new and innovative technology in drug delivery, for preventing HIV/AIDS and STIs. For this reason, novel techniques and appropriate materials were employed in this study which produced a robust IBPD with the following important attributes: a) the IBPD could remain stable in simulated human vaginal fluid (pH 4.5) and in simulated human seminal fluid (pH 7.4) for more than 30 days; b) the IBPD was substantially vaginally bioadhesive both *ex vivo* and *in vivo;* c) the IBPD could provide controlled release rate of the microbicide-ARV ensuring the potential for prolonged protection in women; and d) the IBPD was non-toxic. Thus, the novel developed controlled release microbicide-ARV loaded IBPD may significantly contribute in solving the problems currently faced in drug delivery technology development and may lead to the following: i) provide an alternative preventive measure in the transmission of HIV/AIDS; ii) reduce the burden of HIV/AIDS; iii) improve the quality of life with subsequent macroeconomic benefits; iv) reduce the cost of medication; v) develop products locally; vi) create intellectual property; vii) overcome the challenges of current intravaginal drug delivery systems; and viii) a versatile IBPD may be suitable for the treatment of other diseases burdening developed and developing countries.

8.2 Recommendations

Although prevention of HIV infection is an overwhelming task, a human, female-controlled anti-HIV intravaginal microbicide delivery system points to promising future for success in

the fight against HIV-1 with the eventual eradication of the HIV pandemic. The development of an ideal anti-HIV intravaginal microbicide delivery system that will pass through all clinical trial phases will be achieved through focused and endeavored scientific efforts. The work presented in this thesis provides a significant contribution towards this goal.

Local delivery of an anti-HIV intravaginal microbicide to the vaginal compartment at the correct time and concentration is a key requirement, and this presents a formidable challenge to overcome if the potential for microbicides in preventing HIV infection is to be realized. This research has identified priority challenges and opportunities for pre-competitive research in intravaginal anti-HIV microbicide delivery for the prevention of STIs and HIV infection. Much needed further research should gain greater interactive-based understanding of the physicochemical and physicomechanical properties of biomaterials (in addition to polymers), microbicidal agents and anti-HIV drugs. Such studies will discover how these properties can be modified within the human vaginal biological environment so as to determine how optimal efficiency towards prevention of STIs and HIV infection can be achieved. It will also be of great importance to conduct *in vivo* human-oriented clinical trials to validate and compare the stability, viability and efficacy of the IBPD.

The developed IBPD may be applied not only for preventing STIs and HIV but also in other areas like hormone replacement therapy, contraception, infections, infertility, and other female-related conditions, as an alternative to oral or parenteral administration. One such example is the everlasting problem that has been incurred so far in the treatment of Candidiasis (from *Candida albicans*) due to resistance complications. The great stability of the IBPD in the vagina, its high bioadhesivity and therefore unquestionable substantial retention, and the superior ability to control drug release rate over a prolonged period may make it a gold standard in this endeavor. Needed at this point in time is to streamline the

developed IBPD by undertaking more directed studies aimed at realizing its best clinical applicability. Such attempts should involve a study on irritation potential of this device which goes beyond the histology work but also involves looking at the liberation of any cytokines like IL-1, alpha IL-6 and IL-8 in the skin tissue. Furthermore, it may be necessary to look for pharmacodynamic disease models to which this device can be applied in order to finally assess the benefit of this approach which will be a very good preclinical model.

Finally, this study does not only provide encouraging results for the delivery of PSS and AZT but also provides a novel systematic approach for the development of delivery systems for other microbicide-ARVs. It is therefore highly recommended that the approach employed in this study for the development of a microbicide-ARV delivery system for the prevention of STIs and HIV infections be adopted as a design tool for other similar bioactive systems.

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APPENDICES

APPENDIX A

ABSTRACTS OF PAPERS PUBLISHED/SUBMITTED FROM THIS THESIS

APPENDIX A1

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In vitro and *ex vivo* bioadhesivity analysis of polymeric intravaginal caplets using physicomechanics and computational structural modeling

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ABSTRACT

The *in vitro* and *ex vivo* bioadhesivity of polyacrylic acid (PAA)-based intravaginal caplets was explored from a physicomechanical and chemometrical structural modeling viewpoint. An Extreme Vertices Mixture Design was constructed for analyzing the bioadhesivity of 11 matrices that were optimized. Two sets of crosslinked PAA-based matrices comprising either allyl-sucrose (AS-PAA) or allyl-penta-erythritol (APE-PAA) were explored. Powders were compressed into caplet-shaped matrices and rotational rheological analysis was performed on hydrated polymeric blends. Caplets were evaluated for bioadhesiveness using a simulated vaginal membrane (SVM) with optimized caplets further tested using freshly excised rabbit vaginal tissue. The SVM and caplets were hydrated in simulated vaginal fluid before bioadhesivity testing using a texture analyzer to determine the rupture force between the membranous substrates and hydrated caplets. Computational and molecular structural modeling deduced transient sol–gel mechanisms, chemical interactions and inter-polymeric interfacing during caplet-substrate bioadhesion. Peak adhesive force (PAF) and work of adhesion (AUC_{FD}) values for the APE-PAA caplets (1.671 ± 0.232 N; 0.0010 ± 0.0002 J) were higher than the AS-PAA caplets (1.168 ± 0.093 N; 0.00030 ± 0.0001 J) revealing superior bioadhesiveness. Similarly, rheological analysis revealed APE-PAA blends with higher viscosity and shear stress values (9×10^5 mPa/180 Pa). The optimized APE-PAA matrices adhered appreciably to rabbit vaginal tissue (PAF = 0.883 ± 0.083 N; $AUC_{FD} = (0.0003 \pm 3.5355) \times 10^{-5}$ J). Results strongly suggest that the approach may be useful for assessing the bioadhesivity of intravaginal matrices on *ex vivo* rabbit vaginal tissue with data further supported by molecular structural analysis and energy-dependant bioadhesivity modeling.

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APPENDIX A2

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Review Article

A Review of Current Intravaginal Drug Delivery Approaches Employed for the Prophylaxis of HIV/AIDS and Prevention of Sexually Transmitted Infections

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Abstract. The objective of this review is to describe the current status of several intravaginal anti-HIV microbicidal delivery systems these delivery systems and microbicidal compounds in the context of their stage within clinical trials and their potential cervicovaginal defence successes. The global Human Immuno-Deficiency Virus (HIV) pandemic continues to spread at a rate of more than 15,000 new infections daily and sexually transmitted infections (STIs) can predispose people to acquiring HIV infection. Male-to-female transmission is eight times more likely to occur than female-to-male transmission due to the anatomical structure of the vagina as well as socio-economic factors and the disempowerment of women that renders them unable to refuse unsafe sexual practices in some communities. The increased incidence of HIV in women has identified the urgent need for efficacious and safe intravaginal delivery of anti-HIV agents that can be used and controlled by women. To meet this challenge, several intravaginal anti-HIV microbicidal delivery systems are in the process of been developed. The outcomes of three main categories are discussed in this review: namely, dual-function polymeric systems, non-polymeric systems and nanotechnology-based systems. These delivery systems include formulations that modify the genital environment (e.g. polyacrylic acid gels and *lactobacillus* gels), surfactants (e.g. sodium lauryl sulfate), polyanionic therapeutic polymers (e.g. carageenan and carbomer/lactic acid gels), proteins (e.g. cyanovirin-N, monoclonal antibodies and thromspondin-1 peptides), protease inhibitors and other molecules (e.g. dendrimer based-gels and the molecular condom). Intravaginal microbicide delivery systems are providing a new option for preventing the transmission of STIs and HIV.

KEY WORDS: HIV/STIs; intravaginal drug delivery systems; microbicides; nanostructures; polymers; prophylaxis.

APPENDIX A3

Submitted

Investigation of the Physicochemical and Physicomechanical Properties of an Optimized Intravaginal Bioadhesive Polymeric Device in the Pig Model

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Abstract
The purpose of this study was to develop and evaluate the bioadhesivity, diffusivity, thermal stability and the *in vitro* drug release an Intravaginal Bioadhesive Polymeric Device (IBPD) loaded with 3'-azido-3'-deoxythymidine (AZT) and polystyrene sulfonate (PSS). Biodegradable and biocompatible polymers namely, modified polyamide 6,10, poly(lactic-co-glycolic acid), polyacrylic acid, polyvinyl alcohol and ethylcellulose were blended with model drugs AZT and PSS (separately and in combination) as well as radio-opaque barium sulfate ($BaSO_4$) and then compressed into robust caplet devices on a tableting press at a pressure of 5 tons. One set of devices was coated with 2%$^{w}/_{v}$ PAA while the other remained uncoated. Thermal analysis was performed on the constituent polymers as well the IBPD employing temperature modulated differential scanning calorimetry. The micro-environmental pH changes within simulated vaginal fluid due to the degrading constituents of the IBPD were monitored using a Multi- Purpose Titrator (MPT-2) instrument. Textural profile analysis indicated that the mucoadhesivity of the PAA-coated devices (3.699±0.464N; 0.0098±0.0004J) was higher than that of the uncoated devices (1.198±0.150N; 0.0019±0.0001J). In addition, $BaSO_4$ facilitated x-ray imaging revealed that the IBPD adhered to pig vaginal tissue over the experimental period of 30 days *in vivo*. Controlled drug release kinetics was obtained over 72 days, and this was attributed to the superior physicomechanical properties of the polymer blend selected. A characteristic bi-phasic response in pH was within a range of 3.5-5.5 was maintained indicating that the IBPD did not significantly alter the pH within the simulated vaginal fluid. This was certainly enabled by PLGA upon breaking down into its constituent units, i.e. lactic and glycolic acids. During a 24-hour permeation study, an increase in drug flux for both AZT (0.84 $mg.cm^{-2}.h^{-1}$) and PSS (0.72 $mg.cm^{-2}.h^{-1}$) was realized up to 12 hours and thereafter a steady-state was reached. This indicated that a large proportion of AZT and PSS were retained within the vaginal tissue beyond steady-state. The permeation and dissolution dynamics were mechanistically deduced based on a chemometric and molecular structure modeling approach. Overall, results suggested that the IBPD may be sufficiently bioadhesive with desirable physicochemical and physicomechanical stability for use as a prolonged intravaginal drug delivery device.

Keywords: Intravaginal drug delivery, Microbicidal polymeric device, Mucoadhesivity, Controlled release, Physicochemical and physicomechanical characterization

APPENDIX A4

Submitted

In Vivo Drug Content Analysis and Histopathological Evaluation of an Intravaginal Bioadhesive Polymeric Device in Pig Tissue

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Abstract

The purpose of this study was to determine the concentration of drug in plasma and vaginal tissues from a pig model administered with a 3'-azido-3'-deoxythymidine (AZT) and Polystyrene Sulfonate (PSS)-loaded Intravaginal Bioadhesive Polymeric Device (IBPD), intended to be used as homeo-prophylactic agent against HIV and STIs. In addition, histopathological evaluation was undertaken on the vaginal epithelium to assess the potential for toxicity. Biocompatible polymers namely modified polyamide 6,10, poly(lactic-co-glycolic acid), polyacrylic acid, polyvinylalcohol and ethylcellulose were blended with model drugs AZT and PSS, compressed into caplet shaped IBPDs and then intravaginally administered into the pig model. The study employed twenty pigs which were divided into four groups of five each (i.e. control, placebo, AZT-containing and the PSS-containing group). The IBPDs were deeply inserted into the posterior fornix of the vagina of group 2, 3 and 4 pigs using a novel applicator under anesthesia. Plasma samples (10mL) were taken from the jugular vein of each pig at day 0, 3, 7, 14, 21 and 28. AZT and PSS were extracted via Solid Phase Extraction (SPE) and subjected to Ultra Performance Liquid Chromatography (UPLC) analysis. At day 28 each pig was euthanized to remove the vaginal tissue which was digested with subtilisin, extracted and subjected to AZT and PSS content analysis. Histopathological evaluation was conducted on vaginal epithelium, lamina propria as well as submucosa and vaginal wall. The mean drug concentration in the vaginal tissue at day 28 was 1.2148±0.062mg/mL (N=5) for AZT and 1.4004±0.071mg/mL (N=5) for PSS, while the plasma concentration was 0.332±0.014mg/mL (N=5) for AZT and 0.256±0.013mg/mL (N=5) for PSS. This is an indication that the majority of the drug was retained in the vaginal tissue. For each pig, the vaginal tissue drug concentration at three vaginal sites (namely anterior, middle and posterior) was approximately the same ($P>0.05$). On average, PSS presented with the highest vaginal tissue drug concentration at the 28th day. These results may be attributed to the hydrophobicity and high matrix resilience of the polymers employed coupled with the presence of strong intermolecular forces in PSS. This findings correlated well (R^2=0.99) with the results obtained from AZT/ PSS content analysis in blood samples from day 1-28 where AZT ranged between 0.012-0.332mg/mL and PSS between 0.009-0.256 (N=5). The histopathological events were negative to mild levels in most cases. AZT and PSS concentration in the pig blood and vaginal tissue and the histopathological findings suggests that the developed caplets may be suitable for intravaginal drug delivery that may be useful for the prevention of female-related disease conditions.

Keywords: Histopathological evaluation, HIV/AIDS, STIs, Microbicidal drug delivery system, UPLC analysis.

APPENDIX A5

Submitted

Application of Artificial Neural Networks for the Elucidation of an Optimized Synergistic Polymer Combination for Effective Performance of an Intravaginal Bioadhesive Polymeric Device.

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Abstract
This study aimed at elucidating the synergistic polymer combination that govern the matrix integrity, controlled release kinetics and the release mechanisms of the loaded drugs in an Intravaginal Bioadhesive Polymeric Device (IBPD), using Artificial Neural Networks (ANN) approach. Fifteen formulations derived from hydrophilic and hydrophobic polymer combinations were blended with the model drugs 3'-azido-3'-deoxythymidine (AZT) and Polystyrene Sulfonate (PSS) and then compressed into caplet shaped devices on a Manesty D3B 16 station tableting press (Manesty D3B L249LQ, Liverpool, England) at a compression pressure of 5 tons. In process validation tests were performed to ensure that the IBPDs had desirable quality attributes in terms of matrix hardness, uniformity in mass and friability. Matrix erosion test was conducted to the formulations at pH 4.5 to assess their matrix integrity. *In vitro* drug release evaluation was conducted on the AZT and PSS-loaded IBPDs in simulated vaginal fluid (pH 4.5; 37°C) and samples were analysed by UV spectroscopy (Cecil Instruments, Cambridge, England). Artificial Neural Networks (ANN) was used to enable the selection of the appropriate polymers for developing the IBPD. Robust devices with mass uniformity=800±0.48mg, matrix hardness of BHN values ranging from 2.687-4.981N/mm^2 and an average friability of 0.21% were produced. The tested formulations presented with good matrix integrity with matrix erosion percentage ranging from 1.21-7.68. Zero order release kinetics was obtained over a prolonged period ranging from 24 hours to 72 days. Polymers employed were polyamide 6,10, poly(lactic-co-glycolic acid), polyethylene oxide, polyacrylic acid, carrageenan, ethycellulose, polyvinylalcohol, xanthan gum, gelatin, beeswax, Eudragit® S100 and Eudragit® RS 100. ANNs elucidated that among these polymers a combination comprised of modified polyamide 6,10, poly(lactic-co-glycolic acid), polyvinylalcohol, poly(acrylic acid) and ethycellulose was the most appropriate polymer combination for formulation optimization of the IBPD. This combination presented with the highest degree of matrix integrity that contributed highly to the good drug release kinetics obtained. Results revealed a highly satisfactory fit for the input variables (R^2 = 0.99) for matrix erosion. A linear correlation between the matrix loss and drug release was also realized (R^2 = 0.99).

Keywords: Artificial neural networks, Controlled drug release, Micro-environmental pH, Matrix integrity, Matrix erosion, Optimization.

APPENDIX A6

Submitted

Preformulation Investigations for the Development of a Lead Intravaginal Bioadhesive Polymeric Device

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Abstract
The purpose of this study was to screen various polymers through extensive preformulation investigations to ultimately obtain a lead polymer combination that can be employed in designing desirable Intravaginal Bioadhesive Polymeric Device (IBPD). Hydrophilic and hydrophobic polymers at different combinations were blended and compressed into caplet shaped devices at a force of 5 tons. One of the hydrophilic polymers was a modified synthetic product of polyamide 6,10 ($_m$PA 6,10). The devices were subjected to in-process validation tests and thereafter to preformulation investigational screening using a One Variable at a Time (OVAT) approach. A series of swelling tests were conducted on 62 formulations that were derived from 18 polymers, to investigate which formulation had the optimal swelling at both simulated and seminal vaginal fluids. Each formulation was weighed, immersed into both vaginal and semen fluids and then placed in an orbital shaking incubator, maintained at 20rpm (37°C) for 24 hours. After 24 hours each device was removed from the orbital shaking incubator, gently blotted on filter paper and re-weighed. The swelling behavior was determined in terms of the equilibrium swelling ratio (ESR) which was the critical indicator of the formulation's matrix stability (i.e. the degree of matrix robustness), and was used as a screening parameter for each formulation. The developed devices were sufficiently strong and robust with a mean hardness of 286±0.01N, mean weight 600±0.48mg and a mean friability of 0.31±0.04% which was within the limits. Through OVAT approach, 15 suitable formulations (out of 62) with ESRs ranging from 0.011-0.084. Through extensive screening of the developmental formulations using an OVAT approach, robust and strong devices with substantial matrix integrity were produced, as evidenced by the swelling equilibrium ratios. Fifteen lead formulations with minimal swelling tendencies were obtained. F62 which is comprised of $_m$PA 6,10, (150mg), PLGA (400mg), EC (200mg), PVA (25mg) and PAA (25mg) appeared to be the most suited in terms of minimal swelling capacity and highest stability. Overall, the results suggested that PA 6,10, PLGA, EC, PVA and PAA at an appropriate weight ratio may be suitable for development of an IBPD.

Keywords: OVAT, Intravaginal Bioadhesive Polymeric Device (IBPD), Developmental design, Differential scanning calorimetry, Matrix swelling.

APPENDIX B

ABSTRACTS OF CONFERENCE PROCEEDINGS

APPENDIX B1

In Vitro and Ex-vivo Evaluation of a Polystyrene Sulfonate-loaded Vaginal Polymeric Platform through Porcine Vaginal Tissue

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Purpose

To develop and evaluate a Vaginal Polymeric Device (VPD) loaded with polystyrene sulfonate (PSS) for intravaginal drug delivery. The drug diffusion dynamics from the PSS-loaded devices through pig vaginal tissue was also assessed.

Methods

Biodegradable and biocompatible polymers namely polyamide 6,10 (PA 6,10), poly(lactide-co-glycolide) (PLGA) and polyacrylic acid (PAA) were blended with model drug PSS and compressed into robust matrices on a tableting press. One set of devices was coated with 2%$^{w}/_{v}$ PAA while another remained uncoated. Temperature modulated differential scanning calorimetry (TMDSC) was performed on the constituent polymers as well as on the VPD to assess the thermal behavioral transitions. Mucoadhesivity and diffusivity studies as well as drug content analysis employing UV spectroscopy was also conducted.

Results

Textural profile analysis indicated that the mucoadhesivity of the coated VPDs, as determined from the peak adhesive force (3.699±0.0464N) and the area under the force-distance profile (0.0098±0.0004J) was higher compared to that of the uncoated devices (1.198±0.150N; 0.0019±0.0001J). All polymers displayed multi-transitional thermal behaviors with varying glass transition temperatures (T_g). The T_g values were 163°C for PA 6,10, 45-55°C for PLGA and 90°C for PAA. The thermal stability of PA 6,10, PLGA and the VPD was confirmed by TMDSC analysis signifying the suitability of the polymers to produce controlled drug delivery. Drug release profiles revealed that PSS was delivered in a controlled and prolonged manner over 72 days. This was attributed to the hydrophobicity and highly compressive properties of PLGA coupled with superior matrix resilience conferred by PA 6,10. A rapid increase in the drug flux of PSS was initially realized and continued constantly up to $t1_{2hours}$ (72.17±13.7%) prior to reaching a maximum flux at $t_{24hours}$, which indicated that a large proportion of PSS was retained within the vaginal tissue.

Conclusions

Robust VPDs were produced that confirmed the ability of PA 6,10 and PLGA to control the release and diffusivity of PSS. The VPDs were sufficiently mucoadhesive facilitating their use for intravaginal drug delivery.

APPENDIX B2

Development of a Highly Sensitive Assay Method for the Determination of AZT in Porcine Plasma

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Purpose

To develop a highly sensitive quantitate assay method for the determination of 3'-azido-3'-deoxythymidine (AZT) in the porcine plasma employing Ultra Performance Liquid Chromatography (UPLC) with methylparaben (MP) as an internal standard.

Methods

Standard solutions of AZT (0.025-10µg/mL) and MP (5µg/mL) were prepared in a binary solvent comprising water/acetonitrile (60:40) to yield an initial concentration of 0.1mg/mL. Solid Phase Extraction (SPE) was used to extract AZT from the plasma samples using Oasis® HLB cartridges conditioned with 1mL each of methanol and deionized water. Acetonitrile (1mL) was added to 600µL of freshly thawed porcine plasma, vortexed and centrifuged at 13,000rpm for 6 minutes at 24°C. The supernatant (650µL) was spiked with 1mL AZT solution at varying concentrations. SPE cartridges were washed with 5%$^{v}/_{v}$ methanol (1mL) in deionized water and analytes were eluted with 0.01M KH_2PO_4 (550µL) followed by absolute methanol (2mL). The eluant was evaporated under N_2 gas and the extracted sample was reconstituted with absolute methanol (100µL), mixed with MP (400µL) and subjected to UPLC analysis. AZT content was analyzed with a UPLC phenyl column (1.7µm; 2.1×50mm). A gradient method was used with the binary mobile phase (water/acetonitrile) varying from 60:40 at t_0, 5:95% at $t_{1\text{-}2.6min}$, and 60:40 at $t_{3.5\text{-}3.6min}$ with an injection volume=2µL, a flow rate=0.5mL/min and UV detection set at 267nm.

Results

AZT and MP were eluted at 3.7±0.5min and 1.5±0.3min. Assay method validation revealed that the intra and inter-day precision and accuracy were satisfactory (R^2=0.99). The signal to noise ratio at the lower limit of quantification (25ng/mL) was >10.8. For validation of the linear regression model, the weighting factors were selected to be proportional to the reciprocal of the standard deviations which was optimal under least square estimation with aR^2>0.99. Three dimensional chromatographic peak analysis revealed no interfering peaks at the retention times within the UV range of 200-400nm. The accuracy of the assay method was evaluated from standard curves with mean recoveries ranging from 96.4-102.5%.

Conclusions

A UPLC gradient method was validated for determining AZT content in porcine plasma. The assay method developed was sufficiently accurate and suitable for further pharmacokinetic studies.

APPENDIX B3

In Vivo Morphological and Histopathological Evaluation of a Vaginal Polymeric Device in a Porcine Model

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Purpose

To determine the concentration of drug in plasma and vaginal tissues from a porcine model administered a Polystyrene Sulfonate (PSS)-loaded Vaginal Polymeric Device (VPD). In addition, histopathological and morphological evaluation was undertaken on the vaginal mucosa to assess the potential for toxicity.

Methods

Biocompatible polyamide 6,10 (PA 6,10), ethylcellulose (EC) and polyacrylic acid (PAA) were blended with model drug PSS and compressed into caplet shaped VPDs and intravaginally administered in the porcine model. Fifteen pigs divided into 3 groups (control, placebo and PSS-loaded) of 5 pigs each. The VPDs were inserted into the posterior fornix of the vagina using a novel applicator under anaesthesia. Plasma samples (10mL) were taken from the jugular vein of each pig at day 0, 3, 7, 14, 21 and 28. PSS was extracted via SPE and subjected to Ultra Performance Liquid Chromatography (UPLC) analysis on a C_{18} (1.7µm; 2.1×100mm) column with a binary isocratic mobile phase comprising methanol/water (50:50), a flow-rate=0.2mL/min, an injection volume=1.7µL and UV detection set at 244nm. At day 28 each pig was euthanized to remove the vaginal tissue which was digested with subtilisin, extracted and subjected to PSS content analysis using UPLC. Histopathological and morphological evaluation involved the epithelial histological lesions, assessment of the lamina propria as well as submucosa and vaginal wall.

Results

PSS content in the porcine vaginal tissue from day 0-28 ranged between 68-77% (N=5) indicating that PSS was substantially retained within the vaginal tissue. This was attributed to the hydrophobicity of the polymers employed coupled with the presence of strong intermolecular forces of PSS. This finding correlates with results obtained from PSS content analysis in blood samples where PSS content was minimal and ranged between 19-26% (N=5). Histopathological and morphological evaluation revealed that hyperplasia, exocytosis, exudates on the mucosal surface, ulceration, polymorphonuclear infiltration and perivascular ranged from negative and mild-moderate levels.

Conclusions

PSS concentrations in porcine blood and vaginal tissue as well as the histopathological and morphological findings suggested that the VPD may be suitable for intravaginal drug delivery.

APPENDIX B4

In vivo Drug Content Analysis and Histopathological Evaluation of an Intravaginal Polymeric Platform in a Pig Model

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Purpose

To determine the drug concentration in both plasma and vaginal tissue in a pig model inserted with a zidovudine (AZT)-loaded Intravaginal Polymeric Platform (IPP) for the prophylaxis and/or prevention of HIV and STIs. In addition, histopathological and morphological evaluation was undertaken on the vaginal mucosa to assess the potential for toxicity.

Methods

Biocompatible polymers namely PLGA, ethylcellulose (EC) and PAA were blended with model drug AZT, compressed into caplet shaped IPPs and then intravaginally inserted into the pig model. Fifteen pigs divided into 3 groups (control, placebo and AZT-loaded IPPs) of 5 pigs each were employed for this study. The IPPs were inserted into the posterior fornix of the vagina (group 2 and 3) using a novel applicator under anesthesia. Plasma samples (10mL) were taken from the jugular vein of each pig at day 0, 3, 7, 14, 21 and 28. AZT was extracted via Solid Phase Extraction (SPE) and subjected to Ultra Performance Liquid Chromatography (UPLC) analysis using a phenyl column (1.7µm; 2.1×50mm). A gradient method was used with a binary mobile phase (water/acetonitrile) varying from 60:40 at $t_{o,}$ 5:95 at $t_{1\text{-}2.6min,}$ and 60:40 at $t_{3.5\text{-}3.6min}$ with an injection volume of 2µL, a flow rate=0.5mL/min and UV detection set at 267nm. At day 28 each pig was euthanized to remove the vaginal tissue which was digested with subtilisin, extracted and then subjected to AZT content analysis using UPLC. Histopathological and morphological evaluation involved assessment of the epithelial histological lesions, the lamina propria, as well as submucosa and vaginal wall.

Results and Discussion

AZT content in the porcine vaginal tissue from day 0-28 ranged between 56-67% (N=5) indicating that AZT was substantially retained within the vaginal tissue. This was attributed to the high matrix integrity of the IPP that was imposed by the networked microstructure that was formed by the hydrophobic and hydrophilic polymers employed. This finding correlates (R^2=0.99) with the results obtained from AZT content analysis in blood samples where AZT content was minimal and ranged between 22-37% (N=5). Histopathological and morphological evaluation revealed that hyperplasia, exocytosis, exudates on the mucosal surface, ulceration, polymorphonuclear infiltration and perivascular ranged from negative to mild-moderate levels.

Conclusions

AZT concentration in the plasma and vaginal tissue as well as the histopathological and morphological findings suggests that the developed IPP may be suitable for intravaginal drug delivery.

APPENDIX B5

2008 AAPS Annual Meeting

Development of an X-ray Detection Method for Hydroxyapatite-Loaded Matrices for Intravaginal Insertion in a Pig Model

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Purpose.

To develop and evaluate the influence of hydroxyapatite (HA) within drug-loaded radio-opaque matrices for intravaginal insertion in a pig model.

Methods.

Hydrophobic and hydrophilic polymers were blended with model drug zidovudine (AZT) and radio-opaque HA. Two different blends (with and without HA) were then compressed into matrices at a pressure of 8.5 tons. The matrices were undercoated with shellac at a concentration of 4mg/matrix and thereafter coated with 2%w/v polyacrylic acid (PAA) to prevent irritation during insertion and to ensure adherence to the vaginal tissue. The Brinell Hardness Number (BHN) was computed from textural profile analysis. The glass transition temperature (T_g) (ramped at 10°C/min to 200°C) was determined on the powdered matrices. *In vitro* drug release studies were performed to assess the effect of HA on matrix integrity. The HA-containing matrices were inserted into the vagina of pig models and thereafter monitored by X–ray imaging at 2, 4, 6 and 8 days after insertion to assess the bioadhesivity and degradation pattern of the matrices in the vagina.

Results.

BHN values were higher for the HA-containing matrices ($4.615\pm0.3 Nmm^2$). HA resulted in a variation of the T_g ranging from 51-55°C due to the added matrix integrity imparted by the highly crystalline HA. Likewise, HA-containing matrices displayed superior control of AZT release for up to 7 days in a first-order manner compared to 2 days achieved for matrices without HA. This indicated that HA was able to minimize the rate of matrix disentanglement and erosion thus prolonging and controlling the quantity of AZT released. X-ray imaging facilitated the detection of the matrices in the vagina for a period of 7 days. Sequential degradation of the matrices was traced through X-ray images obtained due to the inherent radio-opaqueness of HA.

Conclusion.

The highly crystalline HA displayed the ability of controlling the release of AZT. Furthermore, the presence of HA in the formulation was shown to be suitable for detecting the matrices in the vagina and therefore this approach could be employed for the assessment of intravaginal drug delivery systems in a pig model.

APPENDIX B6

2008 AAPS Annual Meeting

Evaluation of the Bioadhesive Potential of a Multi-configured Polymeric Matrix for Intravaginal Drug Delivery

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Purpose.

The purpose of this study was to explore the bioadhesivity potential of a multi-configured polymeric matrix and to develop a bioadhesion test method for an intravaginal drug delivery system employing a Mixture Extreme Vertices statistical design.

Methods.

Two sets of polymer-based matrices comprising two different crosslinked polyacrylic acid (PAA) grades namely allyl-sucrose crosslinked polyacrylic acid (AS-PAA) and allyl-penta-erythritol crosslinked polyacrylic acid (APE-PAA) were used. Rheological analysis of the two matrices was performed at 25±1.0°C, a flow analysis time of 200s and a controlled shear rate (CR) of $5.0s^{-1}$ ranging from 0.0-$500s^{-1}$. Powders were blended, granulated and compressed into matrices and the optimized formulation was tested and evaluated on both a simulated vaginal membrane (dialysis membrane; Mw=12,000-14,000g/mol) and freshly excised vaginal tissue from a rabbit model. The simulated vaginal membrane and the matrices were hydrated for 30 minutes in simulated vaginal fluid (pH4.5; 37°C), before bioadhesivity testing using a texture analyser. Bioadhesivity was determined by measuring the peak adhesive force (PAF) and work of adhesion i.e. Area Under the Force-Distance Curve (AUC_{FD}) required to split the adhesive bonds between the simulated vaginal membrane and the matrices. A similar test was repeated on freshly excised vaginal tissue from the rabbit model.

Results.

For both matrices, textural profiling displayed similar results between the PAF and AUC_{FD}. PAF and AUC_{FD} values for the APE-PAA matrices (1.671±0.232N and 0.0010±0.0002J) were higher than those of AS-PAA matrices (1.168±0.093N and 0.0030±0.0001J), revealing the superior bioadhesiveness of the APE-PAA matrices. Likewise, rheological analysis revealed a similar trend resulting in APE-PAA matrices having higher shear stress values (180Pa) as compared to AS-PAA (58Pa). Justifiably, this observation may be a reason for the obtained differences in bioadhesivity between the two matrices. The optimized matrix of APE-PAA adhered well to the freshly excised rabbit vaginal tissue (PAF=0.883±0.0827N; AUC_{FD}=0.0003±3.5355 $X10^{-5}$J).

Conclusion.

PAA polymers have shown great potential for intravaginal solid drug delivery system development. Results from the bioadhesivity testing in this study is a promising approach that may be useful for measuring the bioadhesivity of intravaginal drug delivery systems on freshly excised vaginal tissue from the rabbit model.

APPENDIX B7

2008 AAPS Annual Meeting

Combination of Biosorbable, Relatively Low and High Compressible Polymers in Controlled Drug Delivery

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Purpose.

The purpose of this study was to assess the potential of developing a controlled drug delivery system employing a polymeric composite of biosorbable, low and high compressible polymers for subcutaneous implantation.

Methods.

Highly compressible polymers namely poly(lactic-co-glycolic acid) (PLGA) and ethylcellulose (EC) were combined with relatively low compressible polymers such as gelatine (GL), beeswax (BWX) and xanthan gum (XG) and compressed at 8.0 tons into AZT–loaded tablet matrices using a Carver press. In-process validation tests involving hardness, friability and mass uniformity analysis were performed to ensure the reproducibility of the matrices. Hardness was represented by the Brinell Hardness Number (BHN) using a textural analyser. A friability of 1% was set as the upper limit of acceptability. In vitro matrix erosion and drug release studies were performed. Each formulation was immersed in 100mL PBS (pH7.0; 37°C), placed in an orbital shaking incubator, rotated at 50rpm. The mass loss (%) was determined after 24 hours of hydration and samples taken at pre-determined time interval were analyzed by UV spectroscopy for AZT content.

Results.

The matrices were uniform in mass (800±0.48mg). Textural analysis confirmed BHN values ranging from 4.587-4.881N/mm^2 while friability was 0.29% demonstrating desirable matrix compatibility and compressibility. All formulations presented with <5% matrix mass loss, confirming controlled erosion kinetics. The high compressibility of PLGA and EC coupled with the ability of GL, BWX and XG to form relatively thickened gel-like macrostructures, contributed to the controlled erosion and BHN values obtained. Controlled AZT release representing first-order kinetics was obtained over a period of 48 hours primarily due to the hydrophobic nature of PLGA and EC coupled with PLGA being a crosslinked high molecular mass polymer. Likewise, GL, BWX and XG resulted into non-collapsible swelling which imposed the veracity of the matrix networked-structure, attributing to the observed drug release kinetics.

Conclusion.

Robust matrices capable of controlling drug release over 48 hours were produced. Results of this study indicate that the potential of compressing hydrophobic polymers with polymers that form relatively thickened gel-like macrostructures may produce controlled drug release formulations suitable for subcutaneous drug delivery.

APPENDIX B8

Release Rate Modulation and *Ex Vivo* Diffusion of Zidovudine from a Stealth Bioadhesive Polymeric Matrix Through Porcine Vaginal Tissue

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Introduction:
The purpose of this study was to develop and evaluate a bioadhesive zidovudine (AZT)-loaded radio-opaque polymeric matrix for intravaginal drug delivery. The diffusion dynamics of the AZT-loaded matrix through porcine vaginal tissue was also assessed.

Methods:
Biodegradable and biocompatible polymers namely ethylcellulose (EC) and polyamide 6,10 (PA 6,10) were blended with model drug AZT and compressed into robust matrices using a Carver Press at 15 tons. One set of matrices were coated with 2%$^{w}/_{v}$ PAA while another remained uncoated. Thermal analysis was performed by Differential Scanning Calorimetry (DSC) on the constituent polymers to assess the physicomechanical properties. Biodhesivity testing was conducted on both sets using a Textural Analyzer. Fresh porcine vaginal tissue (1.5±0.1mmin thickness and 15±1mm in diameter) was employed for the diffusion studies. Diffusivity was measured using Franz Diffusion Cells with simulated vaginal and plasma fluids (pH 4.5 and ph 7.4; 37°C) for the donor and receiving compartments respectively over 24 hours and computing the steady state flux of AZT through the vaginal tissue ($J_s=Q_r/A_t$). AZT release analysis was performed by placing the matrices in closed jars containing 100mL of simulated vaginal fluid at 20rpm at 37±0.5°C over 14 days in a shaking incubator. Samples were analyzed by UV spectroscopy.

Results:
Textural profile analysis indicated that the bioadhesivity of the coated matrices (PAF=3.699±0.0.464N; AUC_{FD}=0.0098±0.0004J) were superior to the uncoated matrices (PAF=1.198±0.150N; AUC_{FD}=0.0019±0.0001J). These results were substantiated by the chemometric models generated and further supported by *in vivo* bioadhesive testing. X-ray imaging facilitated by the inclusion of $BaSO_4$ in the matrices revealed the devices to be adherent to the pig vaginal tissue for over 30 days. The polymeric matrices had a substantial matrix strength and firmness (T_g=133.0°C and 45.0°C for EC and PA 6,10 respectively) that could control and prolong the quantity of AZT released thus resulting in a 12 day controlled AZT release pattern representing zero-order kinetics. These results can be attributed to the hydrophobic nature and high compressibility properties of EC coupled with high matrix resilience properties of PA 6,10. Pertaining to the AZT diffusion behaviour, an initial sharp increase of AZT influx was realized at the onset that increased consistently up to 12 hours ($Js=0.1547mg.cm^{-2}h^{-1}$), thereafter decreasing steadily which indicated that potential drug transport mechanisms within the vaginal tissue were saturated with AZT and thus a large proportion of AZT was retained within the vaginal tissue.

Conclusion:
The matrices were sufficiently bioadhesive for intravaginal drug delivery. The ability of EC and PA 6,10 to control the release of AZT was also confirmed. Diffusion studies indicated that the polymeric matrices may be suitable for prolonged intravaginal drug delivery.

APPENDIX B9

The Influence of an Innate Moisture-Protective Shell on Drug Release from a Composite Polymeric Matrix

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Introduction:
Drug release from polymeric platforms can be controlled and extended by having an outer coating which is substantially impermeable to the entrance of an environmental fluid. The purpose of this study was therefore to assess the influence of a moisture-protecting agent namely shellac on drug release from polymeric platforms for controlled drug delivery.

Methods:
Hydrophilic and hydrophobic polymers were blended with modal drug zidovudine (AZT) and compressed into caplet shaped polymeric platforms on a Manesty D3B machine using 9X22mm oblong tooling at a compression pressure of 25tons. One batch of caplets were coated with PAA ($2\%^{w}/_{v}$) while the other batch was undercoated with shellac at a concentration of 4mg/tablet and thereafter coated with PAA. Rheological analysis on PAA as the coating was performed. *In vitro* drug release studies were performed to evaluate and compare the effect of coating on AZT release. Platforms were immersed in 100mL simulated vaginal fluid (pH 4.5; 37°C) and then placed in an orbital shaking incubator maintained at 20rpm. Samples (5mL) were withdrawn at specific time intervals over a period of 30 days and analyzed by UV spectroscopy at 254nm.

Results:
The shellac-coated platforms displayed an extended controlled AZT release pattern as compared to the uncoated platforms (26 vs. 20 days of constant AZT release) due to the inherent moisture protecting effect of shellac and its ability to act as a natural plasticizer. Rheological analysis revealed that PAA was transformed into a mucilage-like substance with short flow rheological dynamics ($\eta=900\times10^{-3}$mPas; $\tau=180$Pa) portraying its potential as a coating agent. This may also be associated with the high degree of crosslinking within PAA.

Conclusion:
This study revealed that shellac as an undercoating and PAA as a surface coating may be employed effectively for controlling the rate of AZT release from the polymeric platforms. Furthermore, shellac has revealed the potential to be used as a suitable matrix component for extended release of AZT.

APPENDIX B10

P47

A comparison between the bioadhesivity potential of two novel polymer-based matrices for intravaginal drug delivery

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Purpose

Hydrophilic polymers adhere to mucosal surfaces as they attract water from the mucus layer on the epithelial surface. Various kinds of adhesive forces such as hydrogen bonding between the adherent polymers and mucus are involved in mucoadhesion at the molecular level. Polyacrylic acid (PAA) polymers have demonstrated to create tenacious bonds with the mucus membrane resulting in strong bioadhesion, a property that renders them as carriers for transmucosal drug delivery systems. This study compares the bioadhesive effects of formulations containing among other polymers, PAA 974 and PAA 934 in a simulated vaginal mucosal environment.

Methods

A Mixture Design Extreme Vertices Formulation Template was used to produce various formulations of polymers which included the ethylene oxide and acrylic acid type polymers. The study consisted two sets of experiments with the first involving PAA 934 and the second PAA 974. Powders were blended using an Erweka cube blender. The blends were then granulated and compressed into tablets using a Beckmann hydraulic press at a force of 3 tons. Bioadhesive testing was then conducted by measuring the force (measured as bioadhesive strength) required to break the adhesive bond between the simulated vaginal membrane (i.e. dialysis flat sheet membrane, Mw. 12-14,000) and the tablet as well as the work of adhesion i.e. the area under the Force Distance Curve (AUC). The simulated membrane and the sample tablets were hydrated for 30 mins in simulated vaginal fluid (pH 4.5), before conducting tests on a Texture Analyser (TAXT plus Stable Microsystems, UK). Two sets of experiments (i.e. with PAA 934 and 974) were then optimized on Minitab® experimental design software and then subjected to similar testing.

Results

For both sets of experiments (i.e. Carbopol 934 and 974), textural profiling showed that there was a direct correlation between the force of adhesion and work of adhesion. Values for bioadhesivity and work of adhesion for PAA 974 formulations were higher than 934. The increase in bioadhesivity was 46.8% while the increase in work of adhesion was 79.1%. Either, the optimized matrix force of adhesion results obtained by Minitab® experimental design software, presented a significantly higher bioadhesivity value for PAA 974 in which the extent of increase in bioadhesivity was 14.1%. The desirability (D) values were 1 and 0.9 respectively. This can be associated with the higher degree of cross-linking in PAA 974 as compared to 934 as a result of the type of cross-linking agent used. Carbopol 934 is cross-linked with Allyl sucrose while Carbopol 974 is crosslinked with allyl-penta-erythritol which has superior cross-linking ability.

APPENDIX B11

P48

Development of a method to investigate the *ex vivo* bioadhesivity of a novel polymer-based platform on freshly excised rabbit vaginal tissue

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Purpose

Ensuring prolonged contact of a drug with a body tissue, by using bioadhesive polymers, can significantly improve the performance of many drugs. Such improvements range from better prevention and treatment of local pathologies to improved bioavailability and controlled release to enhanced patient compliance. The aim of this study therefore, was to develop a bioadhesion test method for intravaginal drug delivery systems, employing novel bioadhesive hydrophylic polymers and freshly excised rabbit vaginal tissue.

Methods

The polymer-based intravaginal drug system was prepared by using various bioadhesive hydrophylic polymers such as Ethylene Oxide and Carageenan. An adult female New Zealand White (NZW) rabbit weighing 3.5kg was euthanized with sodium pentobarbitone (200mg/kg). The pelvic canal of the rabbit was opened by dissecting through the symphysis pubis of the pelvis and exposing the intra-abdominal vaginal tract (the vestibulum). The external vaginal tract was carefully dissected from the surrounding tissues and then removing the vagina. An incision was made through the vaginal canal to expose the inner lining, kept in an airtight vial and immediately subjected to bioadhesivity testing. Firstly, the vaginal membrane was secured on a Texture Analyzer (TAXT plus Stable Microsystems, UK) probe. Then a sample tablet produced using a Mixture Design Extreme Vertices Formulation Template was fixed on the Texture Analyzer platen after being exposed to a vaginal simulated fluid (pH 4.5) 30 minutes before conducting the test. The parameter settings were: Pre-test speed 2.00mm/sec, test speed 2.00mm/sec, post test speed 10.0mm/sec, applied/contact force 0.49033N, return distance 4.00mm/sec and contact time 5 secs. Bioadhesive testing was then conducted in triplicate using a highly sensitive Textural Analyser by measuring the force (measured as bioadhesive strength) required to break the adhesive bond between the vaginal membrane and the tablet, as well as the work of adhesion i.e. the area under the Force Distance Curve (AUC).

Results

The bioadhesivity was 0.907±0.066N while the work of adhesion was 0.137±0.002N/Sec. The results portrayed a direct correlation between the force of adhesion and the work of adhesion. The optimized matrix obtained by Minitab® experimental design software which had a desirability (D) value of 1, disclosed that there was a close correlation between the predicted (1N) and the experimental (0.907±0.066N) bioadhesive force results. These results highly convince that the method developed may be useful for measuring the bioadhesivity of intravaginal drug delivery systems on the vaginal membrane.

APPENDIX B12

Theme: Science in Therapeutics

ST-O-2

A Resident Homeo-Prophylactic Intravaginal Polymeric Caplet for the Prevention of HIV/AIDS and STIs

Valence Ndesendo

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School of Therapeutic Sciences

Purpose: To design and develop a rate-modulated zidovudine (AZT)-loaded intravaginal bioadhesive polymeric caplet for the prevention of STIs and HIV/AIDS.
Methods: Polymeric caplets were prepared by directly compressing a blend of polyamide 6,10, PLGA, ethylcellulose, polyvinyl alcohol, polyacrylic acid, barium sulphate and AZT as a model drug. Bioadhesivity testing was performed by measuring the peak adhesive force (PAF) and work of adhesion (AUCFD) from textural profiles. In vitro drug release studies were performed using simulated vaginal fluid (pH 4.5; 37°C). In vivo bioadhesivity testing was performed on a large white pig model. X-ray imaging employing radiopaque $BaSO_4$ was used to assess the bioadhesivity and degradation pattern of the caplets in the pig vagina.
Results: Textural profiles revealed relatively high PAF (3.6992±0.464N) and AUCFD (0.0098±0.0004J) values which indicated that the caplets were substantially bioadhesive. AZT was released in a rate modulated manner over 8 days. X-ray imaging enabled the detection of the caplets as well as their sequential degradation and spreading in the aginal canal.
Conclusions: Results suggest that the vaginal caplets may be a feasible strategy for developing a drug delivery system that has the potential to prevent STIs and HIV/AIDS by intravaginal administration.

APPENDIX B13

An *In vitro, Ex vivo, In vivo* Analysis as well as Histopathological and Histomorphological Evaluation of an Intravaginal Microbicidal Bioadhesive Polymeric Device for the Prevention of HIV and STIs.

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Introduction:
The global HIV/AIDS epidemic calls for the need to search ideal microbicides for curbing transmucosal transmission of HIV and STI pathogens. The aim of this study therefore was to develop an Intravaginal Bioadhesive Polymeric Device (IBPD) loaded with zidovudine (AZT) and polystyrene sulfonate (PSS) for the prevention of HIV and STIs.

Methods:
Suitable polymers were blended with model drugs AZT, PSS, barium sulfate ($BaSO_4$) then compressed into IBDPs, and thereafter subjected to *in vitro* drug release analysis, *ex vivo* drug flux analysis and *ex vivo* and *in vivo* bioadhesivity testing. Furthermore, the IBPDS were also subjected to *in vivo* drug content analysis as well toxicity evaluation in the pig model

Results:
Controlled drug release was obtained over a period of 72 days. A suitable *ex vivo* drug flux and *ex vivo* bioadhesivity was obtained. The IBPDs could adhere to the vaginal tissue over 30 days. Most of the drug was retained in the vaginal tissue (77%) with only little quantity crossing to the blood (22%). Histopathological and histomorphological evaluation indicated that the IBPDs were non-toxic.

Conclusions:
Results suggest that the developed IBPDs may be suitable for use as a localized intravaginal drug delivery system for the potential prevention of HIV infection and STIs.

Ethical clearance No. 2007/25/05.

APPENDIX C

ADDITIONAL OUTPUTS FROM COLLABORATIVE RESEARCH

APPENDIX C1

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Nanotechnological applications for the treatment of neurodegenerative disorders

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ABSTRACT

Nanotechnology employs engineered materials or devices that interact with biological systems at a molecular level and could revolutionize the treatment of neurodegenerative disorders (NDs) by stimulating, responding to and interacting with target sites to induce physiological responses while minimizing side-effects. Conventional drug delivery systems do not provide adequate cyto-architecture restoration and connection patterns that are essential for functional recovery in NDs, due to limitations posed by the restrictive blood–brain barrier. This review article provides a concise incursion into the current and future applications of nano-enabled drug delivery systems for the treatment of NDs, in particular Alzheimer's and Parkinson's diseases, and explores the application of nanotechnology in clinical neuroscience to develop innovative therapeutic modalities for the treatment of NDs.

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APPENDIX C2

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Review

Trends in the Molecular Pathogenesis and Clinical Therapeutics of Common Neurodegenerative Disorders

Yahya E. Choonara [1], Viness Pillay [1,*], Lisa C. du Toit [1], Girish Modi [2], Dinesh Naidoo [3], Valence M.K. Ndesendo [1] and Sibongile R. Sibambo [1]

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Abstract: The term neurodegenerative disorders, encompasses a variety of underlying conditions, sporadic and/or familial and are characterized by the persistent loss of neuronal subtypes. These disorders can disrupt molecular pathways, synapses, neuronal subpopulations and local circuits in specific brain regions, as well as higher-order neural networks. Abnormal network activities may result in a vicious cycle, further impairing the integrity and functions of neurons and synapses, for example, through aberrant excitation or inhibition. The most common neurodegenerative disorders are Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis and Huntington's disease. The molecular features of these disorders have been extensively researched and various unique neurotherapeutic interventions have been developed. However, there is an enormous coercion to integrate the existing knowledge in order to intensify the reliability with which neurodegenerative disorders can be diagnosed and treated. The objective of this review article is therefore to assimilate these disorders' in terms of their neuropathology, neurogenetics, etiology, trends in pharmacological treatment, clinical management, and the use of innovative neurotherapeutic interventions.

Keywords: Parkinson's disease; Alzheimer's disease; Amyotrophic Lateral Sclerosis; Huntington's disease; neuropathology; amyloid-β protein; Tau; Huntingtin; α-Synuclein; neurotherapeutics; drug delivery

APPENDIX C3

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Design, biometric simulation and optimization of a nano-enabled scaffold device for enhanced delivery of dopamine to the brain

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Box–Behnken design
Sprague–Dawley rat model

ABSTRACT

This study focused on the design, biometric simulation and optimization of an intracranial nano-enabled scaffold device (NESD) for the site-specific delivery of dopamine (DA) as a strategy to minimize the peripheral side-effects of conventional forms of Parkinson's disease therapy. The NESD was modulated through biometric simulation and computational prototyping to produce a binary crosslinked alginate scaffold embedding stable DA-loaded cellulose acetate phthalate (CAP) nanoparticles optimized in accordance with Box–Behnken statistical designs. The physicomechanical properties of the NESD were characterized and *in vitro* and *in vivo* release studies performed. Prototyping predicted a 3D NESD model with enhanced internal micro-architecture. SEM and TEM revealed spherical, uniform and non-aggregated DA-loaded nanoparticles with the presence of CAP (FTIR bands at 1070, 1242 and 2926 cm^{-1}). An optimum nanoparticle size of 197 nm (PdI = 0.03), a zeta potential of −34.00 mV and a DEE of 63% was obtained. The secondary crosslinker $BaCl_2$ imparted crystallinity resulting in significant thermal shifts between native CAP (T_g = 160–170 °C; T_m = 192 °C) and CAP nanoparticles (T_g = 260 °C; T_m = 268 °C). DA release displayed an initial lag phase of 24 h and peaked after 3 days, maintaining favorable CSF (10 μg/mL) versus systemic concentrations (1–2 μg/mL) over 30 days and above the inherent baseline concentration of DA (1 μg/mL) following implantation in the parenchyma of the frontal lobe of the Sprague–Dawley rat model. The strategy of coupling polymeric scaffold science and nanotechnology enhanced the site-specific delivery of DA from the NESD.

APPENDIX C4

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Chemometric, physicomechanical and rheological analysis of the sol–gel dynamics and degree of crosslinking of glycosidic polymers

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Abstract

The influence of calcium (Ca^{2+}), zinc (Zn^{2+}) and barium (Ba^{2+}) ions on the sol–gel interconversion dynamics, degree of crosslinking and the matrix resilience of crosslinked alginate gelispheres was determined. The dependent compositional and operational variables of crosslinking make it a challenging task to optimize the degree of crosslinking and the physicomechanical properties of alginate gelispheres. The combinatory approach of textural profiling, assessing pertinent rheological descriptors and chemometric model analysis of the sol–gel interconversion mechanisms and energy paradigms involved during crosslinking, hydration and erosion of gelispheres was explored. Molecular structural modelling of the gelispheres provided a mechanistic understanding of the sol–gel interconversion phenomena and their influence on the degree of crosslinking, the hydrational dynamics and gelisphere formation. Rheological analysis revealed offset yield point values of 6.1 mg ml^{-1} and 8.0 mg ml^{-1} were computed from fitted regression curves for determining the crosslinker concentration required for combinatory crosslinkers such as Ca/Zn/Ba ions and Ba/Zn, respectively. The influence of hydration on the erosion was a direct function of the gelispheres physicomechanical strength. Textural profiling characterized the gelisphere matrices for their resilience. The various crosslinkers interacted with monomeric units at varying intensities. Ba-crosslinked gelispheres were brittle with dense polymeric networks. Zn-crosslinked gelispheres produced permeable resilient matrices when hydrated and Ca-crosslinked gelispheres demonstrated intermediate resilience with greater G/M ratio alginate grades. Chemometrical analysis explicated a potential link between several phenomena such as the type of crosslinkers employed, the static shear-rate viscosity attained, the matrix resilience and the associated sol–gel mechanisms and energy paradigms of crosslinked gelispheres.

(Some figures in this article are in colour only in the electronic version)

APPENDIX C5

The influence of polyamide 6,10 synthesis variables on the physicochemical characteristics and drug release kinetics from a monolithic tablet matrix

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Abstract

This study investigated the influence of solute-solvent quotients on the physicochemical properties and release kinetics of two amitryptyline-loaded polyamide 6,10 (PA 6,10) monolithic matrices, Formulations A and B (FA and FB). The molecular mass, crystallinity, structural elucidation and thermo-transitions were assessed using mass spectrophotometry, X-ray diffraction, Fourier-Transform Infra-red spectroscopy and differential scanning calorimetry. Surface morphologies of the matrices and physicomechanical strength were captured using scanning electron microscopy and textural analysis. Drug release, distension and matrix erosion were evaluated using mathematical modeling. FA and FB displayed overall drug release fractions of 0.58 and 0.92 with 55% and 30% of matrix remaining over 24 hours respectively. The indentation diameters (FA=1.51mm; FB=2.39mm), deformation energies (FA=0.02J; FB=0.03J) and Brinell Hardness Numbers (FA=17.88N/mm^2; FB=14.45N/mm^2) were divergent. SEM revealed irregular matrix surfaces with varying pore distributions. Minimal shifts in the structural backbone of PA 6,10 and semi-crystallinity was noted from FTIR and XRD analysis. Multiple reversible and irreversible thermal transitions with molar masses of FA=345.2g/mol and FB= 307.2g/mol were obtained. Drug release followed zero-order kinetics regulated by matrix relaxation ($k_2 > k_1$). In conclusion, the physicochemical properties of FA and FB were reversibly influenced by differences in the PA 6,10 solute-solvent quotient employed during development.

Keywords: Polyamide 6,10; monolithic matrix; amitryptiline hydrochloride, physicochemical; kinetic modeling, controlled release, solute-solvent quotients.

For Peer Review Only

APPENDIX C6

The simultaneous *in vitro* characterization of poly(lactic co-glycolic acid) and poly(glucuronide)-rich nanoparticles employing various sol-gel synthetic wet chemical processing strategies

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Abstract

This study focussed on the formulation and evaluation of polymeric nanoparticles adopting various preparation approaches and attempts to explicate the mechanisms of nanoparticle formation employing molecular modeling and chemometrical computations. Nanoparticles were formulated using three different approaches such as an Emulsification/Solvent Evaporation (ESE), Emulsification/Surfactant/Solvent Evaporation (ESSE) and Ionic Gelification (IG) approaches. The ESE approach comprised the emulsification of an aqueous and organic solution, while sorbitan monooleate was added as a surfactant during the ESSE approach. Cation-induced crosslinking of hydrophilic alginate was employed for the IG approach. Fourier Transform Infrared (FT-IR) analysis was performed to elucidate any changes in the structural backbone of the native polymers due to nanoparticle formation. The size and morphology of nanoparticles were analysed by Zetasize analysis and Scanning Electron Microscopy (SEM) with photomicrographs taken at several magnifications. Step-wise molecular simulation models revealed the mechanisms of nanoparticle formation to occur via solvation, surface interactions, crosslinking/precipitation initiation and surface-volume minimization with sphericalization and interlaced network formation. The size distribution of the nanoparticles were manipulated by the surfactant introduction The addition of sorbitan monooleate prevented coalescence of particles, resulting in stable nano-emulsions with distinct particle morphologies and particle sizes and zeta potential values in the region of 200nm and -40mV, respectively. The IG approach also produced stable nano-emulsion with a higher yield of nanoparticles with superior size and stability control of confined nanoparticles. Both the ESSE and IG approaches were found to be suitable for producing stable nano-emulsions that may potentially be employed for the novel delivery of various drug molecules.

Keywords: Polymeric nanoparticles, alginate, poly(lactide-co-glycolide), polymeric characterization, surfactant, crosslinking, emulsification, drug delivery

APPENDIX C7

Applications of Nanotechnology in Parkinson's and Motor neurone diseases

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Abstract

Parkinson's and motor neurone diseases are ruthless nerve disorders. While the etiology of these diseases is not yet fully known the drugs so far available for treating these conditions are still not as effective and often have serious side-effects. The greatest challenge in treating these conditions lies in the difficulties involved in delivering drugs to the brain which is due to the resistance imposed by arachnoid tight junctions, choroid plexus and blood brain barrier (BBB). Treatment strategies, such as use of alternative drugs (e.g. Levo dopa instead of dopamine), viral vectors, gene, and lipid therapy have not been as successful, due to either the inability to cross the BBB, or development of side effects or triggering of immune responses. Nanotechnology poses to be a likely solution to these challenges. Thus far, nanotechnology has enabled the creation of smart nanomaterials and microdevices that can deliver drug molecules (e.g. dopamine) to the nervous system conveniently. This review article addresses various avenues in which nanotechnology has been involved in the management of Parkinson's and Motor neurone disease to date, with specific emphasis on nano-based therapeutic modalities that can limit and reverse neuropathological disease states, support and promote functional regeneration of damaged neurons, provide neuroprotection, as well as facilitate the delivery of drugs and small molecules across BBB. Nano-surgery as well as the diagnosis of Parkinson's and motor neurone diseases through nano-based techniques has also been discussed.

Keywords: Parkinson's disease, Motor neurone disease, Dopamine, Brain delivery, Nano-based drug delivery systems.

APPENDIX C8

Review: Advances in the Approaches for Enhancement of Oral Drug Bioavailability

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Abstract

In conscious and co-operating patients, oral drug delivery remains the preferable route of drug administration. However, not all drugs possess the desirable physicochemical and pharmacokinetic properties which favour oral administration mainly due to poor bioavailability. This has in some cases led to the choice of other routes of administration, which may compromise the convenience and increase the risk of non-compliance. Poor bioavailability has necessitated the administration of higher than normally required oral doses which often leads to economic wastages, risk of toxicity, erratic and unpredictable responses. The challenge over the years has been to design techniques that will allow oral administration of most drugs, irrespective of their properties and yet achieve a therapeutic systemic availability. This will be a worthy achievement since over 90% of therapeutic compounds are known to possess oral bioavailability limitations. In this review, an attempt has been made to explore various approaches that have been used in the recent years to improve oral drug bioavailability, including physical and chemical means. Design of prodrugs to bypass metabolism or enhance solubility as well as modification of formulation techniques such as use of additives, permeation enhancers, solubilizers, emulsifiers and non aqueous vehicles have been discussed. Pharmaceutical application of nanotechnology, which is an emerging area in drug delivery, has also been addressed. This review sought to assess each method aimed at enhancing the oral bioavailability of drugs in terms of the purpose, scientific basis, limitations, commercial application as well as the areas for further research improvement.

Keywords: Absorption, Bioavailability, Cytochrome P450, Efflux transporters, Enzymes, Inhibitors, Permeation enhancers, P-glycoproteins, Pre-systemic metabolism

APPENDIX C9

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Abstract:

Membrane technology is broadly applied in the medical field. The ability of membranous systems to effectively control the movement of chemical entities is pivotal to their significant potential for use in both drug delivery and surgical/medical applications. An alteration in the physical properties of a polymer in response to a change in environmental conditions is a behavior that can be utilized to prepare 'smart' drug delivery systems. Stimuli-responsive or 'smart' polymers are polymers that upon exposure to small changes in the environment undergo rapid changes in their microstructure. A stimulus, such as a change in pH or temperature, thus serves as a trigger for the release of drug from membranous drug delivery systems that are formulated from stimuli-responsive polymers. This article has sought to review the use of stimuli-responsive polymers that have found application in membranous drug delivery systems. Polymers responsive to pH and temperature have been extensively addressed in this review since they are considered the most important stimuli that may be exploited for use in drug delivery, and biomedical applications such as in tissue engineering. In addition, dual-responsive and glucose-responsive membranes have also been addressed as membranes responsive to diverse stimuli.

Keywords: Membranous drug delivery systems, stimuli-responsive polymers, dual responsive membranes, glucose responsive membranes, pH, temperature, "On-off" gating mechanisms.

APPENDIX C10

A Modified Crosslinked Oramucosal Lyophilized Wafer System for Gradual Drug Delivery

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Abstract
The aim of this study was to develop a polymeric wafer system targeted for gradual oramucosal drug delivery. The mucoadhesive polymer pectin was crosslinked with various salts such as calcium chloride, barium chloride and zinc sulphate for the production of the wafers and subjected to three methods of lyophilization. A simultaneous qualitative and quantitative analysis of the wafer surface morphology and porosity was undertaken. In addition, vibrational transitions, the thermal behavior as predicted by temperature modulated differential scanning calorimetry, textural attributes, and *in vitro* drug release behavior of the highly water soluble model drug, diphenhydramine HCl, from the various wafer systems were systematically characterized. The techniques employed yielded wafers of varying porosities. Crosslinked pectin wafers generally demonstrated a smaller pore size (having a mean diameter of 107.63Å compared to 180.53Å for non-crosslinked pectin wafers); due to the highly interconnected matrix structure afforded by the formation of inter- and intra-molecular crosslinks between the pectin chains. The drug release capabilities of the wafers varied markedly with the approach employed in the production of the wafers. Non-crosslinked wafers released 82.5% of drug within 30 minutes. Wafers that included a crosslinking step in their formulation design showed a more gradual release of drug. Furthermore, wafers crosslinked after the lyophilization process controlled drug release more efficiently than those that were crosslinked before lyophilization. Vibrational and thermal analysis confirmed the crosslinking effect and resultant drug release capabilities of the wafer systems. There was a strong negative correlation between the normalized pore size of the wafers and their resilience (R^2=0.99). It was established that Å), possessed the propensity to form a number of ionic bridges between pectin monomers in intra- and inter-molecular associations producing an interconnected wafer matrix, culminating in wafers of high porosity (0.048cm^2/g) on lyophilization.

Keywords: Oramucosal, wafer matrix, mucoadhesive polymer, gradual drug release, lyophilization, crosslinking, porosity, physicochemical, physicomechanical

APPENDIX C11

Levodopa Delivery Systems: Advancements in Delivery of the Gold Standard

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Abstract

Background:
Despite the fact that Parkinson's disease (PD) was discovered almost 200 years ago, its treatment and management remains an immense challenge since progressive loss of dopaminergic nigral neurons, motor complications experienced by the patients as the disease progresses and drawbacks of pharmacotherapeutic management still persists. Various therapeutic agents have been employed in the management of PD, including levodopa (L-dopa), selegiline, amantadine, bromocriptine, entacapone, pramipexole dihydrochloride and more recently istradefylline and rasagiline. Of all agents, L-dopa though the oldest, remains the most effective. L-dopa is easier to administer, better tolerated, less expensive and is required by almost all PD patients. However, L-dopa's efficacy in advanced PD is significantly reduced due to metabolism, subsequent low bioavailability and irregular fluctuations in its plasma levels. To date, significant strides have been made to improve the delivery of L-dopa in order to enhance its bioavailability and reduce plasma fluctuations as well as motor complications experienced by patients purportedly due to pulsatile stimulation of the striatal dopamine receptors.

Objective:
The ultimate aim was to critically assess the attempts made thus far directed at improving L-dopa absorption, bioavailability and maintenance of constant plasma concentrations, including the drug delivery technologies implicated.

Methods:
Drug delivery systems that have been instituted for the delivery of L-dopa include immediate release formulations, liquid formulations, dispersible tablets, controlled release formulations, dual release formulations, microspheres, infusion and transdermal delivery, among others. In this review, the L-dopa loaded drug delivery systems developed over the past three decades were elaborated on.

Conclusion:
This review highlights the fact that neuropharmaceutics is at a precipice, which is expected to spur investigators to take that leap to enable the generation of innovative delivery systems for the effective management of PD.

Keywords: Bioavailability, Conventional dosage forms, Drug delivery systems, Levodopa, Microspheres, Motor complications, Neuropharmaceutics, Nanotechnology, Parkinson's disease, Pulmonary delivery, Transdermal delivery.

APPENDIX C12

Review Article: Recent Advances in the Design of Drug-loaded Polymeric Implants for the Treatment of Solid Tumors

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Abstract

The effective treatment of solid tumors still continues to be a great challenge to clinicians despite the development of novel drugs. In order to improve the clinical efficacy of the existing chemotherapeutics agents, researchers have considered the possibility of local treatment at the site of the solid tumor. The greatest advantage of this localised delivery is the significantly fewer side effects experienced by the patient. In the past, the peri- or intra- tumoral delivery of chemotherapeutics agents was mainly based on implants that used to be inserted surgically into the affected region. In the recent years, *in situ* forming implants have attracted considerable interest. These are polymeric systems which are injected as solutions into the tumor site using commercially available syringes and needles. The injected solution forms an implant at the tumor site as a result of local environmental stimuli and hence removes the need for surgical implantation. However, while these implants have been shown to improve the treatment of various solid tumors, the ideal implant is yet to be formulated. To date, it is only a few implants that are biodegradable and able to deliver the chemotherapeutic agent over a prolonged period of time. Many of these implants also have an undesirable initial burst release effect. This review summarises the attempts that have so far been made in the development of polymeric implants for the treatment of solid tumors.

Keywords: Chemotherapy, Environmental stimuli, Implant, Polymeric systems, Solid tumors.

APPENDIX C13

A review on oral drug delivery systems comprising altered geometric configurations for controlled drug delivery

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Abstract

Recent pharmaceutical research has focused on controlled drug delivery as an advantage over conventional methods. Adequate controlled plasma drug levels, reduced side effects as well as improved patient compliance are some of the benefits that these systems may offer. Controlled delivery systems that can provide zero-order drug delivery have the potential for maximizing efficacy while minimizing dose frequency and toxicity. Thus, zero-order drug release is ideal in a large area of drug delivery which has therefore led into the development of various technologies with such drug release patterns. Systems such as multilayered tablets and other geometrically altered devices have been created to perform this function. One of the principles of multilayered tablets involves creating a constant surface area for release. Polymeric materials play an important role in the functioning of these systems. Technologies so far developed include among others, Geomatrix® multilayered tablets which utilizes specific polymers that may act as barriers to control drug release; Procise® which has a core with an aperture that can be modified to achieve various types of drug release, core-in-cup tablets where the core matrix is coated on one surface while the circumference forms a cup around it, donut-shaped devices which have a hole in the middle, Dome Matrix® and “release modules assemblage” which can offer alternating drug release patterns. This review discusses the novel altered geometric system technologies that have been developed to provide controlled drug release, also focusing on polymers that have been employed in such developments.

Keywords:

Controlled drug delivery, geometrically altered devices, multilayered tablets, Polymeric materials, “Release modules assemblage”.

APPENDIX C14

Pharmaceutical Applications of Electro-Spinning

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Abstract

Electro-spinning of polymers is a unique technology which produces very fine fibres using electrostatic repulsive forces obtained from applying an electrical potential to a liquid. The resultant fibres have much smaller diameters in comparison with fibres obtained using different methods. Electro-spun fibres have found application in many and varied fields such as drug and gene delivery, tissue engineering, wound dressing, electronics, filtration as well as absorption and adsorption. This review will cover the various processing parameters which play a role in electro-spinning, including the applied voltage, solution flow rate, solution viscosity, solvents, solution conductivity, capillary-to-collector distances and the influence of surfactants. A main focus will be on the application of electro-spun fibres in drug delivery, where researchers have already investigated the use of these fibres in transdermal delivery systems, long-term implants and grafts. Different methods of drug incorporation will be discussed as well as numerous polymers which have been successfully utilised in this field, including cellulose acetate, poly(ε-caprolactone), poly(ethylene oxide), poly(vinyl alcohol), gelatin, poly(d,l-lactide-co-glycolide), poly(lactic acid), poly(l-lactic acid), polyurethane, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), and poly(acrylic acid).

Keywords: Electro-spinning, drug delivery, electrospun fibres, electrospun scaffolds, parameters,

APPENDIX C15

Design and Development of Pediatric Wafers for the Treatment of Pediatric HIV/AIDS.

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Abstract

The purpose of this study was to fabricate pediatric wafers containing 3'-azido-3'-deoxythymidine (AZT) as an antiretroviral (ARV) drug for the treatment of HIV/AIDS in pediatric patients. Oramucosal drug delivery systems have many advantages over the other routes mainly because of the ability to circumvent the pitfalls of traditional drug delivery systems by directly entering the systemic circulation via the internal jugular vein. A 5% hydroxypropylcellulose solution and a separate 1% carbopol solution were allowed to homogenize, these solutions were then combined in a ratio of 1:1. Approximately 200mg of AZT needed to be delivered to each cylindrical well of the wafer mould. Other excipients employed included mannitol and lactose as bulking agents, sodium starch glycolate as a disintegrant and glycine as an anti-collapsing agent. Cylindrical wells were lubricated with a mineral oil. 1.5mL of the final solution was transferred into each well under continuous stirring. The formulation was then frozen for a period of 24 hours at negative 60ºC. Thereafter, the formulation was lyophilized for a period of 48 hours. Extensive *in vitro* and e*x-vivo* studies including disintegration test, determination of DLC and DEE, drug release analysis and bioadhesivity testing were conducted on the wafer systems. An optimal formulation consisting of a ratio of 1:2 (HPC/C934), glycine (0.03g) as an anti-collapsing agent and sodium starch glycolate (4%w/w) as a disintegrant was obtained. This optimal formulation had a disintegration time as short as 6.7 seconds, a high DLC (190.4mg) and a desirable DEE (95.175%). A zero order drug release kinetics was realized (optimal formulation) and almost the whole quantity of drug was released within 10 seconds. The developed wafers were substantially bioadhesive. The Peak Adhesion Force and the work of adhesion (AUC_{FD}) for the optimal wafer system were PAF=0.193±0.004N and AUC_{FD}=0.104±0.006J respectively. The developed pediatric a wafers may be suitable for the treatment of pediatric HIV/AIDS.

Keywords: Bioadhesivity, HIV/AIDS, Drug loading capacity, Drug entrapment efficiency, Disentergration time.

APPENDIX C16

Fabrication, characterization and optimization of MTX-loaded PLA/MAA nanoparticles

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Abstract The purpose of this research was to develop poly (DL-lactide) (PLA)/Eudragit S 100 (ES 100) nanoparticles (NP) with the potential to serve as carrier systems for methotrexate (MTX) an anticancer drug. The nanoparticles were prepared by double emulsion solvent evaporation technique. A 3-Factor Box Behnken design was applied for preparing various nanoparticle formulations. The size of the nanoparticles varied from 211 nm to 378.3 nm and the nanoparticle recovery varied from 36.8 mg to 86.2 mg. Most formulations showed a poly-dispersity index (PdI) value less than 0.5 indicating that the nanoparticle size distribution was homogenous within the formulations. The drug loading capacities (*DLC*) of the nanoparticles were very low with the maximum being 12 % for the optimized formulation. In order to improve the drug loading capacity of the optimized formulation, we adsorbed the drug onto the nanoparticles matrix by incubating the nanoparticles in a concentrated MTX solution. By so doing, we successfully improved drug loading to 98 %. Fourier-Transform Infrared (FTIR) studies showed that MTX was present in the nanoparticles. The nanoparticles showed an initial burst release with 50% of MTX released in 24 hours, thereafter the release was prolonged for 84 hours. Scanning electron micrographs revealed almost spherical solid nanoparticles with a smooth surface and transmission electron micrographs showed the presence MTX within the nanoparticles.

Keywords: Poly (DL-lactide) (PLA), Eudragit S 100 (ES 100), Methotrexate (MTX) and Drug loading capacity (*DLC*)

APPENDIX C17

Modulation of Nicotine Release from a Gelisphere–Loaded Compressed External Polymeric Matrix

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Abstract

The purpose of this study was to develop a prolonged-release drug delivery device for site-specific delivery of a newly researched neuroprotective agent, namely nicotine. The device was formulated as a novel reinforced crosslinked composite polymeric system with the potential for implantation into the substantia nigra pars compacta of the brain to provide site-specific drug delivery for the treatment of Parkinson's disease. Polymers with biocompatible and bioerodible characteristics were selected to incorporate nicotine within electrolyte-crosslinked alginate- hydroxyethylcellulose gelispheres compressed within a release-rate modulating external polymeric matrix, comprising either hydroxypropylcellulose, polyethylene oxide and poly(lactic-co-glycolic) acid to further attenuate nicotine release. The degradation and erosional behavior of the discs following exposure to simulated CSF are also conducted to elucidate their influence on drug release. Kinetic modeling of drug release data was performed to understand the predominant mechanisms involved in controlling nicotine release from the device. Zero-order drug release was observed for a period of 50 days in simulated cerebrospinal fluid from the device comprising poly(lactic-co-glycolic) acid as the external matrix.

Keywords: Alginate gelispheres, textural analysis, crosslinked matrices, kinetic modeling, PLGA discs, prolonged release, BHN, powder flow properties

APPENDIX C18

The simultaneous *in vitro* analysis of poly(lactic co-glycolic acid) and poly (glucuronide)-rich alginate nanoparticles: A formulation approach

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Abstract
The purpose of this study was to formulate stable polymeric nanoparticles within a desirable particle size range suitable to be employed as carriers for drug delivery. Four approaches were employed namely an Emulsion-Solvent Evaporation (ESE), Emulsification-Surfactant-Solvent Evaporation (ESSE), Ionic Gelification (IG) and Ionic Surfactant- Gelification (ISG). The ESE approach involved the emulsification of an aqueous and organic solution, while Span 80 was added as a surfactant for the ESSE approach. Cation-induced crosslinking of hydrophilic alginate was employed for the IG approach with a modification involving the addition of Span 80 for the ISG approach. Fourier Transform Infrared (FT-IR) analysis was performed to elucidate changes in the structural backbone of the native polymers due to nanoparticle formation. The particle size, zeta potential and the polydispersion index (PdI) were determined using a ZetaSizer NanoZS instrument (Malvern Instruments (Pty) Ltd., Malvern, Worcestershire, U.K). The surface morphology of the nanoparticles was analysed by Scanning Electron Microscopy (SEM) with photomicrographs taken at several magnifications. The ESE approach produced agglomerated particles with an average size of 357nm, a PdI= 0.444 and a zeta potential value of -32.9mV. The ESSE approach formed non-agglomerated, smooth nanoparticles within a size range of 101-275.5nm with a zeta potential in the region of -25.4mV and a PdI value ranging between 0.173-0.486 that was dependant on the surfactant concentration. The IG approach resulted in particles with an average size of 3075nm as compared to the ISG approach that revealed particles within the nano-scale range of between 165.1-173.6nm, a PdI=0.605-0.628 and a zeta potential value in the region of -60.9mV. SEM revealed that the ESE approach produced spherical nanoparticles with substantial agglomeration as compared to the ESSE approach in which the degree of smoothness and reduction in coalescence and agglomeration increased as the concentration of the surfactant (Span 80) increased up to a concentration of 2.5%$^{v}/_{v}$. In both cases, the process of nanoparticle formation was related to the interfacial area generated by emulsion formation and reduction of globule size due to rapid solvent evaporation. The emulsification process and the stability of emulsion globules were the most significant factors for controlling the particle size. The IG approach produced a lower yield but spherically shaped nanoparticles with striations on the particle surface potentially due to the effect of crosslinking during nanoparticle formation. IR spectra revealed that the polymeric backbone of the constituent polymers was maintained though minor frequency vibrational changes were noted, indicative of superficial morphological transitions. Overall, the addition of a surfactant had a significant influence on nanoparticle formation in terms of shape, size, zeta potential and PdI. In conclusion, the ESSE approach was found to be the most suitable for producing a stable emulsion for nanoparticle formation with desirable particle sizes and zeta potential values. The size of the PLGA nanoparticles obtained in this study favours their use as drug delivery carriers.

Keywords: Nanoparticles, Poly(lactide-co-glycolide), Surfactant, Emulsification, Particle size, Zeta potential, Polydispersion index and Drug delivery

APPENDIX C19

2008 AAPS Annual Meeting

A biodegradable Doughnut-Shaped Minitablet for sustained intraocular release of ganciclovir

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Purpose.

To evaluate the toxicity and potential of using a biodegradable doughnut-shaped minitablet (DSMT) for intraocular sustained release of ganciclovir (GCV) in a rabbit eye model.

Methods.

Specially designed core-rod tooling was used to directly compress GCV-loaded PLGA matrices into doughnut-shaped minitablets (5×2mm). Six New Zealand White Albino rabbits were used for *in vivo* drug release studies. A DSMT was implanted through the pars plana of the right eyes. Vitreous samples from the left eyes were used as controls. GCV concentrations from vitreous samples obtained from euthanized rabbits at 3, 7, 14, 28, 48 and 70 days after implantation was measured by Ultra Performance Liquid Chromatography. Enucleated test and control eyes were histopathologically examined to ascertain the effect of the DSMT device on ocular tissues. Eyes were fixed in 10% buffered formalin for 24 hours, then bisected and processed in their entirety for histological assessment employing a hemotoxylin and eosin staining technique.

Results.

Semi-logarithmic plots revealed that GCV concentrations in the vitreous initially exceeded the therapeutic range (ED_{50}) for viral suppression in the human eye (0.10-2.75µg/mL) and thereafter remained within the therapeutic window for a period of 70 days after implantation. In contrast with previous *in vitro* release studies, the *in vivo* study displayed a reduced initial burst with 20% GCV released at day 3 followed by a steady phase of diffusional drug release of 69% after 70 days. Histological studies revealed a progressive inflammatory response centered predominately around the ciliary body and in the posterior chamber. An initial acute inflammatory response was noted with haemorrhage, oedema and neutrophilic infiltration that became chronic with lymphocytes, eosinophils, plasma cells and fibroblasts. The haemorrhage and oedema subsided by day 28. After 70 days, a minimal residual inflammatory response was noted and of significance there was no evidence of sympathetic ophthalmitis, chronic fibrosis or damage to ocular tissue.

Conclusion.

Results revealed that no ocular tissue toxicity was observed as a result of intraocular implantation of the DSMT device and therefore may be promising for the sustained intravitreal delivery of GCV.

APPENDIX C20

2008 AAPS Annual Meeting

Design and Characterization of a Pore-Regulated Matrix for Transmucosal Drug Delivery

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Purpose.

To fabricate a pore-regulated matrix, characterize its physicochemical and physicomechanical properties for applications in transmucosal drug delivery employing phenytoin sodium and porcine buccal mucosa as a model drug and mucosa respectively.

Methods.

The pore-regulated matrix (8×5mm) was produced by lyophilization utilizing combinations of cellulose, polyacrylic acid and amino-acid based polymers. Inorganic excipients were employed as dispersants in a polar protic co-solvent system. A texture analyzer fitted with either a cylindrical or flat-tipped steel probe were used for measuring bioadhesivity and matrix rigidity respectively. Fresh porcine buccal mucosa (0.9±0.1mm thick and 15±1mm in diameter) was employed for the bioadhesivity and permeation tests. Permeation was measured using Franz Diffusion Cells with simulated saliva (pH 6.8; 37ºC) and plasma (pH 7.4; 37ºC) as the donor and receiving compartments respectively over 8 hours. The flux of drug through the mucosa was then calculated; [J_{drug} =(Δct/Δt)V/S]. Drug-loading capacity (DLC) was calculated (Da/Dt×100). Drug release analysis was performed by immersing the formulation in 25mL simulated saliva in closed 100mL jars, at 20rpm and maintained at 37ºC over 8 hours in a shaker bath. Surface morphology was evaluated using SEM (20keV, 200x magnification).

Results.

Formulations adhered to the porcine buccal mucosa (1.03±0.01N). Matrix rigidity was 4.94±0.08N/mm which reflected physicochemical stability and resistance of the matrix to deformation. Permeation of phenytoin through the porcine buccal mucosa into the receiving compartment was evident (J_{drug}=1.40×10^{-4}mg/cm^{2}sec). A DLC value of 94.51±2.47% was achieved from which 88.92±2.83% of phenytoin was released over 8 hours following a pseudo-zero-order pattern. SEM images revealed a complex internal microporous structure with irregular diffusion channels and pore diameters ranging from 20-60µm.

Conclusion.

The investigated physicochemical and physicomechanical properties revealed the potential of the pore-regulated matrix to be applied for prolonged transmucosal drug delivery.

APPENDIX C21

The influence of pore morphology on the matrix integrity, drug loading and drug release from a porous delivery platform

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Purpose.

To investigate the influence of pore morphology on the matrix integrity, drug loading capacity and drug release from a porous drug delivery platform employing phenytoin sodium as a model drug.

Methods.

Interphase homogenization guided through a Box-Behnken experimental design was employed for the preparation of 15 porous drug delivery platforms. The effect of pore morphology on the matrix integrity, drug loading capacity and drug release behaviour was assessed. Each phenytoin-loaded porous platform (1.2×0.4cm) was formulated from a co-particulate, homogenous polymeric blend using specialized polystyrene moulds and lyophilization. The physicomechanical properties were elucidated in terms of matrix firmness (MF), matrix fracture energy (MF_e) and matrix resilience (MR) utilizing a texture analyzer. The nature of pores and interconnections were evaluated employing SEM. The Drug-Loading Capacity (DLC) was computed using the relationship; $D_0/D_t \times 100$. A closed system containing 25mL of PBS (pH 6.8; 37°C), placed in a shaker bath set at 20rpm was utilized for drug release studies over 8 hours.

Results.

The individual masses of the 15 formulations averaged 121.95±5.08mg. Mass distribution relative to porosity varied between formulations. The platforms demonstrated a resistance to deformation despite their porous nature (MF values ranged between 3.67±1.1-5.98±0.4Nmm-1; MF_e=0.02±0.005-0.06±0.01J; MR=1.16±0.6-3.35±1%). SEM showed a variety of pore distributions (scattered, dense and aligned) and interconnections (rigid, web-like, thread-like and spongy). The DLC values showed desirable flexibility in drug-loading. A general trend observed was that formulations displaying scattered pore distributions and web-like/spongy interconnections had lower DLC values (51.29±5.19%) as compared to dense/aligned pore distributions and thread-like/rigid interconnections which had higher DLC values (93.22±8.54%). Phenytoin was released over 8 hours in a pseudo-zero-order or first-order manner which was dependant on the pore morphology. A cumulative release of phenytoin ranging between 81.22±5.51-91.48±1.2% was obtained over the 8 hour period.

Conclusion.

The impact of pore morphology on the matrix integrity, drug loading capacity and drug release was established. Results from this study may be promising for the development of porous drug delivery platforms.

APPENDIX C22

2008 AAPS Annual Meeting

Formulation and evaluation of a buoyant prolonged release drug delivery system

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Purpose.

To formulate and evaluate a buoyant sustained-release polymeric drug delivery system and to assess the physicomechanical properties in relation to the drug release dynamics.

Methods.

Two variants of the buoyant polymeric system were prepared encompassing chitosan (CHT), sodium alginate (ALG) and polyacrylamide (PAAm). Diphenhydramine HCl (DPH) (aqueous solubility=1g/mL) was incorporated as a model drug. Both variants namely CHT-ALG-PAAm and ALG-PAAm underwent redox-initiation in a ratio of 4:1 (g/mL). The solutions were agitated at room temperature until homogenous and underwent lyophilization and subsequent drug release studies in SGF (pH 1.2; 37°C). Samples were analyzed with UV spectrophotometry. Physicomechanical analysis was conducted by textural profile analysis (TA.XT*plus*, Stable Microsystems, UK) to evaluate the matrix resilience and energy absorbed (EA) in the dry (t_0 hours) and hydrated states (t_5 hours).

Results.

Both variants demonstrated sustained release of DPH. Inclusion of chitosan (CHT-ALG-PAAm) (N=3) resulted in considerably higher drug release with 91±6.08% at t_5 hours, compared to formulations without chitosan (ALG-PAAm) (N=3) (52±2.61%). However, a 'burst' effect was observed with ALG-PAAm at t_1 hours. At t_0 hours, ALG-PAAm had an EA value of 11.21±1.45N.mm compared to 14.72±1.75N.mm of CHT-ALG-PAAm. This provided an indirect indication of matrix porosity. Lower EA values indicated smaller pore sizes and a greater swelling ability which allowed an increase in drug diffusion through the matrix. At t5 hours, CHT-ALG-PAAm had an EA value of 1.5±0.11N.mm and ALG-PAAm had a value of 1.77±0.04N.mm. Both formulations remained buoyant for the duration of the study. A matrix resilience of 56±2.04% at t_0 hours was obtained by both formulations. At t_5 hours ALG-PAAm had an average resilience of 18±0.57% compared to CHT-ALG-PAAm which had a resilience of only 10±1.52%.

Conclusion.

Both formulations demonstrated potential as a prolonged release buoyant drug delivery system with the appropriate modification of the polymeric constituents.

APPENDIX C23

2008 AAPS Annual Meeting

Evaluation of the Disintegration Characteristics of a Multi-Configurational Oramucosal Drug Delivery System

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Purpose.

A rapidly disintegrating multi-configurational polymeric delivery system was fabricated and subjected to in vitro testing to assess its ability to rapidly and effectively deliver drug to the oramucosa.

Methods.

Standard solutions of chitosan (9.00–10.50%w/w) and diphenhydramine-loaded cellulosic polymer (9.00–0.50%w/w) were homogenously combined and lyophilized to produce multi-configurational Low Density Matrices (LDM) according to a face-centered central composite statistical design. Disintegration testing was conducted using a Texture Analyzer (TA) (Stable Microsystems, UK), fitted with a 5kg load cell (N=69). LDMs were attached to the flat-ended cylindrical probe of the TA and lowered at a constant pre-determined force into 5mL of simulated saliva (pH 6.75; 37°C). Disintegration rate and the distance traveled by the probe were monitored with typical Time-Distance profiles generated. LDM matrix energy absorbed and matrix tolerance was also assessed. Samples hydrated with 5mL of simulated saliva were analyzed using a Modulated Advanced Rheometer System maintained at 37°C.

Results.

The multi-configurational matrices demonstrated rapid disintegration (29.77±5.43s), at an average disintegration rate of 0.121±0.130mm/sec. Onset of disintegration was instantaneous (1.52±0.33s). Disintegration resulted in the formation of a bioadhesive gel able to withstand shear rates exceeding $100s^{-1}$. Matrix energy absorption (8.546±8.829N.mm) was calculated as an indirect determinant of LDM porosity and stability. A similarity between matrix energy absorption and disintegration was observed, with increasing energy absorption leading to increasing disintegration rates. Changes in the concentrations and ratios of the LDM constituents, especially that of the cellulosic component, were shown to impact the energy absorbed, subsequently altering the disintegration profile, as well as the integrity of the resultant gel. Matrix tolerance, indicative of overall LDM strength was calculated as 1.133±1.487N/mm and correlated closely with the onset of disintegration. All TA profiles generated distinctly demarcated the multi-configurational structure of the LDM.

Conclusion.

The multi-configurational LDM demonstrated rapid disintegration into a bioadhesive gel capable of adhering to the oramucosa for enhanced drug delivery.

APPENDIX C24

2008 AAPS Annual Meeting

In vitro evaluation of the drug release, swelling and erosion dynamics of a layered multi-disk tablet matrix

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Purpose.

To evaluate the drug release kinetics, erosion and swelling dynamics from a directly compressed layered multi-disk tablet matrix.

Methods.

Matrices comprising two drug-loaded disks where compressed between three polymeric layers of HPMC, pectin and a HPMC/pectin blend. Diphenhydramine HCl (solubility ~1g/mL) was used as a model drug. Ethylcellulose and HPMC were employed for the drug-loaded discoid matrices. Drug release studies were performed using USP dissolution apparatus II comprising 900mL PBS (pH 6.8;37°C). Matrices were subjected to dissolution for 12 hours at 50rpm. Swelling and erosion studies were conducted under similar conditions. At regular time intervals samples were removed and gravimetrically analyzed to determine the hydration dynamics of swelling and erosion.

Results.

Drug release displayed a biphasic pattern with an initial burst phase at t_1 hour, followed by controlled diffusional drug release over the remaining 12 hours. Matrices with pectin as a top layer, displayed a lag phase of 4 hours after the initial burst. Erosion followed a linear increase where matrices with pectin as a top layer displayed the highest erosion (42±0.15%) while HPMC matrices displayed the lowest (21±0.12%). Swelling studies revealed a 55-155% range at t_1 hour, indicating that the formulations began to swell immediately upon exposure to the dissolution media. Matrices with pectin as a top layer however, displayed an increase in swelling up to t_6 hours, after which swelling decreased and then increased once again in a pulsatile manner. This is comparable with the erosion of the pectin top layer, exposing the first drug disk to the dissolution media, allowing the disk to swell and drug to be released. The second disk did not release immediately due to the higher swelling ratio of the surrounding HPMC layer, thus contributing to a lag phase between drug release.

Conclusion.

The layered multi-disk formulations displayed biphasic release with a drug-free period between two release phases that may be suitable for pulsatile drug release.

APPENDIX C25

Physical characterization of a novel composite membranous system for rate-modulated drug delivery

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Purpose.

To determine the physicochemical and physicomechanical properties of a composite polymeric membranous system by means of textural profile analysis, thermal investigations and porosity studies to establish the efficacy and stability of the membrane as a potential drug delivery system.

Methods.

A novel crosslinking polymerization reaction approach between polymethacrylate and polyethylene glycol was employed to develop three composite polymeric membranous systems. The membranous systems were prepared by producing a polymeric latex and subsequently crosslinking in a rate-controlled manner resulting in a hydrogel. Upon air drying under a fume hood, at 21°C for 7 days, the hydrogel solidified into a membranous system. Textural profiling was conducted to determine the physicomechanical parameters (TA.XT*plus*, Stable Microsystems, UK). DSC analysis was used for stability evaluation (DSC1 STAR^e system; Mettler Toledo, Switzerland). To postulate the possible mechanisms of drug release from the membrane the surface area and porosity was measured (Micromeritics ASAP 2020, GA, USA).

Results.

Upon subjection of the 3 composite membranes to textural analysis, a resiliency ranging from 28-35% and rigidities between 9.334-16.894N/mm were noted, with no deformation or damage to the structural integrity. DSC revealed similar thermal plots for the three composite systems. Membrane samples were inserted into 40µL aluminum crucibles and underwent thermal analysis with a temperature gradient between -35°C- 400°C ramped at 10°C/minute. Results revealed an average glass transition temperature (T_g) of 40°C and a melting point of 65°C. Samples thermally decomposed at 260°C and were observed as charred residues after analysis. Single point surface area (at P/P_o) indicated a low surface area of 0.324:0.066m^2/g. Average pore diameters were 334.573Å. Stability at standard storage conditions was noted from results of the T_g and textural profiling analysis. Mesopores were present that may allow for rate-modulated drug delivery. Drug-loading may also determine the efficiency of drug release through the mesoporous structure and optimization of the T_g and physicomechanical properties may produce a desirably robust membranous system.

Conclusion.

The composite membranous system developed in this study has the potential for rate-modulated drug delivery based on the inherent physicochemical and physicomechanical properties of the system.

APPENDIX D

ANIMAL ETHICS CLEARANCE CERTIFICATE

AESC 3

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2007/25/'05

APPLICANT:	VMK Ndesendo
SCHOOL:	Pharmacy and Phrmacology
DEPARTMENT:	
LOCATION:	Medical School
PROJECT TITLE:	**An In Vivo study of the Novel Controlled Release Intravaginal Bioadhesive Device in Rabbits**

Number and Species

80 NZW rabbits adult 7 month - 4kg

Approval was given for to the use of animals for the project described above at an AESC meeting held on 20070327. This approval remains valid until 20090327

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

pilot study only approved

Condition: Assess retention of placebo capsule in vagina of rabbit. Monitor for vaginal discharge for signs of infection and inflammation. Assess on this basis and come back for more animals.

Signed: _____

Date: 08/05/07 (Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____

Date: 8/5/07 (Registered Veterinarian)

cc: Supervisor: #

UNIVERSITY OF THE WITWATERSRAND OFFICE OF THE DEPUTY REGISTRAR (RESEARCH) JOHANNESBURG

The rabbit model was changed to pig model due the pig vagina more closely resembling that of the human (see Modifications and Extensions to Experiments approval next page).

1. **AESC 2008**

Please note that only typewritten applications will be accepted. Should additional space be required for section "I" and/or "j", please use the back of this form.

ANIMAL ETHICS SCREENING COMMITTEE

MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: VALENCE M.K. NDESENDO

b. Department: PHARMACY AND PHARMACOLOGY

c. Experiment to be modified / extended **AESC NO:**

Original AESC number	2007	25	05
Other M&E's :			

d. Project Title: An in Vivo Study of the Novel Controlled Release Intravaginal Bioadhesive Device

e.	Number and species of animals **originally approved**:		80 Rabbits
f.	Number of additional animals **previously allocated on M&Es**:		
g.	Total number of animals **allocated** to the experiment to date:		10 Rabbits 4 pigs
h.	Number of animals **used** to date:		10 Rabbits 4 pigs
i.	Specific modification / extension requested: Twenty large white pigs (minimum weight 35kg) are requested for the above titled project. 5 pigs will be used for testing the effect of the formulation without the drugs (i.e. the placebo), 10 for detecting and assessing the sequential degradation of the devices in the vagina through X-ray imaging as well as for analyzing the amount of AZT and PSS (separately) in the vagina tissue at the end of the study. The remaining 5 pigs will be the control group. The details are as shown in the attached schematic flow diagram (Figure 1).		
	Motivation for modification / extension: By incorporating a radiopaque material namely $BaSO_4$ in the formulation and mobilizing the devices (just after insertion) using a radiopaqued sponge material (to allow proper adherence), it has being ascertained that the vaginal polymeric devices can keep in the pig vagina through X-ray imaging, for the intended period of study. This is the rationale for requesting for 20 pigs for the main study.		

Date: 13th June, 2008 Signature: .....

RECOMMENDATIONS: Approved

Date: 13/06/08 Signature: .....
Chairman, AESC