

CONFIGURATION OF A MULTI-LAYERED MULTI-DISK TABLET FOR SPECIALIZED DRUG DELIVERY

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Pharmacy

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October, 2011

DECLARATION

I, Zaheeda Khan, declare that this dissertation is my own work. It has been submitted for the degree of Master of Pharmacy in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this or any other University.

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This day of October 2011

RESEARCH OUTPUTS

A. Publications

- 1) Zaheeda Khan, Viness Pillay, Yahya E. Choonara, and Lisa C. du Toit (2009). Drug delivery technologies for chronotherapeutic applications. *Pharmaceutical Development and Technology*, 14, 602-612 (Abstract in Appendix A1).

B. Poster presentations

- 1) Zaheeda Khan, Viness Pillay and Yahya E. Choonara. Evaluation of the drug release dynamics from a polyconcentric disk-loaded matrix. Presented at 28th Annual Conference of the Academy of Pharmaceutical Sciences, 4-7 September 2007, Cape Town (Abstract in Appendix B1).
- 2) Zaheeda Khan, Viness Pillay, Yahya E. Choonara and Lisa du Toit. Evaluation of the drug release dynamics from a layered disk tablet. Presented at the 29th Annual Conference of the Academy of Pharmaceutical Sciences, 22-26 September 2008, Rustenburg (Abstract in Appendix B2).
- 3) Zaheeda Khan, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit and Valence Ndesendo. *In vitro* evaluation of the drug release, swelling and erosion dynamics of a layered multi-disk tablet matrix. Presented at the 2008 American Association of Pharmaceutical Scientists (AAPS) Annual Meeting and Exposition, 16-20 November 2008, Atlanta, United States of America (Abstract in Appendix B3).
- 4) Zaheeda Khan, Viness Pillay, Yahya E. Choonara and Lisa du Toit. *In vitro* evaluation of the drug release, swelling and erosion dynamics of a layered disk tablet. Presented at Faculty Research Day and Postgrad Expo, University of Witwatersrand, 20 August 2008 (Abstract in Appendix B4).
- 5) Zaheeda Khan, Viness Pillay, Yahya E. Choonara and Lisa du Toit. A novel multi-layered discoidal compressed system for use in chronotherapeutic disorders. Presented at the 2009 5th ICPPS conference, 23-26 September 2009, Potchefstroom (Abstract in Appendix B5).

- 6) Zaheeda Khan, Viness Pillay, Yahya E. Choonara and Lisa du Toit. The Influence of Polymer Type on the Drug Release Kinetics from a Novel Disk Layered Tablet system. Poster presented at the 2009 American Association of Pharmaceutical Scientists (AAPS) Annual Meeting and Exposition, 8-12 November 2009, Los Angeles, United States of America (Abstract in Appendix B6).

C. Podium presentations

- 1) Zaheeda Khan, Viness Pillay, Yahya E. Choonara and Lisa du Toit. A novel layered tablet matrix for application in chronotherapeutic drug delivery. Podium presentation at the School of Therapeutic Sciences Research Day, University of Witwatersrand, 12 August 2009 (Abstract in Appendix C1).
- 2) Zaheeda Khan, Viness Pillay, Yahya E. Choonara and Lisa du Toit. A Discoidal Multi-Layered Compressed Matrix for Use in Chronotherapeutic Disorders. Podium presentation at the 2009 5th ICPPS conference, Young Scientist Competition, 23-26 September 2009, Potchefstroom (Abstract in Appendix C2).

PATENT FILED

A chronotherapeutic pharmaceutical dosage form. Inventors: Viness Pillay, Yahya E. Choonara, Zaheeda Khan and Seshni Sewlall. United States Patent Application 2009/005832 filed on 3 June 2009 and published on 23 December 2009 (WO/2009/153635) (Abstract in Appendix D1).

SUMMARY

Chronotherapy is a form of therapy where treatment is administered according to a schedule that corresponds to an individual's biological clock. Research demonstrates that the body's natural processes follow a 24-hour pattern, or circadian rhythm. In addition, symptoms of disease fluctuate according to this 24-hour pattern. These diseases, termed chronotherapeutic disorders may include amongst other disorders, hypertension, cardiovascular disease and asthma. Common therapy for these disorders involves the use of controlled zero-order release formulations. Here, the same quantity of drug is released over a period of time. Although beneficial, these formulations are not ideal in the treatment of chronotherapeutic disorders. Treatment of these disorders aims to release drug at specific periods, only when it is required, such that therapy coincides with the body's natural rhythm. Ideally, drug should be released in pulses with two or more pulses released from the dosage form. In this manner, the patient is exposed to drug only when required, reducing the number of dosages, reducing side-effects and ultimately increasing patient compliance. Therefore, the aim of this research was to develop a Multi-Layered Multi-Disk Tablet (MLMDT) that incorporates two drug-loaded disks enveloped by three polymeric layers. The proposed system, to be used in the treatment of chronotherapeutic disorders, is designed to provide a lag phase and then two pulses of drug release separated by a "switch-off" phase. During the "switch-off" phase no drug is released from the system.

Initially, preliminary screening studies were performed on various polymeric materials to assess their effectiveness to generate the desired drug release profile. Of the numerous polymer combination and ratios, only a few were relevant and were subsequently tested further. From the preliminary studies it was ascertained that the composition of disk 2 was critical in generating the "switch-off" phase separating the two pulses. Artificial Neural Networks (ANN); a computational technique that simulates the thinking process of the human brain was employed for optimization. Results from this technique outlined the polymer combination suitable for the optimized MLMDT. The optimized formulations were subjected to friability, hardness and uniformity of mass analysis as well as swelling, erosion and magnetic resonance imaging techniques to observe and confirm the performance of the MLMDT during dissolution. In addition, textural analysis, computational modeling and temperature modulated differential scanning calorimetry techniques were used to elucidate any incompatibilities or complexes formed. *In vitro* drug release analysis revealed that the MLMDT generated a lag phase followed by two pulses of drug release over the 24 hour period. The two pulses were separated by a "switch-off" phase.

To confirm data obtained during preclinical *in vitro* testing, animal studies were undertaken using the Large White Pig model. Pigs were dosed with conventional products and the optimized MLMDT. Blood samples collected over a 24 hour period were analyzed using Ultra Performance Liquid Chromatography to determine the drug concentration in blood. Drug concentration analysis from conventional products revealed increasing plasma concentrations up to 2 hours followed by a steady decline in concentration while the developed MLMDT displayed two pulse drug release separated by a "switch-off" phase.

ANIMAL ETHICS DECLARATION

I hereby confirm that the following study entitled “Configuration of a Multi-Layered Multi-Disk Tablet for Specialized Drug Delivery” had received the approval from the Animal Ethics Screening Committee of the University of the Witwatersrand with ethics clearance number 2007/56/04. (Appendix E).

ACKNOWLEDGEMENTS

The completion of this work would not have been possible without the help, support and encouragement received from various people. Firstly, I would like to thank my heroes, my parents, without whom this work may not have been possible. Thank you for your unconditional love, constant encouragement and for believing in me during the toughest times. You pointed me in the right direction when I lost my way. Mum, you are my pillar of strength and I can only pray that I too will someday be blessed with your courage, patience and strength. Dad, you are my living example of “mind over matter”. Your persistent perseverance through adversity will always be an inspiration to push myself to the limit.

To my sisters, Shameelah and Anisa, thank you for your support and help over the past few years. To my late grandmother, I cannot thank you enough for molding me into the person I am today.

Zain, my husband, my best friend and confidante, thank you for supporting and encouraging me through all the tears, late nights and failed experiments. You were the shining light during some of the darkest times in my life and for this I will always be grateful.

My deepest gratitude goes to my supervisor, Professor Viness Pillay. Thank you for advice, support and encouragement during this study. You opened my eyes to the world of research and taught me to see the world through a scientific eye. To my co-supervisor, Doctor Yahya E. Choonara, thank you for your advice and for always having a solution to every problem.

My esteemed gratitude goes to Doctor Valence M.K. Ndesendo. No words can express my gratitude for all your help, advice and prayers. Thank you for selflessly giving up your time to assist me. You are truly an angel and I pray that you have success in all your future endeavors.

My sincere appreciation goes to Ndidi Ngulukwa and Pradeep Kumar for all the help and also for the encouragement and advice. I am indebted to my fellow drug delivery colleagues, Ameena Wadee, Ahmed Seedat, Caragh Murphy, Deshika Reddy,

Shivaan Cooppan, Priya Bawa, Samantha Pillay, Sheri-Lee Harrilal, Sajida Suleman, Yasien Docrat, Rubina Shaikh, Clare Dott, Yusuf Dawood, Kovanya Moodley Oluwatoyin Kolawale, Bongani Sibeko, Lisa C. du Toit, Pius Fasinu for the help, encouragement, advice and most of all the good times. A special thanks goes to the technical staff, the late Elizabeth, Florence, Kleinbooie Mohlabi, Bafana Themba, Lehlohonolo Tebogo Chandu and Sello Ramarumo for the endless help.

My deepest appreciation goes to the staff at the Central Animal Service, Dr. Leith C.R. Meyer, Sister Mary-Ann Costello, Patrick Selahle, Ammelia Rammekwa, Lorrain Setimo and Kershnee Chetty for your assistance and guidance throughout this study.

I would like to extend my heartfelt thanks to Tabassum and Sultaana Shaik. The strength of friendship is determined by adversity and through every good or bad time I could always count on your advice, support and encouragement. I am truly blessed to have you in my life.

I would also like to express my special gratitude to my colleagues Elsabe Cilliers. Thank you for the advice and support over the past few months. You have brightened the darkest of days and provided me with hope in periods of despair.

This work would not have been possible if it were not for the funders of this research, The Technology Innovation Agency (TIA) of South Africa and the National Research Foundation (NRF) of South Africa. In addition my sincere gratitude goes to all at The Wits Foundation, Tata Africa and especially Mr. Raman Dhawan for the special scholarship awarded.

Lastly, and most importantly, I would like to thank the Almighty for all the blessings that have been bestowed on me.

DEDICATION

This work is dedicated to my father Nadirshah and my mother Nazley, for teaching me the value of education and the importance of perseverance.

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CHAPTER ONE

INTRODUCTION AND MOTIVATION FOR THIS STUDY

1.1 Background

Drug may be administered into the body via various routes. These include oral, parenteral, buccal, nasal, rectal, pulmonary, transdermal and ocular routes of administration. Of these, the oral drug route is the most common and allows for non-invasive delivery of drug. Oral formulations are easy to manufacture, convenient and affordable resulting in an increase in patient compliance (Bredenberg et al., 2003). To further facilitate patient compliance controlled release oral formulations are preferred. These systems allow for constant or variable drug release. Constant controlled release preparations that provide zero-order release are able to maintain a desired drug concentration over a prolonged period of time. In recent years, extensive emphasis has been placed on constant drug release (Löbenberg et al., 2005). With these systems drug release is maintained within a therapeutic window over a prolonged period, thereby reducing peak-to-trough fluctuations and ultimately decreasing side-effects and the dosage frequency (Figure 1.1).

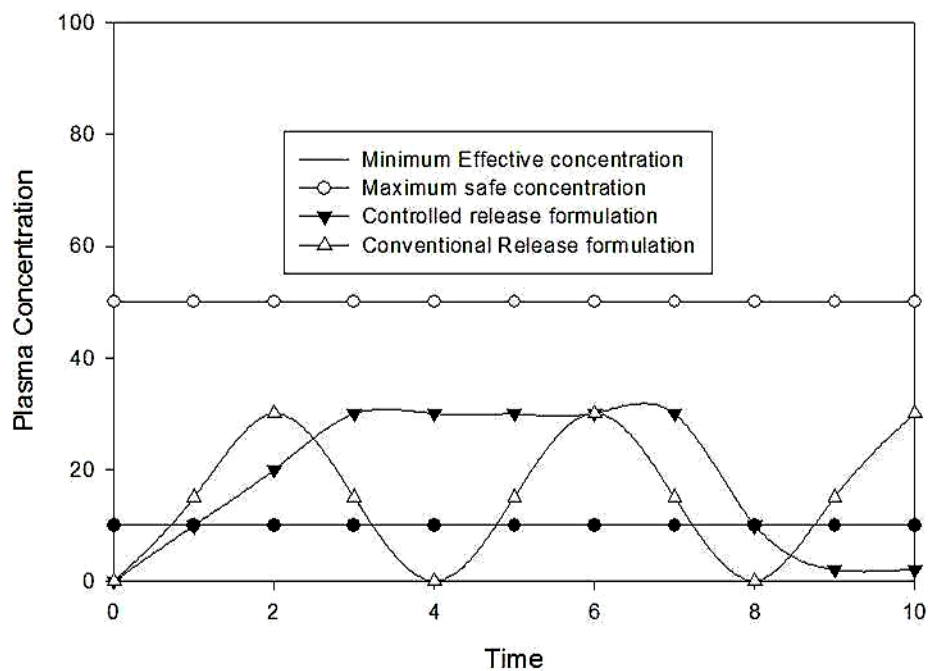


Figure 1.1: Difference in peak plasma concentrations between a conventional release formulation and a controlled release formulation (Adapted: Gothoskar et al., 2004).

Although these devices have proven to be extremely valuable, there are certain diseases when constant prolonged release is not desirable. These diseases are termed chronotherapeutic disorders and would benefit from chronotherapy, which involves timing drug application to achieve optimal therapeutic success. It is known that humans follow a 24-hour cycle known as circadian rhythms, which are controlled by a biological clock and work on a daily time scale. Using the concept of circadian rhythms, it is possible to treat chronotherapeutic disorders via specialized drug delivery systems (Hassan and Haefeli, 2010).

Chronotherapeutics is a branch of therapy that involves synchronizing drug application in a manner that matches circadian rhythms in order to achieve optimal therapeutic success (Landau, 1991). A major objective of chronotherapy is to deliver drug in higher concentrations during the times of greatest need. Conventional zero-order controlled release drug delivery systems used in the treatment of chronotherapeutic conditions are not designed to complement the circadian rhythm. In order to achieve optimal success, drug release ought to match the body's circadian rhythm and should occur after predetermined time delays such that drug release within a 24-hour period is in synchrony with the biological determinants of the disease (Pawar and Awasthi, 2010). Therefore, to meet these requirements pulsatile drug release is beneficial so that drug may be released after a lag-phase at predetermined time intervals (Kalantzi et al., 2009; Kadam and Gattani, 2010; Kumar et al., 2010). Pulsatile drug delivery offers advantages such as, extended day-time or night-time activity, reduced side-effects, reduced dosing frequency and dose size, improved patient compliance and lower treatment costs. Therefore, this study proposes to design a multi-layered multi-disk tablet (MLMDT) for use in chronotherapeutic disorders. The MLMDT is intended to produce a lag-phase and two pulses of drug release which are separated by a "switch-off" phase to coincide with the body's natural circadian rhythm. During this "switch-off" phase drug no drug should be released.

1.2 Rationale and Motivation for the Study

Patient non-compliance is seen as a major drawback in the treatment of disease. A few causes of patient non-compliance include side-effects, tolerance and the cost of medication. In addition, the frequency of dosing plays a role as patients forget to take medication. The proposed MLMDT aims to address these issues. The timing of drug administration such that drug is only released when required in a 24-hour period, implies that the patient is not continually exposed to drug thereby reducing side-effects.

Furthermore as the body is not constantly exposed to the drug, tolerance to a drug can be avoided. The aim of the MLMDT is to produce a lag phase followed by two pulses of drug release equivalent to administering two doses. This, together with the use of low cost materials reduces the medication costs. Also the dosing frequency is reduced to once a day increasing patient compliance.

The proposed chronotherapeutic drug delivery system should aim to deliver drug after a predetermined time interval, deliver drug when it is most needed in a 24-hour period and reduce drug release when not needed (e.g. when the patient is asleep). A schematic depicting the desired release profile is represented in Figure 1.2.

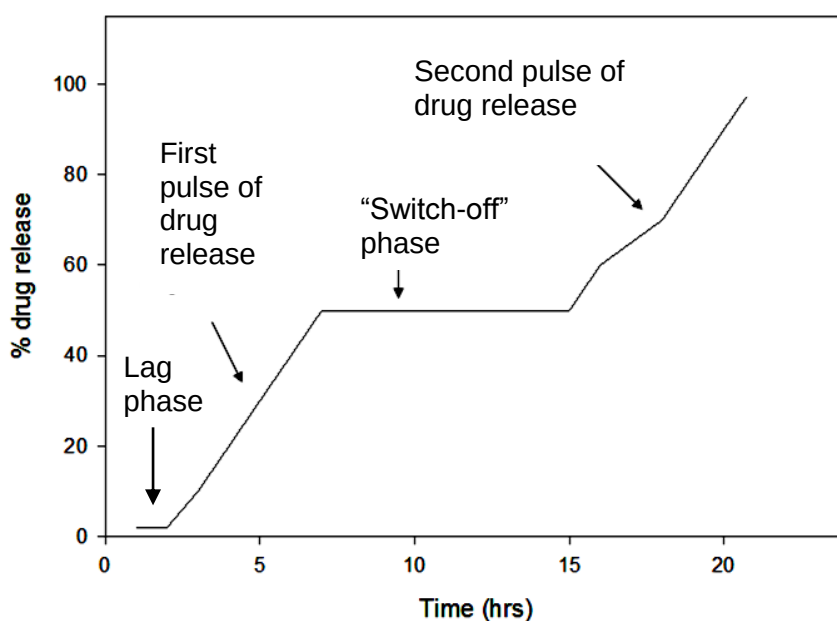


Figure 1.2: Schematic depicting the desired release profile.

To achieve this desired release profile requires a complex interplay between a variety of polymers. As two pulses of release are required, the first pulse is required to release after a lag phase of approximately 2 hours whereas the second pulse is required to release after approximately 12-15 hours (Figure 1.2). Drug release in this manner may provide therapeutic blood levels similar to those produced by two smaller doses reducing the number of doses required, thereby reducing side-effects improving patient compliance and reducing cost of treatment. Furthermore, this release pattern ensures that drug is delivered during the period when it is most needed.

The proposed MLMDT comprises two drug-loaded disks, enveloped by three polymeric drug-free barrier layers (Figure 1.3). The barrier layers control the rate at which drug is

released from the disks. It is envisaged that the MLMDT will be dosed every evening (approximately 6pm) and will release drug after a lag phase. Thereafter, drug release will cease as the barrier layers protect the second disk from release. The second pulse will be released at approximately 4am and drug release will continue till the next dose thus providing pulsatile drug release that takes circadian variation into consideration and minimizes toxic effects.

The design may apply to any drug that is used to treat chronotherapeutic conditions, as well as allowing for more than one drug to be combined in a single drug delivery system. In this study theophylline (THP) and diltiazem HCL (DTZ) were employed as model drugs.

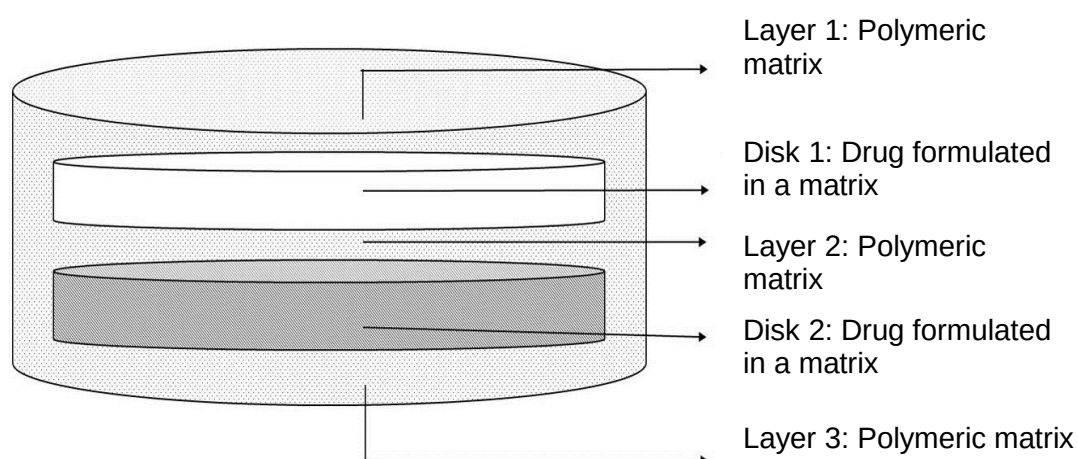


Figure 1.3: Schematic of the MLMDT comprising of two drug disks enveloped by a polymeric matrix.

Even though THP preparations have been replaced with newer drugs in the treatment of asthma they may still be useful in the treatment of nocturnal asthma and COPD (Kraft and Martin, 1995; Caramori and Adcock, 2003; Gothaskar et al., 2004; Mastiholimath et al., 2007; Kadam and Gattani, 2010). THP also serves as a model drug where circadian variability and chronobiology may affect the release of a drug (Pollock and Olanoff, 1990). A study conducted by Jonkman et al., in 1984 demonstrated that plasma concentration after 12 hours of a morning dose of THP were lower than that of an identical preparation dosed at night. This phenomenon was also observed when intravenous THP was used. It was concluded that a circadian pattern was responsible for lengthening the half-life during the evening hours (Jonkman et al., 1984).

Chronotherapeutic dosing of THP once daily in the evening between 6 p.m. and 7 p.m., attenuates nocturnal symptoms and early morning bronchoconstriction without the deterioration of asthma control at other times of the day. This regimen has been found to be clinically superior to conventional twice-daily dosing (Welsh et al., 1986; Grossman, 1988; Martin et al., 1989). A single-blind, randomized, crossover study compared equivalent doses of once-daily THP tablets with twice-daily THP tablets in patients who repeatedly experienced 20% or greater declines in PEFr during the night. The overnight decrease in FEV₁ was 12% after the once-daily preparation compared with 34% after the twice-daily preparation (Martin et al., 1989). In addition, evidence suggests that THP dosed at night may have a positive cumulative impact on lung function. A trial compared a once-daily, controlled-release THP preparation given in the morning or evening with twice-daily THP therapy (Grossman, 1988). The outcome of this trial proved the once-daily evening THP dosing produced significantly higher morning PEFrs and fewer night time awakenings compared with the twice-daily THP regimen. Thus, a once-daily evening dosage regimen is effective in controlling nightly asthma symptoms without compromising daytime control of asthma.

Chronotherapy with THP has proven effective in COPD patients with nocturnal worsening of lung function. Improved and more stable spirometric performances have been documented in patients treated with once-daily THP chronotherapy. A study was conducted on patients who received a placebo, once daily dose of controlled release THP at 10 p.m. or a twice daily dosage of extended release THP at 8 a.m. and 8 p.m. Results found that patients receiving the once daily dosage demonstrated significant improvement as compared to the other two groups (Rivington et al., 1988), indicating that extended release THP appears to be efficacious both objectively and subjectively in patients with asthma or COPD.

1.3 Aim and Objectives of the Study

The aim of this study was to focus on *in vitro* and *in vivo* development and evaluation of a novel MLMDT for use in two specific chronotherapeutic disorders, namely asthma and hypertension. As previously mentioned, the MLMDT should produce the desired release profile which exhibits pulsatile drug delivery after an initial lag phase.

In order to accomplish this aim the following specific objectives were outlined:

- Evaluating the physicochemical, physicomechanical and micromeritic properties of polymeric materials for potential use in the formulation.
- Undertaking compatibility studies between the model drugs and the polymers to determine if any unfavorable interactions exist between the materials.
- Assessing the *in vitro* drug release rates using simulated gastric and intestinal media.
- Undertaking bio-erosion and swelling studies.
- Performing in-process validation testing by determining the friability, uniformity of mass and hardness.
- Performing *in vivo* animal studies, to determine the drug release and plasma profile.

1.4. Overview of this Dissertation

Chapter One provides an overview into the concept of chronotherapy as well as highlighting the importance of this concept in disease. The rationale and motivation of the study is explained and the aims and objectives are summarized.

Chapter Two provides an introduction into the concept of chronotherapy and explains the role of chronotherapy in disease. Furthermore, current chronopharmaceuticals are discussed.

Chapter Three describes preliminary studies which includes the formulation and development of the MLMDT as well as *in vitro* studies and in-process validation.

Chapter Four focuses on optimization of the formulation using Artificial Neural Networks. Various experiments on the optimized formulation are also described including compatibility studies and analyses of physicochemical and physicomechanical properties of the MLMDT.

Chapter Five outlines the *in vivo* animal studies undertaken in the large white pig model. In addition, a method of analyses and plasma-drug profiles are explained.

Chapter Six presents the conclusions and recommendations for future work.

CHAPTER TWO

LITERATURE REVIEW OF CURRENT DRUG DELIVERY TECHNOLOGIES EMPLOYED TO TREAT CHRONOTHERAPEUTIC DISORDERS

2.1 Introduction

During the 1700s Jean-Jacques d'Ortous de Mairan was the first researcher to take note of the 24-hour patterns occurring in the movement of a plant. Since then research has been conducted to prove the presence of the 24-hour cycle. The 24-hour cycle that takes place in physiological processes of all human beings is termed the circadian rhythm. Other biological rhythms include the ultradian (length of hours, minutes or seconds), infradian (length of days or months), circatrigintan (the female menstrual cycle) and circannual rhythms (seasonal variation) (Gherghel et al., 2004; Haus, 2007).

The term circadian rhythm was first coined by Halberg and Stephens in 1959 and literally means "about-a-day" (Eriguchi et al., 2003; Gherghel et al., 2004). It was then found that the human biological clock is coordinated by the suprachiasmatic nucleus located at the base of the hypothalamus (Lévi, 2001). The suprachiasmatic nucleus is a paired nucleus situated above the optic nerve. Light that passes through the retina conducts impulses to the retino-hypothalamic tract that in turn regulates nine genes (Table 2.1.) that create 24-hour variations in cellular physiological processes (Dunlap, 1999; Shearman et al., 2000; Gherghel et al., 2004). The suprachiasmatic nucleus inherently possesses a pacemaker which produces endogenous electrical activity thus allowing variations in the suprachiasmatic nucleus to arise in the absence of external stimuli (Gherghel et al., 2004). The biological rhythms generated play a role in controlling biological functions that include the autonomic nervous system, endocrine system and immune system (Figure 2.1) (Eriguchi et al., 2003).

The circadian clock essentially comprises three components: an input pathway (photoreceptors and projections of retinal ganglion cells), circadian pacemakers that generate the circadian signal and an output pathway that couples the pacemakers to effector systems manifesting into circadian physiology and behavior (Aronson et al., 1993; Ohdo, 2007; Ohdo, 2010).

Table 2.1: Genes associated with the bodies' circadian rhythm.

Genes responsible for circadian rhythms	
PER 1	CLOCK
PER 2	BMAL1
PER 3	TIM
CLOCK	CRY1
BMAL1	CRY2
TIM	tau

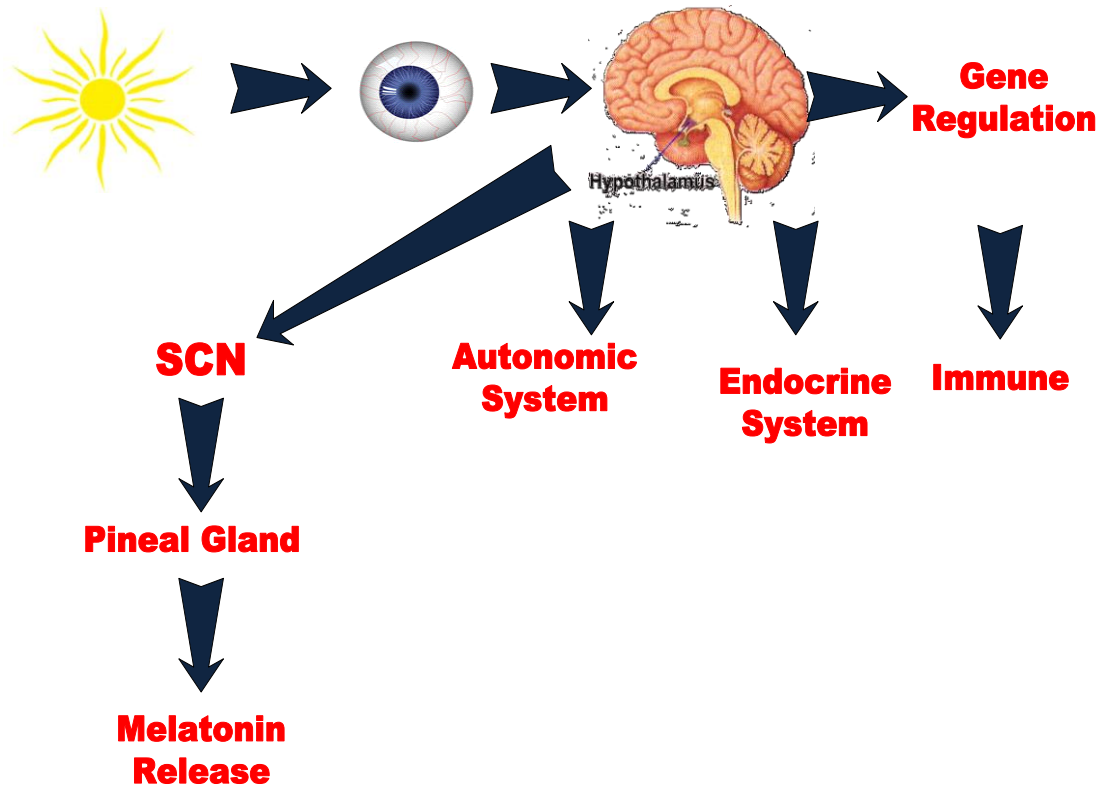


Figure 2.1: Schematic depicting the influence of light on the hypothalamus affecting gene regulation, melatonin release and the autonomic, endocrine, and immune system.

The circadian clock behaves like a multifunctional timer in order to regulate homeostatic systems within the human body (Ohdo, 2007; Ohdo, 2010). The most frequently used methods to assess the circadian rhythms in humans are:

- plasma melatonin release
- core body temperature measurement
- plasma cortisol level
- dim light melatonin onset

Melatonin production starts rising in the evening and thus is used as an indication of the circadian phase (Lewy et al., 1999). Collection of an individual's core body temperature is an easy measurement of circadian rhythm. Body temperature rises during the day with the maximum temperature occurring at 5 p.m. This is followed by a nocturnal decline is observed with a minimum temperature occurring at 5 a.m. Cortisol is released in a rhythmic manner and declines throughout the day. A nocturnal "quiet phase" occurs during the evening where low concentrations are released. This is followed by a rise occurring in the second half of the night leading towards a morning maximum (Hofstra et al., 2008).

Circadian rhythms govern nearly all functions in the body, including sleep and activity, hormone levels, appetite and those functions responsible for pharmacokinetic parameters (Lemmer, 1991; Lemmer, 1996, Ohdo, 2007). Furthermore, hormones such as cortisol, rennin, growth hormone and aldosterone also display daily fluctuations (Lemmer, 1996; Ohdo, 2010). The secretion of growth hormone peaks during sleep with aldosterone and cortisol levels typically peaking during the morning (Elliot, 2001). A list of hormones and biological functions that are known to follow a circadian rhythm are highlighted in Table 2.2.

Table 2.2.: The effect of circadian rhythmicity on the secretion of hormones and biological functions (Lemmer 1990, Lemmer, 1996, Youan 2004).

Circadian Rhythmicity	Hormone/Biological Functions
Late at night/early in sleep	• Gastric Acid Secretion • White Blood Cell Count • Calcitonin Gene-Related Protein • Atrial Natriuretic Peptide
Peaks during sleep	• Growth Hormone • Thyroid stimulating Hormone • Melatonin • Prolactin • Follicle Stimulating Hormone • Adrenocorticotrophic Hormone • Luteinizing Hormone
Peaks during the morning	• Cortisol • Renin • Angiotensin • Vascular Resistance • Platelet Aggregation • Blood Viscosity
Peaks at noon	• Hemoglobin • Insulin Release
Peaks in the evening	• Triglycerides • Urinary Diuresis • Cholesterol

A thorough knowledge of the circadian clock helps in understanding the 24-hour biological clock as well as the pathophysiology and symptoms of disease. Also the pharmacokinetics and pharmacodynamics of various drugs can be further understood. Information on the circadian rhythms and their influence on disease states may assist formulation scientists in developing innovative approaches for drug delivery and other treatment modalities.

2.2 The Role of Circadian Rhythms in Disease

Diseases that follow the daily biological rhythms are known as chronotherapeutic disorders. The symptoms of these disorders are exacerbated or more prominent at certain times during the day. The biological systems known to follow a circadian rhythm are described hereunder.

2.2.1 The cardiovascular system

Several functions of the cardiovascular system such as the heart rate, stroke volume, fibrinolytic activity, platelet aggregability, cardiac output and blood flow follow a

circadian rhythm (Kraft and Martin, 1995; Smolensky, 1995; Lemmer, 1996; Takeda and Marmora, 2010). Blood pressure and heart rate in both normotensive and hypertensive patients are higher during the morning hours (6 a.m. to 12 p.m.) than any other time of the day due to a decrease in sympathetic output occurring at night while asleep (Prisant, 2001; Smith, 2001; Lemmer, 2006; Hermida et al., 2007; Smolensky and Peppas, 2007; Portaluppi and Hermida, 2007; Hermida and Ayala, 2009). Upon waking, the systolic blood pressure rises rapidly by 20-25mmHg and diastolic blood pressure by 10-15mmHg (Smolensky and Peppas, 2007). Gherghel et al. (2004) expressed the extent of the drop in blood pressure during the night in the region of 10-20%.

It is further described that approximately two thirds of the world's population present with a blood pressure drop of this magnitude during the night and are known as dippers. The remaining one third present with a blood pressure drop of <10% and are known as non-dippers. These factors, in conjunction with the prolonged exposure to a higher blood pressure level seen in non-dippers, contributes to an increase in cardiovascular diseases such as myocardial infarctions, angina and strokes during the early hours of the morning (Kraft and Martin, 1995; Smolensky and Portaluppi, 1999; Smith, 2001; Douglas, 2002; Portaluppi and Hermida, 2007). Douglas, (2007) reported that there is a 40% higher risk of a heart attack, a 29% increased risk of cardiac death and a 49% increased risk of stroke between 6 a.m. and 12 p.m. in the morning. Conversely, vasospasms in Prinzmetal angina and congestive heart failure symptoms are common during sleep (Gallerani et al., 1992; Kraft and Martin, 1995; Smolensky and Portaluppi, 1999). Furthermore, the diagnosis of hypertension is based on the assessment of blood pressure when a patient presents at the doctor during the daytime. However, blood pressure fluctuates during the day and a single measurement during the day is not representative of the systolic and diastolic blood pressure throughout the day. The use of 24-hour blood pressure ambulatory monitoring technology is required to establish a correct diagnosis (Smolensky and Peppas, 2007; Portaluppi and Smolensky, 2010).

2.2.2 The respiratory system

Lung function is also affected by circadian rhythms. Lung function in healthy patients dips during the early hours of the morning. This change is more pronounced in asthmatic patients. The decrease in lung function can vary between 25-50% (Kraft and Martin, 1995). Airway resistance in asthmatic patients increases progressively at night (Martin and Banks-Schlegel, 1998; Burkioka et al., 2002; Sutherland, 2005;

Mastiholimath et al., 2007; Shiohira et al., 2009). At night the lungs are more sensitive to bronchoconstrictors such as acetylcholine and histamine (Lemmer, 1996; Gwen, 2002; Smolensky et al., 2007). A survey conducted by Turner-Warwick (1988) demonstrated that of 7600 asthmatic patients interviewed, 74% awoke from sleep with asthmatic symptoms. Treatment of asthma involves the inhalation of corticosteroids that directly inhibits inflammatory cells. A study conducted on individuals affected with asthma ascertained that dosing an inhaled corticosteroid once daily at 3 p.m. was as effective as four times daily dosing (Pincus et al., 1995). In another study, dosing with inhaled corticosteroids at 8 a.m. and 5:30 p.m. were compared to four times daily dosing. The outcome of the study proved that dosing at 5:30 p.m. was as effective as dosing four times daily, however dosing at 8 a.m. did not produce results comparable to four times daily dosing (Pincus et al., 1997). Optimal dosing of inhaled corticosteroids was thus between 3:00-5:30p.m. These observations highlight the significance of dosing medication in synchrony with the body's circadian rhythms.

2.2.3 The skeletal system

Patients suffering from rheumatoid arthritis are inclined to experience pain symptoms during the morning due to an increase in the concentration of interleukin-6 and C-reactive protein. Patients with rheumatoid arthritis also display circadian rhythms in joint pain and finger swelling. These debilitating symptoms are in phase with the circadian rhythms of pain (Cutolo, 2003). In contrast, osteoarthritic patients have less pain during the morning compared to the rest of the day (Youan, 2004, Sher et al., 2007). Timing the treatment of arthritis is important as non-steroidal anti-inflammatory drugs are also affected by the timed daily scale. Studies have demonstrated that a large dose taken twice daily is more effective than four smaller doses, provided that one of the doses is taken at night (Kraft and Martin, 1995).

2.2.4 The gastrointestinal system

Gastric acid secretion follows a circadian rhythm with a rapid surge of gastric acidity for at least one hour at midnight resulting in a decrease in the gastric pH level (Fass, 2004; Castilla-Guerra et al., 2009; Bahammam et al., 2010; Hsieh and Ohman, 2010; Kaya et al., 2010; Reddy and O'Neill, 2010; Rosenwasser, 2010; Sewlall et al., 2010; Diskmeis and Foulkes, 2011; Stalder et al., 2011). This change in pH may result in circadian modifications of drug ionization according to its properties (Van Herwaarden et al., 1999; Farup et al., 2001). A previous study conducted revealed that gastric emptying rates for meals eaten at 8 p.m. was on average 50% slower than the same meal eaten at 8 a.m. (Goo et al., 1987). This observation has implications in the

pharmacokinetics of orally administered drugs. The drug disintegration, dissolution and absorption may also be slower at night. In addition, the increase in acid secretion may exacerbate duodenal ulcers in affected patients (Sanders and Moore, 1998; Youan, 2004) and consequently a once daily dosage in the evening has been advocated (Lemmer, 1991; Youan, 2004; Roy and Shahiwala, 2009).

2.2.5 The role of circadian rhythms in glaucoma

In the United States open angle glaucoma is the second leading cause of blindness (Wax et al., 2002). Primary open angle glaucoma is characterized by atrophy of the optic nerve, increased intraocular pressure and visual defects (Wax et al., 2002; Gherghel, 2004). The main cause of primary open angle glaucoma is an increased intraocular pressure due to a reduction in aqueous humor drainage. The secretion of aqueous humor follows a circadian rhythm with a greater quantity secreted during the day compared to the night as the two hormones known to be responsible for secretion, namely cortisone and epinephrine/adrenalin, display concentrations that are lower at night than during the day (Jacob et al., 1996).

2.2.6 The role of circadian rhythms in cancer

The risk of developing cancer as well as its treatment has links with circadian rhythmicity. Cell proliferation and metabolism as well as apoptosis and DNA repair are under circadian control (Matsuo et al., 2003; Gery et al., 2006). In other studies it has been suggested that women who worked night-shifts for long periods of time had a higher risk of developing breast, colorectal and endometrial cancer (Keith et al., 2001; Schernhammer et al., 2001; Davis et al., 2006; Hansen et al., 2006; Stevens, 2006; Viswanathan et al., 2007; Pushkala and Gupta, 2009). Similarly, in men shift-work was associated with an increase in the risk of developing prostate cancer (Kubo et al., 2006; Conlon et al., 2007). In the treatment of cancer to achieve efficacious therapeutic outcomes patients often receive treatment where the dosage of drug is near the maximum tolerated dose. As a result, there is always the possibility of toxicity to normal cells. Due to normal cell physiology following a 24-hour clock, synchronizing the dosing schedule of chemotherapeutic agents to match the circadian rhythm of cells may result in a reduction in the cytotoxicity of these agents. The extent of toxicity and the efficacy of more than thirty different anticancer drugs are influenced by circadian dosing time (Mormont and Lévi, 2006; Lévi et al., 2007; Innominato et al., 2010). For example, platinum analog complexes such as cisplatin, carboplatin and oxaliplatin are best tolerated near the middle of the nocturnal activity span of laboratory mice whereas 5-fluorouracil is best tolerated when it is administered 12 hours earlier than the platinum

analogs (Boughattas et al., 1989; Lévi et al., 2007). In another example, a study was conducted where the survival rates of patients suffering from acute lymphoblastic leukemia were measured. Eighty percent of patients were alive and disease free after 5 years when dosed with 6-mercaptopurine and methotrexate during the evening and only forty percent of patients survived when dosed with the same medication during the morning (Lévi et al., 2007). Other anticancer drugs affected by circadian dosing time include arabinofuranosylcytosine, 5-fluorouracil deoxyribonucleoside (FUDR), doxorubicin, melphalan, cisplatin and oxaliplatin (Lévi, 1997; Focan et al., 2000).

2.2.7 Chronotherapy and sleep

The circadian system plays a vital role in the sleep-wake cycle. A disruption in the circadian timing system or a misalignment between the endogenous circadian timing and the external 24 hour social and physical environment can lead to circadian rhythm sleep disorders. To correct this disorder, timed exposure to bright light is often advocated. Melatonin also proves useful either alone or in combination with bright light exposure (Barion and Zee, 2007; Lack et al., 2009; Dodson and Zee, 2010).

2.2.8 Circadian rhythms and normal thyroid function

The hypothalamic-pituitary-thyroid axis follows a complex time structure. Briefly, the hypothalamus releases thyrotropin releasing hormone (TRH) which stimulates the pituitary to release thyroid stimulating hormone (TSH). This stimulates the thyroid to release thyroid hormones. TSH is released in pulses, however these pulses are not equally distributed throughout the day, rather they group together during the evening and night peaking between 2 a.m. and 4 a.m. (Brabant et al., 1990; Samuels et al., 1990). This pulse release may be essential for regular thyroid function and a change in this regular nocturnal variation may lead to hypothyroidism (Samuels et al., 1990; Innominato et al., 2010).

2.2.9 Cortisol and circadian rhythmicity

Cortisol release like the release of thyroid hormones is controlled by a complex set of influences. The hypothalamus releases corticotrophin-releasing hormone (CRH) which acts on the pituitary to release adrenocorticotrophic hormone (ACTH) which stimulates the adrenal cortex to release cortisol. The SCN inputs information that releases CRH in a pulsatile and periodic manner leading to the pulsatile release of ACTH which in turn leads to pulsatile release of cortisol. ACTH release peaks during the late night and early morning (Hillhouse and Grammatopoulos, 2006). Release then declines during the day with smaller pulses during the evening and night (Linkowski et al., 1993). Due

to the complex and sensitive nature of this pathway, timing of corticosteroid treatment is crucial. When suppression of the secretion of adrenocorticotrophic hormone or cortisol is required (as in the treatment of congenital adrenal hyperplasia), treatment should be administered in the evening which is prior to the circadian rise in ACTH release. Administration at this time results in a greater inhibition of the pituitary than any other time of the day or night (Moeller, 1985). Alternatively when corticosteroid therapy is required for inflammation, medication should be dosed in the morning as it leads to minimal adrenal suppression and fewer side-effects. When twice a day dosing is required, dosing should take place at 3 p.m. (Beam et al., 1992; Pincus et al., 1995; Smolensky et al., 1999, Takahashi et al., 2010).

2.3 Chronotherapy and Drug Delivery

An in-depth knowledge of circadian rhythms is of great importance when administering a drug delivery system as numerous drugs display variations in their pharmacokinetics and pharmacodynamics. For example, a drug delivery system administered in the morning may not have the same pharmacokinetic properties when administered at night. This can be attributed to the gastric ion concentration, stomach emptying rate and blood flow to the gastrointestinal tract, hepatic bile function, renal blood flow, glomerular filtration, and tubular function that follows a 24-hour rhythm (Smolensky and Portaluppi, 1999; Smolensky and Peppas, 2007). The pharmacokinetic properties of numerous drugs are affected by circadian rhythms, as listed in Table 2.3. Chronotherapeutics does not necessarily have to include new drug molecules, but instead established drugs may be incorporated into innovative drug delivery systems or devices that can be employed to improve their efficacy. This can be accomplished by unequal morning and evening dosing of a 12-hour drug delivery system, superior timing of a once-a-day formulation or by development of novel drug delivery systems that are able to release drug over a 24-hour period in synchrony with the biological rhythms (Smolensky and Peppas, 2007).

Table 2.3: Drugs affected by circadian rhythm (Adapted: Lemmer, 1996).

• Class of Drug	• Drug	• Class of Drug	• Drug	• Class of Drug	• Drug
• Beta-Blockers	• Acebutolol • Atenolol • Bisoprolol • Metoprolol • Nadolol • Propranolol • Bevantolol • Bopindolol • Sotalol • Labetolol • Mepindolol • Nebivolol • Nadolol • Oxprenolol • Pindolol • Timoptolol • Carvedilol	• Analgesics and anesthetics	• Acetylsalicylic Acid • Ketoprofen • Paracetamol • Indomethacin • Piroxicam • Morphine • Ibuprofen • Fentanyl • Flurbiprofen • Metamizole • Pranoprofene • Tenoxicam • Carticaine • Lidocaine • Fentanyl • Halothane	• Antiasthmatics	• Theophylline • Aminophylline • Orciprenaline • Isoprenaline • Terbutaline • Budesonide • Adrenaline • Bambuterol • Metacholine • Methylprednisolone • Adrenaline • Dexamethasone
• Diuretics	• Indapamide • Torsemide • Furosemide • Xipamide • Piretanide	• Cancer	• Cisplatin • Cyclosporin • 5-Fluorouracil • Methotrexate • Oxyplatin • Folinic acid • Doxorubicin • Busulphan	• Miscellaneous	• Digoxin • Clonidine • Prazosin • Phentolamine • Indoramine • Sodium nitroprusside
• Calcium Channel Blockers	• Amlodipine • Nifedipine • Verapamil • Nisoldipine • Diltiazem • Nicardipine • Nitrendipine • Laridipine • Isradipine	• Angiotensin converting enzyme (ACE) Inhibitors	• Enalapril • Captopril • Perindopril • Lisinopril • Quinapril • Benazepril • Trandolapril • Spirapril • Delapril	• Psychotropics	• Diazepam • Haloperidol • Midazolam • Lorazepam • Amitriptyline • Lithium • Carbamazepine • Valproic Acid • Phenylpropanolamine • Clomipramine
• AT ₁ -Receptor Antagonists	• Irbesartan • Losartan	• Antibiotics	• Ampicillin • Gentamycin	• Nitrates	• Glyceryl-trinitrate • Isosorbide-mononitrate • Isosorbide-dinitrate
• Gastrointestinal Tract (GIT) Drugs	• Cimetidine • Ranitidine • Omeprazole • Lansoprazole • Famotidine • Nizatidine	• Antihistamines	• Terfenadine • Cyproheptadine • Clemastine	• Cholesterol Lowering Drugs	• Bezafibrate • Clofibrate • Simvastatin

2.4 Overview of Chronopharmaceutical Technologies

One of the first commercially available oral products to employ the use of chronotherapy is the long-acting bronchodilator Uniphyll[®] (THP) (Purdue Frederick Co., Connecticut, USA). Chronopharmaceutical technologies currently available include: CONTIN[®] (Purdue Pharma, Ontario, Canada), Chronotropic[®] and Pulsincaps[®] (Catalent Pharma Solutions, New Jersey, USA). CEFORM[®] (Biovail Corporation, Ontario, Canada), TIMERx[®] (Penwest Pharmaceutical Company, Connecticut, USA), OROS[®] (DURECT Corporation, California, USA), CODAS[®] (Elan Corporation, Florida, USA), Egalet[®] (Egalet a/s, Copenhagen Denmark) and Diffucaps[®] (Eurand Pharmaceuticals, Pennsylvania, USA). Table 2.4 defines the significant applications of the described technologies in oral drug delivery (Youan, 2004).

Table 2.4: Chronopharmaceuticals currently on the market.

Trade Name	Drug	Chronopharmaceutical technology	Rationale for use in chronotherapy
Cardizem [®] LA	Diltiazem HCl Verapamil HCl	CEFOrm [®]	Hypertensive management to reduce morning blood pressure surge
Covera [®] HS	Verapamil HCl	OROS [®]	Hypertensive management to reduce morning blood pressure surge
InnoPran [®] XL	Propranolol	Diffucaps [®]	Hypertensive management to reduce morning blood pressure surge
Uniphyll [®]	Theophylline	CONTIN [®]	Asthma management to reduce early morning bronchoconstriction
Verelan [®] PM	Verapamil	CODAS [®]	Hypertensive management to reduce morning blood pressure surge

2.4.1 CONTIN[®]

CONTIN[®] technology (Purdue Pharma, Ontario, Canada), involves combining a drug and hydrophilic polymer followed by selective hydration with a polar solvent and fixation through a higher aliphatic alcohol. A molecular coordination complex is formed between a cellulose polymer and a substituted aliphatic alcohol. The result is a

complex that can be utilized as a matrix for controlled drug release. The complex has a uniform porosity which may be varied (Leslie, 1986). This technology is employed in the long-acting bronchodilator Uniphy[®]. Although CONTIN[®] technology functions as a conventional sustained release matrix system research suggests that evening administration demonstrates an improved lung function as compared to patients taking Uniphy[®] twice daily. When taken at night, Uniphy[®] causes THP blood levels to peak during the early hours of the morning when lung function is known to dip, making this the first commercial product to be indirectly used a chronopharmaceutical (Leslie, 1986, Arkinstall, 1988).

2.4.2 Pulsincaps[®]

Pulsincaps[®] technology (Catalent Pharma Solutions, New Jersey, USA) consists of a water insoluble capsule body filled with drug. The capsule body is sealed with a swellable hydrogel plug. The plug comprises polymers such as poly (methacrylates), hydroxypropylmethylcellulose (HPMC), polyvinyl alcohol (PVA), polyvinyl acetate, polyethylene oxide (PEO), saturated polyglycolate d-glycerides, glyceryl monooleate and pectin (PEC) (Shweta et al., 2006; Gazzaniga et al., 2008). The capsule is coated with an enteric layer that dissolves upon reaching the small intestine. Upon exposure of the capsule to the intestinal fluid, swelling of the plug takes place which creates a lag-time. The plug then expands and is pushed outward and drug release occurs. The dimension or length of the plug and its point/depth of insertion determines the lag-time before drug release (Ross et al., 2000; Verma and Garg, 2001; Shweta et al., 2006; Anal, 2007; Patel et al., 2007, Gazzaniga et al., 2008). This technology allows one or more minitables, coated tablets, solutions, or multiparticulates to be loaded within the capsule body (Shweta et al., 2006).

2.4.3 Chronotopic[®]

The Chronotopic[®] system provides delayed, time-dependent pulsatile delivery as well as colon specific release. The system comprises a drug-loaded core coated with HPMC. The HPMC undergoes a glassy-rubbery transition when in contact with aqueous fluid. Drug is then released across the gel-layer either by diffusion and/or erosion. The onset of action and lag-time are controlled by the thickness and viscosity grade of the HPMC employed during formulation (Sangalli et al., 2001; Sangalli, 2004; Patel et al., 2007). Furthermore, the application of a gastro-resistant film onto the polymer coated core of the system overcomes the variability in gastric transit-time and allows for colon-specific drug release (Gazzaniga et al., 1994; Gazzaniga et al., 1995; Sangalli et al., 2001; Shweta et al., 2006). The film allows the system to stay intact until

reaching the intestine where it eventually erodes and exposes the HPMC layer to the intestinal fluid, thus allowing the system to be pH-responsive. The tablets are prepared by first granulating the drug with a range of excipients which is then compressed. A mixture of HPMC and poly (ethylene glycol) (PEG) solutions are then sprayed onto the core and allowed to dry. A coating of Eudragit[®] is then applied onto the outer surface of the tablets (Sangalli et al., 2001).

2.4.4 CEFORM[®]

CEFORM[®] technology (Biovail Corporation, Ontario, Canada) involves the production of microspheres that are uniform in shape and size. The microspheres have a diameter ranging from 150-180µm and have a high drug-loading capacity. They may be applied in a wide variety of drug delivery systems including capsules suspensions, tablets, effervescent tablets and sachets (Verma and Garg, 2001; Youan, 2004; Shivakumar et al., 2006). This technology involves subjecting biodegradable polymers or bioactive agents to a combination of temperature, thermal gradients, mechanical forces, flow and flow-rates during processing. CEFORM[®] technology has been used to develop the chronopharmaceutical Cardizem[®] LA which is a once-daily DTZ formulation (Youan, 2004).

2.4.5 TIMERx[®]

Penwest Pharmaceutical Company (Danbury, Connecticut, USA) has developed SyncroDose[™] using Penwest's TIMERx[®] patented matrix. This enables drugs to be delivered after predetermined lag-times to coincide with the body's circadian rhythm or to allow drugs to be delivered to different sites within the gastrointestinal tract. The developed SyncroDose[™] tablet consists of an inner core of drug and a surrounding compression coating (Figure 2.2). The TIMERx[®] matrix system comprises xanthan gum, a semi-synthetic bacterial polysaccharide and locust bean gum and a plant polysaccharide. These polysaccharides interact synergistically with secondary and tertiary components (Tobyn et al., 1995; Mu et al., 1996). A ratio of between 1:3 or 3:1 of xanthan gum to locust bean is preferably employed. A possible mechanism for the interaction between the xanthan gum and locust bean involves interfacing between the helical regions of the xanthan gum and the unsubstituted regions of the locust bean. This interaction allows for a high-strength gel similar to the effects of crosslinking in hydrogels.

The tablet core is prepared by wet granulation and then compressed. The TIMERx[®] matrix is prepared by blending the polymers and inert filler after which the powders are wet-granulated. The moistened mass is dried and milled into granules. Compression coating techniques are then used to enclose the core (Tobyn et al., 1995). The SyncroDose[™] technology is able to release drug after a predetermined lag-phase with maximum plasma levels achieved in 5 hours. Upon contact with gastric fluid the combination polysaccharides interact and swell to form a taut gel with a gradual eroding core from which drug is released (Kelly et al., 1996; Verma and Garg, 2001; Patel et al., 2007). While the outer polysaccharide core may swell, the inner drug core remains intact. As the tablet moves through the gastrointestinal tract through the pylorus and into the duodenum, the outer swellable coating undergoes erosion. After approximately 3.5 hours erosion of the coating is complete, exposing the drug core to the intestinal environment where drug is released. Inclusion of a surfactant into the drug core allows for immediate release of drug. Alternatively, the core may be formulated as a controlled release matrix allowing for sustained release. The swelling of the matrix also results in a reduction in the bulk density, thus providing buoyancy and allowing the gel-mass to float on the stomach contents. Furthermore, depending on the size of the original tablet matrix, the gel-mass may swell to a degree that allows the system to obstruct the opening of the pylorus. The physicochemical properties of the polymers used, for example xanthan gum may be considered to be self-buffering (Tobyn et al., 1995). As a result the xanthan gum is not sensitive to pH variations along the gastrointestinal tract. Xanthan gum also has mucoadhesive properties allowing the drug delivery system to be retained in the stomach.

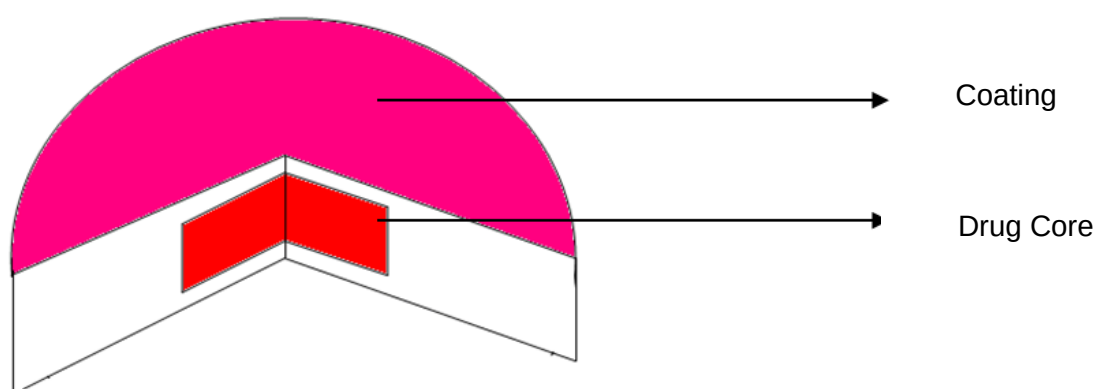


Figure 2.2: Diagrammatic illustration showing Penwests Synchrondose[™] formulation using the patented TIMERx[®] matrix (Adapted from www.penwest.com).

2.4.6 OROS®

An OROS® osmotic pump patented by DURECT Corporation (California, USA) utilizes an osmotic mechanism to provide pre-programmed, controlled drug delivery. This drug delivery system comprises a drug compartment, a push compartment and a semi permeable membrane (Figure 2.3). The drug compartment is made up of drug and PEO granulated with a solution of poly (vinyl pyrrolidone) (PVP). The push compartment comprises PEO, HPMC, sodium chloride and black ferric oxide which is granulated with HPMC. The drug compartment and the push compartment are then compressed into a bi-layered core. The entire core or only the drug compartment is sub-coated with a 95% hydroxyethylcellulose (HEC) and 5% PEG solution. This facilitates a drug-free interval of 2-5 hours. Thereafter, the sub-coated drug core is coated with a semi-permeable membrane comprising 60% cellulose acetate, 35% hydroxypropylcellulose (HPC) and 5% PEG. An orifice is then drilled into the outer and inner cores to connect the drug layer with the exterior of the drug delivery system. Finally the osmotic pump is dried for 96 hours to remove residual solvent and a further 2 hours to remove any excess moisture.

Drug release takes place due to the influx of fluid across the semi-permeable membrane into the push compartment. The increase in osmotic pressure forces drug to be diffused through the microscopic orifice. This drug delivery system provides a uniform non-varying rate of drug release allowing blood concentrations to be maintained within narrower limits for longer periods (Swanson et al., 1987). In addition, the release mechanism is less vulnerable to changes within the gastrointestinal tract due to the semi-permeable membrane being permeable to only water thus facilitating pH-independent drug release (John, 1990). This technology was employed in formulating the chronopharmaceutical, Covera-HS® (verapamil), an antihypertensive. The product facilitates delayed overnight drug release to prevent the dangerous surge in blood pressure that happens during the early morning. While this system has been applied to products, it has its limitations. Manufacturing has proved to be complicated with the need for a laser-drilled hole in the semi-permeable coating. In addition, clogging of the hole may limit drug release. Drying time also poses a challenge as the drug delivery system requires 4 days of drying time.

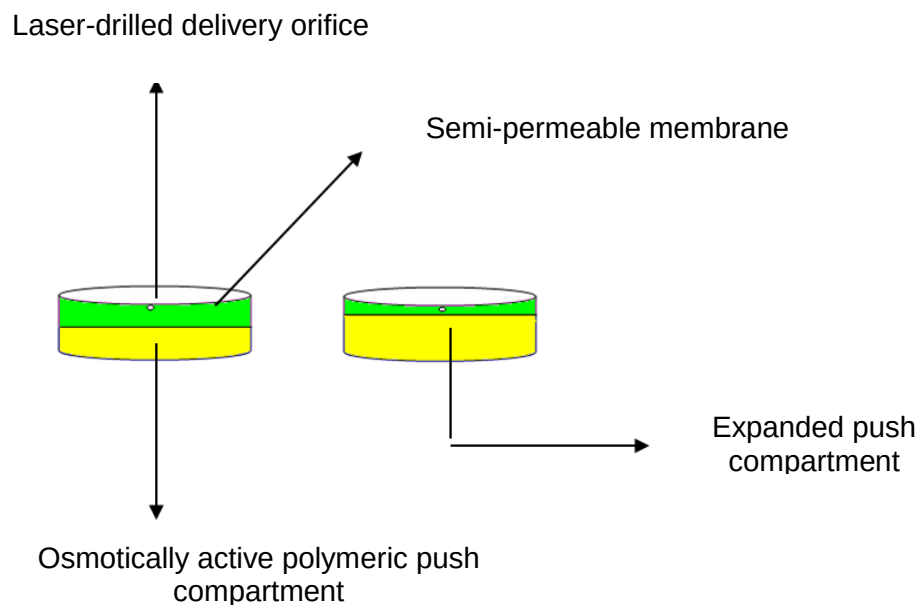


Figure 2.3: Schematic portraying the mechanics of OROS®.

2.4.7 CODAS®

Chronotherapeutic Oral Drug Absorption System (CODAS®) manufactured by Elan Corporation (Florida, USA) is a multiparticulate drug delivery system that provides a delayed onset of drug release, resulting in release that more accurately compliments circadian rhythms. This drug delivery system comprises a drug core and a multilayered membrane surrounding the core. Both the core and the multilayered membrane comprise water soluble and water insoluble polymers. When the bead is exposed to water, the water-soluble polymer dissolves and drug diffuses through the pores present in the coating (Figure 2.4). The water-insoluble polymer acts as a barrier and maintains the release of the drug (Kendall et al., 1980; Youan, 2004). The CODAS® technology has been applied to the chronopharmaceutical Verelan® PM (verapamil). This formulation is designed to release verapamil 4-5 hours after ingestion. When taken at bed-time, a maximum plasma concentration is achieved in the early hours of the morning when blood pressure is known to rise (Smith, 2001).

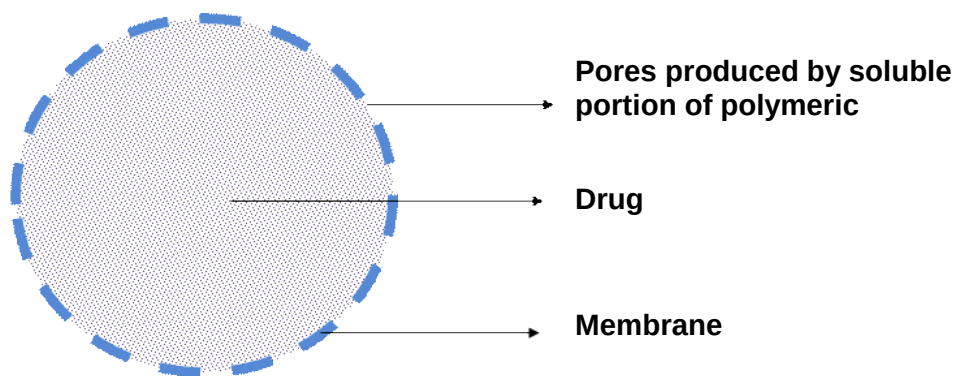


Figure 2.4: Diagrammatic representation illustrating the concept of CODAS[®] technology.

2.4.8 Diffucaps[®]

Diffucaps[®] manufactured by Eurand Pharmaceuticals (Pennsylvania, USA) is a multiparticulate drug delivery system that delivers drug according to the body's circadian rhythms (Roos, 2002). Drug is layered onto a neutral core which may comprise an inert particle such as sugar spheres, crystals or granules. An inert binder is used to bind the drug particles to the inert core (Figure 2.5). Examples of such binders include PEO, HPMC, HPC and PVP. Drug particles are dissolved or suspended in a 5-10 % solution of binder. The drug core is then coated with a plasticized enteric coating and thereafter coated with a mixture of water insoluble and enteric polymers. Examples of water insoluble polymers include ethylcellulose (EC), polyvinyl acetate and methacrylate polymer such as Eudragit[®] RS. Enteric polymers include cellulose acetate phthalate, hydroxypropylmethylcellulose phthalate and shellac. In order to separate the various layers a thin layer of HPMC is typically used (Prisant et al., 2000). The beads produced are small (<1mm in diameter) and by combining different beads, a combination release profile can be achieved. An organic acid comprising a membrane layer may optionally be added between the second and third coating in order to further modulate the lag-time. This technology also allows combining two or more drugs in order to increase patient compliance and has been used to produce a chronopharmaceutical product known as InnoPran[®] XL.

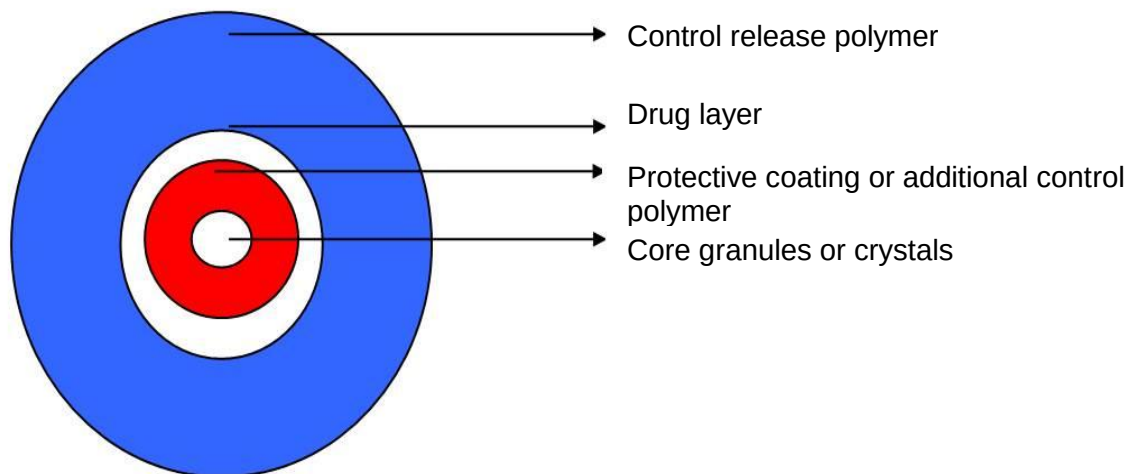


Figure 2.5: Schematic diagram illustrating the various layers surrounding a Diffucaps[®] bead (Adapted from www.eurand.com/Tecnologies/Controlling-Drug-Release/Diffucaps).

2.4.9 Egalet[®]

Egalet[®] (Egalet a/s, Copenhagen, Denmark) technology uses erosion instead of diffusion to control drug release. This technology has potential in chronotherapeutics and consists of an impermeable shell with two lag plugs enclosing a core of drug (Figure 2.6) (Washington and Wilson, 2006). The lag-phase is dependent on the length and the composition of the plugs. The impermeable shell comprises EC and cetostearyl alcohol, whereas the plug consists of PEG and PEO (Washington and Wilson, 2006). The matrix is designed to erode upon contact with the gastrointestinal fluid, though gastric fluid should not diffuse in the system until the point of release. This reduces hydrolysis and luminal enzymatic activity. A balance should exist between erosion and diffusion such that the surface area exposed to the dissolution media is constant ensuring zero order drug release (Washington and Wilson, 2006). This design also allows for more than one drug to be combined in a single drug delivery system. Varying the outer plug different release profiles may be achieved.

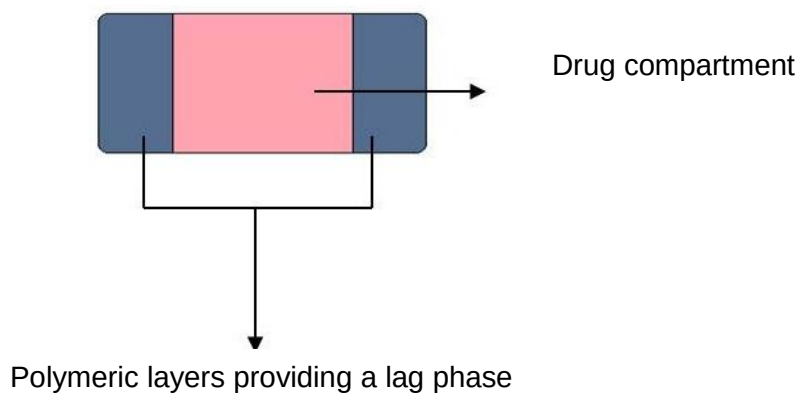


Figure 2.6: Schematic outlining the mechanics of the Egalet® Time Release System (Adapted from www.egalet.com/technology_timerelease.aspx).

2.4.10 CHRONOSET™

CHRONOSET™, an ALZA Corporation (California, USA) product is an OROS® drug delivery system that is designed to deliver more than 80% of drug within 15 minutes in a time or site specific fashion (Wong et al., 1995; Dong et al., 1999). This technology protects the drug from chemical and enzymatic degradation before release. In addition, the timing of drug release is unaltered by stomach contents. The CHRONOSET™ drug delivery system comprised of two compartments i.e. the drug vessel and the osmotic cap (Figure 2.7). On exposure to aqueous media, water enters the osmotic cap via the rate-controlling membrane leading to expansion of the osmotic compartment. This expansion exerts a force against the drug vessel enabling the two compartments to detach exposing drug to the dissolution media enabling release of the entire dose. Polymers utilized in this dosage form include ethylene-co-vinyl acetate copolymer, water-permeable blends of polycaprolactone and flux enhancers.

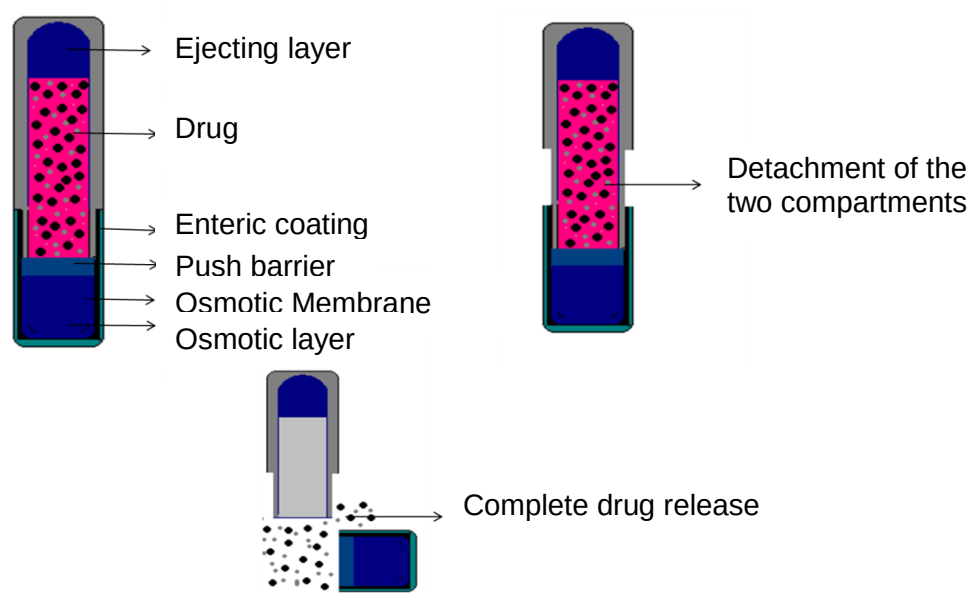


Figure 2.7: Schematic depicting the release of drug from the CHRONOSET™ drug delivery system.

2.5 Emerging Advances in Oral Chronotherapeutic Drug Delivery Technology

The search for innovative drug delivery systems applicable to chronotherapy is ensuing with increasing intensity. Below are some promising drug delivery devices that may have application in chronotherapy. Lee et al., (1999) developed a HPMC tablet matrix comprising an inner drug core and an outer drug coating. The inner drug core was prepared using direct compression of the drug and polymer. The core was then coated with a drug containing aqueous-based polymeric Eudragit® RS30D dispersion. The tablet was able to provide a biphasic release profile with an initial zero-order release followed by a second sustained phase of drug release. The tablet was ineffective in providing a lag-phase.

Dashevsky and Mohamad (2006) introduced a pulsatile rupturable drug delivery system consisting of a drug core, a swelling layer comprising a super-disintegrant and binder, and an insoluble polymeric coating comprising ethylcellulose. Drug was layered onto sugar pellets followed by layering with either 5% suspension of croscarmellose sodium, low-substituted HPC or sodium starch glycolate in a 2% HPC solution. The beads were coated with aqueous ethylcellulose dispersion. Fluid entered the system and triggered swelling causing the outer membrane to rupture resulting in drug release.

Drug release comprised a lag-time that was determined by the coating layer followed by rapid drug release indicating sigmoidal drug release.

Efentakis et al., (2006) developed a drug delivery system based on core-in-cup technology. The device comprised of a drug containing core tablet an outer impermeable layer and a top cover. The bottom and the circumference wall comprised an inactive material which surrounded the drug core. The core comprised cellulose acetate propionate. The top layer comprised a soluble polymer such as sodium carboxymethylcellulose, sodium alginate and PEO. Upon contact with dissolution media, the top cover absorbs fluid and swells creating a barrier between the dissolution media and the inner drug containing core. The time taken for the swollen layer to erode determines the lag-time. Once the top layer has eroded drug release occurs. Drug release from this formulation displays a lag-phase between 50-245 minutes depending on the type of polymer and drug used. Drug is then rapidly released so that drug is released in a sigmoidal manner.

Karavis et al., (2006) developed a bilayered tablet that provided a lag-phase followed by drug release. The bi-layered tablet makes use of felodipine and PVP as a drug core and a HPMC/PVP blend surrounding the core. The combination of HPMC and PVP served to enhance the mucoadhesive properties of the tablet. Upon exposure to dissolution media the outer layer disintegrated exposing the core to the gastric contents thus allowing the release of drug. The lag-time was dependent on the swelling/erosion of the HPMC/PVP matrix. Varying the ration of the HPMC to PVP affected the lag-time.

Sher et al., (2007) made use of floating and pulsatile technology in order to develop a chronotherapeutic drug delivery system that may be employed in the treatment of cancer. This was achieved with no excipient, low-density, microporous beads loaded with ibuprofen. Drug was loaded onto porous Accurel MP 1000[®] microparticles using melt and solvent evaporation techniques. The microparticles were tested for 6 hours in pH 1.2 followed by 3 hours in pH 7.2. Drug release proved to be minimal while floating in the stomach followed by a burst of drug release at a pH of 7.2. Similar results were obtained by Badve et al., 2007 where hollow porous calcium pectinate beads were developed. *In vivo* studies conducted on rabbits demonstrated promising results with gastric floatation lasting for 5 hours.

A novel drug delivery system based on a “tablet in capsule device” capable of producing up to three pulses of drug release, has been developed by Li et al., (2008). The device comprises a water soluble cap, impermeable capsule body, and two multi-layered tablets which are filled within the capsule body and sealed with a water-soluble cap. The capsule is also filled with lactose and a modulating barrier layer (Figure 2.8).

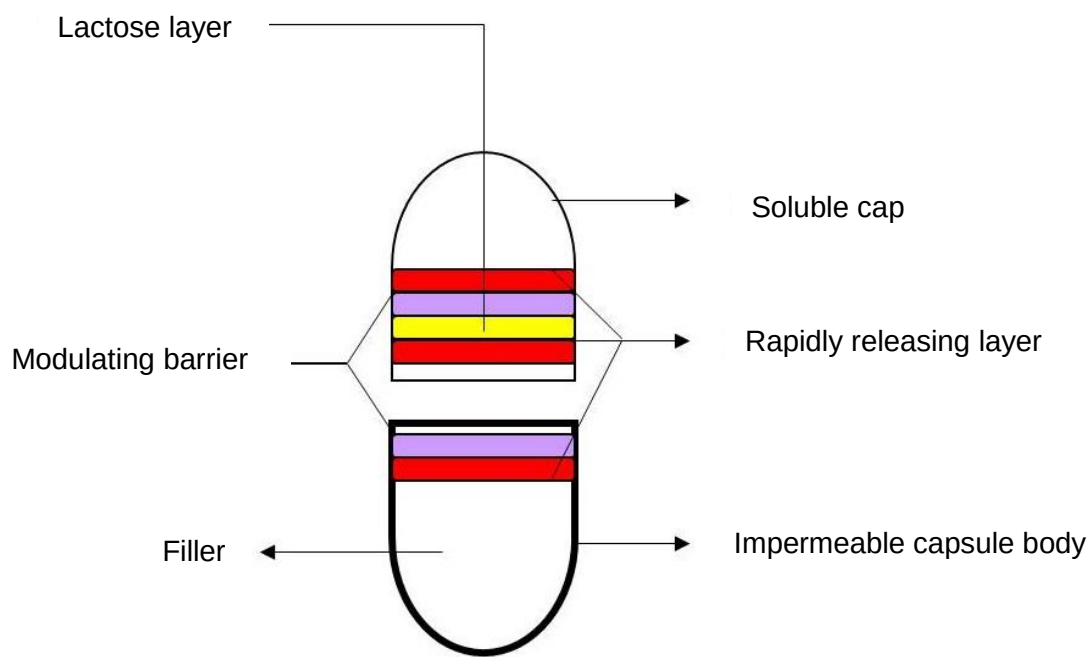


Figure 2.8: Diagrammatic representation of the tablet in capsule device (Adapted: Li et al., 2008).

The impermeable capsule comprises EC while the rapid release layer comprises crospovidone and lactose. HPMC, sodium alginate, carbopol and carboxymethylcellulose were evaluated for use as the modulating barrier, with alginate and HPMC displaying the best results. The drug release profile achieved by this device displays three phases of drug release within a 12 hour period. By manipulating the quantity of polymer in the barrier layer, the lag-phase between the pulses can be controlled. A difference in lag-time was observed when the tablets were inserted into various locations within the capsule body. Although this technology allows for the combining of three doses into a single system, the manufacturing process might prove time consuming as each component of the device is prepared separately.

Enteric-coated microspheres were developed for colonic drug delivery by Maestrelli et al., (2008). These calcium pectinate microspheres may be applied to chronotherapeutic disorders as well as for local treatment of the colon. The

microspheres were formed by homogeneously dissolving PEC in distilled water and then adding drug. The dispersion was then passed through a nozzle into a solution of calcium chloride. The microspheres were formed immediately by ionotropic gelation. Coating of the microspheres involved immersing the spheres in a solution of Eudragit® S100 and allowing them to dry by solvent evaporation. Drug release studies were performed in pH 1.1, 6.6 and 7.4. Results obtained from the study displayed negligible drug release at pH 1.2, <10% drug release in simulated intestinal fluid followed by complete release in the colon. Though this drug delivery device produces an initial lag-phase it does not provide pulsatile drug release.

Chitosan microspheres were developed by Jose et al., (2011) with the purpose of creating a chronotherapeutic colon specific DTZ dosage form for use in the treatment of stable angina. The microspheres were developed by emulsion cross-linking of chitosan and coated with Eudragit S-100 by solvent evaporation techniques. DTZ was encapsulated within the microspheres. Non-coated microspheres displayed a burst release whereas coated microspheres displayed controlled release suggesting that the Eudragit® coating protects the DTZ from release below a pH of 7.

These technologies do have their limitations mainly due to their complicated manufacturing procedures. In addition, multiparticulates have not been extensively tested in humans where they may behave in a very different manner to that exhibited *in vitro*. In terms of the drug release profiles achieved only the “Tablet in Capsule Device” was able to provide more than one pulse of drug release.

2.6 Parenteral Chronotherapeutic Drug Delivery Systems

Examples of parenteral drug delivery systems that are indicated for chronotherapeutic use include the chronomodulated infusion pumps: Melodie® (Laboratoire Aguettant, Lyon, France), Synchromed® (Medtronic, Minneapolis, USA) and Rhythmic® (Micrel Medical Devices, Athens, Greece). With these pumps frequency, timing and order of drug administration can be programmed.

2.7 Concluding Remarks

The concept that the body is in a constant homeostatic equilibrium is no longer true according to the literature. Evidence suggests that the bodies' internal clock alters homeostatic control during a 24 hour period leading to fluctuations in certain integral processes such as blood pressure control, cortisol release and gastric acid secretion. With the identification of numerous conditions that follow a circadian rhythm, a superior understanding of chronotherapy is required in the medical and pharmaceutical fields. As numerous drugs are influenced by the daily time-scale, it is disconcerting that so few chronopharmaceuticals exist on the market. To date, no drug delivery system is able to satisfy all the requirements of chronotherapeutics. The search for innovative drug delivery systems applicable to chronotherapy is ensuing with increasing intensity. Extensive research in this field is a necessity in order to improve patient outcomes with research going as far as human studies.

CHAPTER THREE

PRELIMINARY STUDIES INTO THE DEVELOPMENT OF THE MULTI-LAYERED MULTI-DISK TABLET

3.1 Introduction

The use of hydrophilic matrices in controlling the release rate of drugs from solid dosage forms has become increasingly popular. Examples include Matsuo et al., 1996; Roy and Rohera, 2002; Wu et al., 2005; Conti et al., 2006; Jamzad and Fassihi, 2006; Khairuzzaman et al., 2006; Hardy et al., 2007. The use of such water swellable polymers allows for the formation of a viscous gel layer as the surface of the polymer hydrates when exposed to dissolution media. The viscous gel layer is a diffusional barrier that retards further water uptake and the release of the dissolved drug. Verma et al., (2004), have reported that the gel layer formation and its stability define the kinetics of drug delivery from matrix systems. This is controlled by the concentration, viscosity and chemical structure of the polymer. Conti et al., (2006), have reported that cellulose ethers are probably the most commonly used water soluble polymers in pharmaceutical text. Cellulose ethers have the advantage of being non-toxic, cost effective, having good compressibility and swelling characteristics and are able to accommodate a large percentage of drugs. Furthermore, they are FDA approved and as such can contribute to shorter approval times.

To formulate a successful hydrophilic system a polymer needs to be chosen that will wet, hydrate and swell to form a gel layer fast enough to prevent disintegration of the tablet. The rapid formation of a gel layer is critical to prevent wetting of the interior and disintegration of the tablet core. As water penetrates the system, the outer layer gels and swells while the core remains dry. The region between the gel layer and dry core is known as the rubbery region (Figure 3.1). Once the gel layer forms it controls the penetration of additional water into the tablet. The gel layer dissolves due to a decrease in the viscosity of this gel and a new inner layer must replace it and must be cohesive and continuous enough to retard the influx of water and control drug diffusion. Although gel strength is controlled by polymer viscosity and concentration, polymer chemistry also plays a significant role. HPMC is thought to encourage a strong tight gel formation compared to other cellulosic polymers. As a result, drug release has been

sustained longer with HPMC than with the same quantity of methylcellulose, HEC, HPC and carboxymethyl cellulose. Thus, HPMC has been chosen for use in the rate controlling outer layers of this drug delivery system.

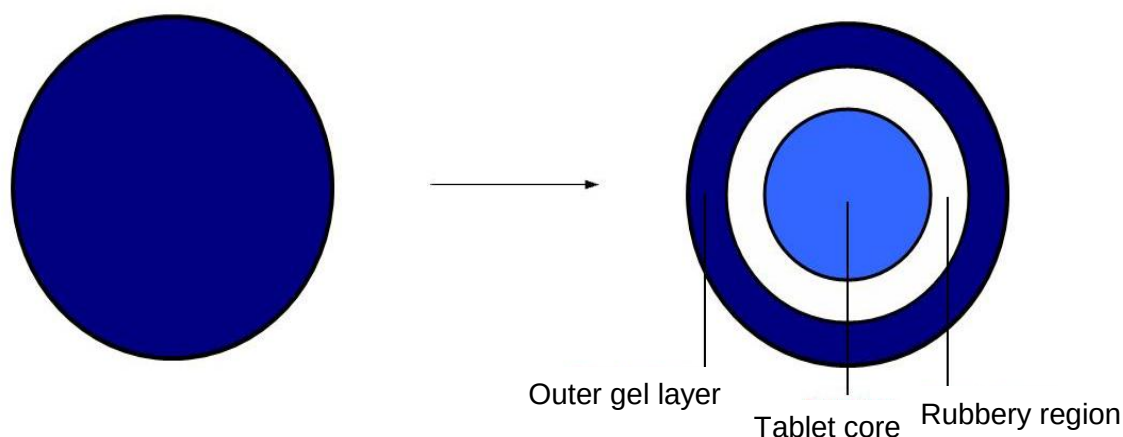


Figure 3.1: Schematic depicting a matrix tablet at a) T_0 and b) after exposure to dissolution media.

Of the cellulose ethers, HPMC is the most widely studied hydrophilic swellable polymer (Li et al., 2005; Khairuzzaman et al., 2006). HPMC is derived from alkali-treated cellulose by reacting with methyl chloride and propylene oxide. It is non-ionic and minimizes interactions when used in acidic, basic or other electrolyte systems. It offers the convenience of swift hydration, excellent compressibility, superior gelling and low toxicity.

Other cellulose ethers used in this study include EC and HEC. HEC is a cellulose of short to very long chain length that is non-ionic in nature thus allowing pH-independent drug release. EC is a hydrophobic cellulose in which the backbone is based on repeating anhydroglucose units. EC offers the benefits of being non-toxic, inert and stable at various pH values. Uses include coating (Li et al., 2010; Rujivipat and Bodmeier, 2010; Terebesi and Bodmeier, 2010; Zhao et al., 2010), pellets and spheres (Frohoff-Hülsmann et al., 1999; Kranz and Gutsche, 2009; Shi et al., 2009), tablets (Mäki et al., 2006; Abdel-Rahman et al., 2009; Quinten et al., 2009).

This chapter addresses the selection of polymers that led to the development of the MLMDT. A comparative analysis of polymers is performed as these tests are critical in obtaining the desired release profile. In-process validation of the developed MLMDT's was assessed determining the suitability of the devices components.

3.2. Material and Methods

3.2.1 Materials

Polymers utilized in the study include: HPMC (Methocel[®] K4M CR, Colorcon Limited, Kent, United Kingdom), HPC (Klucel[®], EF Pharm, Hercules Inc., North Carolina, USA), HEC (viscosity 4000mPas, Merck Schuchardt, Hohenbrunn, Germany), PEO (Polyox N12K[®], molecular weight approximately 1×10^6 g/mol, viscosity range 400-800cps, Dow Chem. Corp., Michigan, USA), poly(lactic-co-glycolic acid) (PLGA) (Resomer RG504, molecular weight 55,000g/mol, Boehringer Ingelheim GmbH, Ingelheim, Germany), EC (48-49.5% ethoxy content, viscosity 11cP, Sigma-Aldrich, Missouri, USA), PEC Cu 701 (Degree of esterification 38%, molecular Weight 80, Herbstreith and Fox, Neuenburg Germany), Alginate (Protanal[®] LF 10/60, FMC BioPolymer, Haugesund, Norway), PVA (molecular weight 49,000g/mol, Sigma-Aldrich, Missouri, USA). The model drugs THP (solubility 1-5mg/mL) and DTZ (solubility > 50%) were purchased from Sigma-Aldrich (Missouri, USA). Surelease[®] (EC aqueous dispersion) and Sureteric[®] (polyvinyl acetate phthalate) (PVAP) (Colorcon Limited, Kent, United Kingdom) were employed as granulating fluid. Other excipients include Avicel[®] (AVC) (Microcrystalline cellulose, FMC BioPolymer, Haugesund, Norway), Lactose and Sodium Bicarbonate (Saarchem, Krugersdorp, South Africa). Lactose was used as a bulking agent in Disk 1.

3.2.2 Preparation of Disk 1 (D1)

Lactose and either THP (15mg) or DTZ (18mg) were measured, blended and directly compressed using a Karnavati Mini Press II (Rimek Products, Gujarat, India) loaded with a 10mm in diameter punch and die. The quantity of drug in this disk comprised 30% of the total drug utilized in the MLMDT.

3.2.3 Preparation of Disk 2 (D2)

Quantities of drug (70% of total drug utilized in the entire device) and polymer were granulated by wet granulation, with Surelease[®], Sureteric[®] or a combination of these used as a granulating fluid (where applicable). Where no granulating fluid was utilized, polymer and drug were directly compressed. Surelease[®] was prepared as per manufacturer instruction by measuring a 60% w/v of the solution and adding 40% w/v of deionized water (Milli-Q Millipore, Massachusetts, USA). The solution was then agitated for 40 minutes prior to use. Sureteric[®] was reconstituted to a 15% solid suspension using deionized water (Milli-Q Millipore, Massachusetts, USA) to which 0.33% of an antifoaming agent was added. The suspension was agitated for 30

minutes prior to use. Drug and polymer were blended using a cube blender (Erweka Apparatebau, Heusenstamm, Germany) to which the granulating fluid was then added to produce a wet mass. The wet mass was then passed through a 2000mm aperture sieve and collected. The granules were placed in an oven at 37°C until dry. The granules were weighed and compressed using a Karnavati Mini Press II.

3.2.4 Preparation of the final MLMDT

A schematic of the MLMDT is highlighted in Figure 1.3, Section 1.2, Chapter 1. Briefly, the outer drug-free polymeric layers comprised three barrier layers (L1, L2 and L3) in which the disks were enveloped. DTZ formulations contained sodium bicarbonate (where applicable). Table 3.1 and 3.2 expands on the specific quantities utilized. In order to combine the device, L1 was added to a flat-faced 13mm punch and die and after leveling out the powder D1 was inserted and then centered using the tip of a needle. The next layer (L2) was then incorporated and leveled followed by the placement and centering of D2 and lastly L3. The punch was then inserted and the device was compressed at 5 tons using a Beckman hydraulic tablet press (Beckman Instruments, Inc., California, USA). To minimize processing variables, all the devices were produced under identical conditions.

		D1	D2		Barrier Layers		Granulating Agent	
Formulation	Lactose (mg)	Polymer (70mg)	L1 (50mg)	L2 (100mg)	L3 (200mg)	Surelease [®] (EC) ^b	Sureteric [®] (PVAP) ^b	
1	30	EC	Alginate	PEO	PLGA	-	-	
2	30	PLGA	PEC	HEC	HEC	-	-	
3	30	PLGA	PEC	PEO	PEO	-	-	
4	30	HPMC	PEC	HPMC	HPMC	-	-	
5	30	EC	PEC	HPMC	HPMC	-	-	
6	30	EC	PEC	Alginate	Alginate	-	-	
7	30	PLGA/PVA (1:3)	PEC	HPMC	HPMC	1mL	-	
8	145	EC	PEC	HPMC	HPMC	1mL	-	
9	145	PVA	PEC	HPMC	HPMC	1mL	-	
10	145	EC/HEC (1:1)	PEC	HPMC	HPMC	1mL	1mL	

Table 3.1: Mass constituents utilized in the preformulation of the THP^a-loaded MLMDT's.
^b

Total quantity loaded=50mg with 15mg loaded in D1 and 35mg loaded in D2.

^b: 1mL of Granulating Agent was utilized per formulation

Formulation	Disk One	Disk Two	Barrier Layers			Granulating Agent	
	Lactose (mg)	Polymer (84mg)	L1 (150mg)	L2 (100mg)	L3 (200mg)	Surelease [®] (EC) ^b	Sureteric [®] (PVAP) ^b
11	142	EC	PEC	HPMC	HPMC	-	1mL
12	142	HEC/EC (1:3)	PEC	HPMC	HPMC	1mL	-
13	142	HEC/EC (4:1)	PEC	HPMC	HPMC	1mL	-
14	142	EC	PEC	HPMC	HPMC	1mL	-
15 ^c	142	HEC/EC (1:3)	PEC	HPMC	HPMC	1mL	-

Table 3.2: Mass constituents utilized in the preformulation of the DTZ^a-loaded MLMDT's.

^a: Total quantity of drug loaded=60mg with 18mg loaded in the first disk and 42mg loaded in the second.

^b: 1mL of Granulating Agent was utilized per formulation

^c: Sodium Bicarbonate added in D2.

3.2.5 *In vitro* drug release studies

The *in vitro* drug release studies were carried out using USP dissolution apparatus II (Erweka, Heusenstamm, Germany) equipped with paddles. Dissolution was performed in 900mL simulated human gastrointestinal fluid (SHGF) pH 1.2 (Table 3.3) (Lee et al., 1999) for the first 2 hours and simulated human intestinal fluid (SHIF) pH 6.8 (Table 3.3) (Giannola et al., 2007) for the remainder of the study. Where sodium bicarbonate was used, formulations were tested in pH 1.2 due to floatation of the formulation. The devices were placed on a ring wire-mesh assembly as formerly carried out by Pillay and Fassihi (1999). The wire mesh fits into the lower portion of the glass vessels and prevents the device from sticking to the bottom of the vessel, allowing the full surface area of the device to be exposed to the dissolution medium. Dissolution was carried out at a speed of 50 rpm and at a temperature of $37 \pm 0.5^\circ\text{C}$. Sampling of 5mL was carried out every 1 hour for 12 hours and thereafter at the 24th hour. The withdrawn amount of sample (i.e. 5mL) was replaced with an equal amount of the simulated fluid such that the volume of the simulated fluid in the dissolution medium remained constant. The drug content was analyzed by Ultra Violet (UV) spectrophotometer (Hewlett Packard 8453, Boeblingen, Germany) at $\lambda_{280\text{nm}}$ for THP and $\lambda_{238\text{nm}}$ for DTZ and computed from a standard linear curve of drug in SHGF and SHIF ($R^2 > 0.99$).

Table 3.3: Constituents used to prepare the simulated human gastric fluid (SHGF) and simulated human intestinal fluid (SHIF).

Component	SHGF (pH 1.2) ^a (Quantity in 1L)	SHIF (pH 6.8) ^b (Quantity in 1L)
KH ₂ PO ₄	-	0.144g
Na ₂ HPO ₄	-	0.795g
NaCl	2.0g	9.000g
HCl (Conc)	7mL	-

^aSimulated human gastric fluid (Lee et al., 1999)

^bSimulated human intestinal fluid (Giannola et al., 2007)

3.2.6 Construction of calibration curves for spectrophotometric determination of THP and DTZ

Calibration curves for THP and DTZ was constructed using a known series of concentrations of THP and DTZ (0 to 0.025mg/mL). Using simulated fluid as a blank, the absorbance of each solution was measured and a linear curve was plotted with the observed absorbance on the y-axis and concentration (mg/mL) on the x-axis. The correlation coefficient was also calculated.

3.2.7 Assessment of the friability of the disks and the MLMDT

Friability was performed on both disks as well as the compressed MLMDT. A sample of 10 units was examined to ensure reproducibility of the tablet making process. Analyses were determined 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 analytical digital balance (Mettler, Model AE 240, Griefensee, Switzerland) with readings recorded to 2 decimal places. The friability was expressed in terms of weight loss and was calculated in % (% $\pm$ SD) of the initial weight.

3.2.8 Assessment of the powder and granule flowability

The flowability of both the granules and the combination of powders used for both disks was studied. Carr's index, angle of repose and Hausner ratios were used to determine powder flow. Carr's index and Hausner ratio were calculated from the bulk and tapped density via the tapping method. A cylinder, to which a known quantity of powder or granules was added, was subjected to a fixed number of taps. The final volume after tapping was recorded and calculated using the equations below:

$$\text{Carr's index (\%)} = \frac{\text{Tapped-Poured density}}{\text{Tapped density}} \times 100$$

Equation 3.1

$$\text{Hausner Ratio} = \frac{\text{Tapped density } (\rho_{\max})}{\text{Poured density } (\rho_{\min})} \times 100$$

Equation 3.2

The angle of repose (θ) was calculated using the fixed funnel method. A glass funnel was secured with its tip positioned at a fixed height (H) above graph paper placed on a horizontal surface. The sample was poured through the funnel until the apex of the conical pile touched the tip of the funnel. The angle of repose was calculated using the formula below:

$$\tan \theta = \frac{H}{R}$$

Equation 3.3

where θ is the angle of repose and R is the radius of the conical pile.

3.3 Results and Discussion

3.3.1 Calibration curves for the determination of drug concentration

Standard solutions of both model drugs, THP and DTZ, were prepared by separately mixing known concentration of each drug in simulated gastric fluid. The standard solutions employed in preparing the calibration curve of the drugs were obtained by serial dilutions with a final concentration ranging between 0.005-0.025mg/mL. Figure 3.2 illustrates the calibration curves for THP and DTZ employing a UV spectrometer at $\lambda_{280\text{nm}}$ for THP and $\lambda_{238\text{nm}}$ for DTZ.

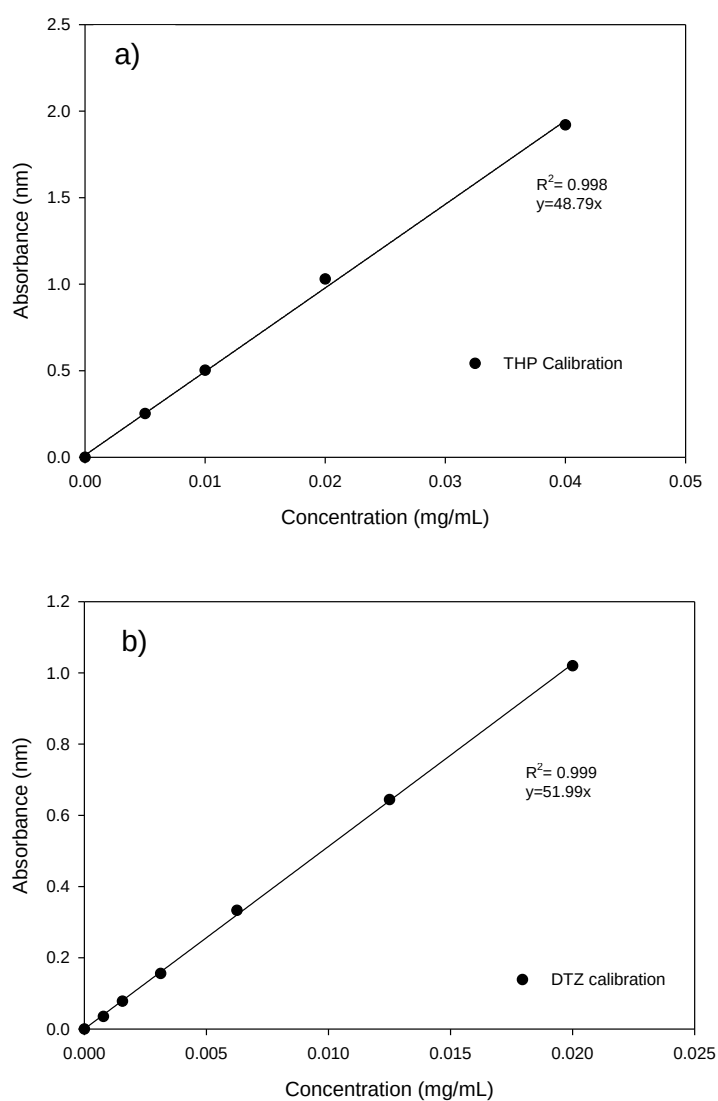


Figure 3.2: Calibration curves for a) THP at 280nm and b) DTZ at 238nm (N=3; SD <0.01 in all cases).

3.3.2 *In vitro* drug release analysis from THP-loaded MLMDT's

Drug is released after a lag-phase from D1 corresponding to the first pulse of release. The remaining layers, L2 and L3, control the rate of release from D2. These layers should ideally protect drug from release for approximately 8-12 hours to allow for the “switch-off” phase, and then only should drug be released from D2. Theoretically release should not occur from both disks at the same time. A schematic of this process is highlighted in Figure 3.3.

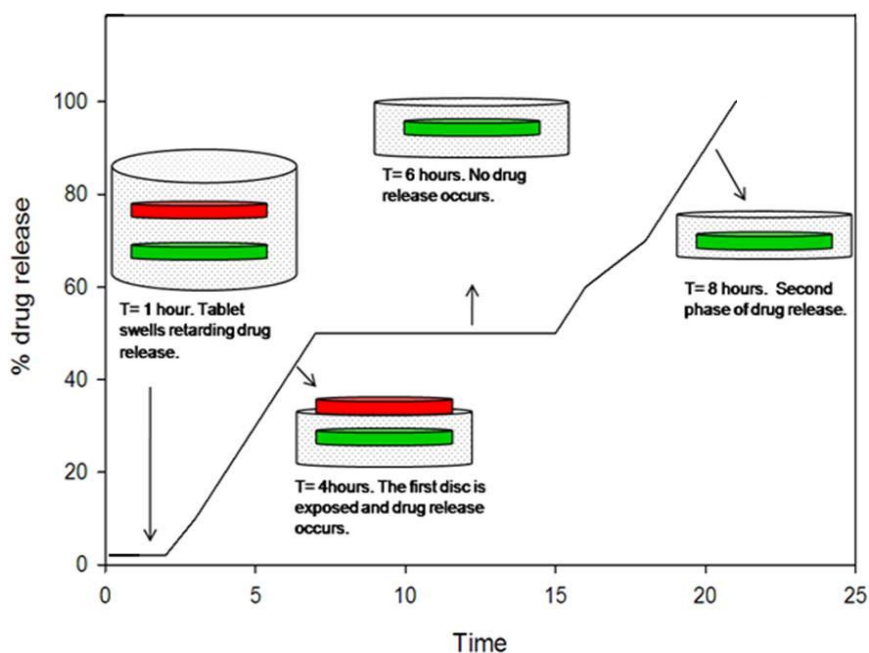


Figure 3.3: Schematic depicting the ideal drug release profile required from the MLMDT.

Figure 3.4 (a-c) depicts the drug release profiles from the 10 THP formulations. All release profiles display biphasic release with an initial lag-phase. The first pulse provides rapid drug release while the second pulse displays sustained release. The rate at which drug is released during the second pulse is governed by the L2, L3 and D2. F5 and F8 (Figure 3.4a) displayed minimal drug release from D2 (38% and 32% respectively) due to the hydrophobic nature of EC which impedes the release of drug. Furthermore, combining EC with Surelease[®] (F8) resulted in no drug release from D2 suggesting that this combination was too hydrophobic.

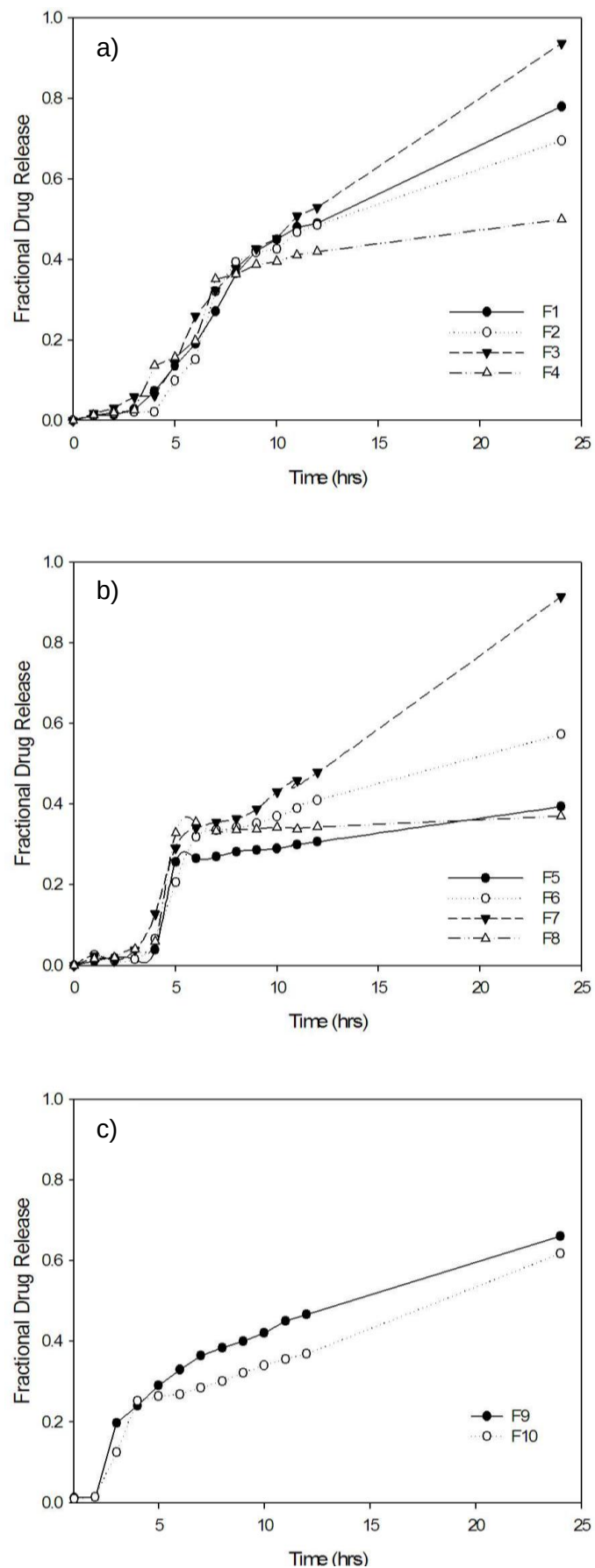


Figure 3.4: Drug release profiles of THP-loaded MLMDT's: a) formulation 1 to 4; b) formulation 5 to 8 and c) formulation 9 and 10 (N=3; SD <0.47 in all cases).

3.3.3 *In vitro* drug release analysis from DTZ-loaded MLMDT's

Results from drug release studies performed on DTZ-loaded MLMDT's are presented in Figure 3.5a-b. F11 displayed biphasic release kinetics whereas F12 presented zero-order release indicating that both disks released drug simultaneously. F13 (Figure 3.5a) displayed no drug release between T=5 and T=6 hours. However, drug release was incomplete over the 24 hours (<60%). F14 displayed an initial pulse of drug release, but thereafter only displayed cumulative release of 40% in the remaining 24 hours implying that the polymer combination used was ineffective. F15 (Figure 3.5b) included the electrolyte sodium bicarbonate with EC and the hydrophilic HEC granulated with Surelease® in D2. The inclusion of sodium bicarbonate is based on work published by Pillay and co-workers (1999) which demonstrated that the inclusion of sodium bicarbonate gave rise to *in situ* ionic interactions resulting in the inhibition of drug dissolution due to a "matrix-stiffening" effect. A "switch-off" phase of three hours was observed with over 70% of drug release over the full 24 hours. Thus, the inclusion of sodium bicarbonate with the combination of a hydrophobic and hydrophilic polymer was sufficient to retard drug release so that a "switch-off" phase is achieved while at the same time allowing for adequate drug release over the 24 hour period. With the remaining formulations, the polymer combinations utilized proved to be ineffective in producing the desired release profile. In order to produce this profile, polymers used in D2 are required to retard drug release for approximately 8-12 hours. Therefore, the polymer combination employed plays an important role in producing the desired release profile.

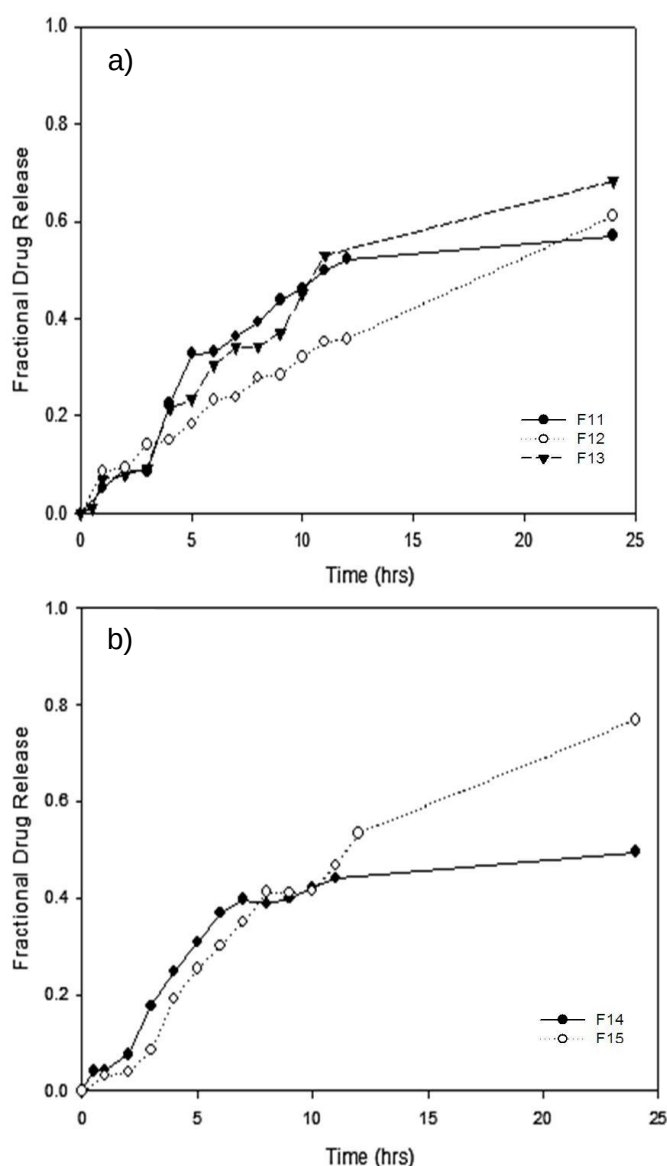


Figure 3.5: Drug release profiles of DTZ-loaded MLMDT's: a) formulation 11-13 and b) formulation 14 and 15 (N=3, SD<0.02 in all cases).

3.3.4 Analysis of the disk and MLMDT friability

Table 3.4 illustrates the friability (expressed as a percentage) of D1, D2 and the complete MLMDT device. Formulations 1-7 demonstrated values ranging between 11.0 and 12.1% for D1 indicating the disks were extremely friable. To decrease the friability, the quantity of lactose incorporated into D1 was increased from 30mg to 145mg (F8-F15). This decreased the friability to within the acceptable range of <1% and had no significant impact on drug release. Friability values of D2 all fall within the desired range. Granulated disks (Formulation 6 onwards) revealed lower friability than the directly compressed disks (Formulation 1-6). Friability values of the complete MLMDT all met the necessary requirements and fall within the acceptable range.

Table 3.4: Table displaying the friability values of the fifteen formulations.

Formulation	D1 (%±SD)	D2 (%±SD)	MLMDT (%±SD)
1	11.251 ± 0.015	0.621 ± 0.014	0.475 ± 0.005
2	12.015 ± 0.021	0.611 ± 0.009	0.675 ± 0.009
3	11.425 ± 0.016	0.651 ± 0.015	0.401 ± 0.011
4	11.502 ± 0.024	0.677 ± 0.019	0.367 ± 0.017
5	12.102 ± 0.017	0.658 ± 0.017	0.354 ± 0.014
6	11.963 ± 0.024	0.641 ± 0.012	0.322 ± 0.017
7	11.547 ± 0.020	0.511 ± 0.011	0.360 ± 0.021
8	0.854 ± 0.027	0.498 ± 0.021	0.346 ± 0.018
9	0.795 ± 0.014	0.533 ± 0.009	0.351 ± 0.014
10	0.80 ± 0.029	0.554 ± 0.017	0.339 ± 0.010
11	0.815 ± 0.014	0.578 ± 0.018	0.351 ± 0.019
12	0.837 ± 0.012	0.521 ± 0.023	0.301 ± 0.012
13	0.797 ± 0.014	0.503 ± 0.007	0.332 ± 0.008
14	0.812 ± 0.013	0.435 ± 0.006	0.329 ± 0.014
15	0.785 ± 0.011	0.448 ± 0.009	0.321 ± 0.013

3.3.5 Analysis of the powder and granule flowability

Table 3.5 displays the Carr Index and Hausner ratio of the disks used in the formulations. Poor flowability of D1 was displayed in F1-F6 as both the Carr Index and the Hausner Ratio are out of the normal range (< 18 and < 1.25 respectively). The increase in lactose (F8 onwards) decreased the values which suggested improved flowability. Values for D2 all fell within the acceptable range with granulated blends (F8 onwards) displaying enhanced flowability due to the larger particle surface area.

Table 3.5: Carr Index and Hausner ratio of D1 and D2 in the various formulations.

Formulation	Carr Index		Hausner Ratio	
	D1	D2	D1	D2
1	26.31 ± 0.320	12.33 ± 0.394	1.963 ± 0.008	1.073 ± 0.006
2	28.21 ± 0.442	11.73 ± 0.493	1.895 ± 0.006	0.959 ± 0.005
3	26.94 ± 0.393	13.01 ± 0.470	1.937 ± 0.007	1.092 ± 0.007
4	29.12 ± 0.462	12.32 ± .349	1.824 ± 0.004	1.003 ± 0.006
5	28.22 ± 0.511	11.90 ± 0.462	1.876 ± 0.005	1.041 ± 0.008
6	27.39 ± 0.401	12.67 ± 0.369	1.927 ± 0.006	1.087 ± 0.006
7	28.39 ± 0.367	8.69 ± 0.476	1.909 ± 0.009	0.946 ± 0.004
8	17.79 ± 0.509	6.90 ± 0.432	1.187 ± 0.008	0.917 ± 0.007
9	17.63 ± 0.439	6.37 ± 0.561	1.197 ± 0.005	0.987 ± 0.005
10	17.99 ± 0.591	7.21 ± 0.532	1.190 ± 0.007	0.902 ± 0.005
11	17.93 ± 0.466	6.27 ± 0.632	1.101 ± 0.006	0.910 ± 0.004
12	17.21 ± 0.407	7.93 ± 0.497	1.078 ± 0.003	0.937 ± 0.003
13	18.02 ± 0.397	7.43 ± 0.367	1.117 ± 0.006	0.952 ± 0.004
14	17.91 ± 0.409	8.13 ± 0.567	1.121 ± 0.003	0.993 ± 0.005
15	17.29 ± .0428	6.37 ± 0.497	1.167 ± 0.004	0.949 ± 0.006

3.4 Concluding Remarks

A range of polymers were initially utilized to determine the effects of the polymer on drug release. Results from dissolution studies displayed one pulse of drug release suggesting that drug release occurred simultaneously from both disk. Thus, in order to produce the second pulse, release from D2 should be delayed. This delay is dependent on the polymers in D2. The combination of a hydrophobic and hydrophilic polymer in D2, such as EC and HEC proved to be advantageous as it slowed drug release in THP-loaded formulations, but still allowed for near complete drug release after the 24 hours. The use of a granulating fluid was also necessary as it impedes drug release. Due to the high solubility of DTZ, both disks released drug simultaneously even with the inclusion of the hydrophobic EC. The inclusion of sodium bicarbonate however, decreased the rate at which drug release occurred. This was attributed to *in situ* ionic interactions due to a “matrix-stiffening” effect. Friability, Carr Index and Hausner Ratio tests indicated that increasing lactose in D2 improved the friability of the disks and the flowability of the powders.

CHAPTER FOUR

IN VITRO STUDIES AND OPTIMIZATION OF THE MULTI-LAYERED MULTI-DISK

TABLET

4.1 Introduction

Successful development of a drug delivery system requires the delivery of the active ingredient in a controlled manner. In the design and characterizing of a drug delivery system, computer programs are becoming exceedingly popular (Ghaffari et al., 2006). Artificial neural networks (ANN) were introduced into the pharmaceutical field by Hussain et al. and coworkers in 1991. Since then ANN models were utilized in the prediction of drug release profiles, optimization of formulations and predicting process variables (Chen et al., 1999; Ibrić et al., 2002; Vaithiyalingam and Khan, 2002; Barmapalexis et al., 2010). This model also eliminates trial and error which proves to be time-consuming and inaccurate. With ANNs there is no need to assume an underlying data distribution. In addition, ANNs are applicable to highly non-linear multivariate problems (Sando et al., 2005). ANN's are computer programs that are designed to replicate the learning process of the human brain (Chen et al., 1999; Sun et al., 2003). The most recognized ANN types are: Multilayer Perceptron (MLP), radial basis function networks, linear networks, Kohonen networks and Bayesian networks (probabilistic and generalized regression) (Ibrić et al., 2002).

The MLP overcomes the limitation of the single-layer perceptron by the addition of one or more hidden layer(s) (Ghaffari et al., 2006). MLPs are layered feed forward networks typically trained with static back propagation. These networks have found their way into countless applications requiring static pattern classification. Their main advantage is that they can approximate any input/output map. The key disadvantages are that they train slowly, and require lots of training data typically three times more training samples than network weights. Generalized Feed Forward (GFF) networks are a generalization of the MLP such that connections can jump over one or more layers. In theory, a MLP can solve any problem that a GFF network can solve. In practice, however, GFF networks often solve the problem much more efficiently. This study utilizes a GFF model to predict the release of drug from the device when the composition and quantity of polymer in the second disk is varied.

The aim of this chapter is to utilize ANNs to generate an optimized formulation and to evaluate the physicochemical and physicomechanical properties of the generated optimized formulations.

4.2 Materials and Methods

4.2.1 Materials

Materials employed include those mentioned in Chapter 3, Section 3.2.1.

4.2.2 Preparation of the MLMDT device

The MLMDT was prepared according to the method provided in Chapter 3, Section 3.2.2.1. Table 4.1 depicts the mass of the constituents of the MLMDT.

Table 4.1: Mass constituents utilized in the preparation of the MLMDT device.

	DTZ-Loaded MLMDT	THP-Loaded MLMDT
PEC Barrier Layer (L1)	150mg	150mg
HPMC Middle Barrier Layer (L2)	150mg ¹	100mg
HPMC Outer Barrier Layer (L3)	250mg ¹	200mg
Disk 1	160mg	160mg
Disk 2	160mg	160mg
Total Weight of Device	870mg	770mg

¹ The DTZ-loaded MLMDT includes an additional 100mg of sodium bicarbonate in the HPMC layers.

4.2.3 Evaluation of the prepared disks and MLMDT

Friability, hardness, thickness and uniformity of mass analyses were performed on both the disks and the final MLMDT. A sample of 10 units was examined to ensure reproducibility of the tablet making process. Hardness analyses were carried out on a Hardness Tester (Pharma Test, Hainburg, Germany) while friability was determined using the same instruments, parameters and limits as described in Chapter 3, Section 3.2.2.6. A digital caliper was used to determine the thickness of the disks and the final MLMDT's.

4.2.4 Computational modeling to obtain an optimized formulation using the Artificial Neural Networks approach

Comparative polymer studies conducted in Chapter 3, Section 3.2.2.1 highlighted the important polymers (HPMC, EC and HEC) and combinations thereof that would ultimately lead to the desired release profile. Important data generated from the above analysis indicated that drug release from D2 proved to be the most variable and therefore was fit into the ANN model to generate an optimized formulation. Based on

data generated in Chapter 3, Section 3.2.2.1, 21 formulations with variations in the quantities of HPMC, EC and HEC were evaluated for drug release. Table 4.2 depicts 11 THP formulations while Table 4.3 illustrates 10 DTZ formulations. Although HPMC proved to be a suitable candidate when formulating disks for the THP devices, this was not the case for DTZ formulations and was thus omitted. Sodium Bicarbonate was evaluated for its effect on drug release in DTZ-loaded formulations and was studied in D2 and in L2 and L3.

Table 4.2: Table depicting the polymers, granulating and bulking agents used in D2 of THP-loaded formulations.

Formulation	HEC (mg)	EC (mg)	HPMC (mg)	Granulating Agent		Bulking Agent (lactose) (mg)
				Surelease [®] (mL)	Sureteric [®] (mL)	
1	-	-	70	-	-	-
2	70	-	-	0.5	0.5	-
3	-	70	-	-	1	-
4	-	70	-	1	-	-
5	-	50	-	1	-	30
6	-	35	-	1	-	35
7	70	-	-	1	-	-
8	-	23	-	1	-	47
9	-	-	70	1	-	-
10	35	35	-	1	-	-
11	14	56	-	1	-	-

Table 4.3: Table depicting the polymers, granulating and bulking agents used in D2 of DTZ-loaded formulations.

Formulation	HEC (mg)	EC (mg)	Surelease [®] (mL)	Sodium Bicarbonate		
				D2 (mg)	L2 (mg)	L3 (mg)
12	-	84		-	-	-
13	21	63	1	-	-	-
14	21	63	1	50	-	-
15	21	63	1	-	50	50
16	21	63	1	50	50	50
17	21	63	1	-	100	100
18	21	63	1	100	100	100
19	21	63	1	50	-	50
20	42	42	1	-	-	-
21	21	63	1	50	50	-

The data generated was fit into an ANN model, specifically the MLP, so as to generate an optimized formulation. MLPs are layered feed-forward networks typically trained with static back propagation. These networks have found their way into countless applications requiring static pattern classification. Their main advantage is that they can approximate any input/output map. The key disadvantages are that they train slowly and require lots of training data (typically it utilizes three times more training samples than network weights). The GFF network used in this study is a generalization of the MLP such that connections can jump over one or more layers (Figure 4.1). In theory, a MLP can solve any problem that a GFF network cannot solve. In practice, however, GFF networks often solve the problem much more efficiently.

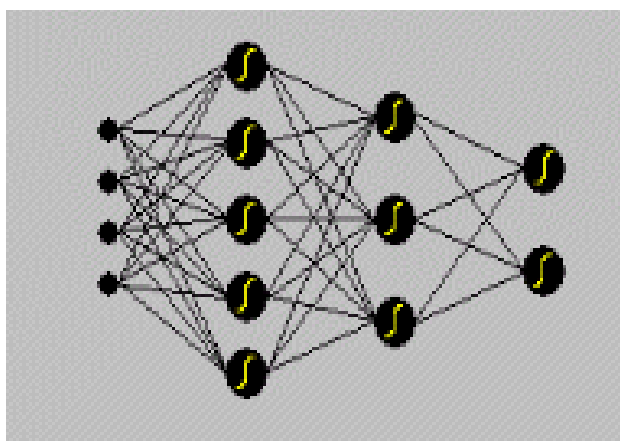


Figure 4.1: Diagram representing a Multilayer Perceptron (MLP) with two hidden layers.

For the hidden and output layers, a genetic algorithm with the Sigmoid Axon transfer function and Conjugate Gradient learning rule were employed. A maximum of 10 000 epochs were run on NeuroSolutions Version 4.32 (NeuroDimension Inc., Florida, USA) to ensure optimal training of data.

4.2.5 *In vitro* drug release analysis

The *in vitro* drug release studies were carried out using a USP dissolution apparatus II (Erweka, Heusenstamm, Germany) equipped with paddles as described in Chapter 3, Section 3.2.2.4. Digital images of the devices at certain dissolution times were captured using a digital camera (Samsung NV15) to obtain changes of the devices during dissolution by recording the top view of the MLMDT.

4.2.6 Polymer swelling studies

The rate of test water uptake was determined by the equilibrium weight gain method as formerly carried out by Efentakis et al., (2000). The MLMDT's were weighed and placed in a USP dissolution apparatus II as described in Section 3.2.2.2 over a period of 24 hours. At regular intervals the MLMDT's were removed, blotted with tissue paper to remove the excess fluid and reweighed. The percentage water uptake, which is the degree of swelling, was estimated at each time point using Equation 4.1:

$$\% \text{ Water uptake} = \frac{(W_s - W_i)}{W_p} \times 100 \quad \text{Equation 4.1}$$

where W_s is the weight of the swollen matrix at time, t , W_i is the initial weight of the matrix, and W_p is the weight of polymer in the matrix. The polymer swelling or water uptake data are a mean of three determinations.

4.2.7 Matrix erosion studies

Erosion studies were determined under conditions identical to those described in Chapter 3, Section 3.2.2.2. At pre-determined time intervals the MLMDT's were removed and dried to constant weight in a hot air oven. The percentage matrix erosion (E) at time, t , was estimated from Equation 4.2:

$$\text{Matrix erosion (\%)} = \frac{(W_i - W_t)}{W_i} \times 100 \quad \text{Equation 4.2}$$

where W_i is the initial starting weight of the matrix and W_t is the weight of matrix subjected to erosion, for time, t . The matrix erosion data are a mean of three determinations.

4.2.8 Magnetic Resonance Imaging of the MLMDT's performance

A magnetic resonance system with digital MARAN-iP System configured with a DRX2 HF Spectrometer console (Oxford Instruments Magnetic Resonance, Oxon, UK) equipped with a compact 0.5 Tesla permanent magnet stabilized at 37°C and a dissolution flow through cell was employed for the viewing of the mechanical behaviors of the matrices. After duly configuring, optimizing the shims and probe tuning, the cone-like lower part of the cell was filled with glass beads to provide laminar flow at 16mL/min of the solvents employed. The matrices were placed in position each time within the cell which in turn was positioned in a magnetic bore of the system and magnetic resonance images were acquired every 30 minutes over 13 hours with MARAN-iP version 1.0 software. The image was acquired after setting the frequency offset and testing gain employing RINMR version 5.7 under continuous solvent flow conditions. MARAN-iP software comprises image acquisition software and image analysis software. The image acquisition parameters are depicted in Table 4.6.

Table 4.4: Image acquisition parameters applied during magnetic resonance imaging using MARAN-iP.

Parameter	Value
Requested gain (%)	4.17
Signal strength	68.92
Average	2
Matrix size	128
Repetition time (ms)	1000.00
Spin Echo Tau (ms)	6.80
Image acquired after	60min
Total scans	64

4.2.9 Determination of polymeric structural variations using Fourier

Transmission Infrared spectroscopy

The molecular structure of the native polymers, the drug-polymer granulated blend and the compressed drug-loaded MLMDT were analyzed using Fourier Transmission Infrared spectroscopy (FTIR) (Perkin Elmer Spectrum 100 Series, Beaconsfield, United Kingdom) to elucidate any variations in vibrational frequencies and subsequent polymeric structure as a result of drug-polymer or even polymer-polymer interactions. Changes in the polymeric backbone may affect the physicochemical and physicomechanical properties of the polymer and as such any changes need to be determined. All analyses were performed in triplicate.

4.2.10 Determination of deformation energy and Brinell Hardness Number

Textural analysis was used to evaluate energy of deformation and indentation hardness which was converted to Brinell Hardness Number (BHN). A calibrated Texture Analyzer (TA.XTplus, Stable Microsystems, Surrey, England) fitted with a flat-tipped steel probe (2mm diameter) was employed for energy of matrix deformation and a ball probe (2mm diameter) was utilized for indentation hardness. Hardness was measured as the force (N) required to indent the matrices to a set distance (mm). This force was then converted to BHN using Equation 4.3. Data was captured at a rate of 200 points per second via Texture Exponent Software (Version 3.2). The employed settings are described in Table 4.4. All experiments were conducted in triplicate.

$$\text{BHN} = \frac{2F}{\pi D(D^2 - d^2)^{1/2}} \quad \text{Equation 4.3}$$

where F is the force generated from indentation, D the diameter of spherical probe indenter (3.175 mm), and d the indentation depth (1.563mm).

Table 4.5: Textural analysis settings employed for the determination of deformation energy and Brinell Hardness Number.

Parameter	Settings	
	Deformation Energy	Brinell Hardness Number
Pre-test speed	1mm/sec	1mm/sec
Test speed	0.5mm/sec	0.5mm/sec
Post-test speed	10mm/sec	1mm/sec
Compression force	40 N	40 N
Trigger Type	Auto	Auto
Trigger Force	0.05N	0.01N
Load cell	5kg	5kg

4.2.11 Determination of surface morphology using Scanning Electron

Microscopy

The surface morphology of the MLMDT's was observed using Scanning Electron Microscopy (SEM). Samples were mounted onto stubs and sputter coated with gold in a vacuum evaporator (Module™ Sputter Coater, SPI Supplies, Pennsylvania, USA) and then photographed using a scanning electron microscope (Phenol™ Fei Company, Oregon, USA).

4.2.12 Atomistic molecular structural mechanics simulations

Molecular mechanics computations in vacuum, which included the model building of the energy-minimized structures of multi-polymer complexes, were performed using the HyperChemTM 8.0.8 Molecular Modeling System (Hypercube Inc., Gainesville, Florida, USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, Cambridge, UK) (Kumar et al., 2011). The structures of EC, HEC, PEC and AVC (4 saccharide units each) were built from standard bond lengths and angles using sugar builder module on HyperChem 8.0.8. The oligosaccharide length for the polysaccharide chain was determined on the basis of equivalent grid surface area covered by the polysaccharide so that the inherent stereo-electronic factors at the interaction site can be perfectly optimized. The set of low-energy conformers that were in equilibrium with each other was identified and portrayed as lowest energy conformational model. The structure was built up with natural bond angles as defined in the Hyperchem software. The generation of the overall steric energy associated with the energy-minimized structures was initially executed initially via energy-minimization using MM+ force field and the resulting structures were again energy-minimized using the Amber 3 (Assisted Model Building and Energy Refinements) force field. The conformer having the lowest energy was used to create the polymer-polymer complexes. A complex of one molecule with another was assembled by disposing them in a parallel way, and the same procedure of energy-minimization was repeated to generate the final models: EC-HEC and PEC-AVC. Full geometry optimizations were carried out in vacuum employing the Polak–Ribiere conjugate gradient method until an RMS gradient of 0.001kcal/mol was reached. Force field options in the AMBER (with all hydrogen atoms explicitly included) and MM+ (extended to incorporate non-bonded cut-offs and restraints) methods were the HyperChem 8.0.8 defaults. For calculations of energy attributes, the force fields were utilized with a distance-dependent dielectric constant scaled by a factor of 1. The 1-4 scale factors are following: electrostatic 0.5 and van der Waals 0.5. Invariant factors common to mathematical description of binding energy and substituent characteristics have been ignored.

4.2.13 Determination of the thermal behavior of the polymeric constituents

Thermal analysis was performed on the granulated polymers and their compressed versions in order to assess thermal behavior using Temperature Modulated Differential Scanning Calorimetry (TMDSC) (Mettler Toledo DSC1, STAR_e System, Switzerland). Thermal transitions were evaluated in terms of the glass transition temperature (T_g) measured as the reversible heat flow due to variation in the magnitude of the C_p -complex values (ΔC_p), melting temperature (T_m) and crystallization temperature (T_c)

peaks that were consequences of irreversible heat flow corresponding to the total heat flow. The temperature calibration was accomplished with a melting transition of 6.7mg indium. Samples of 7mg were weighed on perforated 40µL aluminium pans and ramped within a temperature gradient of 50-250°C under a constant purge of N₂ in order to diminish oxidation. The instrument parameter settings employed comprised a sine segment starting at 50°C with a heating rate of 1°C/min at an amplitude of 0.8°C and a loop segment incremented at 0.8°C and ending at 250°C. The instrument parameter settings employed are described in Table 4.5.

Table 4.6: Temperature modulated differential scanning calorimetry settings employed for thermal analysis.

Segment Type	Parameter Setting
SINE	
Start	50°C
Heating Rate	1°C/min
Amplitude	0.8°C
Period	0.8°C
LOOP	
To segment	1
Increment	0.8°C
End	220 ^a /250°C ^b
Count	212 ^a /249°C ^b

^a: DTZ

^b: THP

4.3. Results and Discussion

4.3.1 Assessment of the physical properties of the MLMDT's

The devices were uniform in mass, each having an average weight of 99.6±0.6 (Table 4.7). The thickness ranged from 1.78±0.02 to 5.18±0.03mm while friability was measured at an average of 0.5±0.03% (i.e. within the set limit 1%), demonstrating desirable matrix compressibility.

Table 4.7: Friability, thickness and mass uniformity for the DTZ and THP-loaded formulations (N=10).

Formulation	Friability (%)	Thickness (mm)	Uniformity of mass ¹ (%)
THP MLMDT	0.3	4.82±0.03	99.6±0.4
THP lactose disk	0.9	1.78±0.02	99.4±0.6
THP polymer disk	0.5	2.08±0.02	99.6±0.4
DTZ MLMDT	0.3	5.18±0.03	99.7±0.3
DTZ lactose disk	0.8	1.78±0.02	99.9±0.1
DTZ polymer disk	0.4	2.14±0.04	99.5±0.5

¹ Expressed as a percentage of the theoretical weight

4.3.2 *In vitro* drug release studies

Based on previous preliminary studies, drug release profiles were separated into four phases namely, the lag-phase, the first pulse of drug release, the “switch-off” phase and second pulse of drug release. Consequently, the rate release constant (k) for each phase was calculated based on the power law expression (Equation 4.4) describing drug release from simple swellable matrix systems.

$$M_t/M_\infty = k_1 t^n$$

Equation 4.4

where M_t/M_∞ is the fraction of drug released at time t , k is the release rate constant and n is the release rate exponent.

The rate constant pertaining to the initial lag-phase, first phase of drug release, “switch-off” phase and second phase of drug release were termed k_1 , k_2 , k_3 and k_4 respectively. Ideally k_3 should be 0. Table 4.8 highlights the rate constants of the 21 chosen formulations.

Table 4.8: The rate constants of the various formulations.

Formulation	k_1 (lag phase)	k_2 (first pulse)	k_3 (switch-off phase)	k_4 (second pulse)
1	0.031	0.089	0.042	0.026
2	0.034	0.069	0.040	0.026
3	0.018	0.049	0.034	0.015
4	0.038	0.092	0.049	0.020
5	0.018	0.040	0.039	0.028
6	0.056	0.071	0.048	0.033
7	0.018	0.047	0.034	0.026
8	0.022	0.045	0.031	0.020
9	0.018	0.053	0.036	0.029
10	0.021	0.044	0.034	0.030
11	0.024	0.051	0.029	0.031
12	0.048	0.075	0.049	0.021
13	0.042	0.062	0.051	0.030
14	0.017	0.12	0.063	0.029
15	0.021	0.095	0.032	0.025
16	0.019	0.092	0.025	0.032
17	0.013	0.062	0.021	0.027
18	0.027	0.055	0.034	0.020
19	0.010	0.067	0.035	0.022
20	0.038	0.010	0.057	0.036
21	0.019	0.090	0.042	0.021

Drug release profiles indicate that the formulations displayed an initial lag phase (Figure 4.2 and 4.3) due to the barrier layers surrounding the disks. Thereafter, a rapid

increase in drug release was observed which is attributed to erosion of L1 and the subsequent exposure of the D1 to the media. After complete release of drug from D1, drug release from all formulations decreased and then gradually increased. The release of drug from D2 is controlled by the barrier layers and the type of polymer utilized. Due to the highly hydrophobic nature of EC, formulations containing pure EC (F3, F4, F5, F6 and F12) displayed poor drug release (<50%) over the 24-hour period (Figure 4.2a, b and Figure 4.3a). Formulations that employed hydrophilic polymers (F1, F2, F7 and F9) demonstrated higher rates of drug release although instead of a “switch-off” phase, biphasic release was observed (Figure 4.2a and c). Utilizing both hydrophobic and hydrophilic polymers (F10-F21) gave rise to an overall increase in the rate of drug release as compared to utilizing hydrophobic polymers on their own.

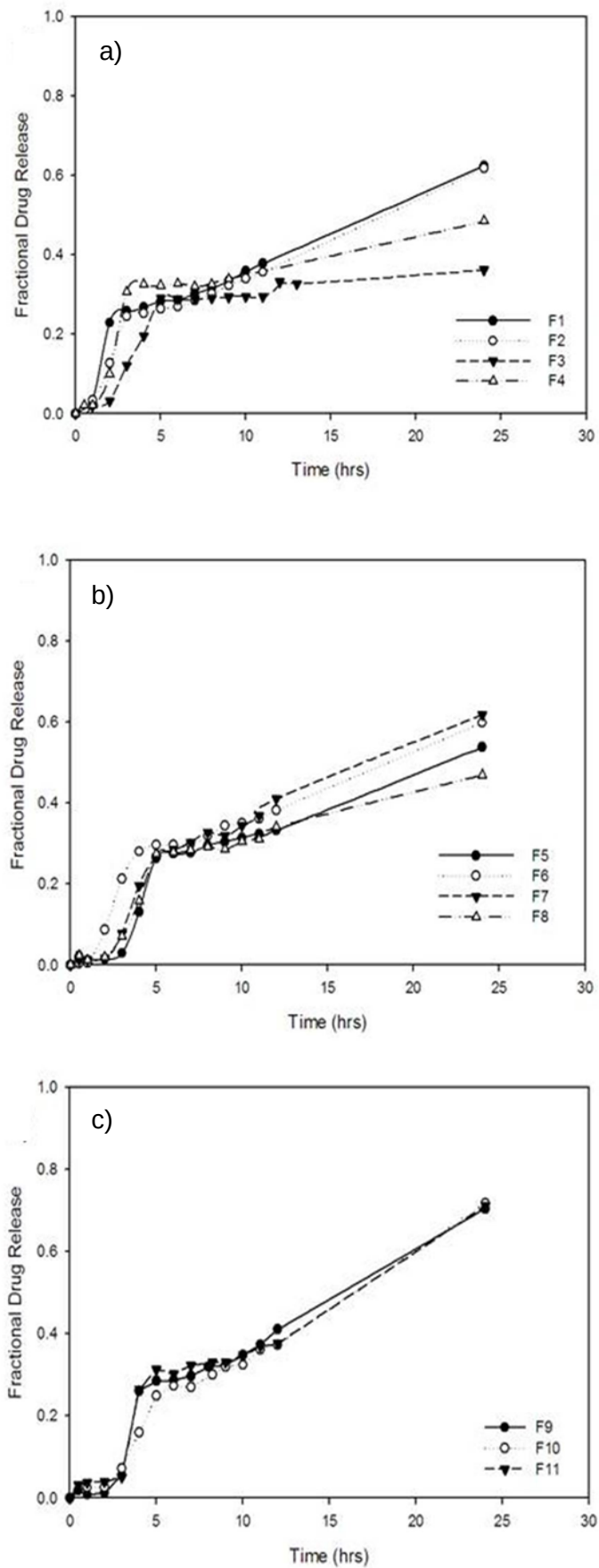


Figure 4.2: Release profiles depicting THP-loaded MLMDT's: a) formulation 1 to 4; b) formulation 5 to 8 and c) formulation 9 to 11 10 (N=3; SD <0.22 in all cases).

4.3.3 The effect of sodium bicarbonate on drug release from DTZ-loaded MLMDT's

DTZ is known for its high water solubility (>50% at 25°C) making controlled release a challenge. This is demonstrated in Formulation 12 (Figure 4.3a and b) where DTZ is combined with the EC. Despite the hydrophobic nature of EC, release occurs simultaneously from both disks resulting in biphasic release. To counteract this problem the electrolyte sodium bicarbonate was incorporated into the formulation. Sodium bicarbonate is known to control the release rate of DTZ as demonstrated by Pillay and Fassihi (1999). Sodium bicarbonate was utilized in varying quantities in D2, L2 and L3 (Table 4.3) in Formulations 14, 15, 16, 17, 18, 19 and 21. Drug release analysis from these formulation demonstrate that the rate of drug release is considerably lower (Figure 4.3) than formulations without sodium bicarbonate.

Based on the drug release profiles sodium bicarbonate should ideally be placed in D2, L2 and L3 to produce the “switch-off” phase. Furthermore, utilizing high concentrations (more than 150mg) of sodium bicarbonate per formulation reduced drug release significantly resulting in incomplete drug release after the 24 hour period (F17 and F18). Formulations that served as controls i.e. had no sodium bicarbonate (F12, F13 and F20) displayed biphasic release instead of two pulse release further substantiating the “matrix-stiffening” effect of sodium bicarbonate.

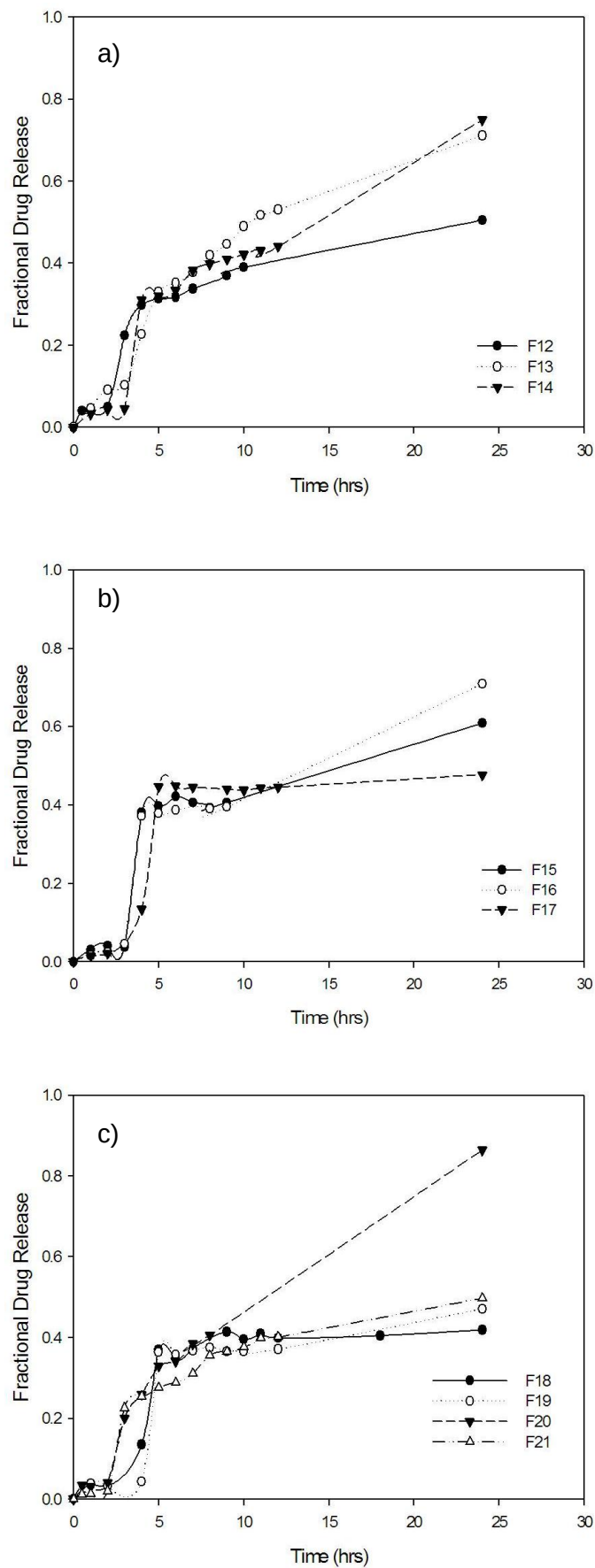


Figure 4.3: Typical release profiles illustrating drug release from DTZ-loaded MLMDT's a) formulation 12 to 14; b) formulation 15 to 17 and c) formulation 18 to 21 (N=3; SD <0.35 in all cases).

4.3.4 Employment of an ANN approach to generate an optimized formulation for both THP and DTZ-loaded MLMDT's

Results obtained from the ANN model including the average of the mean square error (MSE) (Figure 4.4) values for all the training runs and the best network run out of 10,000 epochs are highlighted in Table 4.9 and 4.10 respectively. The correlation coefficient of $R^2=0.98$ (Table 4.11) determined from the comparison between the desired output and actual output of k_3 (Figure 4.5) suggests that the training model utilized was extremely efficient.

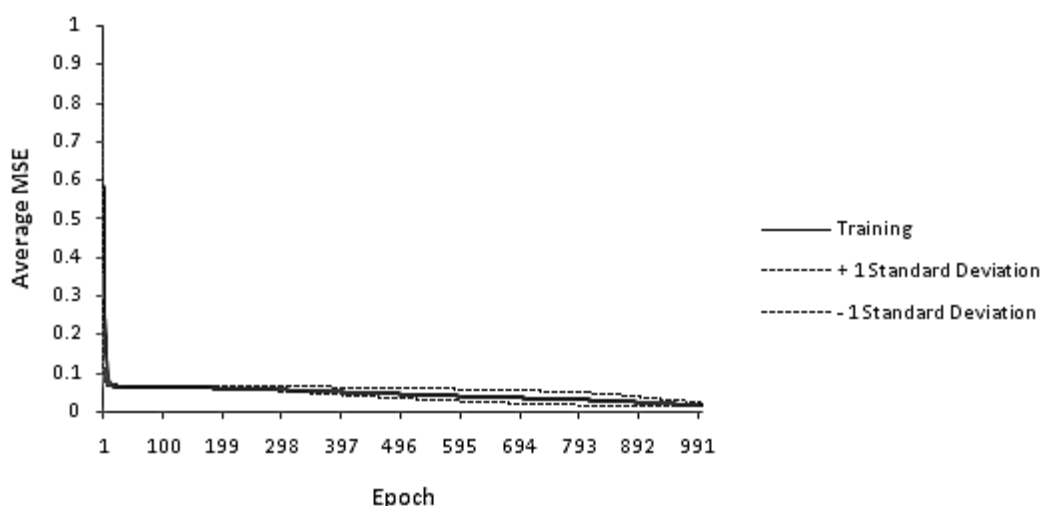


Figure 4.4: Average mean square error (MSE) with standard deviation (SD) boundaries for 10,000 epochs.

Table 4.9: Results obtained from ANN modeling.

All Runs	Training Minimum	Training Standard Deviation
Average of Minimum MSEs	0.016	0.0059
Average of Final MSEs	0.016	0.0059

Table 4.10: Table depicting best network run.

Best Network	Training
Run #	2
Epoch #	1000
Minimum MSE	0.0098
Final MSE	0.098

Table 4.11: Table depicting optimized ANN parameters.

Performance	K_3
MSE	4.51E-06
NMSE	0.056
MAE	0.0015
Min Abs Error	0.00011
Max Abs Error	0.0053
Correlation coefficient R^2	0.98

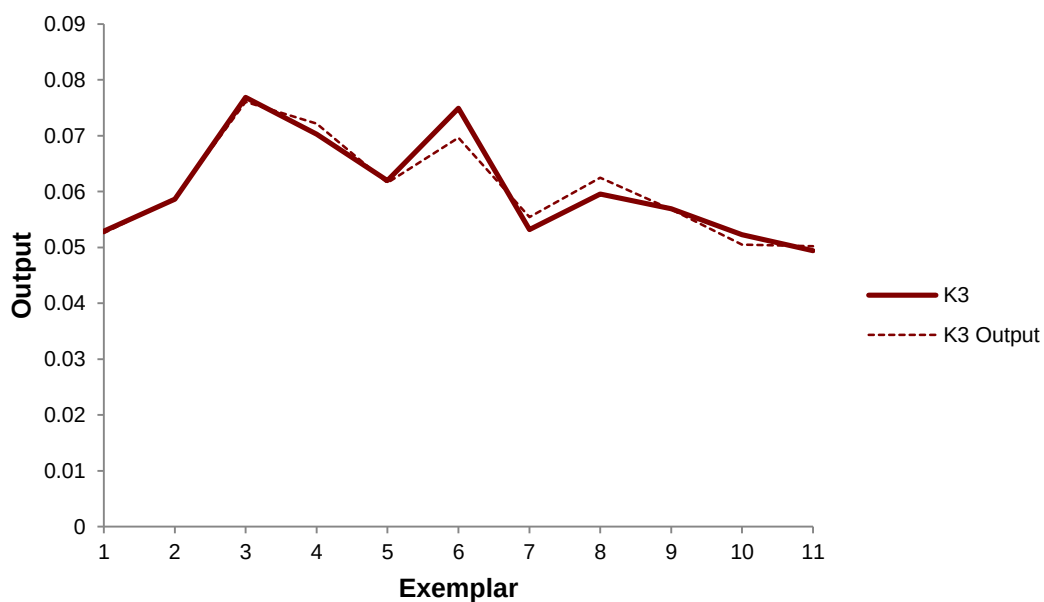
MSE: Mean square error

NMSE: Normalized mean square error

MAE: Mean absolute error

Min Abs Error: Minimum absolute error

Max Abs Error: Maximum absolute error

**Figure 4.5:** A diagrammatic profile depicting the desired and actual network output.

Results from sensitivity testing suggest that the quantity of HEC in D2 has the greatest influence on the shape of the drug release profiles (Figure 4.6). This is substantiated in Figure 4.7(a) which demonstrates a linear curve for HEC, suggesting that the concentration of HEC greatly affects the drug release while increasing the concentration of EC has a minor effect on the rate of drug release.

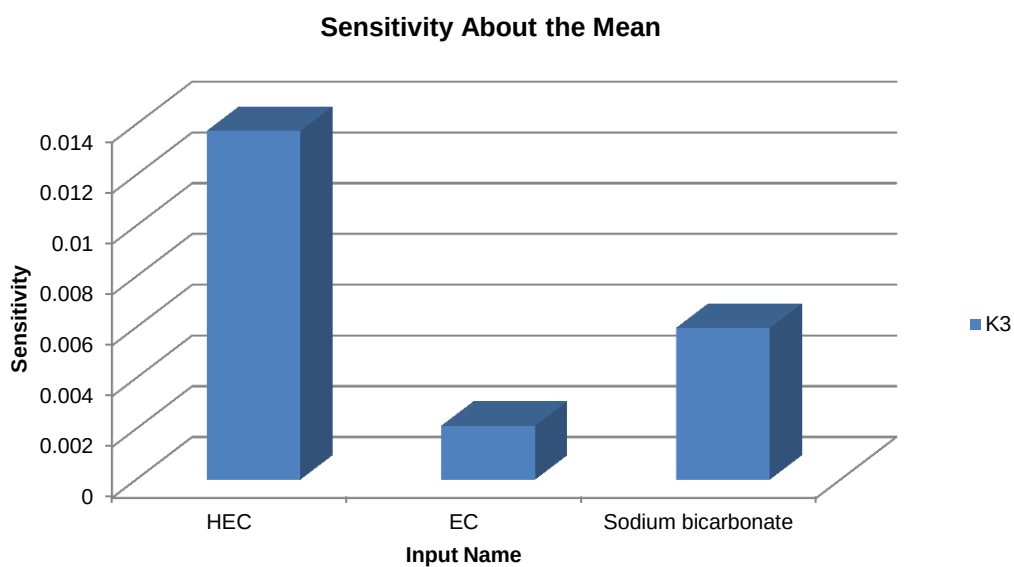


Figure 4.6: A typical bar chart portraying the sensitivity of HEC, EC and sodium bicarbonate on k_3 .

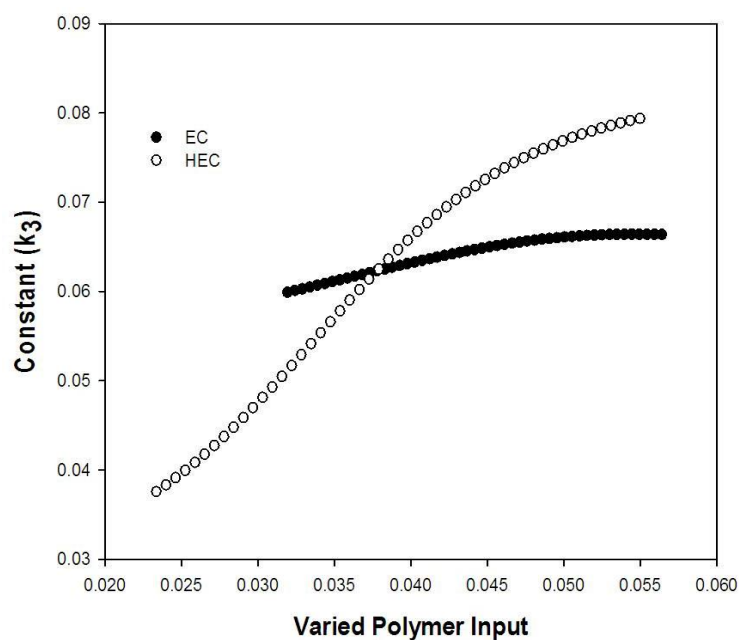
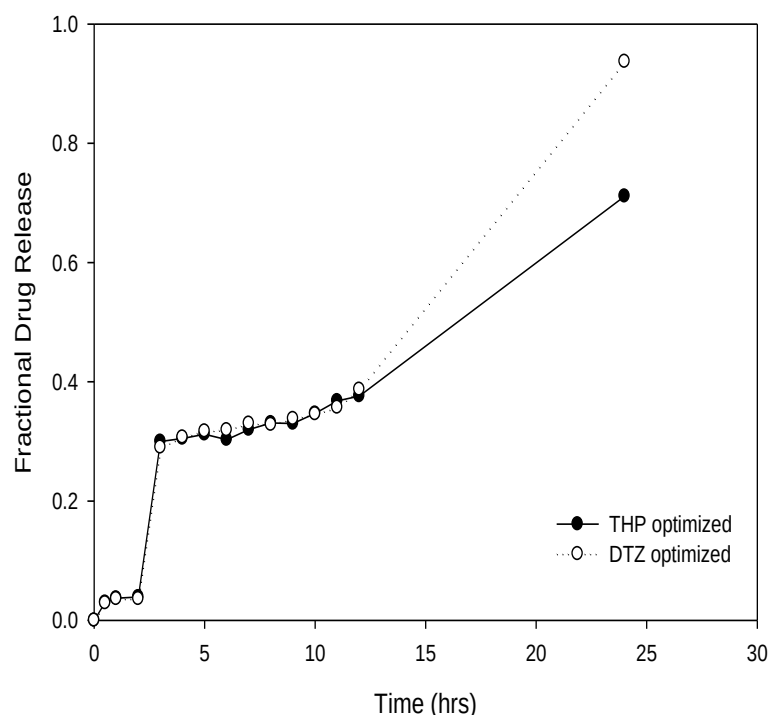


Figure 4.7: Graphical profile depicting the correlation between the polymers on k_3 .

The compositions and quantities of the optimized formulation as determined by the ANN approach are depicted in Table 4.12, while drug release profiles are depicted in Figure 4.8. All further experiments were carried out on the optimized THP-loaded and DTZ-loaded formulations.

Table 4.12: Combination of polymers to produce the optimum k_3 .

Drug	Polymer	Polymer	Ratio	Sodium Bicarbonate		
				D2	L1	L2
THP	HEC	EC	1:3	-	-	-
DTZ	HEC	EC	1:1	50	50	50

**Figure 4.8:** Drug release profiles of optimized THP and DTZ-loaded MLMDT's (N=3; SD <0.21 in all cases).

4.3.5 Matrix swelling and erosion analysis

Swelling and erosional studies were performed on the optimized formulations. Figure 4.9a and b, depicts the relationship between swelling and erosion on THP and DTZ-loaded MLMDT's. The THP-loaded formulation expanded to 80% of the original size within the first two hours with less than 10% of the device displaying erosion. This initial swelling contributes to the initial lag phase observed in the drug release profiles (Figure 4.8). After 2 hours a sharp decrease in the % water intake (i.e. swelling) was observed as erosion of L1 and D1 commenced. At the same time swelling from the exposed areas of L2 commenced. Between 6 and 8 hours swelling and erosion of the device remained constant (Figure 4.10d and e) and by 6 hours erosion of L1 and D2 was complete. After 24 hours a slight decrease in swelling was observed while erosion increased to 60%. The MLMDT was intact after 24 hours which explained the incomplete drug release detected (Figure 4.10g).

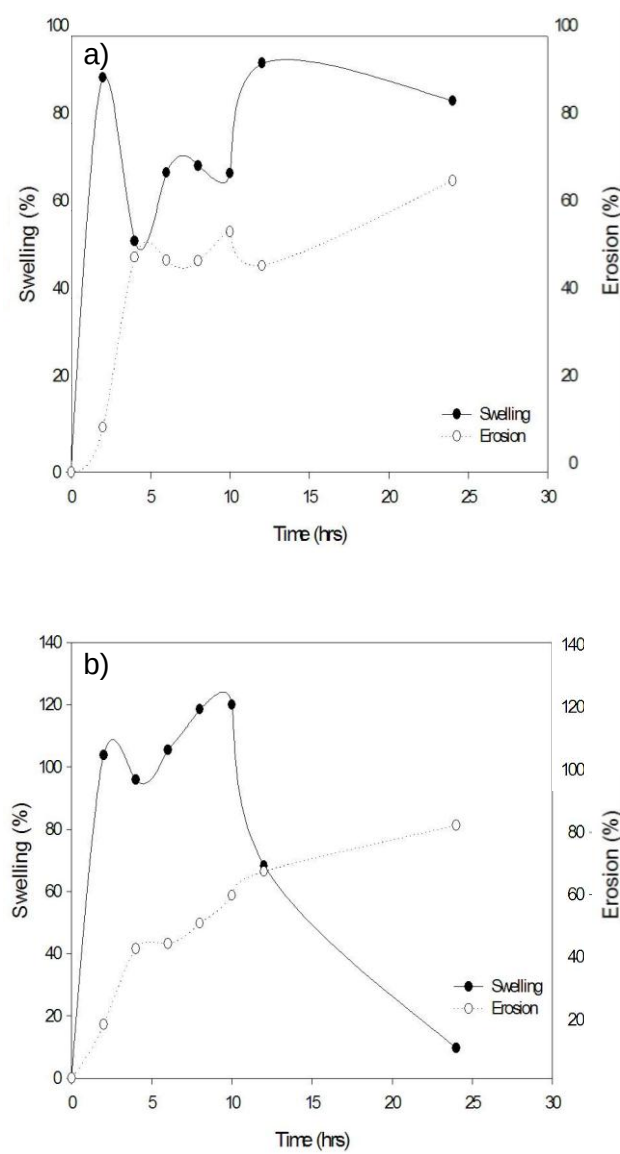


Figure 4.9: Correlation of swelling and erosion profiles of: a) THP-loaded and b) DTZ-loaded MLMDT's (N=3; SD <0.52 in all cases).

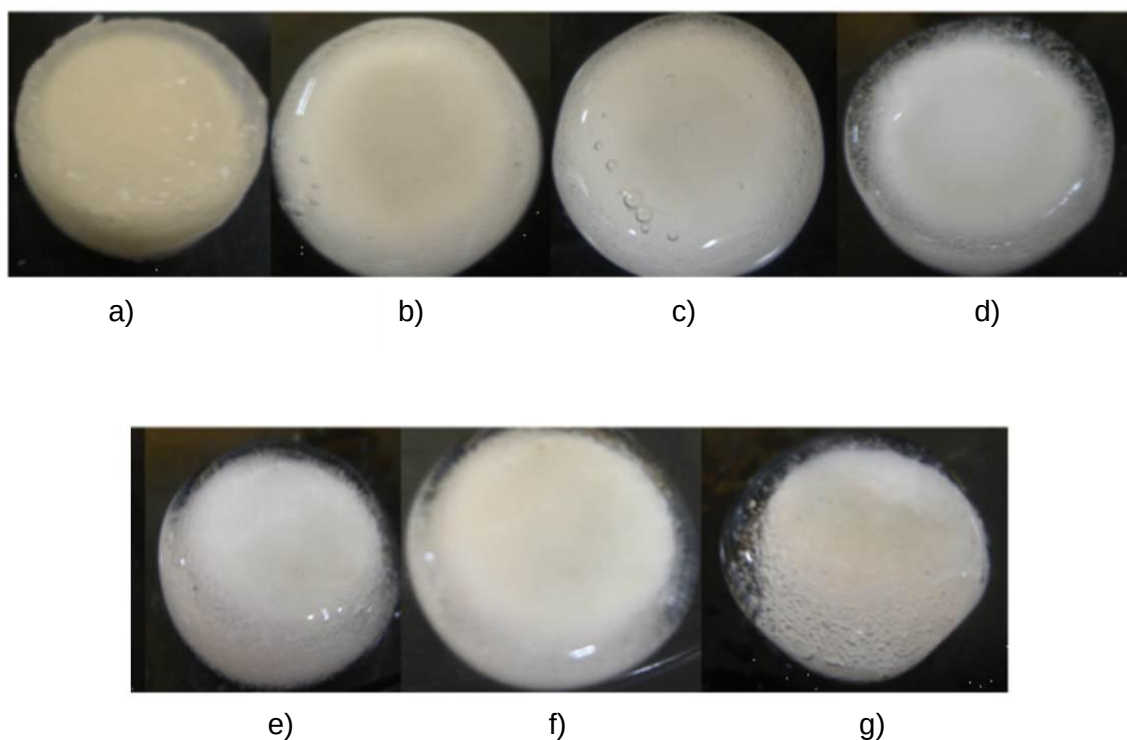


Figure 4.10: Digital images illustrating THP-loaded MLMDT's at: a) 2; b) 4; c) 6; d) 8; e) 10; f) 12 and g) 24 hours.

The addition of an alkaline compound to an acidic drug resulted in a buffering effect which consequently inhibited the release of drug (Pillay and Fassihi, 1999). An increase in swelling was observed within the first two hours contributing to the initial lag phase. Thereafter, there was a drop in the swelling percentage similar to the THP-loaded MLMDT's due to the erosion of L1 and D2.

The DTZ formulation expanded more than 100% in the first 2 hours followed by a constant increase in the swelling as well as the erosion over the next 10 hours (Figure 4.9 and 4.11). Despite an increase in swelling, minimal drug was released between 4 hours and 11 hours confirming that sodium bicarbonate influenced the rate of drug release. After 12 hours a sharp decrease in swelling was observed which corresponds to an increase in drug release. Figure 4.11f depicts the swollen matrix with D2 visible. After 24 hours approximately 20% of the device remained (Figure 4.11g) indicating that the majority of drug was released.

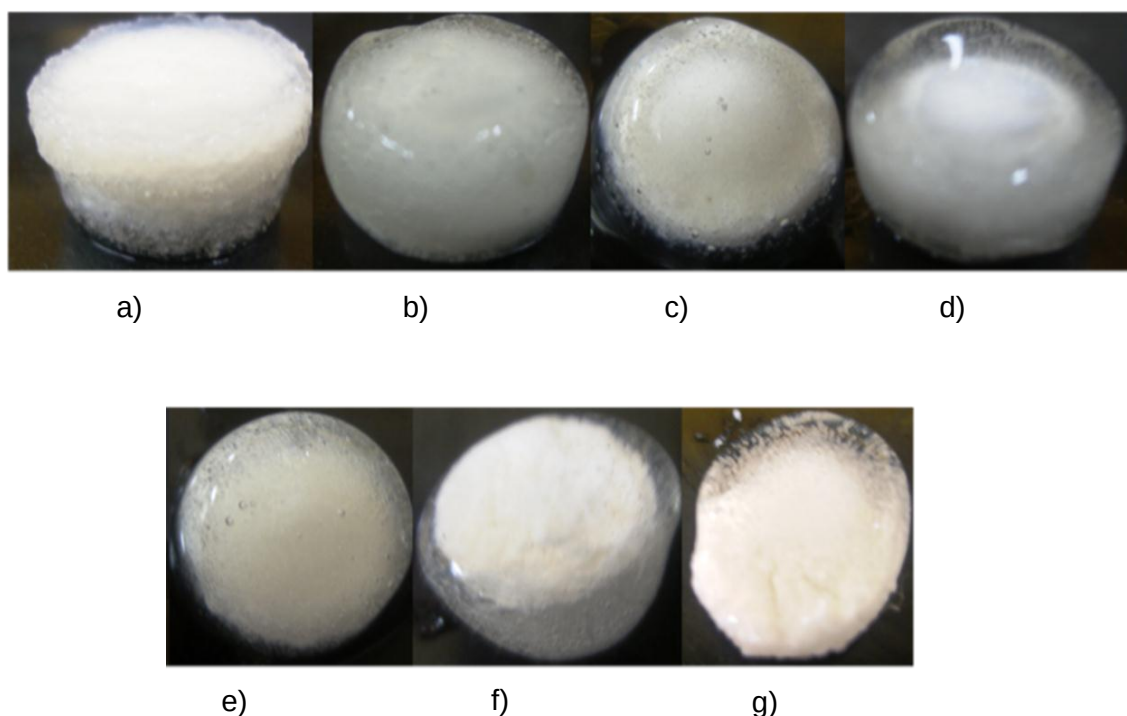


Figure 4.11: Digital images illustrating DTZ-loaded MLMDT's at: a) 2; b) 4; c) 6; d) 8; e) 10; f) 12 and g) 24 hours.

4.3.6 Magnetic Resonance Imaging of MLMDT's performance

Magnetic Resonance Imaging (MRI) covers a range of widely used techniques for studying the distribution and dynamics of a species within a material based on their nuclear magnetic resonance effects (Richardson et al., 2005; Laity et al., 2010). This technique proves to be a powerful tool for following the progression of hydration over a period of time (Mikac et al., 2010). MRI was therefore employed to observe and confirm our postulation for the chronotherapeutic behaviors and performance of the MLMDT's during dissolution studies. Figure 4.12 exhibits the images obtained for the THP-loaded MLMDT's under continuous solvent flow conditions while Figure 4.14 displays the images obtained for DTZ-loaded MLMDT's throughout the image acquisition process under continuous solvent flow conditions. The images displayed were obtained after every thirty minutes. The grey area surrounding the matrix indicates the dissolution medium (simulated gastrointestinal fluid). The black area within the matrix represents the non-hydrated and non-gelled area of the device. As the matrix hydrated, it swelled and gelled which is represented by the white area where the thickness increased over time until the matrix was fully hydrated and gelled.

The layers surrounding the device gelled on exposure to the dissolution media with L1 gelling faster than L2 and L3 (Figure 4.12a-aa). As the device gelled, no drug release

was observed. After 2.5 hours media penetrated the device (Figure 4.13) hydrating the inner layers (4.12f and 4.12g). Based on dissolution data (Figure 4.13) complete drug release from D1 occurred after 3 hours while complete erosion of D1 occurred after 6 hours (4.12m). This observation indicated that the gel at the layer interface was not tight or viscous enough to retard drug release which resulted in drug movement from D1 across the swollen outer layer (L1) into the dissolution media. D2 was protected from the surrounding media due to the highly viscous HPMC contained in L2 and L3 that prevented buffer penetration at the layer-disk interface. D2 was fully visible after 11 hours due to the ingress of buffer into the device. Drug release from D2 occurred thereafter due to the decrease in the viscosity of the surrounding gel after 12 hours (4.12t), thus facilitating drug movement from the core into the media.

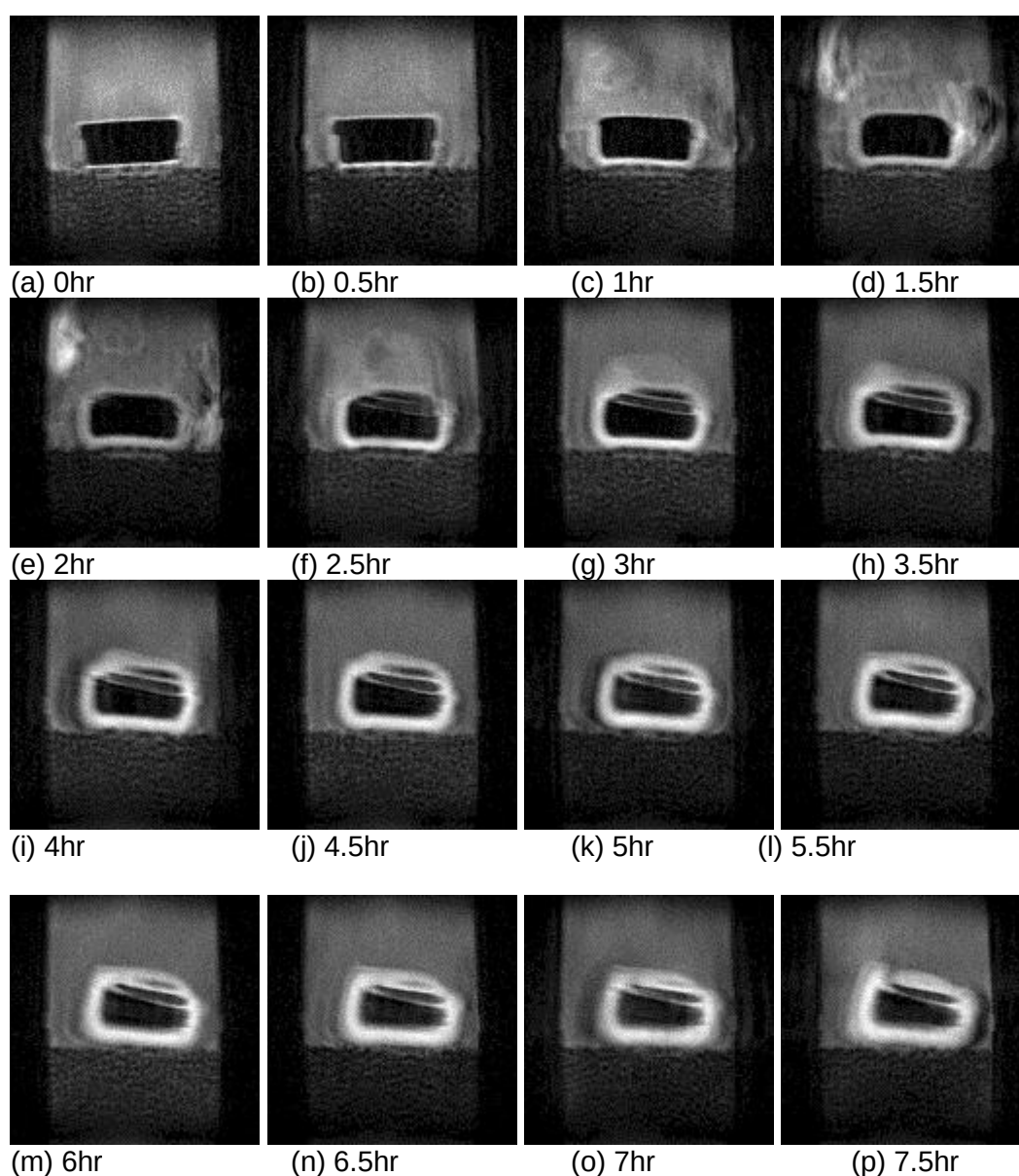


Figure 4.12: MRI depicting the progression of hydration of THP-loaded MLMDT's from 0 to 7.5 hours.

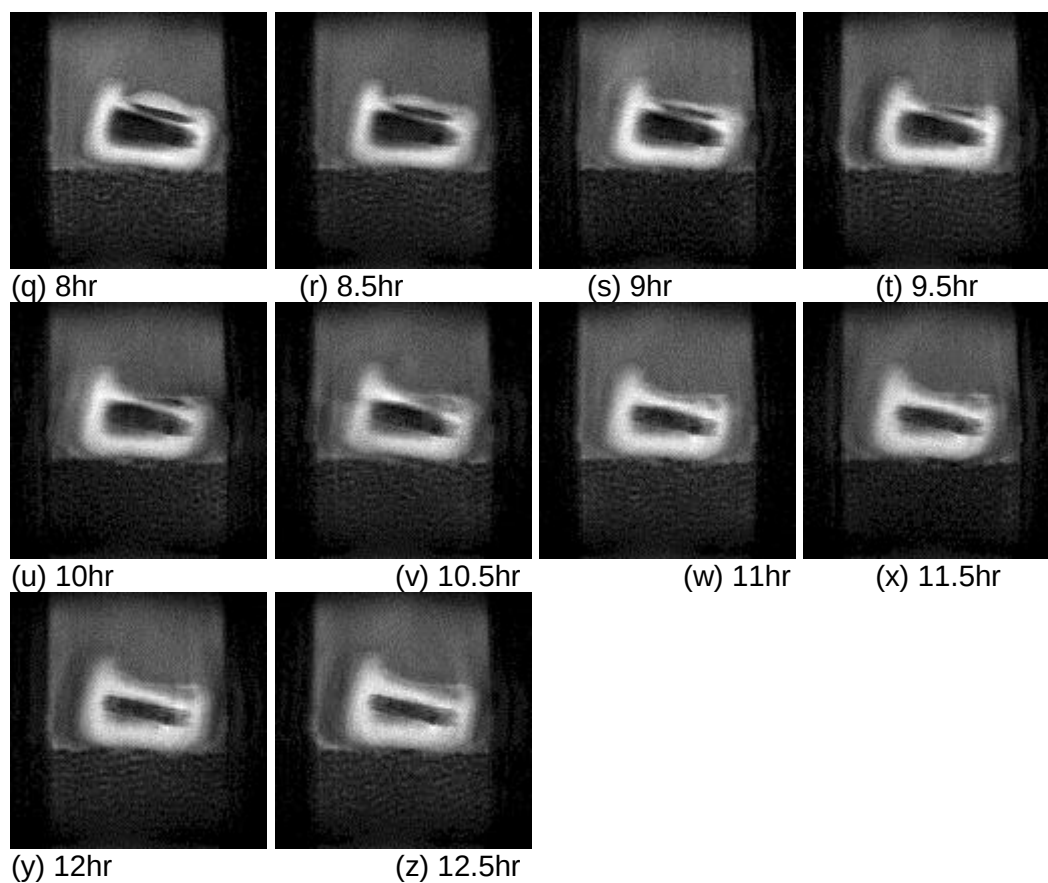


Figure 4.12 (*continued*): MRI depicting the progression of hydration of THP-MLMDT's from 8 to 13 hours.

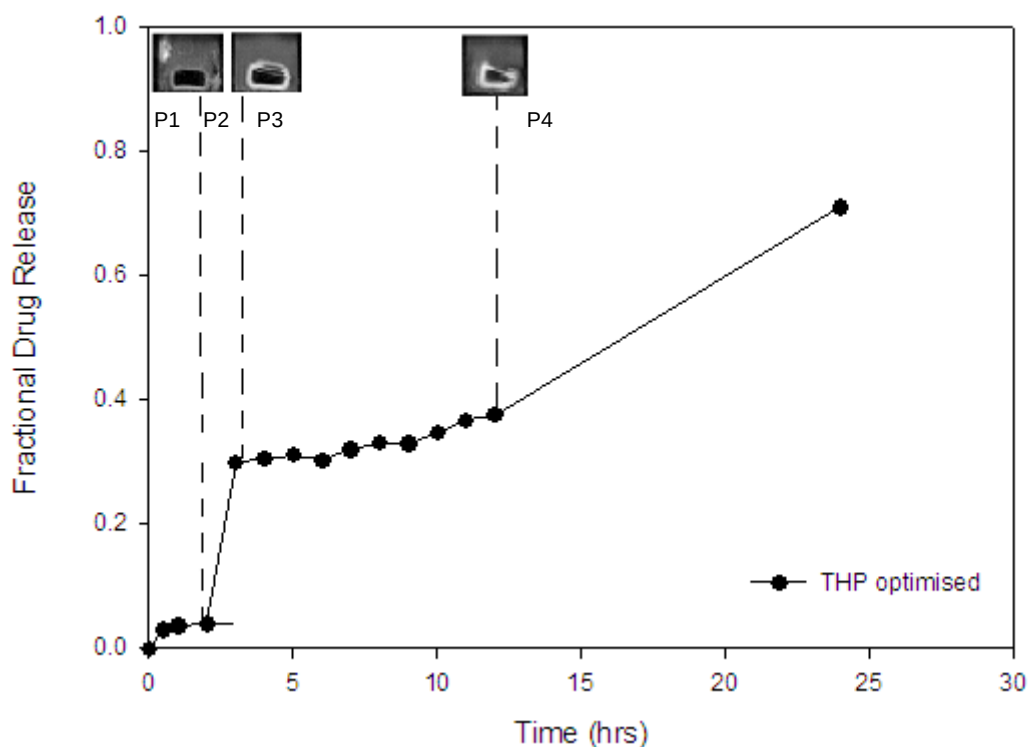


Figure 4.13: Drug release profile detailing the relationship between drug release and the results obtained using MRI from THP-loaded MLMDT's with P1, P2, P3 and P4 representing the lag phase, first pulse of drug release, "switch-off" phase and second phase of drug release respectively.

The DTZ-loaded formulation generated CO_2 due to the inclusion of sodium bicarbonate in the device. This liberation of CO_2 resulted in floatation of the device (4.14a-4.14v). L1 swelled more than the other layers upon contact with the media (4.14b). Penetration of buffer into the device happened after 1 hour (4.14c and d). Disintegration of the PEC layer (L1) occurred after 3.5 hours (4.14d) with complete hydration of L2 and L3 occurring after 8 hours (4.14l). Although the matrix around D2 was hydrated no drug release occurred due to a "matrix-stiffening" effect generated by sodium bicarbonate resulting in a "switch-off" phase (Figure 4.15). This effect diminished after 11 hours (4.14r) and drug release commenced for the remainder of the 24 hours.

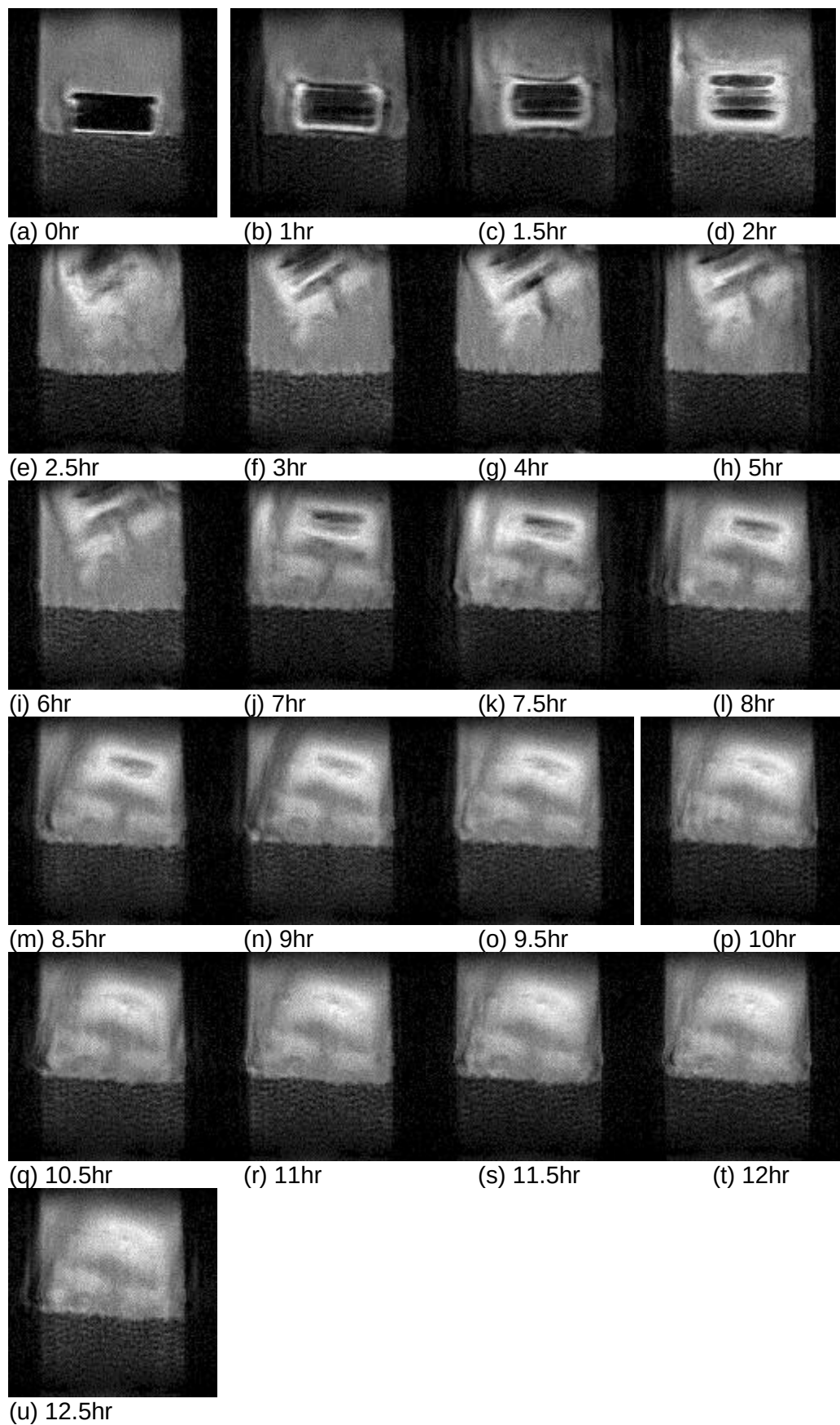


Figure 4.14: MRI depicting the progression of hydration over time from DTZ-loaded MLMDT's.

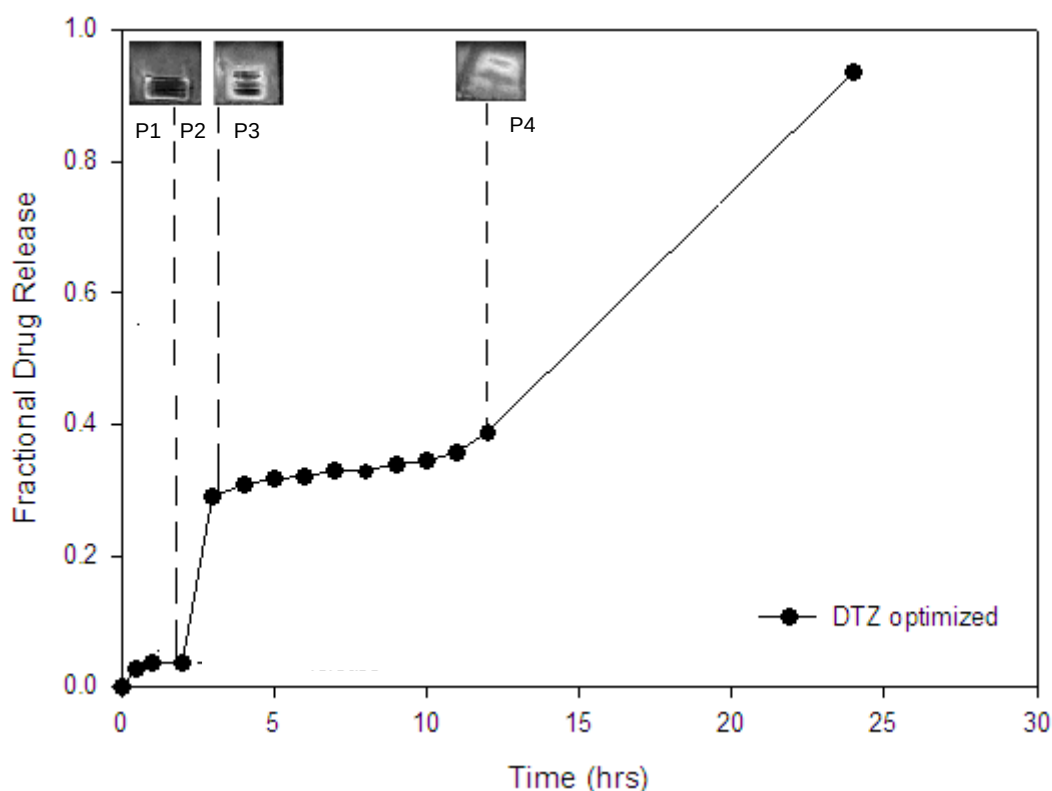


Figure 4.15: Profile portraying the association between drug release and images obtained by MRI from DTZ-loaded MLMDT's with P1, P2, P3 and P4 representing the lag phase, first pulse of drug release, "switch-off" phase and second phase of drug release respectively.

4.3.7 Analysis of the polymeric structural variations using Fourier Transmission Infrared spectroscopy

Fourier Transmission Infrared spectroscopy (FTIR) was carried out on the native polymer and drug as well as the granulated forms (not shown) and compressed forms of the formulation. The spectra were then compared to determine if there were changes in any of the structures. FTIR spectra of both the compressed THP formulation (Figure 4.16a) and the compressed DTZ formulation (Figure 4.16b) displayed no change in the structure of the compressed final MLMDT in comparison with the native polymer and drug. Both figures illustrated the characteristic cellulose bands arising at 3476cm^{-1} and 2934cm^{-1} indicative of EC, HPMC and HEC. Figure 4.16a demonstrates the native THP peaks which were observed at 785cm^{-1} , 1585cm^{-1} , 1715cm^{-1} and 2509cm^{-1} . This indicates the drug remained unchanged during compression. Similarly Figure 4.16b highlights the characteristic DTZ bands observed at 1680cm^{-1} , 1741cm^{-1} and 2363cm^{-1} indicative of the stretching of the carbonyl in the amide group, which was present in the compressed MLMDT. Therefore, the observed spectrum could be regarded as a simple superimposition between the native polymers and drug, suggesting that no interaction occurred between the polymer and the drug.

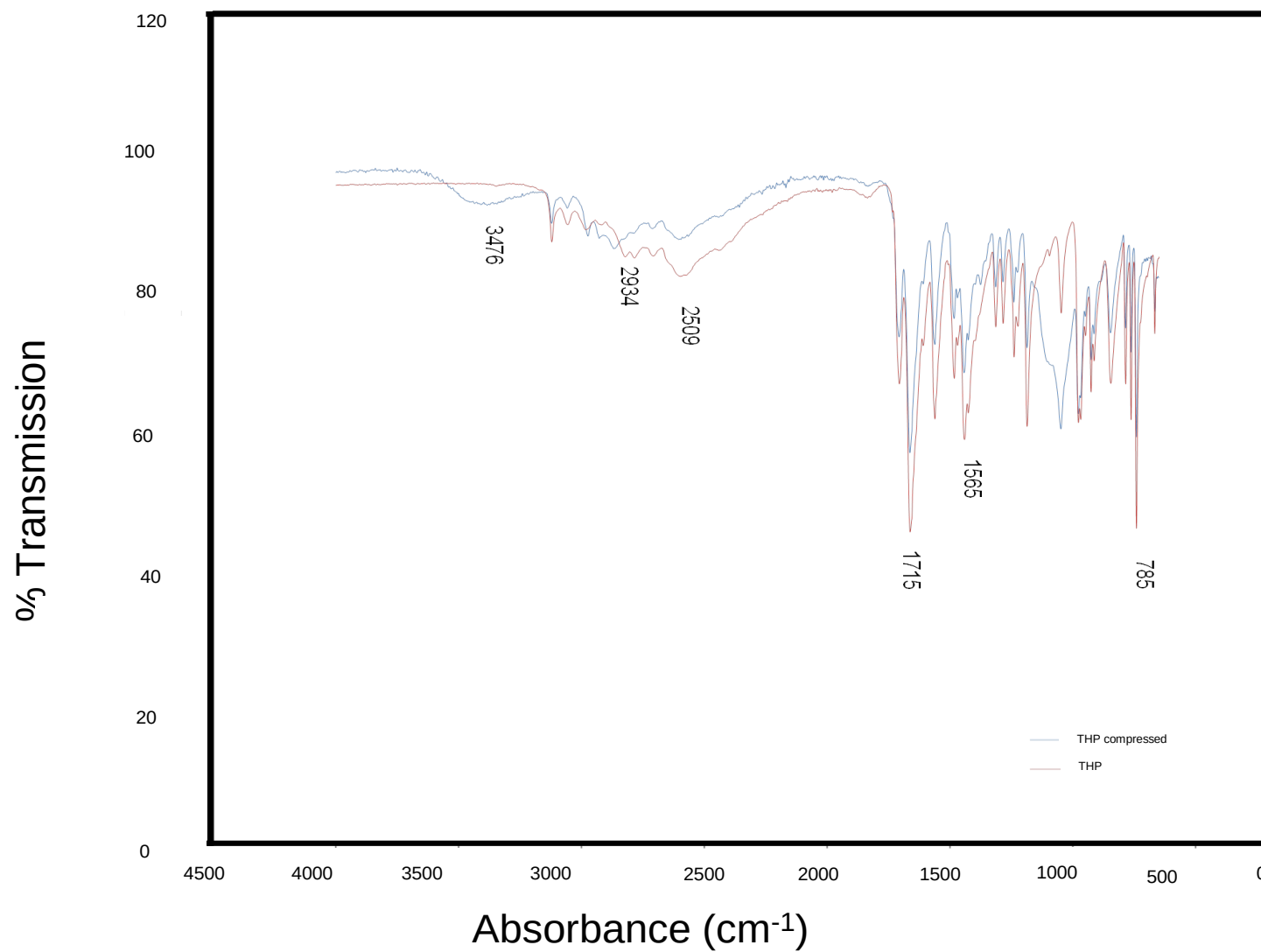


Figure 4.16: FTIR spectra depicting pure THP and compressed THP-loaded MLMDT.

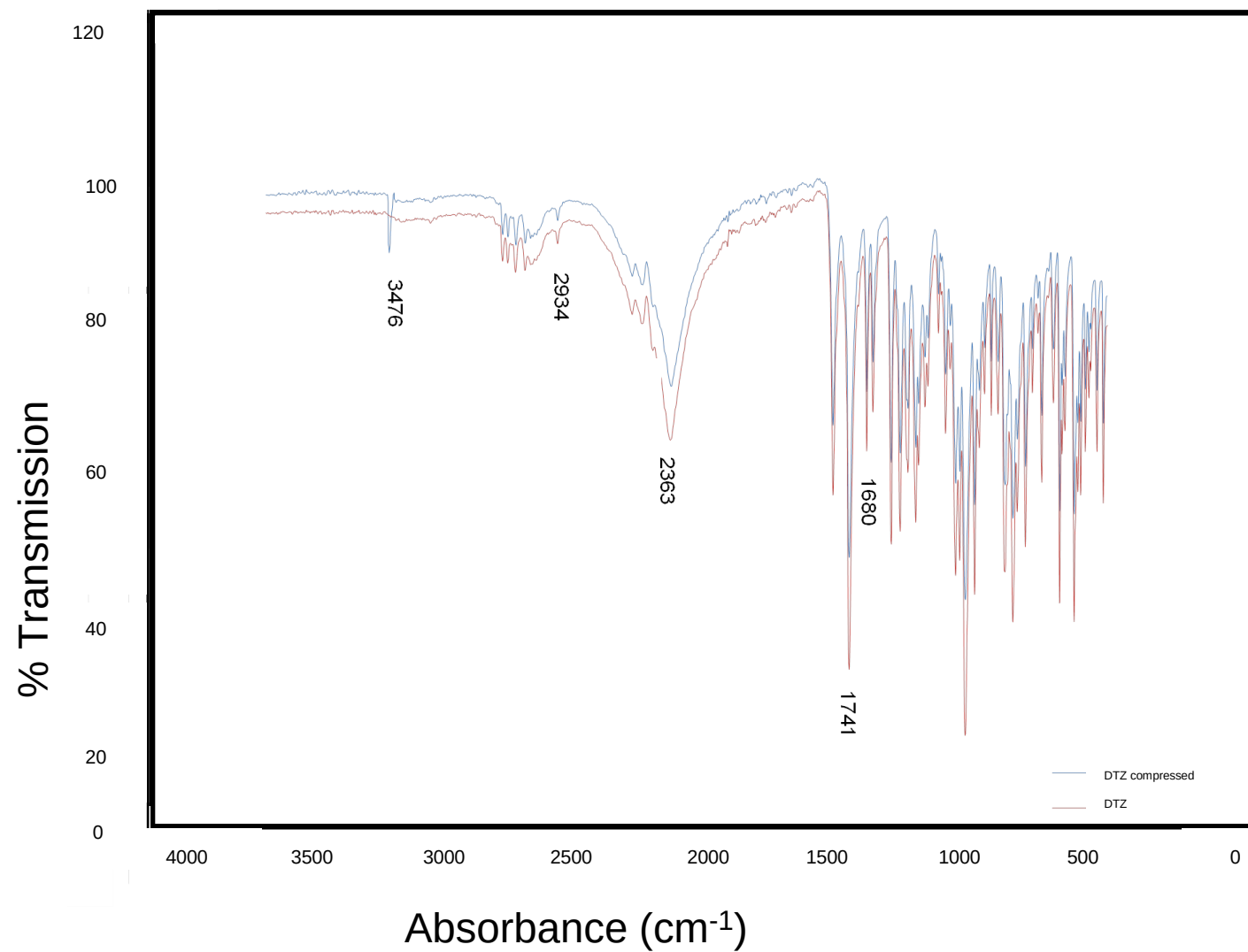


Figure 4.17: FTIR spectra depicting pure DTZ and compressed DTZ-loaded MLMDT.

4.3.8 Determination of deformation energy and Brinell Hardness Number

The MLMDT's comprised different polymers on the top and bottom layers (Figure 4.17) and thus hardness was determined on both the top and bottom layers of the MLMDT. Results show that L3 was considerably harder than L1 (Table 4.13).

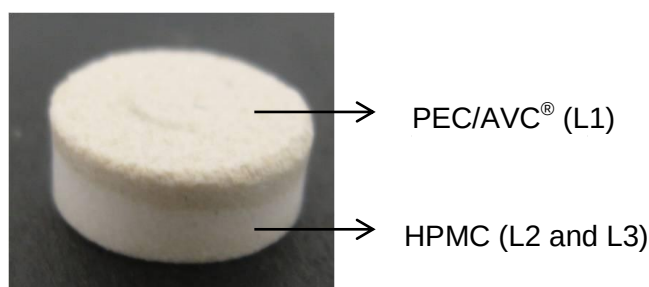


Figure 4.18: A digital image of the MLMDT illustrating the variation between the bottom and top layers of the device as based on the different polymers utilized in the layers.

Table 4.13: Brinell Hardness Numbers values for the MLMDT's.

Formulation	BHN (N/mm ²)
THP MLMDT Layer one	5.35
THP MLMDT Layer three	6.49
DTZ MLMDT Layer one	5.27
DTZ MLMDT Layer three	6.60

Textural analysis was also employed to measure alterations in swelling behavior. Force-displacement profiles for optimized DTZ and THP-loaded formulations were obtained using the Texture Exponent Software (version 2). Figure 4.18a illustrates the force-displacement placement profiles for THP-loaded formulations, while Figure 4.18b displays the profile for DTZ-loaded formulations. The upward curving of the graph suggests the force required to penetrate the swollen matrix, with a smaller force needed to penetrate the gel layer. This force increases once the probe penetrates the dry core. The rapid decline in the curves indicates the retraction of the probe from the swollen matrix (Figure 4.18a and b). THP-loaded formulations expanded significantly within the first two hours followed by a decline in size of the device. This corresponds to the erosion of L1. Swelling progressively increased with the final measurement at 24 hours demonstrating a decline in the overall size. These sequential events coincide with the data obtained from % water uptake (swelling) studies. DTZ-loaded formulations displayed a considerable increase in swelling up to 10 hours and then a decline in swelling as erosion increases. Swelling was greater in the DTZ-loaded formulation compared to the THP-loaded MLMDT. This was attributed to the inclusion of sodium bicarbonate in the DTZ-loaded formulations.

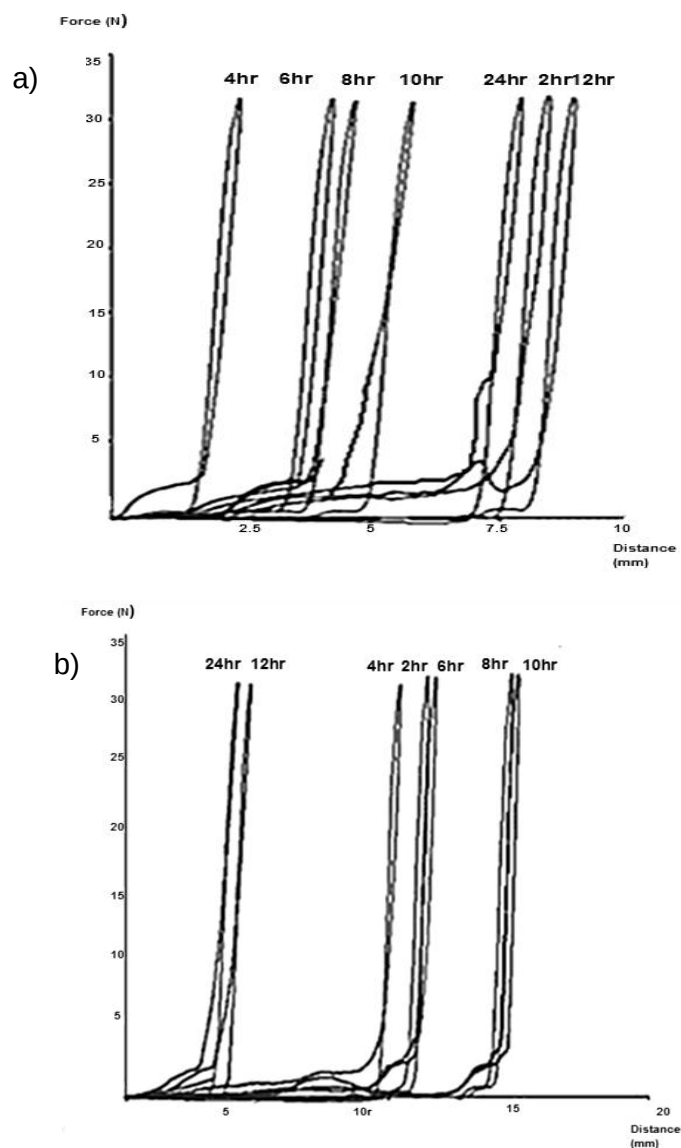


Figure 4.19: Typical force-displacement profiles for: a) THP-loaded MLMDT's and b) DTZ-loaded MLMDT's.

4.3.9 Evaluation of Scanning Electron Microscopy (SEM) imaging

SEM was conducted on the compressed MLMDT to observe the surface morphology of the different layers. As highlighted in Figure 4.19, there is a distinct difference in the surface structure of the L1 and L2. This variation in structure accounts for the different rates of erosion and swelling and consequently the drug release kinetics.

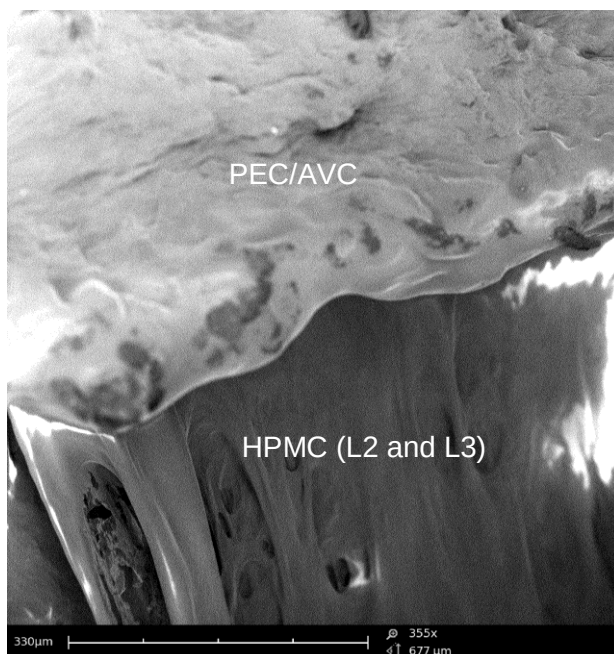


Figure 4.20: SEM image of the side view of the MLMDT illustrating morphology the of the PEC/AVC and HPMC layer (magnification 355x).

4.3.10 Molecular mechanics assisted model building and energy refinements

A molecular mechanics conformational searching procedure was employed to acquire the data employed in the statistical mechanics analysis, to obtain differential binding energies of a Polak–Ribiere algorithm and to potentially permit application to EC-HEC and PEC-AVC polymer composite assemblies. MM+ is a HyperChem modification and extension of Norman Allinger's Molecular Mechanics program MM2 (Warhurst et al., 2003) whereas AMBER, is a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules (Pearlman et al., 1995).

4.3.10.1 Molecular mechanics energy relationship analysis

Molecular mechanics energy relationship (MMER), a method for analytico-mathematical representation of potential energy surfaces, was used to provide information about the contributions of valence terms, noncovalent Coulombic terms, and noncovalent van der Waals interactions for the blended-polysaccharide morphologies and interactions. The MMER model for the potential/steric energy factors in various molecular complexes can be written as:

$$E_{\text{molecule/complex}} = V_{\Sigma} = V_b + V_{\theta} + V_{\varphi} + V_{ij} + V_{hb} + V_{el} \dots \quad \text{Equation 4.5}$$

$$E_{EC} = 72.116V_{\Sigma} = 3.551V_b + 32.878V_{\theta} + 26.457V_{\varphi} + 8.746V_{ij} - 0.209V_{hb} + 0.691V_{el} \quad \text{Equation 4.6}$$

$$E_{HEC} = 130.862V_{\Sigma} = 4.017V_b + 35.115V_{\theta} + 80.816V_{\varphi} + 10.28V_{ij} - 0.231V_{hb} + 0.863V_{el} \quad \text{Equation 4.7}$$

$$E_{EC/HEC} = 115.616V_{\Sigma} = 7.861V_b + 72.277V_{\theta} + 44.159V_{\varphi} + 10.968V_{ij} - 2.578V_{hb} - 17.072V_{el} \quad \text{Equation 4.8}$$

$$[\Delta E_{\text{BINDING}} = -87.362 \text{kcal/mol}]$$

$$E_{PEC} = -25.743V_{\Sigma} = 2.030V_b + 16.827V_{\theta} + 26.87V_{\varphi} + 7.085V_{ij} - 3.399V_{hb} - 75.157V_{el} \quad \text{Equation 4.9}$$

$$E_{AVC} = -4.641V_{\Sigma} = 1.477V_b + 8.718V_{\theta} + 6.145V_{\varphi} + 13.110V_{ij} - 34.094V_{el} \quad \text{Equation 4.10}$$

$$E_{PEC/AVC} = -65.913V_{\Sigma} = 3.824V_b + 28.882V_{\theta} + 40.252V_{\varphi} + 7.804V_{ij} - 4.933V_{hb} - 141.745V_{el} \quad \text{Equation 4.11}$$

$$[\Delta E_{\text{BINDING}} = -35.529 \text{kcal/mol}]$$

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

4.3.10.2 Energy-minimizations involving composite polysaccharide morphologies

The energy minimized structures of the EC-HEC and PEC-AVC following molecular mechanics simulations are depicted in Figure 4.20 and 4.21 and the possible component binding energies to which they will be responsive, are listed in Equations. 4.9-4.11. Molecular modelling studies can account for specific interactions between polymer segments and may provide an estimate of whether two polymers will form a compatible blend. Our theoretical foundation for this method relies upon the thermodynamic factors dominated by the energetics of local interactions between segments of the polymer chains involved in modelling computations (Tiller and Gorella, 1994).

It is evident from the energy value that the EC-HEC complex (representing base disc) is stabilized by a binding energy of $\sim 87 \text{kcal/mol}$ ($\Delta E_{\text{BINDING}} = -87.362 \text{kcal/mol}$; Equations. 4.6-4.8). On a theoretical basis, a necessary condition for the miscibility of a mixture of two polymers is a negative free energy of mixing (Tiller and Gorella, 1994). This energy minimization in case of EC-HEC was inherent from rotation of monosaccharide residues producing strain due to steric interactions which in turn are

relieved by the inclusion of bond length and angle adjustment with respect to all degrees of freedom of the system. As obvious from comparison between Figure 4.18a and b, a lowering of the energy barriers caused a substantial change from the initial starting geometry. The pendent groups (ethyl and hydroxyl ethyl) moved to their nearest minimum downhill of the starting point during minimization, driving the molecule through unfavourable regions. These large steric interactions cause pendent groups to overcome torsional barriers presenting a larger accessible potential energy surface (Perez et al., 1996). This torsional energy stabilization ($\Delta E_{V\phi} \sim 62 \text{ kcal/mol}$) contributed mainly to the finally stabilized geometrical configurations. These energy optimizations are also supported by high magnitude van der Waals interactions ($\Delta E_{Vei} \sim 20 \text{ kcal/mol}$) between the two 4-saccharide unit's sugar molecules. Furthermore, regarding the spatial preference of EC with HEC, as depicted by the dots rendering in Figure 4.18b, a deeper inspection revealed the close proximity of both the molecules in form of H-bonding and closely sharing the van der Waals space. These underlying weak chemical interactions may not cause a structural change in the polymers but may initiate aggregation of the aliphatic chains, as both the polymers here contain aliphatic side chains, creating localized regions with a density and refractive index different from that of the bulk polymers. Additionally, the hydroxyl group induced inter- and intramolecular hydrogen bonding in EC-HEC (≈ 10 times more than the individual polymers) (Figure 4.20b) may influence the hydration process of the EC polymer matrix causing a "notable increase in the amount of drug released over a given period" as explained earlier in the manuscript. This release pattern may be attributed to the modified matrix hydration process of EC (hydrogen bonding induced by the presence of the HEC) and continued retarded release of drug due to the entanglement of saccharide chains which in turn lead to a high BHN of L3 as highlighted in Table 4.13.

Similarly, the molecular mechanistically minimized energy value for PEC-AVC complex (representing L1) is stabilized by a binding energy of $\sim 35 \text{ kcal/mol}$ ($\Delta E_{\text{BINDING}} = -35.529 \text{ kcal/mol}$; Equations. 4.9-4.11). Molecular modelling proved to be a powerful tool for studying the packing of polysaccharides where the models can be built and energies involving chain-chain interactions can be calculated as described by Perez et al, 1996. Here also in case of PEC-AVC, the PEC's interaction with cellulose is calculated at varied helix axis translations and mutual rotational orientations while keeping the helices in van der Waals contact (Figure 4.21a and b). For efficient packing, coupled values of the rotations of the individual chains are also important along with low energy of stabilization. These very rotations caused the formation of bonding interactions in form of torsional energy minimization ($\sim 10 \text{ kcal/mol}$), formation

of H-bonds ($\sim 12\text{kcal/mol}$) and highly stabilized electrostatic interactions ($\sim 30\text{kcal/mol}$) leading to the formation of a rotation axis might even be a screw axis (Figure 4.20a and b). These interactions involving the non-bonded attractive forces may induce dipoles in the complex where the binding energy changes should be proportional to the polarizability of the substituent's, which are in turn may lead to the formation of a dense polymer network responsible for prolonged release of the bioactives (Figure 4.20).

The higher energy of stabilization ($\Delta E_{\text{BINDING}}$) of EC-HEC as compared to PEC-AVC corroborated with the chronotherapeutic strategy explained in this research. PEC-AVC being less stabilized molecular complex is supposed to erode faster than EC-HEC leading to the release of drug from L1 earlier than that from base disc. Hence, the present modeling and computation method involving four polysaccharides provided a justification of using a definite combination of polymers to meet the requirements of a drug delivery system with the desired release profile. This delivery system and subsequent modeling system may act as a template for the future applications employing various drug and polymer combination strategies involving chronotherapeutic and desired release profile requirements.

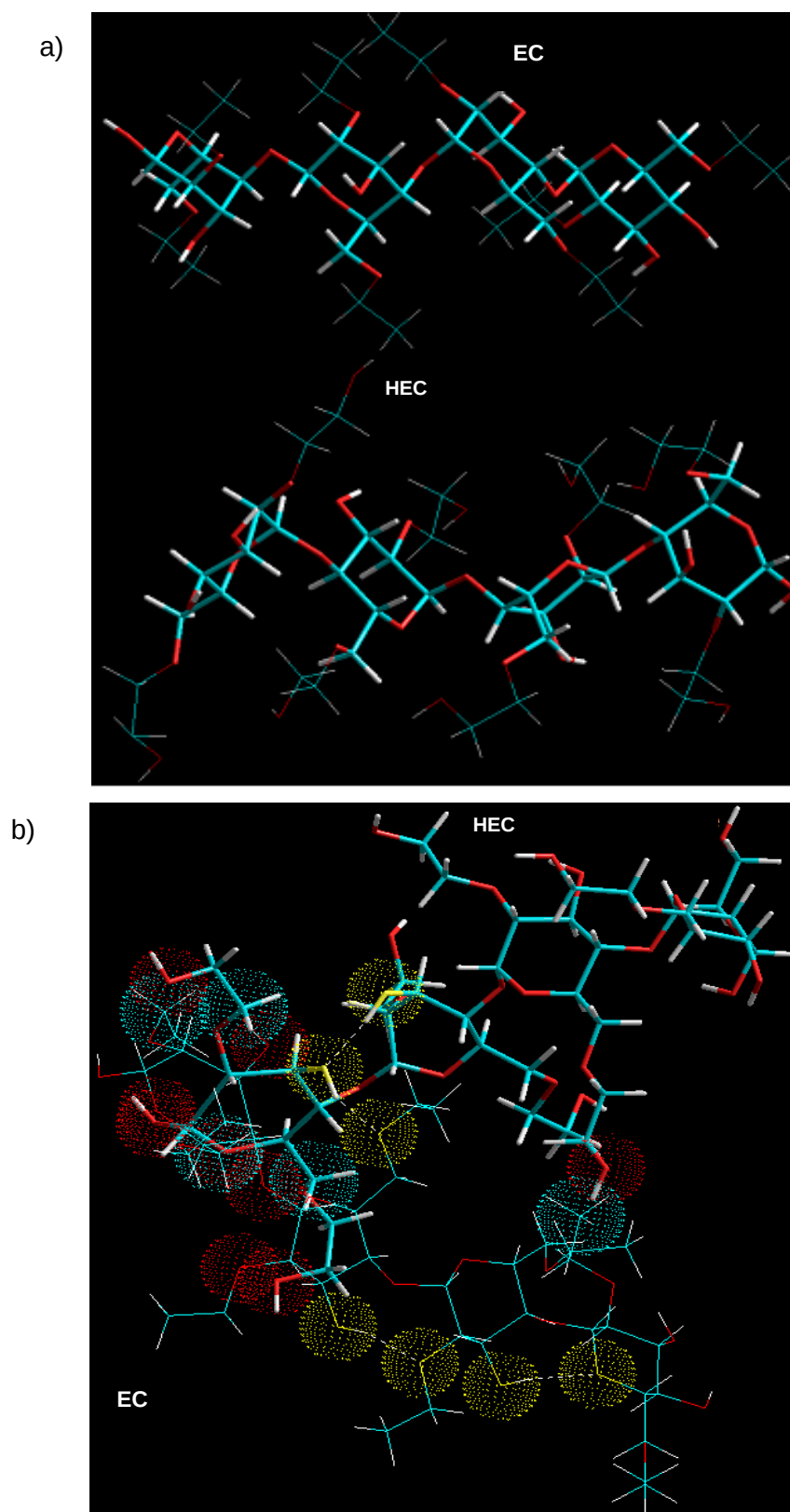


Figure 4.21: Energy minimized geometrically constrained models of: a) ethylcellulose and hydroxyethyl cellulose before complexation; and b) EC-HEC complex derived from molecular mechanics calculations. The atoms in close interaction proximity are emphasized by space filling model (dots) where the yellow dots depict atoms involved in H-bonding. Color codes for elements are: Carbon (cyan), Nitrogen (blue) and Oxygen (red) and Hydrogen (white).

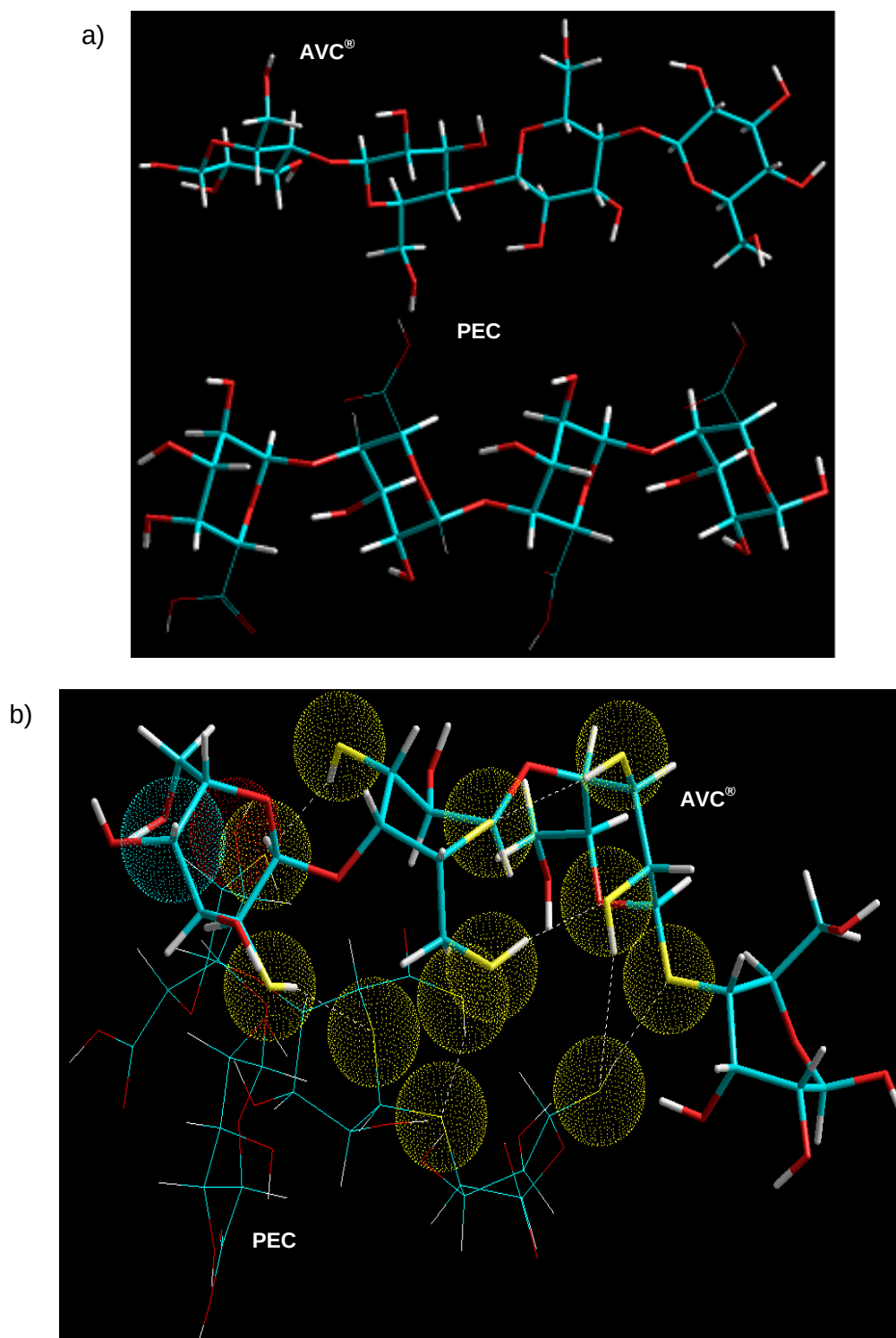
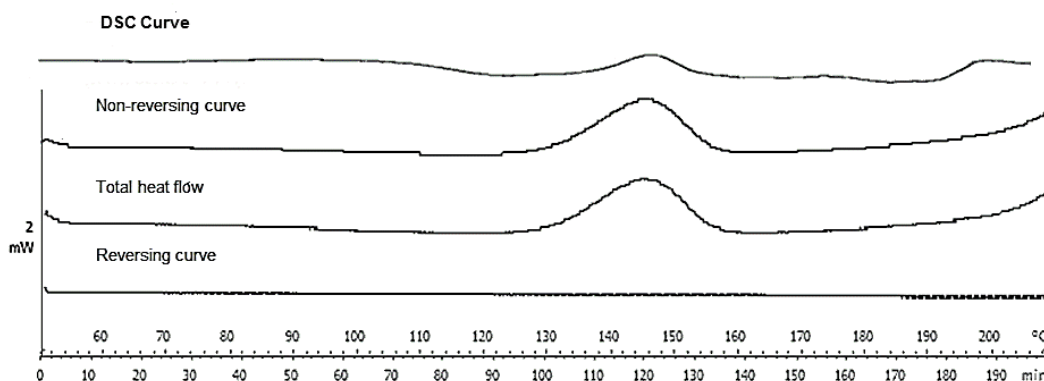


Figure 4.22: Energy minimized geometrically constrained models of a) AVC (cellulose) and PEC before complexation; and b) PEC-AVC complex derived from molecular mechanics calculations. The atoms in close interaction proximity are emphasized by space filling model (dots) where the yellow dots depict atoms involved in H-bonding. Color codes for elements are: Carbon (cyan), Nitrogen (blue) and Oxygen (red) and Hydrogen (white).

4.3.11. Determination of the thermal behavior of the polymeric constituents

DSC analysis of both the polymer blend and the granules revealed a T_c of 130°C indicating the presence of EC (Figure 4.22). Further thermal events are obscured and thus TMDSC analysis was employed. TMDSC provides additional information about time-dependent processes in the sample. TMDSC has the ability to separate reversible glass transition temperatures from overlapping non-reversible relaxation endotherms (Höhne, 1999). TMDSC analysis was conducted on the granules and polymer blend. Figure 4.22a and b illustrates the reversible, non-reversible and total heat flow of THP and DTZ polymer blend and the granules. The T_g of HEC is present at 72°C and 95°C for EC. Based on the information presented in these thermoanalytical curves it may be concluded that no significant interactions are presented. Results obtained from studies of DTZ polymer blends further confirm the results obtained with THP indicating that the drug has no influence on the thermal behavior of the device. This corresponds with results obtained from FTIR analyses.

a)



b)

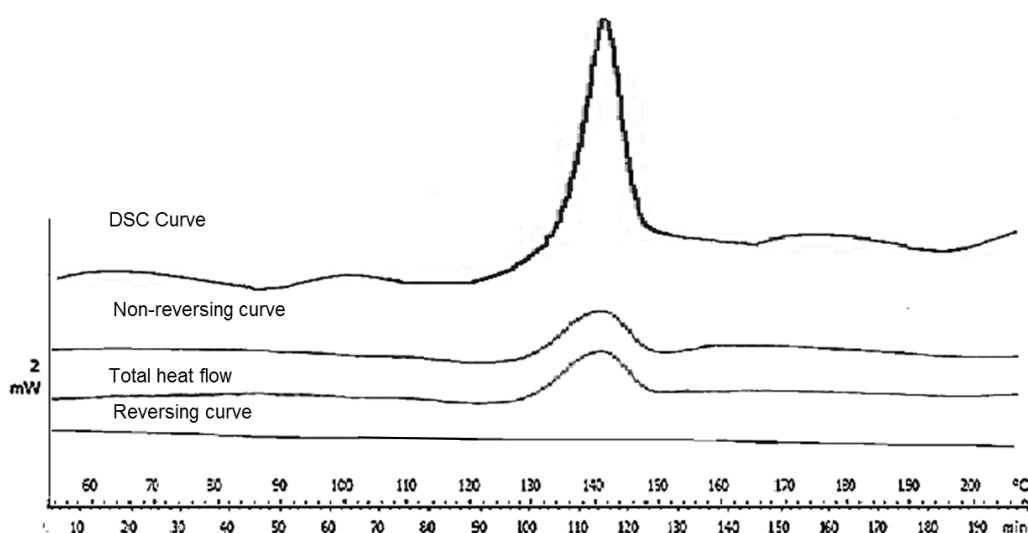


Figure 4.23: Thermogram of a) THP granules and b) DTZ granules depicting the endothermic and exothermic peaks generated.

4.4 Concluding Remarks

The optimized MLMDT was successfully developed. The system comprised two drug-loaded disks enveloped by barrier layers. The optimized formulations included PEC/AVS utilized in L1 and HPMC utilized in L2 and L3. D1 comprised lactose and D2 comprised HEC and EC. DTZ-loaded formulations also comprise of sodium bicarbonate in D1, L2 and L3. Friability, hardness and uniformity of mass were all within the specified limits depicting desirable manufacturing settings. The developed drug delivery system displays the desired release profile with two pulses of drug release, separated by a “switch-off” phase. The “switch-off” phase is dependent on the polymers utilized in the polymer disk, with the concentration of HEC and sodium bicarbonate being the most important factors in producing the “switch-off” phase. MRI results confirmed the data obtained from drug release and swelling and erosion studies. Furthermore, results from DSC highlighted no interaction between the polymers and drug and between the polymers themselves. The experimental results were well corroborated by the Molecular Mechanics Computations. Overall, the MLMDT is an innovative drug delivery system that displays potential as a chronotherapeutic drug delivery system.

CHAPTER FIVE

IN VIVO ANALYSES ON THE OPTIMIZED MULTI-LAYERED MULTI-DISK TABLET IN THE LARGE WHITE PIG MODEL

5.1 Introduction

A crucial step in the formulation of a new drug delivery system is conducting *in vivo* animal studies. Data generated can be used to assess safety and efficacy of a dosage form and more importantly to predict the pharmacokinetics and pharmacodynamics of the system in humans. Over the years studies were conducted in a variety of animal models. These include rats (Miyazaki et al., 1999; Tarvainen et al., 2000, Blanco et al., 2003; Nagahara et al., 2007; Pillay et al., 2009), rabbits (Ugwoke et al., 1999; Latha et al., 2000; Fernandes et al., 2003; Choonara et al., 2006; Ndesendo et al., 2009), dogs (Ishibashi et al., 1999; Löbenberg et al., 2005; Gao et al., 2006; Ghimire et al., 2007; Lavy et al., 2010) and monkeys (Haddish-Berhane et al., 2006).

Though similarities do exist between the gastrointestinal tract (GIT) of animals and humans, no single animal model completely resembles the GIT of humans. The differences between the anatomy and physiology of the GIT explain the variation in the pharmacokinetics and pharmacodynamics of a compound between human and animals (Hildebrand et al., 1991; Dali et al., 2006). Selection of an appropriate animal model largely depends on the dosage form and frequency of administration (Hildebrand et al., 1991). Due to the size of the oral dosage forms and the numerous samples needed, rodents and rabbits are excluded. Dogs, pigs and monkeys are suitable due to similarities in the anatomy of the stomach to humans though the use of dogs and monkeys has been limited due to their restricted availability, high cost and the social pressure of special interest groups. In addition, the size of dogs limits their use as the volume of blood that can be removed is restricted (Kostewicz et al., 1996).

The pig (*Sus scrofa domestics*) has gained popularity as an animal model in biomedical research due to the anatomical resemblance shared with humans. They are large animals enabling multiple blood sampling and share similarities in metabolism, biotransformation (cytochrome P450), feeding patterns dietary habits, kidney function and structure, pulmonary vascular bed structure and respiratory rates (Brunet et al., 2006).

In the previous chapter various formulations were analyzed to determine *in vitro* drug release. The formulations were subjected to optimization using ANN and one optimized formulation for each drug was generated. This chapter deals with *in vitro* comparative studies between the optimized formulations and a conventional system currently on the market as well as *in vivo* testing of these formulations using the large white pig as an animal model.

5.2 Animal Ethics

This study had received the approval from the Animal Ethics Screening Committee of the University of the Witwatersrand with ethics clearance number 2007/56/04. (Appendix E).

5.3 Materials and Methods

5.3.1 Materials

Materials utilized in the tablet making process were the same as in Chapter 3, Section 3.2.1. Methylparaben (MP) (Merck (Pty) Ltd., Darmstadt, Germany) was utilized as an internal standard. Ketamine (Bayer (Pty) Ltd, Isando, South Africa), midazolam (Roche Products (Pty) Ltd, Isando, South Africa), Buprenorphine (Schering-Plough, Rio de Janeiro, Brazil), Carprofen (Pfizer Ltd, Kent, UK), heparin (1000IU/mL), saline (Bodene (Pty) Ltd., Port Elizabeth, South Africa) and isoflurane (Safeline Pharmaceuticals (Pty) Ltd. Johannesburg, South Africa) were employed during the surgical procedure. Double deionized water was obtained from a Milli-Q system (Milli-Q Millipore Massachusetts, USA), while analytical grade acetonitrile and methanol (Rompil-Sp-S™, Cambridge, UK) comprised the mobile phase solvents. All other reagents used were of analytical grade. Theoplus® CR 200 (Altana Madaus, Johannesburg, South Africa) and Zildem CR 180 (Adcock-Ingram, Johannesburg, South Africa), were employed as comparator products.

5.3.2 Preparation of the MLMDT device

The MLMDT's were prepared according to the method described in Chapter 3, Section 3.2.2.1. A digital image illustrating the disk and the layers of the MLMDT is depicted in Figure 5.1.

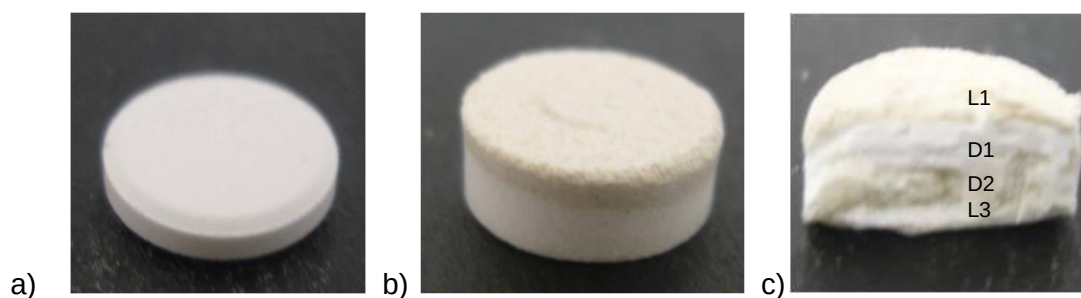


Figure 5.1: Digital photographs of a) the drug loaded disk, b) the fully compressed MLMDT and c) a cross section depicting the various layers.

5.3.3 *In vitro* drug release analysis of the optimized MLMDT and comparator systems

Drug release from Theoplus® CR 200 and Tilazem® CR 180 were compared to that of the optimized THP and DTZ-loaded MLMDT's using USP dissolution apparatus II as described in Chapter 3, Section 3.2.2.4.

5.3.4 *In vivo* animal studies

5.3.4.1 Procurement and pre-surgical preparation of pigs

Five large white female pigs ranging in weight from 30-35kg were sourced from Animal Nutrition and Products Institute of the Agricultural Research Council (Pretoria, South Africa). The animals were housed in standard animal rooms at the Central Animal Service Unit at the University of the Witwatersrand (Johannesburg, South Africa) with access to food and water. Habituation began 7 days pre-surgery through feeding and repeated visits. Habituation and domestication allowed the pigs to adapt to contact and handling by humans (Figure 5.2).



Figure 5.2: Habituation and domestication of the pigs utilized in the study at the Central Animal Service Unit.

5.3.4.2 Insertion of a chronic catheter for blood sampling

The pigs were anaesthetized with ketamine (11mg/kg I.M.) and midazolam (0.3mg/kg I.M.). Buprenorphine (0.05mg/kg I.M.) and carprofen (4mg/kg I.M.) were administered for analgesia and inflammation. The pig was then intubated and anesthesia was maintained with 2% isoflurane in 100% oxygen. Under aseptic conditions, a 7 French gauge double lumen 35cm catheter (CS-28702) (Arrow Deutschland GmdH, Erding, Germany) was surgically inserted into the left jugular vein. The jugular vein was exposed by an incision made dorsal to the jugular groove on the left lateral aspect of the neck. Via blunt dissection, the vein was isolated and the catheter was inserted 10cm into the lumen of the vein. The lumen of the catheter was fastened to the wall of the vein using a purse suture technique. The remaining length of the catheter (25cm) was tunneled subcutaneously, with the use of a trocar, to an exit point cranial to the dorsal aspect of the scapular. The externalized injection ports of the catheter were sutured to the skin of the pig so as to limit excessive movement and bending. The surgical process is depicted in Figure 5.3. Blood was removed via the catheter and the catheter was flushed with heparin saline (1000 i.u. of heparin in 1L of 0.9% saline). Thereafter, the animal was allowed 10 days to recover from the surgical procedure. During this time it was further habituated to the process of blood sampling. Throughout

the study, the catheter was flushed with heparinized saline three times a day to prevent blockage.

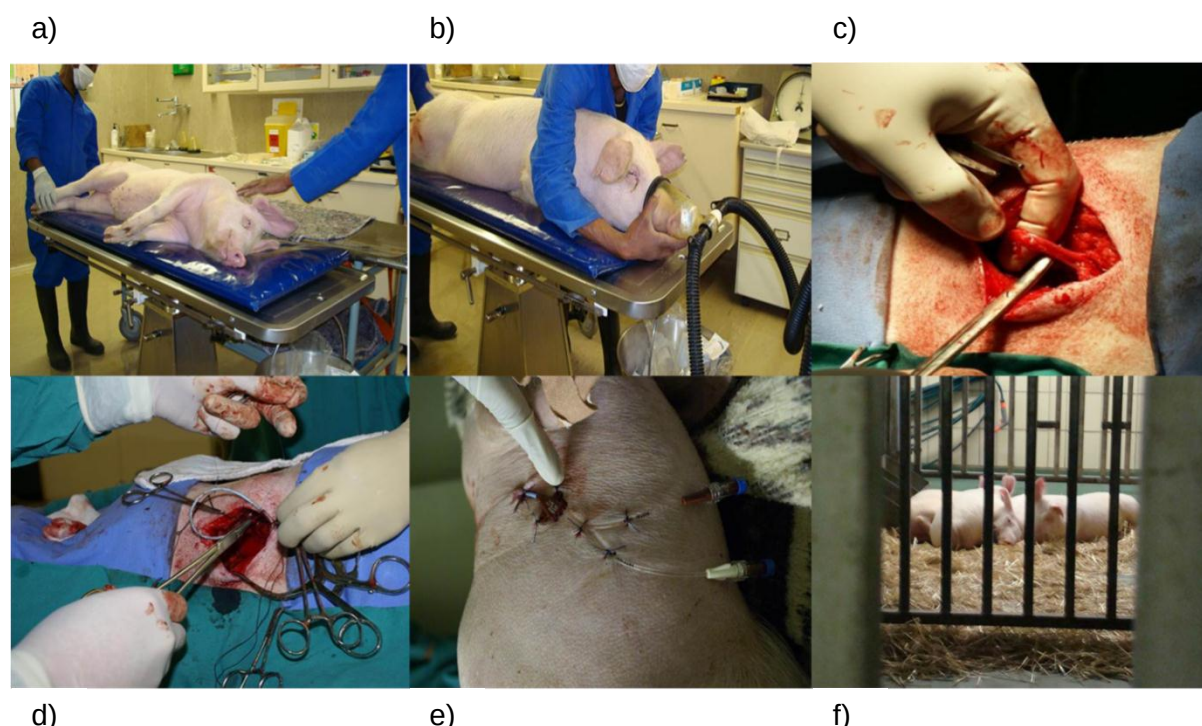


Figure 5.3: Digital photographs depicting: a) sedation and preparation for surgery, b) administration of anesthesia, c) incision into the jugular area permitting the confinement of the jugular vein, d) insertion of the catheter into the jugular vein, e) external ports sutured to the skin of the pig and f) recovery.

5.3.4.3 Oral administration of the MLMDT and conventional systems

Administration of the dosage forms were executed in 5 pigs at a time, dosed on separate occasions with an appropriate wash-out period separating each dosing. The dosage administration was separated into 6 groups (Figure 5.4). Dosing of the pigs commenced 10 days post-surgery allowing the animals to fully recover. Animals were fasted for 12 hours prior to administration with access to water only. Prior to dosing the jugular catheters were flushed with heparinized saline followed by blood sampling to acquire a baseline sample. The pigs were sedated with intramuscular midazolam (0.3mg/kg) and anesthetized with ketamine (4mg/kg). The animal was maintained under anesthesia by gaseous administration of 2% isoflurane in 100% oxygen via a face mask. The animal was then placed in an upright position and intubated with a bore gastric tube (Figure 5.5) through the esophagus and into the stomach. The MLMDT was inserted through the tube and then flushed down to the stomach with $\pm 25\text{mL}$ of water. The animal was then allowed to recover which took approximately 15-25 minutes.

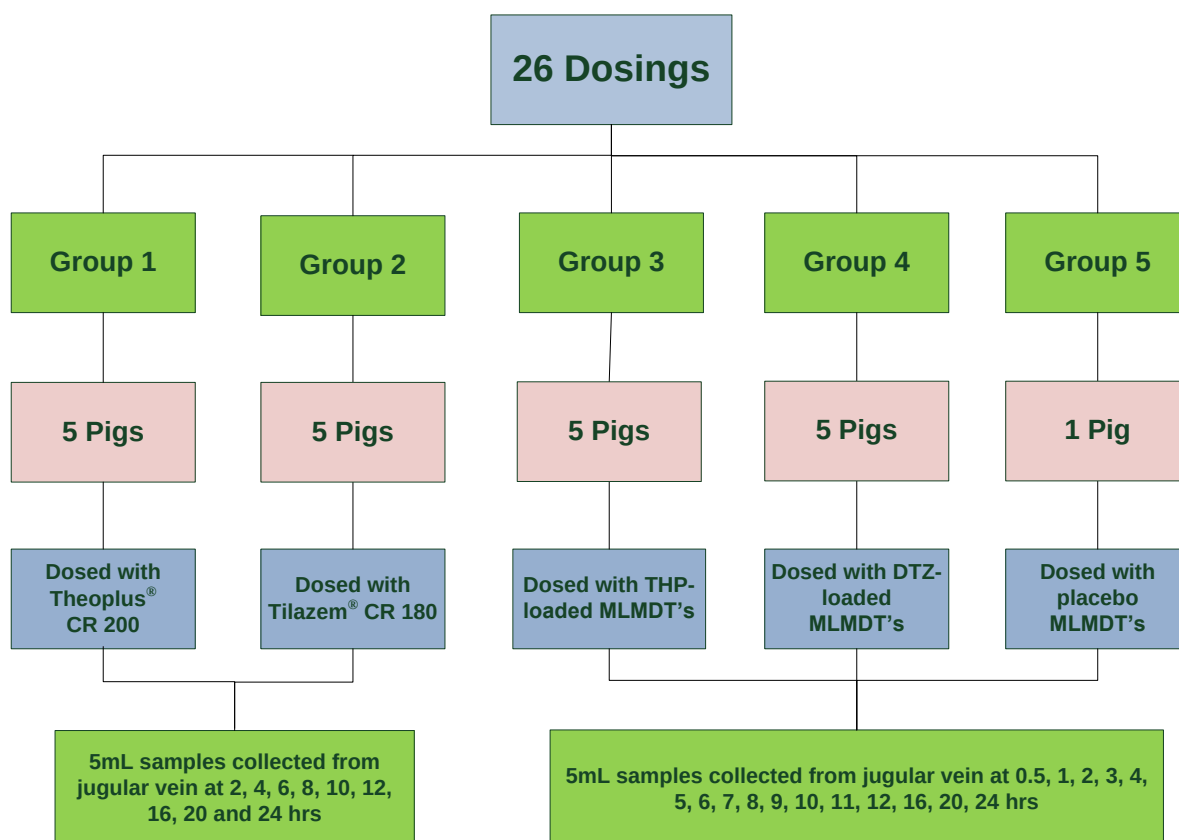


Figure 5.4: Schematic diagram representing the different dosings.



Figure 5.5: Digital image depicting the gastric tube used for administering the MLMDT's.

5.3.4.4 Collection of blood samples

Prior to obtaining the blood, both ports were flushed with 5mL heparinized saline. 5mL of blood was collected at 2, 4, 6, 8, 10, 12, 16, 20 and 24 hours from pigs dosed with conventional formulations (group one and two). Samples collected from group 3, 4 and 5 were obtained at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20 and 24 hours. Blood samples were immediately transferred into heparinized vacutainers (BD Vacutainer®, New Jersey, USA) and centrifuged (Nison Instrument Limited, Shanghai, China) at 10 000rpm for 15 minutes. The clear plasma supernatant was pipetted using an adjustable volume micropipette (Boeco GmbH, Hamburg, Germany) and stored at -70°C until further analysis.

5.3.5 Preparation of standards

Primary stock solutions of THP and DTZ were separately prepared by adding Milli-Q (Millipore®) ultra-pure water to yield a concentration of 0.1mg/mL. The internal standard MP was prepared at a concentration of 5000ng/mL. The stock solutions were stored in polypropylene tubes with screw caps and stored in a refrigerator at 5°C. Working standard solutions of THP and DTZ were prepared separately by diluting the primary solution of each drug with Milli-Q (Millipore®) ultra-pure water to yield solutions with a final concentration range of 20-10 000ng/mL. The internal standard MP was prepared at a concentration of 5000ng/mL. Milli-Q (Millipore®) ultra-pure water and was added to all samples prepared for ULPC analysis. All solutions were filtered with a 0.22µm pore size Cameo Acetate membrane filter (Millipore Co., Massachusetts, USA).

5.3.6 Plasma extraction procedure

A simple yet effective liquid-liquid plasma extraction procedure was developed and applied to both DTZ and THP blood samples. Aliquots (600µL) of sample were transferred into polypropylene tubes followed by the addition of 1mL of acetonitrile in order to precipitate the plasma proteins. The samples were subsequently vortexed for 1 minute and then centrifuged at 3000rpm (Nison Instrument Limited, Shanghai, China) for 20 minutes. Thereafter, 1mL of the supernatant was removed and filtered through 0.22µm Cameo Acetate membrane filters. The filtrate and the internal standard were then combined and transferred into vials for analysis.

5.3.7 Calibration curves and limits of quantification analysis

Calibration curves were developed by spiking blank plasma with MP and various concentrations of either DTZ or THP. The spiked plasma samples were subjected to the extraction method described above. The ratio of the area under the (AUC) (of the

chromatogram) to drug/internal standard was profiled against the corresponding drug concentrations expressed in ng/mL. Linearity equations and correlation coefficients (r_c) were obtained by means of the least square method. The limit of quantification (LOQ) is defined as the concentration which produces chromatographic peaks with heights at least 3 times that of the baseline noise. This concentration was determined for THP as well as DTZ.

5.3.8 Chromatographic conditions for the analysis of blood samples

A Waters® Acquity Ultra Performance Liquid Chromatographic (UPLC) system (Waters Corp., Massachusetts, USA) equipped with a photodiode array (PDA) detector was used to analyze the blood samples. Separation was achieved on an Acquity UPLC BEH RP 18 column (100mm x 2.1mm i.d., 1.7µm particle size) maintained at 25°C. The binary mobile phases and priming solvents are listed in Table 5.1. An injection volume of 2.5µL was injected in the mobile phase at a flow rate of 0.500mL/min. A total run time of 1.5 minutes was required at a wavelength of 238nm for DTZ and 280nm in the case of THP.

Table 5.1: Mobile phases and priming solvent used for the determination of THP and DTZ in plasma samples.

Solvent/Mobile Phase	Composition
Strong wash	Acetonitrile (90% v/v) and water (10% v/v).
Weak wash	Acetonitrile (10% v/v) and water (90% v/v).
Mobile Phase A (55%)	Water
Mobile Phase B (45%)	Acetonitrile

5.3.9 Extraction yield, precision and accuracy of the method

Three duplicate plasma samples (both for THP and DTZ) at 3 different concentrations (20ng/mL, 1000ng/mL and 10 000ng/mL) were prepared and subjected to the extraction procedure mentioned above and injected into the machine. The percentage extraction yield was calculated by comparing the peak areas obtained from extracted analytes to those obtained from standard solutions at the same concentration.

Intraday accuracy was determined by multiple injections (N=3) during a 24-hour period whereas the inter-day accuracy and precision of the method were determined by assaying multiple injections of the above concentrations over 3 consecutive days (N=3) and calculating the percentage deviation from the nominal concentration.

5.4 Results and Discussion

5.4.1 *In vitro* drug release studies from MLMDT and comparator device

In vitro drug release studies were conducted to determine the rate of drug release from optimized drug-loaded MLMDT's and from popular existing THP (Theoplus[®] CR 200) and DTZ (Tilazem[®] CR 180) formulations. Theoplus[®] presents itself as a tablet whereas Tilazem[®] is a capsule loaded with controlled release beads. Upon exposure to the dissolution media swelling of the Theoplus[®] tablet was observed while exposure of the Tilazem[®] to dissolution media resulted in disintegration of the outer gelatin capsule and exposure of the inner controlled release beads. After the 24 hour study, Theoplus[®] had completely disintegrated, while the Tilazem[®] controlled release beads remained intact. A comparison of the rate of drug release between the drug-loaded MLMDT and the comparator products for both THP and DTZ are outlined in Figure 5.6. Observation of Figure 5.6a indicates a burst of drug release from the comparator product followed subsequently by controlled release. During this burst phase close to 40 % of drug is released within the first two hours. Drug release peaks at 8 hours indicating that the patient requires more than one dose in a day to maintain an adequate therapeutic response. The THP-loaded MLMDT exhibits the desired release profile and provides a lag phase and first pulse of drug release. This first pulse continued for duration of 3 hours followed by a "switch-off" phase of approximately 6 hours. Thereafter, a second pulse of controlled drug release for the remainder of the 24 hours was observed. This unique approach to drug delivery results in diminished exposure of the patient to the drug and eradicates the need for multiple dosing which fundamentally decreases side-effects. Drug release from the DTZ conventional produces a similar trend (Figure 5.6b). Tilazem[®] presents with controlled release for the first 12 hours. Thereafter, a 5% increase in drug release between T_{12} and T_{24} is observed. This signifies that the patient may require more than one dose within a 24-hour period. The DTZ-loaded MLMDT displayed the desired release profile with a lag phase and an initial pulse of drug release continuing for approximately 3 hours. This was followed by a "switch-off" phase of 8 hours and subsequently the a second pulse of drug release.

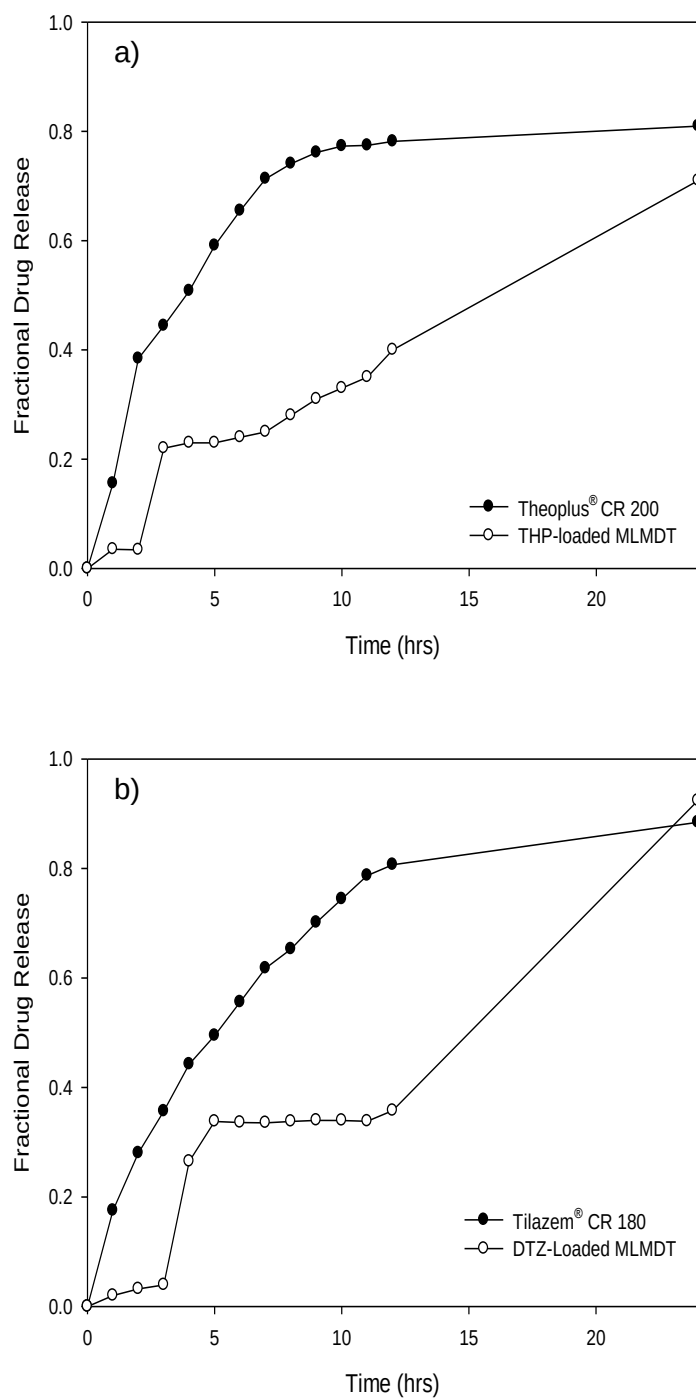


Figure 5.6: Drug release profiles depicting the comparison between a) THP-loaded MLMDT's and the comparator product Theoplus® and b) DTZ-loaded MLMDT's and Tilazem® (N=3, SD<0.071 in all cases).

5.4.2 *In vivo* animal studies

5.3.2.1 Extraction of THP and DTZ from blood samples

An uncomplicated, rapid method for extracting both THP and DTZ from blood samples has been developed. The straightforward process proved to remove plasma constituent interference from the plasma samples. The assay did not demonstrate any interference from plasma components.

5.4.2.2 Chromatograms for THP and DTZ plasma

A typical chromatogram portraying the retention of THP and DTZ combined with the internal standard MP is represented in Figure 5.7 and Figure 5.8. THP exhibits a retention time of 0.424 ± 0.02 minutes while DTZ elutes at 0.435 ± 0.02 minutes. A positive control assay to determine the efficacy of the extraction method was performed by analyzing blank plasma spiked with MP (Figure 5.9). The chromatogram demonstrated no interference peaks at the retention times of both THP and DTZ, indicating that the extraction method was effective.

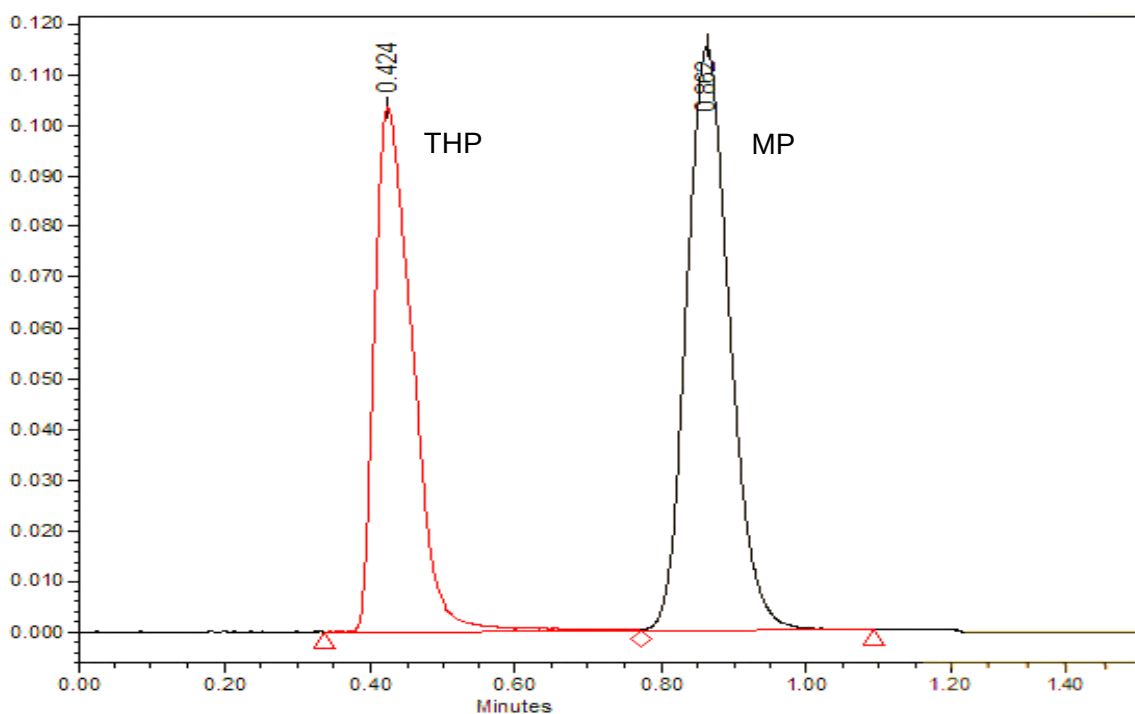


Figure 5.7: Chromatograms depicting the retention times of THP and MP in plasma.

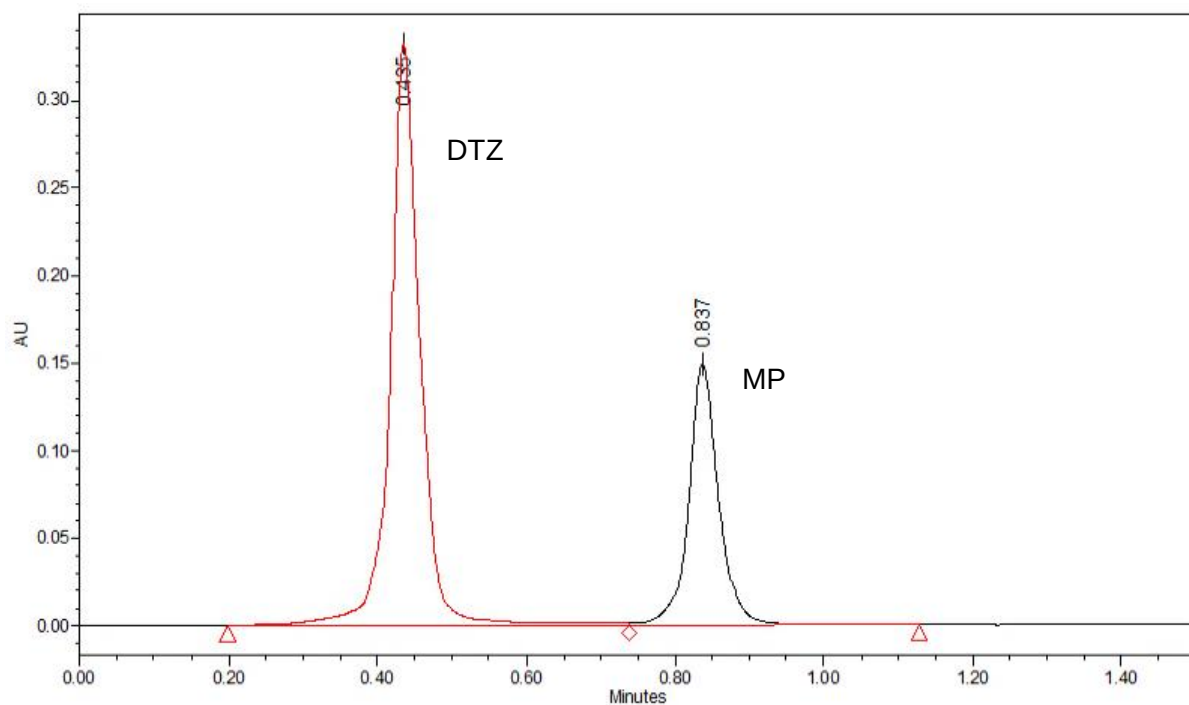


Figure 5.8: Chromatograms depicting the retention times of DTZ and MP in plasma.

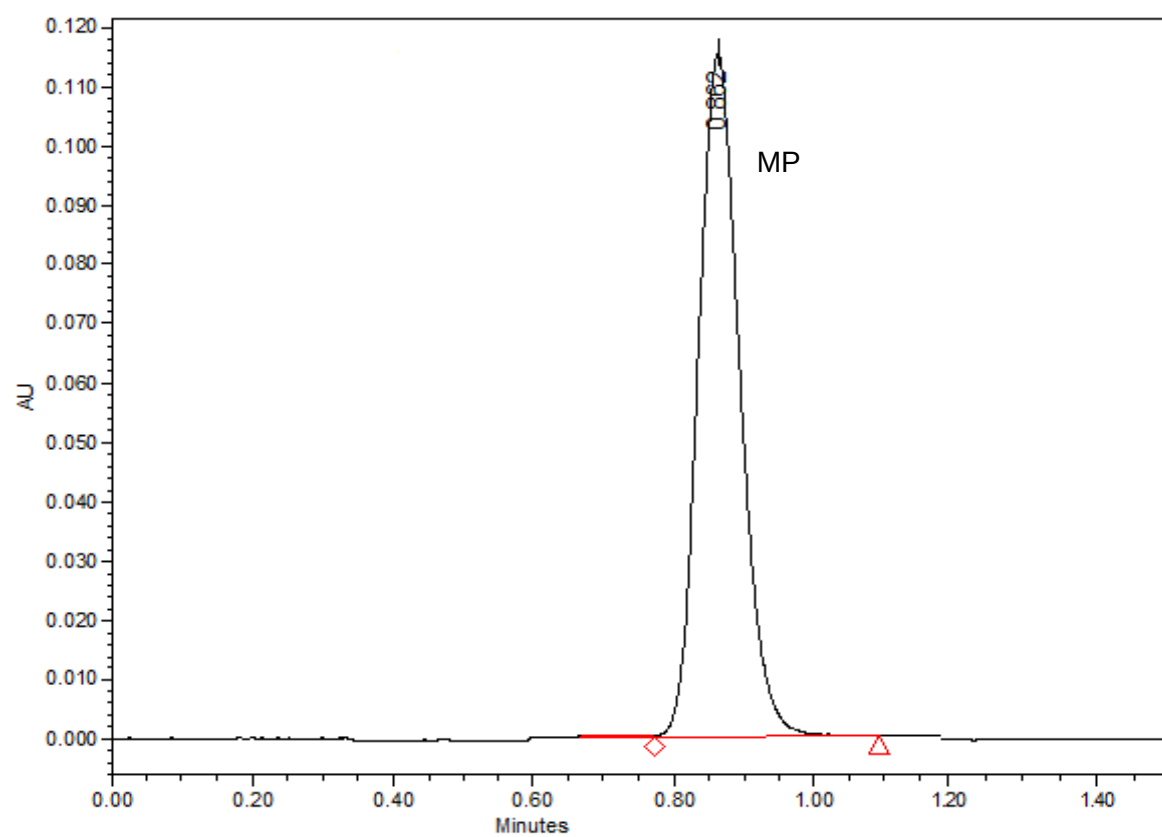


Figure 5.9: Chromatogram obtained from drug-free plasma spiked with MP demonstrating no interference peaks.

5.4.2.3 Calibration curves and lower limit of quantification

Figure 5.10 and Figure 5.11 represents the calibration curve for the quantitative determination of plasma THP and DTZ respectively. Concentrations of both drugs ranged from 20ng/mL -10 000ng/mL and displayed good linearity ($R^2=0.99$). The lower limit of quantification was determined as 19.7ng/mL for THP and 20ng/mL for DTZ.

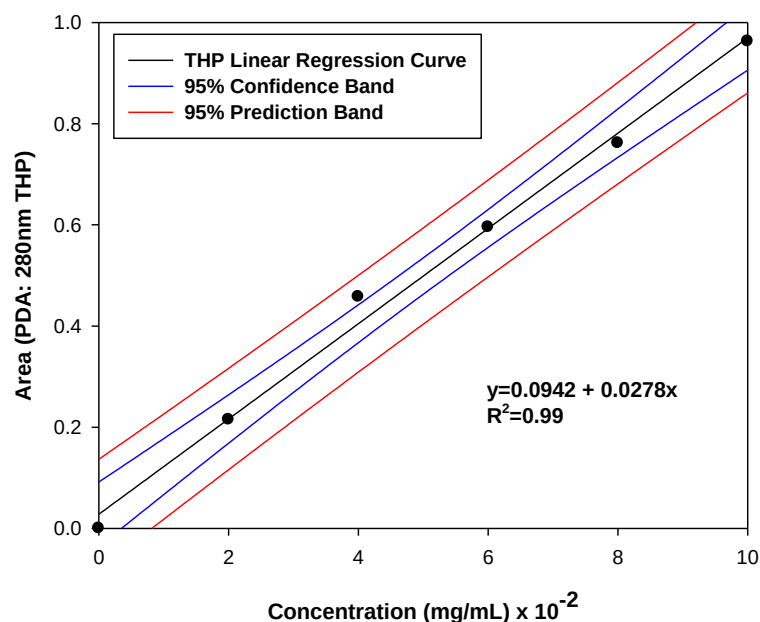


Figure 5.10: Calibration curve for THP in blank plasma 280nm (N=3, SD<0.031 in all cases).

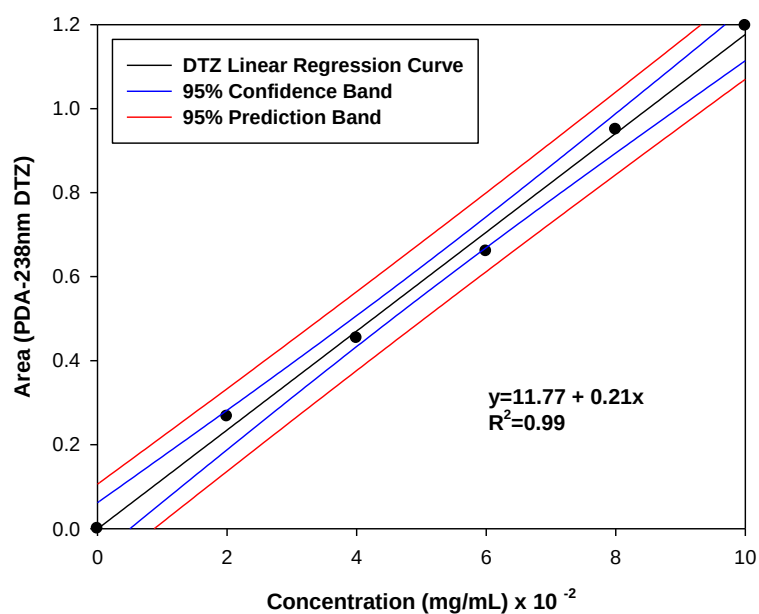


Figure 5.11: Calibration curve for DTZ in blank plasma 238nm (N=3, SD<0.041 in all cases).

5.4.2.4 Method validation for analysis of THP and DTZ in plasma employing UPLC analysis

Results from method validation assays for THP and DTZ are summarized in Table 5.2 and Table 5.3 respectively. Results indicated that the implemented extraction and assay procedure displayed acceptable accuracy and precision.

Table 5.2: Method validation data for THP.

Theoretical plasma THP concentration (ng/mL)	Intra-day		Inter-Day	
	Extraction yield (%)	CV (%)	Extraction yield (%)	CV (%)
20	95.03	0.024	95.42	0.025
1000	97.58	0.020	97.14	0.029
100 000	98.96	0.017	98.90	0.021

Table 5.3: Method validation data for DTZ.

Theoretical plasma DTZ concentration (ng/mL)	Intra-day		Inter-Day	
	Extraction yield (%)	CV (%)	Extraction yield (%)	CV (%)
20	98.57	0.016	98.61	0.022
1000	98.85	0.020	98.70	0.024
100 000	99.06	0.017	98.92	0.030

CV=Coefficient of Variation

5.4.2.5 In vivo drug release analysis

In vivo drug release profiles of the MLMDT's and their conventional comparator products are portrayed in Figure 5.12 and 5.13. The plasma time-concentration profiles display immediate release from both conventional products. Plasma drug concentrations reach a maximum after 2 hours ($T_{max} = 2$ hours). Thereafter, drug concentrations decline as the elimination process commences.

Plasma time-concentration profiles from both THP-loaded and DTZ-loaded MLMDT's display immediate drug release during the first hour. This is followed by a decrease in drug release after 2 hours. This initial burst of drug release corresponds to the first pulse of release emanating from D1 though no initial lag phase is observed. The decrease in drug concentration after 2 hours corresponds to the "switch-off" phase separating the two pulses. The "switch-off" phase is however, not as pronounced as demonstrated from *in vitro* data. Even though no drug is released into the blood during this "switch-off" phase, drug is nonetheless present in the blood and undergoing elimination. As a result, a distinct, pronounced "switch-off" phase is absent. Thereafter,

the second pulse is observed with the THP-loaded MLMDT reaching peak plasma concentration (T_{max}) after 6 hours and DTZ-loaded formulations attaining a maximum concentration of 887.46ng/mL at $T_{max} = 5$ hours. Both drug-loaded MLMDT's displayed two phase release though the “switch-off” phase was not as distinct compared to the *in vitro* data obtained.

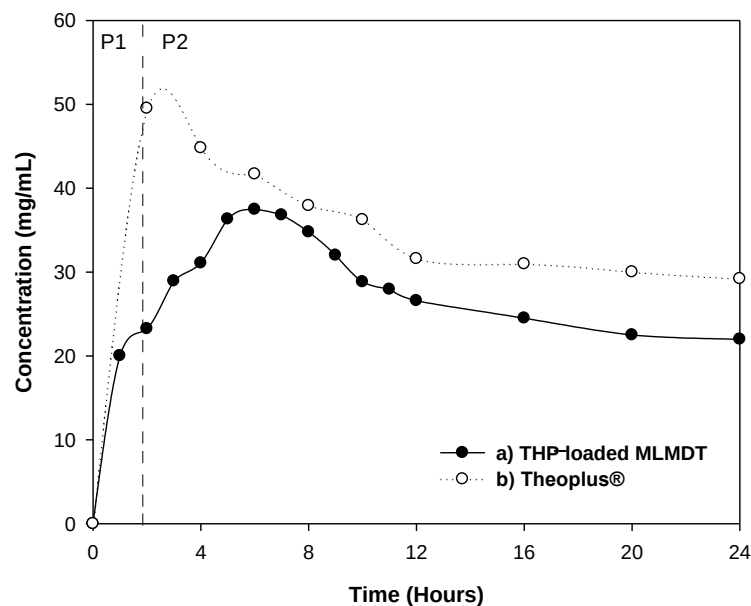


Figure 5.12: Comparison between *in vivo* plasma concentration-time profiles for a) THP (optimized) and b) Theoplus® 200 (conventional) with P1 representing the first pulse of drug release and P2 representing the second pulse of drug release (N=5; SD<0.13 in both cases).

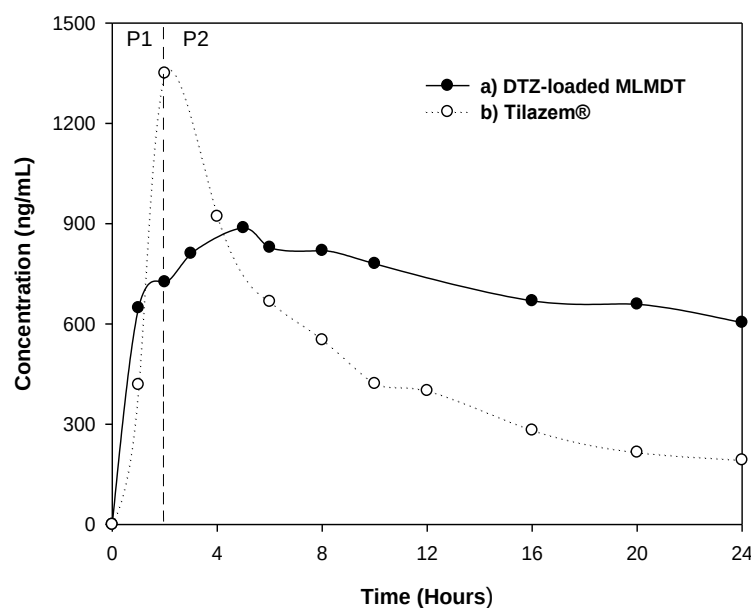


Figure 5.13: Comparison between *in vivo* plasma concentration-time profiles for: a) DTZ (optimized) and b) Tilazem 180 CR (conventional) with P1 representing the first pulse of drug release and P2 representing the second pulse of drug release (N=5; SD<0.14 in both cases).

5.5. Concluding Remarks

The proposed novel MLMDT consists of two drug loaded disks enveloped by drug-free polymeric layers comprising PEC/AVC, HPMC, EC and HEC. THP and DTZ-loaded MLMDT's were evaluated for drug release along with conventional systems namely Theoplus[®] CR 200 and Tilazem[®] CR 180. *In vitro* drug release results revealed two pulses of drug release from the MLMDT's while the conventional systems provided sustained release for 8 and 12 hours from Theoplus[®] ^a and Tilazem[®] respectively. In addition, a method which served to improve blood sampling was developed through the use of chronic implanted jugular catheters. Furthermore, a simple yet efficient extraction and analysis method was developed for both drugs. *In vivo* plasma time-concentration profiles illustrated a first pulse of drug release followed by a slight decrease in drug release after 2 hours and followed by controlled release. Two phase drug release in both THP and DTZ-loaded MLMDT's was observed though the "switch-off" phase was not as prominent as data obtained from *in vitro* analysis. Both Theoplus[®] and Tilazem[®] conventional formulations display increasing plasma concentrations up to 2 hours followed by a steady decline in concentrations. As a result, the proposed MLMDT proved superior to the conventional as two pulse drug release was achieved.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

With the advancement of technology and a better understanding of disease conditions, the concept of chronotherapy still remains largely unexplored. Current chronotherapeutic formulations on the market are not able to time drug release with the body's circadian rhythm leading to unwanted side-effects that may be avoided. As a result, a drug formulation needs to be efficacious, accessible and affordable. In the South African context, an affordable dosage form is welcomed as a large number of patients rely on government funded healthcare.

To address the above challenges, the MLMDT proposed here, aims to decrease side-effects and eliminate multiple dosing during a 24-hour period. This leads to a decrease in the cost of treatment and thus an increase in patient compliance. In addition, the use of low-cost FDA approved polymers and a cost-effective tablet making procedure results in an affordable dosage form.

Preliminary *in vitro* testing allowed for the selection of appropriate polymers to be employed in the MLMDT formulation. Both hydrophobic and hydrophilic polymers were evaluated for drug release in order to obtain the desired release profile. Drug release data depicted a lag-phase followed by phasic release.

Using Artificial Neural Network techniques optimized formulations were determined. Drug release data demonstrated an initial lag-phase followed by two pulses of drug release separated by a "switch-off" phase. The formulations were subsequently evaluated for hardness, friability, swelling, erosion, uniformity in the tablet making process and incompatibilities. All tested parameters fell within the acceptable range.

Drug release from the optimized drug-loaded MLMDT's was then compared to drug release from conventional formulations currently on the market, namely Theoplus[®] CR (THP) and Tilazem[®] (DTZ). The *in vitro* drug release data obtained from the analysis of both conventional formulations demonstrated controlled release over a period 8 hours in the case of Theoplus[®] and 12 hours in the case of Tilazem[®]. Furthermore no pulse

release was observed. Analysis from the drug-loaded MLMDT's illustrated two pulses of drug release with release continuing over the full 24-hour period proving the MLMDT to be superior to the respective conventional products.

The optimized MLMDT and the conventional formulations were also subjected to *in vivo* animal studies using the Large White Pig as an animal model. A convenient method of drawing blood from the pigs was developed which required surgery to catheterize the left vein jugular. External injection points were sutured to the back of the pig. Blood was drawn via these ports at predetermined time intervals, centrifuged and then stored until use. Analyses of the plasma samples were conducted using a developed simple and effective method of plasma extraction. The extracted plasma was subjected to analysis using UPLC to determine drug concentrations.

Plasma time-concentration profiles for both conventional formulations displayed immediate drug release with maximum drug concentrations reached in 2 hours followed by an elimination phase. The MLMDT's demonstrated immediate release during the first 2 hours followed by a "switch-off" phase. Thereafter, a second phase of drug release was observed. This phase demonstrated controlled release over the remaining 24-hours. As a result, the MLMDT proved superior to the conventional in the treatment of chronotherapeutic disorders by affording phasic drug delivery.

6.2 Recommendations

While this drug delivery system has been developed for use in the treatment of chronotherapeutic disorders, it is not exclusive to these diseases as the MLMDT may be loaded with drugs of varying solubilities. The system also allows the versatility of combining two drugs in the same dosage form to treat two disorders. The system may also be used to combine two drugs that are pharmaceutically incompatible in one dosage form or if drug is to be delivered to more than one site along the GIT.

The approach used in this study may also be employed to determine the effect of a change in the size or shape of the disk on drug release. The addition of a third and even fourth disk may also be explored. The additional disks may contain drug or crosslinking agents to further regulate drug release.

Animal studies in the pig model have shown some promise. As a result, testing should now be performed in the human model.

REFERENCES

- 1) Abdel-Rahman, S.I., Mahrous, G.M. and El-Badry, M., (2009). Preparation and comparative evaluation of sustained release metoclopramide hydrochloride matrix tablets, *Saudi Pharmaceutical Journal*, 17, 283-288.
- 2) Anal, A.K., (2007). Time-controlled Pulsatile Delivery Systems for Bioactive Compounds. *Recent Patents on Drug Delivery and Formulation*, 1, 73-79.
- 3) Arkinstall, W.W., (1988). Review of the North American experience with evening administration of Uniphyll tablets a once-daily theophylline preparation in the treatment of nocturnal asthma. *American Journal of Medicine*, 85, 60-63.
- 4) Aronson, B.D., Bell-Pedersen, D., Block, G.D., Bos, N.P.A., Dunlap, J.C. and Eskin, A., (1993). Circadian rhythms. *Brain Research Review*, 18, 315-333.
- 5) Badve, S.S., Sher, P., Korte, A. and Pawar, A.P., (2007). Development of hollow/porous calcium pectinate beads for floating-pulsatile drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 65, 85-93.
- 6) Barion, A. and Zee, P.C., (2007). A clinical approach to circadian rhythm sleep disorders. *Sleep Medicine*, 8, 566-577.
- 7) Bahamman, A., Alrajeh, M., Albabtain, M., Bahamman, S. and Sharif, M., (2010). Circadian pattern of sleep, energy expenditure, and body temperature of young healthy men during the intermittent fasting of Ramadan. *Appetite*, 54, 426-429.
- 8) Barmapalexis, P., Kanaze, F.I., Kachrimanis, K. and Georgarakis, E., (2010). Artificial neural networks in the optimization of a nimodipine controlled release tablet formulation. *European Journal of Pharmaceutics and Biopharmaceutics*, 74, 316-323.
- 9) Beam, W.R., Weiner, D.E. and Martin, R.J., (1992). Timing of prednisone and alternations of airways inflammation in nocturnal asthma. *American Review of Respiratory Disease*, 146, 1524–1530.

- 10) Blanco, M. D., Bernardo, M. V., Teijón, C., Sastre, R.L. and Teijón, J.M., (2003). Transdermal application of bupivacaine-loaded poly (acrylamide (A)-co-monomethyl itaconate) hydrogels. *International Journal of Pharmaceutics*, 255, 99-107.
- 11) Boughattas, N., Lévi, F., Fournier, C., Lemaigre, G., Roulon, A., Hecquet, B., Mathé, G. and Reinberg, A., (1989). Circadian rhythms in toxicities and tissues uptake of oxaliplatin in mice. *Cancer Research*, 49, 3362-3368.
- 12) Brabant, G., Prank, K., Ranft, U., Schuermeyer, T., Wagner, T.O., Hauser, H., Kummer, B., Feistner, H., Hesch, R.D. and von zur Muhlen, A., (1990). Physiological regulation of circadian and pulsatile thyrotropin secretion in normal man and woman. *Journal of Clinical Endocrinology and Metabolism*, 70, 403–409.
- 13) Bredenberg, S., Nyholm, D., Aquilonius, S.M. and Nyström, C., (2003). An automatic dose dispenser for microtablets – a new concept for individual dosage of drugs in tablet form. *International Journal of Pharmaceutics*, 261, 137–146.
- 14) Brunet, B., Doucet, C., Venisse, N., Hauet, T., Hébrard, W., Papet, Y., Mauco, G. and Mura, P., Validation of large white pig as an animal model for the study of cannabinoids metabolism: application to the study of the distribution in tissues. *Forensic Science International*, 161, 169-174.
- 15) Burkioka, N., Sako, T., Tamita, K., Miyota, M., Suyama, H., Igish, T. and Shimizu, E., (2002). Theophylline chronotherapy of nocturnal asthma using bathyphase of circadian rhythm in peak expiratory flow rate. *Biomedicine and Pharmacotherapy*, 55 142-146.
- 16) Caramori, G. and Adcock, I., (2003). Pharmacology of airway inflammation in asthma and COPD. *Pulmonary Pharmacology and Therapy*, 16, 247-277.
- 17) Castilla-Guerra, L., Fernández-Moreno, M.D.C., Espino-Montoro, A. and López-Chozas, J.M., (2009). Ambulatory blood pressure monitoring in stroke survivors: Do we really control our patients? *European Journal of Internal Medicine*, 20, 760-763.

- 18) Chen, Y., McCall, T.W., Baichwal, A.R. and Meyer, M.C., (1999). The application of an artificial neural network and pharmacokinetic simulations in the design of controlled-release dosage forms. *Journal of Controlled Release*, 59, 33-41.
- 19) Choonara, Y.E., Pillay, V., Carmichael, T., and Danckwerts, M.P., (2006). An *in vitro* study of the design and development of a novel doughnut-shaped minitabket for intraocular implantation. *International Journal of Pharmaceutics*, 310, 15-24.
- 20) Conlon, M., Lightfoot N. and Kreiger, N., (2007). Rotating shift work and risk of prostate cancer. *Epidemiology*, 18, 182–183.
- 21) Conti, S., Maggi, L., Segale, L., Machiste, E.O., Conte, U., Grenier, P. and Vergnault, G., (2006). Matrices containing NaCMC and HPMC 1. Dissolution performance characterization. *International Journal of Pharmaceutics*, 333, 136-142.
- 22) Cutolo, M., Seriola, B., Craviotto, C., Pizzorni, C. and Sulli, A., (2003). Circadian rhythms in RA. *Annals of Rheumatic Disease*, 62, 593-596.
- 23) Dali, M.M., Moench, P.A., Mathias, N.R., Stetsko, P.I., Heran, C.L. and Smith, R.L., (2006). A rabbit model for sublingual drug delivery: comparison with human pharmacokinetic studies of propranolol, verapamil and captopril. *Journal of Pharmaceutical Sciences*, 95 37–44.
- 24) Dashevsky, A. and Mohamad, A., (2006). Development of pulsatile multiparticulate drug delivery system coated with aqueous dispersion Aquacoat® ECD. *International Journal of Pharmaceutics*, 320, 124-131.
- 25) Davis, S. and Mirick, D.K., (2006). Circadian disruption, shift work and the risk of cancer: a summary of the evidence and studies in Seattle. *Cancer Causes Control*.17, 539–545.
- 26) Dickmeis, T. and Foulkes N.S., (2011). Glucocorticoids and circadian clock control of cell proliferation: At the interface between three dynamic systems. *Molecular and Cellular Endocrinology*, 331, 11-22.

- 27) Dong, L.C., Wong, P-L S., Yum, S-H. and Pollock, C., (1999). Drug delivery device with minimal residual drug retention. *US patent*, 5,902,605. 1999.
- 28) Dodson, E.R. and Zee, P.C., (2010). Therapeutics for circadian rhythm sleep disorders. *Sleep Med Clinics*. 5, 701-715.
- 29) Douglas, J.G., (2007). Is it Time for Chronotherapy? *American Journal of Hypertension*, 15, 238.
- 30) Douglas, J.G., (2002). Compliance with antihypertensive therapy: Is it time for chronotherapy? *American Journal of Hypertension*, **15**, A238.
- 31) Dunlap, J.C., (2004). Molecular bases for circadian clocks. *Cell*, 96, 271-290.
- 32) Efentakis, M., Koligliati, S. and Vlachou, M., (2006). Design and evaluation of a dry coated drug delivery system with an impermeable cup, swellable top layer and pulsatile release. *International Journal of Pharmaceutics*, 311, 147-150.
- 33) Egalet[®] LTD., (2006). Egalet[®] delayed release. <http://www.egalet.com/index.dsp?area=30.html> [Accessed September 10, 2007].
- 34) Elliot, W.J., (2001). Timing treatment to the rhythm of disease. *Postgraduate Medicine*, 110, 119-129.
- 35) Eriguchi, M., Lévi, F., Hisa, T., Yanagie, H., Nonaka, Y. and Takeda, Y., (2003). Chronotherapy for cancer. *Biomedicine and Pharmacotherapy*, 57, 95-95.
- 36) Eurand. Diffucaps. Eurand. <http://www.eurand.com/Technologies/Controlling-Drug-Release/Diffucaps/>. [accessed September 10, 2007].
- 37) Farup, C., Kleinman, L. and Sloan, S., (2001). The impact of nocturnal symptoms associated with gastroesophageal reflux disease on health-related quality of life. *Archives of Internal Medicine*, 161, 45–52.
- 38) Fernandes, C.M., Ramos, P., Falcão, A.C., Veiga, F.J.B., (2003). Hydrophilic and hydrophobic cyclodextrins in a new sustained release oral formulation of nicardipine: *in vitro* evaluation and bioavailability studies in rabbits. *Journal of Controlled Release*, 88, 127-134.

- 39) Focan, C., Kreutz, F. and Focan-Henrard, D., (2000). Chronotherapy with 5-fluorouracil, folinic acid and carboplatin for metastatic colorectal cancer an interesting therapeutic index in a phase II trial. *European Journal of Cancer*, 36, 341-347.
- 40) Frohoff-Hülsmann, M.A., Schmitz, A. and Lippold, B.C., (1999). Aqueous ethyl cellulose dispersions containing plasticizers of different water solubility and hydroxypropyl methylcellulose as coating material for diffusion pellets: I. Drug release rates from coated pellets. *International Journal of Pharmaceutics*, 177, 69-82.
- 41) Gallerani, M., Manfredini, R. and Ricci, L., (1992). Sudden death from pulmonary thromboembolism: chronobiological aspects. *European Heart Journal*, 6, 305-313.
- 42) Gao, C., Huang, J., Jiao, Y., Shan, L., Liu, Y., Li, Y. and Mei, X., (2006). In vitro release and *in vivo* absorption in beagle dogs of meloxicam from Eudragit® FS 30 D-coated pellets. *International Journal of Pharmaceutics*, 28, 104-112.
- 43) Gazzaniga, A., Lamartino, P., Maffione, G. and Sangalli, M.E., (1994). Oral delayed-release system for colonic specific delivery. *International Journal of Pharmaceutics*, 108, 77-78.
- 44) Gazzaniga, A., Palugan, A., Foppoli, A. and Sangalli, M.E., (2008). Oral pulsatile delivery systems based on swellable hydrophilic polymers. *European Journal of Pharmaceutics and Biopharmaceutics*, 68, 11-18.
- 45) Gazzaniga, A., Sangalli, M.E., Giordano, F., Minghettin, P. and Montanari, L., (1995). A new oral system for time and/or site release specificity (Chronotropic® drug delivery system). *Pharmacological Research*, 31, 315.
- 46) Gery, S., Komatsu, N., Baldjyan, L., Yu, A., Koo, D. and Koeffler, H.P., (2006). The circadian gene *per1* plays an important role in cell growth and DNA damage control in human cancer cells. *Molecular Cell*, 22, 375–382.
- 47) Ghaffari, A., Abdollahi, H., Khoshayand, M.R., Bozchalooi, I.S., Dadgar, A. and Rafiee-Tehrani, M., (2006). Performance comparison of neural network training algorithms in modelling of bimodal drug delivery. *International Journal of*

- 48) Gherghel, D., Hosking, S.L. and Orgul, S., (2004). Autonomic nervous system, circadian rhythms, and primary open-angle glaucoma. *Survey of Ophthalmology*, 49, 491-508.
- 49) Ghimire, M., McInnes, F.J., Watson, D.G., Mullen, A.B. and Stevens. H.N.E., (2007). In-vitro/in-vivo correlation of pulsatile drug release from press-coated tablet formulations: a pharmacoscintigraphic study in the beagle dog. *European Journal of Pharmacy and Biopharmacy*, 2, 515-523.
- 50) Giannola, L. I., De Caro, V., Giandalia, G., Siragusa, M.G., Tripodo, C., Florena, A.M. and Campisi, G., (2007). Release of naltrexone on buccal mucosa: Permeation studies, histological aspects and matrix system design. *European Journal of Pharmaceutics and Biopharmaceutics*, 67, 425-433.
- 51) Goo, R.H., Moore, J.G. and Greenberg, E., (1987). Circadian variation in gastric emptying of meals in man. *Gastroenterology*, 93, 515-518.
- 52) Gothaskar, A.V., Joshi, A.M. and Joshi, N.H., (2004). Pulsatile drug delivery system-a review. *Drug Delivery Technology*, <http://www.drugdeliverytech.com/id/article=250> [accessed 3/08/2008]
- 53) Grossman, J., (1988). Multicenter comparison of once daily Uniphyll tablets administered in the morning or evening with baseline twice daily theophylline therapy in patients with nocturnal asthma. *American Journal of Medicine*, 85, (suppl 1B),11-13.
- 54) Gwen, S.S., (2002). Nocturnal asthma: Mechanisms and management. *The Mount Sinai Journal of Medicine*, 69, 140-147.
- 55) Haus, E., (2007). Chronobiology of hemostasis and inferences for the chronotherapy of coagulation disorders and thrombosis prevention. *Advanced Drug Delivery Review*. 59, 966–984.
- 56) Haddish-Berhane, N., Nyquist, C., Haghighi, K., Corvalan, C., Keshavarzian, A., Campanella, O., Rickus, J. and Farhadi, A., (2006). A multi-scale stochastic drug release model for polymer-coated targeted drug delivery systems. *Journal of Controlled Release*, 110, 314-322.

- 57) Hansen, J., (2006). Risk of breast cancer after night- and shift work: current evidence and ongoing studies in Denmark. *Cancer Causes Control*, 17, 531–537.
- 58) Hardy, I.J., Windberg-Baarup, A., Neri, C., Byway, P.V., Booth, S.W. and Fitzpatrick, S., (2007). Modulation of drug release kinetics from hydroxypropyl methyl cellulose matrix tablets using polyvinyl pyrrolidone. *International Journal of Pharmaceutics*, 337, 246-253.
- 59) Hassan, A. and Haefeli, W.E., (2010). Appropriateness of timing of drug administration in electronic prescriptions. *Pharmacy World and Science*, 32, 162–171.
- 60) Hermida, R.C. and Ayala, D.E., (2009). Chronotherapy with the angiotensin-converting enzyme inhibitor ramipril in essential hypertension improved blood pressure control with bedtime dosing. *Hypertension*, 54, 40-46.
- 61) Hermida, R.C., Ayala, D.E. and Portaluppi, F., (2007). Circadian variation of blood pressure: The basis for the chronotherapy of hypertension. *Advanced Drug Delivery Reviews*, 59, 904-922.
- 62) Hildebrand, H., McDonald, F.M. and Windt-Hanke, F., (1991). Characterization of oral sustained release preparations of iloprost in a pig model by plasma level monitoring. *Prostaglandins*, 41, 473-486.
- 63) Hillhouse, E.W. and Grammatopoulos D.K., (2006). The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. *Endocrinology Review*, 27, 260-86.
- 64) Hofstra W.A., de Weerd, A.W., (2008). How to assess circadian rhythm in humans: a review of literature. *Epilepsy and Behaviour*, 13, 438–444.
- 65) Höhne., G.W.H, (1999). Temperature modulated differential scanning calorimetry (TMDSC) in the region of phase transitions. Part 1: theoretical considerations. *Thermochimica Acta*, 330, 5-54.

- 66) Hsieh, C., Ohman, M.D., (2010). Biological responses to environmental forcing: the linear tracking window hypothesis. *Ecology*, 87, 8, 1932—1938.

- 67) Ibrić, S., Jovanović, M., Djurić, Z., Parojčić, J. and Solomun, L., (2002). The application of generalized regression neural networks in the modelling and optimization of aspirin extended release tablets with Eugdragit® RS PO as matrix substance. *Journal of Controlled Release*, 82, 213-222.

- 68) Innominato, P.F., Lévi, F.A. and Bjarnason, A., (2010). Chronotherapy and the molecular clock: Clinical implications in oncology. *Advanced Drug Delivery Reviews*, 62, 979-1001.

- 69) Ishibashi, T., Hatano, H., Kobayashi, M., Mizobe, M. and Yoshino, H., (1999). In vivo drug release behaviour in dogs from a new colon-targeted delivery system. *Journal of Controlled Release*, 57, 45-53.

- 70) Jacob, E., FitzSimon, J.S. and Brubaker, R.F., (1996). Combined corticosteroid and catecholamine stimulation of aqueous humor flow. *Ophthalmology*, 103, 1303-1308.

- 71) Jamzad, S. and Fassihi, R., (2006). Development of a controlled release low dose class II drug-Glipizide. *International Journal of Pharmaceutics*, 312, 24-32.

- 72) John, V.A., (1990). A structured approach to the development of a controlled-release drug delivery system for a β -adrenoceptor blocking drug. *Journal of Controlled Release*, 11, 307-314.

- 73) Jonkman, J.H.G., VanderBoom, W.J.V., Balant, L.P., Schoenmaker, J., Holtkamp, A., (1984). Chronopharmacokinetics of theophylline after sustained release and intravenous administration to adults. *European Journal of Clinical Pharmacy*, 26, 215-222.

- 74) Jose, S., Prema, M.T., Chacko, A.J., Cinu Thomas, A. and Souto, E.B., (2011). Colon specific chitosan microspheres for chronotherapy of chronic stable angina. *Colloids and Surfaces B: Biointerfaces*, 83, 277-283,

- 75) Kadam, V.D. and Gattani, S.G., (2010). Development of colon targeted multiparticulate pulsatile drug delivery system for treating nocturnal asthma. *Drug Delivery*, 17, 343-351.
- 76) Kalantzi, L.E., Karavas, E., Koutris, E.X. and Bikiaris, DN., (2009). Recent advances in oral pulsatile drug delivery. *Recent Patents on Drug Delivery and Formulation*, 3, 49-63.
- 77) Karavas, E., Georgarakis, E. and Bikiaris, D., (2006). Felodipine nanodispersions as active core for predictable pulsatile chronotherapeutics using PVP/HPMC blends as coating layer. *International Journal of Pharmaceutics*, 313, 189-197.
- 78) Kaya, M.G., Yarlioglues, M., Gunebakmaz, O., Gunturk, E., Inanc, T., Dogan, A., Kalay, N., and Topsakal, R., (2010). Platelet activation and inflammatory response in patients with non-dipper hypertension. *Atherosclerosis*, 209, 278-282.
- 79) Keith, L.G., Oleszczuk, J.J. and Laguens, M., (2001). Circadian rhythm chaos: a new breast cancer marker. *International Journal of Fertility and Womens Medicine*, 4, 238-247.
- 80) Kelly, M.L., Tobyn, M.J. and Staniforth, J.N., (1996). Influence of processing variables on physical properties of TIMERx. *European Journal of Pharmaceutical Science*, 4, 184.
- 81) Kendall, M.J., John, V.A., Quarterman, C.P. and Welling, P., (1980). A single and multiple dose pharmacokinetic and pharmacodynamic comparison of conventional and slow-release metoprolol. *European Journal of Clinical Pharmacology*, 17, 87-92.
- 82) Khairuzzaman, A., Ahmed, S.U., Savva, M. and Patel, N.K., (2006). Zero-order release of aspirin, theophylline and atenolol in water from novel methylcellulose glutarate matrix tablets. *International Journal of Pharmaceutics*, 318, 15-21.
- 83) Kostewicz, E., Sansom, L., Fishlock, R., Morella, A. and Kuchel, T., (1996). Examination of two sustained release nifedipine preparations in humans and in pigs. *European Journal of Pharmaceutical Sciences*, 4, 351-357.

- 84) Kraft, M. and Martin, R.J., (1995). Chronobiology and chronotherapy in medicine. *Disease-a-month*, 41, 506-575.
- 85) Kranz, H. and Gutsche, S., (2009). Evaluation of the drug release patterns and long term solubility of aqueous and organic coated pellets by using blends of enteric and gastrointestinal insoluble polymers. *International Journal of Pharmaceutics*. 380, 112-119.
- 86) Kubo, T., Ozasa, K., Mikami, K., Wakai, K., Fujino, Y., Watanabe, Y., Miki, T., Nakao, M., Hayashi, K., Suzuki, K., Mori, M., Washio, M., Sakauchi, F., Ito, Y., Yoshimura, T. and Tamakoshi, A., (2006). Prospective cohort study of the risk of prostate cancer among rotating-shift workers: findings from the Japan collaborative cohort study. *American Journal of Epidemiology*, 164, 549–555.
- 87) Kumar, K.P.S., Bhowmik, D., Chandira, C.M. and Tripathi, K. K., (2010). Innovations in sustained release drug delivery system and its market opportunities. *Journal of Chemical and Pharmaceutical Research*, 2, 349-360.
- 88) Kumar, P., Pillay, V., Choonara, Y.E., Modi, G., Naidoo, D. and du Toit, L.C., (2011). In silico theoretical molecular modelling for Alzheimer's disease: The nicotine-curcumin paradigm in neuroprotection and neurotherapy. *International Journal of Molecular Sciences*, 12, 694-724.
- 89) Lack, L.C., Wright, H.R. and Bootzin, R.R., (2009). Delayed sleep-phase disorder. *Sleep Medicine Clinics*, 4, 229-239.
- 90) Laity, P.R., Mantle, M.D., Gladden, L.F. and Cameron, R.E., (2010). Magnetic resonance imaging and X-ray microtomography studies of a gel-forming tablet formulation. 74, 109-119.
- 91) Landau, I., Chabaud, A., Cambie, G. and Ginsburg, H., (1991). Chronotherapy of malaria: an approach to malaria chronotherapy. *Parasitology Today*, 7, 350-355.
- 92) Latha, M. S., Lal, A.V., Kumary, T.V., Sreekumar, R. and Jayakrishnan, A., Progesterone release from glutaraldehyde cross-linked casein microspheres: In

vitro studies and in vivo response in rabbits. *Contraception*, 61, 329-334.

- 93) Lavy, E., Prise, U., Soldani, G., Neri, D., Brandriss, N., Chaim, A.B. and Giorgi, M., (2010). Pharmacokinetics of methylphenidate after oral administration of immediate and sustained-release preparations in Beagle dogs. *The Veterinary Journal*, Article in Press.
- 94) Lee, B.J., Ryu, S.G. and Cui, J.H., (1999). Controlled release of dual drug-loaded hydroxypropyl methylcellulose matrix tablet using drug-containing polymeric coatings. *International Journal of Pharmaceutics*, 188, 71-80.
- 95) Lemmer, B., (1990). Implications of chronopharmacokinetics for drug delivery, antiasthmatics, H₂-blockers and cardiovascular active drugs. *Advanced Drug Delivery Review*, 6, 83-100.
- 96) Lemmer, B., (1991). Circadian rhythms and drug delivery. *Journal of Controlled Release*, 16, 63-74.
- 97) Lemmer, B., (1996). The clinical relevance of chronopharmacology in therapeutics. *Pharmacological Research*, 33, 107-115.
- 98) Lemmer, B., (2006). The importance of circadian rhythms on drug response in hypertension and coronary heart disease-from mice to man. *Pharmacology and Therapeutics*, 111, 629-651.
- 99) Leslie, S., (1986). The Contain delivery system: Dosing considerations. *Journal of Allergy and Clinical Immunology*, 78, 768-773.
- 100) Lévi, F. (1997). In: Physiology and Pharmacology of Biological Rhythms. Redfern, P., Lemmer, B., editor. Berlin: Springer-Verlag; *Chronopharmacology of anticancer agents*, 299–33.
- 101) Levi, F., (2001). Circadian chronotherapy for human cancers. *Lancet Oncology*. 2, 307-315.
- 102) Lévi, F., Focan, C., Karaboué, A., de la Valette, V., Focan-Henrard, D., Baron, B., Kreutz, F. and Giacchetti, S., (2007). Implications of circadian clocks for the

- rhythmic delivery of cancer therapeutics. *Advanced Drug Delivery Reviews*, 59, 1015-1035.
- 103) Lewy, A.J., Cutler, N.L. and Sack, R.L., (1999). The endogenous melatonin profile as a marker for circadian phase position. *Journal of Biological Rhythms*. 14, 227–236.
 - 104) Li, G., Han, D., Guan, T., Zhao, X., He, H. and Tang, X., (2010). Isosorbide-5-mononitrite (5-ISMN) sustained-release pellets prepared by double layer coating for reducing 5-ISMN migration and sublimation. *International Journal of Pharmaceutics*, 40, 138-144.
 - 105) Li, B., Zhu, J., Zheng, C. and Gong, W., (2008). A novel system for three-pulse drug release based on “tablets in capsule” device. *International Journal of Pharmaceutics*, 352, 159-164.Li
 - 106) C.L., Martini, L.G., Ford, J.L. and Roberts, M., (2005). The use of hypromellose in oral drug delivery. *Journal of Pharmacy and Pharmacology*, 57, 533–546.
 - 107) Linkowski, P., Van Onderbergen, A., Kerkhofs, M., Bosson, D., Mendlewicz, J. and Van Cauter, E., (1993). Twin study of the 24-h cortisol profile: evidence for genetic control of the human circadian clock. *American Journal of Physiology*, 264, E173–E181.
 - 108) Löbenberg, R., Kim, J.S. and Amidon, G.L., (2005). Pharmacokinetics of an immediate release, a controlled release and a two pulse dosage form in dogs. *European Journal of Pharmaceutics and Biopharmaceutics*, 60, 17-23.
 - 109) Maestrelli, F., Cirri, M., Corti, G., Mennini, N. and Mura, P., (2008). Development of enteric-coated calcium pectinate microspheres intended for colonic delivery. *European Journal of Pharmacy and Biopharmaceutics*, 69, 508-518.
 - 110) Mäki, R., Suihko, E., Korhonen, O., Pitkänen, H., Niemi, R., Lehtonen, M. and Ketolainen, J., (2006). Controlled release of saccharides from matrix tablets, *European Journal of Pharmaceutics and Biopharmaceutics*, 62,163-170.
 - 111) Martin, R.J. and Banks-Schlegel, S., (1998). Chronobiology of asthma. *American*

- 112) Martin, R.J., Cicutto, L.C., Ballard, R.D., Goldenheim, P.D. and Cherniack, R.M., (1989). Circadian variations in theophylline concentrations and the treatment of nocturnal asthma. *American Review of Respiratory Disease*, 13, 475-478.
- 113) Mastiholimath, V.S., Dandagi, P.M., Jain, S.S., Gadad, A.P. and Kulkarni, A.R., (2007). Time and pH dependent colon specific, pulsatile delivery of theophylline for nocturnal asthma. *International Journal of Pharmaceutics*, 328, 49-56.
- 114) Matsuo, M., Arimori, K., Nakamura, C. and Nakano, M., (1996). Delayed-release tablets using hydroxyethylcellulose as a gel-forming matrix. *International Journal of Pharmaceutics*, 138, 225-235.
- 115) Matsuo, T., Yamaguchi, S., Mitsui, S., Emi, A., Shimoda, F. and Okamura, H., (2003). Control mechanism of the circadian clock for timing of cell division *in vivo*. *Science*, 302, 255–259.
- 116) Mikac, U., Sepe, A., Kristi, J. and Baumgartner, S., (2010). A new approach combining different MRI methods to provide detailed view on swelling dynamics of xanthan tablets influencing drug release at different pH and ionic strength. *Journal of Controlled Release*, 145, 247-256.
- 117) Miyazaki, S., Aoyama, H., Kawasaki, N., Kubo, W. and Attwood, D., (1999). In situ-gelling gellan formulations as vehicles for oral drug delivery. *Journal of Controlled Release*, 60, 287-295.
- 118) Moeller, H., (1985). Chronopharmacology of hydrocortisone and 9 alpha-fluorhydrocortisone in the treatment of congenital adrenal hyperplasia. *European Journal of Paediatrics*, 144, 370–373.
- 119) Mormont, M.C. and Lévi, F., (2003). Cancer chronotherapy: Principles, applications, and perspectives. *Cancer*, 97, 155–169.
- 120) Mu, X., Tobyn, M.J. and Staniforth, J.N., (1996). Investigation into the food effect on a polysaccharide dosage form. *European Journal of Pharmacy and Biopharmaceutics*, 4, 184.

- 121) Nagahara, N., Akiyama, Y., Higaki, K. and Kimura, T., (2007). Animal models for predicting potency of oral sustained-release adhesive microspheres in humans. *International Journal of Pharmaceutics*, 331, 46-53.
- 122) Ndesendo, V.M.K., Pillay, V., Choonara, Y.E., Khan, R.A., Meyer, L., Buchmann, E. and Rosin, U. In vitro and ex vivo bioadhesivity analysis of polymeric intravaginal caplets using physicommechanics and computational structural modelling. *International Journal of Pharmaceutics*, 370, 151-159.
- 123) Ohdo, S., (2010). Chronopharmaceutics: pharmaceutics focused on biological rhythm. *Biological and Pharmacy Bulletin*, 33, 159–167.
- 124) Patel, G.C., Patel, J.K. and Patel, M.M., (2007). Specialized chronotherapeutic drug delivery systems. *Pharmainfo.net*, <http://www.pharmainfo.net/reviews/specialized-chronotherapeutic-drug-delivery-systems>, [accessed 21/11/2008].
- 125) Pawar, V. K. and Awasthi R., (2010). Chronotherapy: an approach to synchronize drug delivery with circadian rhythm. *Journal of Chronotherapy and Drug Delivery*, 1, 1-8.
http://chronotherapyjournal.com/yahoo_site_admin/assets/docs/JChrDD_11_2010_002.363195613.pdf [accessed 1/02/2011].
- 126) Pearlman, D.A., Case, D.A., Caldwell, J.W., Ross, W.S., Cheatham, T.E., DeBolt, S., Ferguson, D., Seibel, G. and Kollman, P., (1995). AMBER, a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules. *Computer Physics and Communications*, 91, 1-41.
- 127) Penwest. SyncroDose technical description. Penwest.
http://www.penwest.com/Syncrodose_description.html [Accessed August 3, 2007].
- 128) Perez, S., Kouwijzer M., Mazeau, K. and Engelsent, S.B., (1996). Modelling polysaccharides: Present status and challenges. *Journal of Molecular Graphics*, 14, 307-321.

- 129) Physicians' Desk Reference. (2005). 59th ed. Thomson PDR. 2816.
- 130) Pillay, S., Pillay, V., Choonara, Y.E., Naidoo, D., Khan, R. A., du Toit, L.C., Ndesendo, V.MK., Modi, G., Danckwerts, M.P. and Iyuke, S.E., (2009). Design, biometric simulation and optimization of a nano-enabled scaffold device for enhanced delivery of dopamine to the brain. *International Journal of Pharmaceutics*, 382, 277-290.
- 131) Pillay, V. and Fassihi, R., (1999). Electrolyte-induced compositional heterogeneity: a novel approach for rate-controlled oral drug delivery. *Journal of Pharmaceutical Science*, 88, 1140-1148.
- 132) Pincus, D.J., Humeston, T.R. and, Martin, R.J., (1997). Further studies on the chronotherapy of asthma with inhaled steroids; the effect of dosage on drug efficacy. *Journal of Allergy and Clinical Immunology*, 100, 771-774.
- 133) Pincus, D.J., Szeffler, J., Ackerson, L.M. and Martin, R.J., (1995). Chronotherapy of asthma with inhaled steroids: The effect of dosage timing on drug efficacy. *Journal of Allergy and Clinical Immunology*, 95, 1172-1178.
- 134) Pollock, S.R. and Olanoff, L.S., (1990). Clinical Pharmacology approaches to the assessment of novel drug delivery concepts. *Journal of Controlled Release*, 11, 331-341.
- 135) Portaluppi, F. and Hermida, R.C., (2007). Circadian rhythms in cardiac arrhythmias and opportunities from their chronotherapy. *Advanced Drug Delivery Reviews*, 59, 940-951.
- 136) Portaluppi, F. and Smolensky, M.H., (2010). Perspectives on the chronotherapy of hypertension based on the results of the MAPEC study. *Chronobiology International*, 27, 1652–1667.
- 137) Prisant, L.M., (2001). Hypertension and chronotherapy: Shifting the paradigm. *American Journal of Hypertension*, 14, 277-279.

- 138) Prisant, L.M., Devane, J.G. and Butler, J., (2000). A steady-state evaluation of the bioavailability of chronotherapeutic oral drug absorption system verapamil PM after night time dosing versus immediate- acting verapamil dosed every eight hours. *American Journal of Therapeutics*, 7, 345-351.
- 139) Pushkala, K. and Gupta, P.D., (2009). Prevalence of breast cancer in menopausal blind women. *International Journal of Medicine and Medical Science*, 1, 425–431.
- 140) Quinten, T., Gonnissen, Y., Adriaens, E., De Beer, T., Cnudde, V., Masschaele, B., Van Hoorebeke, L., Siepmann, J., Remon, J.P. and Vervaet, C., (2009). Development of injection moulded matrix tablets based on mixtures of ethylcellulose and low-substituted hydroxypropylcellulose. *European Journal of Pharmaceutical Sciences*, 37, 207-216.
- 141) Reddy, A.B. and O'Neill, J.S., (2010). Healthy clocks, healthy body, healthy mind. *Trends in cell biology*, 20, 36-44.
- 142) Richardson, J.C., Bowtell, J.W., Mäder, K. and Melia, C.D., (2005). Pharmaceutical applications of magnetic resonance imaging (MRI). *Advanced Drug Delivery Reviews*, 57, 1191–1209.
- 143) Rivington, R.N., Calcutt, L., Hodder, R.V., Stewart, J.H. and Aitken, T.L., (1988). Safety and efficacy of once-daily uniphyll tablets compared with twice-daily theo-dur tablets in elderly patients with chronic airflow obstruction. *The American Journal of Medicine*, 85, 48-53.
- 144) Roose, S.P., (2002). Technology: Diffucaps. http://www.eurand.com/tech_custom_diffucaps.html [Accessed September 10, 2007].
- 145) Rosenwasser, A.M., (2010). Circadian clock genes: Non-circadian roles in sleep, addiction, and psychiatric disorders. *Neuroscience and Biobehavioral Reviews*, 34, 1249-1255.

- 146) Ross, A.C., MacRae, R.J., Walther, M. and Stevens, H.N., (2000). Chronopharmaceutical drug delivery from a pulsatile capsule device based on programmable erosion. *Journal of Pharmacy and Pharmacology*, 52, 903-909.
- 147) Roy, D.S. and Rohera, B.D., (2002). Comparative evaluation of rate of hydration and matrix erosion of HEC and HPC and study of drug release from their matrices. *European Journal of Pharmaceutical Sciences*. 16, 193-199.
- 148) Roy, P. and Shahiwala, A., (2009). Statistical optimization of ranitidine HCL floating pulsatile delivery system for chronotherapy of nocturnal acid breakthrough. *European Journal of Pharmaceutical Sciences*, 37, 125-132.
- 149) Rujivipat, S. and Bodmeier, R., (2010). Improved drug delivery to the lower intestinal tract with tablets compression-coated with enteric/nonenteric polymer powder blends. *European Journal of Pharmaceutics and Biopharmaceutics*, 76, 486-492.
- 150) Samuels, M.H., Lillehei, K., Kleinschmidt-Demasters, B.K., Stears, J. and Ridgway, E.C., (1990). Patterns of pulsatile pituitary glycoprotein secretion in central hypothyroidism and hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, 70, 391–395.
- 151) Sanders, S.W. and Moore, J.G., (1998). Gastrointestinal chronopharmacology: Physiology, pharmacology and therapeutic implications. *Pharmacology and Therapeutics*, 54, 1-15.
- 152) Sando, T., Renatus, M., Sabanjo, J. and Sapinhour, L., (2005). Advantages and disadvantages of different crash modelling techniques. *Journal of Safety Research*, 36, 485–487.
- 153) Sangalli, M.E., Maroni, A., Foppoli, A., Zema, L., Giordano, F. and Gazzaniga, A., (2004). Different HPMC viscosity grades as coating agents for an oral time and/or site-controlled delivery system: a study on process parameters and in vitro performances. *European Journal of Pharmaceutics and Biopharmaceutics*, 22, 469-476.

- 154) Sangalli, M.E., Maroni, A., Zema, L., Busetti, C., Giordano, F. and Gazzaniga, A., (2001). In vitro and in vivo evaluation of an oral system for time and/or site-specific drug delivery. *Journal of Controlled Release*, 73, 103-110.
- 155) Schernhammer, E.S., Laden, F., Speizer, F.E., Willett, W.C., Hunter, D.J., Kawachi, I., and Colditz, G.A., (2001). Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *Journal of National Cancer Institute*, 93, 1563–1568.
- 156) Sewlall, S., Pillay, V., Danckwerts, M.P., Choonara, Y.E., Ndesendo, V.M.K. and du Toit, L.C., (2010). A Timely Review of State-of-the-Art Chronopharmaceuticals Synchronized with Biological Rhythms. *Current Drug Delivery*, 7, 370-388.
- 157) Shearman, L.P., Sriram, S. and Weaver, D.R., (2000). Interacting molecular loops in the mammalian circadian clock. *Science*, 288, 1013-1019.
- 158) Sher, P., Ingavle, G., Ponrathnam, S. and Pawar, A.P., (2007). Low density porous carrier based conceptual drug delivery system. *Microporous and Mesoporous Materials*, 102, 290-298.
- 159) Shi, P., Zuo, Y., Shen, J., Zhang, L., Li, Y. and Morsi, Y.S., (2009). Improved properties of incorporated chitosan film with ethyl cellulose microspheres for controlled release. *International Journal of Pharmaceutics*, 375, 67-74.
- 160) Shiohira, H., Fufifi, M., Koizumi, N., Kondoh, M. and Watanabe, Y., (2009). Novel chronotherapeutic rectal aminophylline delivery system for therapy of asthma. *International Journal of Pharmaceutics*, 379, 119-124.
- 161) Shivakumar, H.N., Suresh, S. and Desai, B.G., (2006). Design and evaluation of pH sensitive multi-particulate systems for chronotherapeutic delivery of Diltiazem hydrochloride. *Indian Journal of Pharmaceutical Science*, 68, 781-787.
- 162) Shweta, A., Ali, J., Alka, A., Sanjula, B. and Qureshi, J., (2006). Pulsatile drug delivery systems, an approach for controlled drug delivery. *Indian Journal of Pharmaceutical Science*, 68, 295-300.

- 163) Smith, D.H.G., (2001). Pharmacology of cardiovascular chronotherapeutic agents. *American Journal of Hypertension*, 14, 296-301.
- 164) Smolensky, M.H. and Portaluppi, F., (1999). Chronopharmacology and chronotherapy of cardiovascular medications: Relevance to prevention and treatment of coronary heart disease. *American Heart Journal*, 137, 14-24.
- 165) Smolensky, M.H. and Peppas, N.A., (2007). Chronobiology, drug-delivery, and chronotherapeutics. *Advanced Drug Delivery Reviews*, 59, 823-824.
- 166) Smolensky, M.H., (1995). Medical chronobiology & chronotherapeutics: Concepts and applications. *American Journal of Hypertension*, 8, 7A.
- 167) Smolensky, M.H., Lemmer, B. and Reinberg, A.E., (2007). Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. *Advanced Drug Delivery Reviews*, 59, 852–882.
- 168) Smolensky, M.H., Reinberg, A.E., Martin R.J. and Haus, E., (1999). Clinical chronobiology and chronotherapeutics with applications to asthma. *Chronobiology International*, 16, 539–563.
- 169) Stalder, T., Evans. P., Hucklebridge, F. and Clow, A., (2011). Associations between the cortisol awakening response and heart rate variability. *Psychoneuroendocrinology*, 36, 454-462.
- 170) Stevens, R.G., (2006). Artificial lighting in the industrialized world: Circadian disruption and breast cancer. *Cancer Causes Control*, 17, 501–507.
- 171) Sun, Y., Peng, Y., Chen, Y. and Shukla, A.J., (2003). Application of artificial neural networks in the design of controlled release drug delivery systems. *Advanced Drug Delivery Reviews*, 55, 1201-1215.
- 172) Sutherland, E.R., (2005). Nocturnal asthma. *Journal of Allergy and Clinical Immunology*, 116, 1179-1186.
- 173) Swanson, D.R., Barclay, B.L., Wong, P.S.L. and Theeuwes, F., (1987). Nifedipine gastrointestinal therapeutic system. *American Journal of Medicine*, 83, 3-9.

- 174) Takahashi, M., Ushijima, K., Hayashi, Y., Maekawa, T., Ando, H., Tsuruoka, S.I. and Fujimura, A., (2010). Dosing-time dependent effect of dexamethasone on bone density in rats. *Life Sciences*, 86, 24-29.
- 175) Takeda, N. and Marmora, K., (2010). Cardiovascular disease, chronopharmacotherapy, and the molecular clock. *Advanced Drug Delivery Reviews*, 62, 956-966.
- 176) Tarvainen, T., Nevalainen, T., Sundell, A., Svarfvar, B., Hyrsylä, J., Paronen, P. and Järvinen, P., (2000). Drug release from poly (acrylic acid) grafted poly (vinylidene fluoride) membrane bags in the gastrointestinal tract in the rat and dog. *Journal of Controlled Release*, 66, 19-26.
- 177) Terebesi, I. and Bodmeier, R., (2010). Optimised process and formulation conditions for extended release dry polymer powder-coated pellets. *European Journal of Pharmacy and Biopharmacy*, 75, 63-70.
- 178) Tiller, A.R. and Gorella, B., (1994). Estimation of polymer compatibility from molecular mechanics calculations. *Polymer*, 35, 3251-3259.
- 179) Tobyn, M.J., Staniforth, J.N., Baichwal, A.R. and McCall, T.W., (1995). Prediction of physical properties of a novel polysaccharide controlled release system. *International Journal of Pharmaceutics*, 128, 113-122.
- 180) Turner-Warwick, M., (1998). Epidemiology of nocturnal asthma. *American Journal of Medicine*, 85, 6-8.
- 181) Ugwoke, M.I., Exaud, S., Mooter, G.V.D., Verbeke, N. and Kinget, R., (1999). Nasal mucoadhesive delivery systems of the anti-parkinsonian drug, apomorphine: influence of drug-loading on in vitro and in vivo release in rabbits. *International Journal of Pharmaceutics*, 181, 125-138.
- 182) Vaithiyalingam, S. and Khan, M.A. (2002). Optimization and characterization of controlled release multi-particulate beads formulated with customized cellulose acetate butyrate dispersion. *International Journal of Pharmaceutics*, 234, 179-193.

- 183) Van Herwaarden, M.A., Samson, M. and Smout, A.J., (1999). 24-h recording of intragastric pH: technical aspects and clinical relevance. *Scandinavian Journal of Gastroenterology. Supplement*, 230, 9–16.
- 184) Varma, M.V.S., Kaushal, A.M., Garg, A. and Garg, S., (2004). Factors affecting mechanism and kinetics of drug release from matrix based oral controlled drug delivery systems. *American Journal of Drug Delivery*, 2, 43–57.
- 185) Verma, K.V. and Garg, S. (2001). Current status of drug delivery technologies and future directions. *Pharmaceutical Technology On-Line*, 25(2), 1-14.
- 186) Viswanathan, A.N., Hankinson, S.E. and Schernhammer, E.S., (2007). Night shift work and the risk of endometrial cancer. *Cancer Research*, 67, 10618–10622.
- 187) Warhurst, D.C., Craig, J.C., Adagu, I.S., Meyer, D.J. and Lee, S.Y., (2003). The relationship of physico-chemical properties and structure to the differential antiparasitic activity of the cinchona alkaloids. *Malaria Journal*, 2, 26.
- 188) Washington, N. and Wilson, C.G., (2006). Can oral controlled drug delivery meet the challenges posed by chronotherapeutics? *Drug Delivery Technology*, 6, 1-5.
- 189) Wax, M.B., Camras, C.B., Fiscella, R.G., Girkin, C., Singh, K. and Weinreb, R.N., (2002). Emerging perspectives in glaucoma: optimizing 24-hour control of intraocular pressure. *American Journal of Ophthalmology*, 6, 1-10.
- 190) Welsh, P.W., Reed, C.E. and Conrad, E., (1986). Timing of one-a-day theophylline dose to match peak blood level with diurnal variation in severity of asthma. *American Journal of Medicine*, 80, 1098-102.
- 191) Wong, P.S.L., Theeuwes, F., Larsen, S.D. and Dong, L.C., (1995). Device for administering active agent to biological environment. *US patent*, 5, 312,388.
- 192) Wu, N., Wang, L.S., Tan, D.C.W., Mochhala, S.M. and Yang, Y.Y., (2005). Mathematical modelling and in vitro study of controlled drug release via a highly swellable and dissoluble polymer matrix: polyethylene oxide with high molecular weights. *Journal of Controlled Release*, 102, 569-581.

- 193) Youan, B.B.C., (2004). Chronopharmaceutics: gimmick or clinically relevant approach to drug delivery. *Journal of Controlled Release*, 98, 337-353.
- 194) Zhao, X., Li, G., Zhang, L., Tao, X., Guan, T., Hong, M. and Tang, X., (2010). Preparation and evaluation of nicotinic acid sustained-release pellets combined with immediate release simvastatin. *International Journal of Pharmaceutics*, 400, 42-48.

REVIEW ARTICLE

Drug delivery technologies for chronotherapeutic applications

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Abstract

It has been proven that the body follows a 24-hour cycle called a circadian rhythm. This cycle is coordinated by the suprachiasmatic nucleus and controls nearly all bodily functions including those related to drug delivery. Knowledge of the body's circadian rhythm leads to an improved understanding of diseases and their treatment, known as chronotherapy, such that synchronizing drug application in accordance with the natural rhythm of the body leads to improved disease management and a greater patient therapeutic outcome. Chronotherapeutic diseases include asthma, cardiovascular diseases, glaucoma, rheumatoid arthritis and cancers. In order to treat these diseases numerous chronotherapeutic drug delivery systems have been developed, such that drug is released in the period when it is most needed. This review paper attempts to concisely explicate the role of circadian rhythms in various disease states and furthermore describes the various oral drug delivery technologies that have been employed for the treatment of chronotherapeutic diseases.

Keywords: Chronotherapy; circadian rhythm; chronopharmaceutical; pulsatile drug delivery; polymers; rate-modulated drug delivery

Introduction

During the 1700s Jean-Jacques d'Ortous de Mairan was the first researcher to take note of the 24-hour patterns occurring in the movement of a plant. Since then, research has been conducted to prove the presence of the 24-hour cycle. The 24-hour cycle that occurs in physiological processes of all human beings is termed the circadian rhythm. Related biological rhythms include the ultradian (length of hours, minutes or seconds), infradian (length of days or months), circatrigintan (the female menstrual cycle) and circannual rhythms (seasonal variation).^[1,2] The term circadian rhythm was first coined by Halberg and Stephens in 1959 and literally means "about-a-day".^[1,3] Thereafter, it was found that the human biological clock is coordinated by the suprachiasmatic nucleus located at the base of the hypothalamus.^[4] The suprachiasmatic nucleus is a paired nucleus situated above the optic nerve. Light that passes through the retina conducts impulses to the retino-hypothalamic tract that in turn

regulates nine genes that create 24-hour variations in cellular physiological processes.^[1,5,6] The suprachiasmatic nucleus inherently possesses a pacemaker which produces endogenous electrical activity thus allowing variations in the suprachiasmatic nucleus to occur in the absence of external stimuli.^[1] The biological rhythms generated play a role in controlling biological functions that include the autonomic nervous system, endocrine system and immune system.^[3] The circadian clock essentially comprises three components: An input pathway (photoreceptors and projections of retinal ganglion cells), circadian pacemakers that generate the circadian signal and an output pathway that couples the pacemakers to effector systems manifesting into circadian physiology and behavior.^[7,8] The circadian clock behaves like a multifunctional timer in order to regulate homeostatic systems within the human body.^[8] Circadian rhythms govern nearly all functions in the body, including sleep and activity, hormone levels, appetite and those functions responsible for pharmacokinetic parameters.^[8–10]

Evaluation of the drug release dynamics from a polyconcentric disk-loaded matrix

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Purpose

It has been established that certain diseases are known to follow a circadian rhythm. Most conventional therapies include once-daily preparations that provide sustained zero-order release. However, they are not designed to complement the body's circadian rhythm. This gives rise to the concept of chronotherapy which involves synchronizing drug application in a manner that matches circadian rhythm to achieve optimal therapeutic success. The aim of this study was to evaluate the drug release behavior from a polyconcentric designed disk-loaded matrix for use in treating chronotherapeutic diseases.

Methods

Preparation of the device: Drug-loaded disks were prepared using model drug theophylline. Polymers that were used included various permutations of cellulose-based and polyester-type polymers. Disks were incorporated into tablet matrices by direct compression in alternating layers of either cellulose or polyester-type polymers. Drug release characteristics were studied using a rotating paddle method dissolution apparatus (USP 25 Apparatus 2) with a stirring speed of 50 rpm at $37 \pm 0.5^\circ$ in 900 mL phosphate buffer (pH 6.8) over 24 hours.

Results

Drug release ranged from 9-15% during the 24 hour period when swellable cellulose-based polymers were employed. The reduced release rate occurred as a result of the large diffusion interface created by the hydrated cellulose polymeric layer. However, this did contribute to a lag time which varied between 7-10 hours. The length of the lag time was strongly dependent on the polymer used in the outer layer, for example hydroxypropyl methylcellulose (HPMC) in an outer layer demonstrated a longer lag time as compared to polyethylene oxide (PEO). Further research is currently underway in order to increase the drug release rate over a 24 hour period in an attempt to attain a suitable once-daily drug delivery system for chronotherapeutic disorders.

Evaluation of the drug release dynamics from a layered disc tablet

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Purpose

Although zero order drug release remains a favorable form of drug release, increasing interest has been placed on timed pulsatile drug release, where drug is released at predetermined intervals after a lag phase. This form of drug release is advantages for chronotherapeutic disorders as well as to treat diseases of the colon. The aim of this work is to evaluate drug release from discoid matrices using both a water soluble and insoluble model drug.

Methods

Preparation of the device: Drug-loaded disks were prepared using model drug theophylline and diphenhydramine. Various polymers were employed and included HPMC, EC and pectin. Drug disks were incorporated into tablet matrices by direct compression between alternating layers of the various polymers.

Drug Release Studies: Drug release characteristics were studied using a rotating paddle method dissolution apparatus (USP 25 Apparatus 2) with a stirring speed of 50 rpm at $37 \pm 0.5^\circ$ in 900 mL phosphate buffer (pH 6.8) over 24 hours.

Textural Analysis: Textural analysis was used to determine the swelling characteristics of the hydrated formulations. Tablets were exposed to the same conditions above and at predetermined time interval (2, 6, 8, and 10 hours) tablets were removed and lightly patted with tissue paper to remove excess water. Textural profiling was performed TA.X2plus texture analyzer (Stable Micro Systems, Surrey, England) fitted with a flat-tipped steel probe (2mm diameter).

Erosion Studies: Tablets were exposed to conditions above and at predetermine time intervals (2, 6, 8 and 10 hours) tablets were removed and placed in an oven until a constant weight was achieved. The samples were then gravimetrically analyzed.

Results

Drug release from the theophylline matrices displayed an initial lag phase of three hours during which no drug release occurred. Thereafter the first phase of drug release occurred up to six hours, after which a second lag phase was seen. A second phase of drug release subsequently resumed after 12 hours with 73% drug release occurring after 24 hours. The lag phase seen between the first and second phase of drug release lasted for approximately six hours. Drug release from diphenhydramine matrices differed somewhat, with no lag phase seen initially. Instead, a burst effect is seen with approximately 28% of drug release occurring within the first hour. This is followed by a period between one and five hours where no drug release occurs after which complete drug release is achieved in 12 hours. The burst effect seen with the diphenhydramine formulation is attributable to its high water solubility ($\geq 100\text{mg/ml}$) as compared to theophylline formulation (solubility $1\text{-}5\text{mg/ml}$) where a lag phase is seen initially with drug being released at a slower rate over 24 hours. Textural analysis results showed DPH formulations reaching a diameter of 8.105mm at T_{10} whereas theophylline formulations only reached a diameter of 7.844mm . In addition, after the first two hours the DPH formulation had a greater diameter than the theophylline formulation. Erosion studies show that the rate of erosion in the Theophylline formulations (46.75%) is higher than in DPH formulations (42.65%) after T_{10} . This is comparable to the swelling studies where theophylline formulations have a smaller diameter, suggesting that the theophylline formulations erode to a greater degree than DPH formulations.

Conclusion

Both diphenhydramine and theophylline formulations show pulsatile release. Diphenhydramine formulations, however, do not display an initial lag phase.

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 were 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 ($42 \pm 0.15\%$) while HPMC matrices displayed the lowest ($21 \pm 0.12\%$). 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.

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

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Purpose:

To evaluate the drug release kinetics, erosion and swelling dynamics from a directly compressed layered disk 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 and ethylcellulose and HPMC were employed for the drug discoid matrices. Drug release studies were performed using USP dissolution apparatus II comprising 900mls PBS (pH 6.8; $\pm 37^{\circ}\text{C}$) at 50rpm for 12 hours. Swelling and erosion studies were conducted under similar conditions.

Results:

Drug release displayed biphasic drug release with an initial burst phase followed by controlled release. 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 displayed the highest erosion (42% $\text{SD} \pm 0.15$). Swelling studies showed a range of between 55-155% at t_1 hour. Matrices with pectin displayed an increased in swelling up to 6 hours, after which swelling decreased and then increased. This is comparable to the erosion of the pectin top layer.

Conclusion:

Formulations displayed biphasic release with a drug-free period between the two phases of drug release thus providing pulsatile release.

A Novel Multi-Layered Discoidal Compressed System for Use in Chronotherapeutic Disorders.

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Purpose:

The purpose of this research is to develop a multi-layered multi-disc tablet for use in a chronotherapeutic disorder such as hypertension, asthma, cardiac disease and stomach ulcers. This device should produce biphasic drug release achieved through the use of two drug loaded disks, with the crown disk providing the first phase of drug release and the base disk providing the second phase of drug release.

Methods:

Preparation of the device: To produce a base disk, various ratios of the polymers HEC and EC were granulated with model drug theophylline using an aqueous dispersion of EC as granulating fluid. The granules were then compressed using a Beckman Hydraulic Press into drug disks. The crown disk comprised pure theophylline, with lactose used as a bulking agent. The disks were suspended between layers of HPMC and Pectin using a flat faced punch and die set.

Drug release studies: Drug release studies were performed using USP dissolution apparatus II comprising 900mls PBS (pH 6.8; $\pm 37^{\circ}\text{C}$). Matrices were subjected to dissolution for 24 hours at 50rpm.

FTIR: FTIR analyses was undertaken to assess any possible structural variations in the polymeric backbone, as a result of any interactions of the drugs, excipients, or polymers themselves, in the formulation. Analyses was carried out on the native polymers, the granulation blend and the compressed formulations.

DSC: Differential scanning calorimetry was undertaken to analyse changes, such as glass transition temperature of the polymer, melting point and any interaction between the polymers, excipients or drug during formulation. Samples were run from 0-300°C at a heating rate of 10°C per minute. Analyses were carried out on the native polymers, the granulation blend and the compressed formulations.

Results:

Drug release for all formulations displayed biphasic release with an initial lag phase of approximately 3 hours, followed by a burst of drug release between $t=3$ and $t=5$ hours. Thereafter drug release sustained release is observed for the remainder of the 24 hours with drug release ranging from 70-90%. Formulations with a 2:1 ratio of EC to HEC however, display a drug free period of approximately 5 hours after the initial burst. As a result, this formulation is able to provide two phases of drug release and may have applications in chronotherapeutic drug delivery. FTIR analyses show no significant changes in both the polymeric and drug structure as a result of granulation and compression. This is further motivated by DSC analyses which show no change in the T_g and melting points of the various polymer and drug.

**The Influence of Polymer Type on
the Drug Release Kinetics from a Novel Disk Layered Tablet System**

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Purpose:

To investigate the effect of different polymer blends on drug release from a directly compressed layered disk system and to determine if any interactions are present between the polymers and the drug.

Methods:

The polymers HEC, HPMC and EC were granulated with model drug theophylline using an aqueous dispersion of EC as granulating fluid. The granules were then compressed using a Beckman Hydraulic Press into drug disks. The disks were suspended between alternating layers of HPMC using a flat faced punch and die set. Drug release studies were performed using USP dissolution apparatus II comprising 900mls PBS (pH 6.8; $\pm 37^{\circ}\text{C}$). Matrices were subjected to dissolution for 24 hours at 50rpm. Fourier transform infrared (FTIR) spectroscopy was utilized to determine any structural variations in the polymer backbone as a result of any interactions between the drug and polymers. A textural analysis was used to determine the Indentation hardness and thus the Brinell Hardness Number (BHN).

Results:

Drug release in all formulations displayed biphasic drug release with an initial lag phase of between 6-8 hours. Drug release from formulations comprising HEC displayed the highest drug release (71% SD ± 0.11) followed by HPMC containing formulations (62% SD ± 0.13) and lastly EC formulations with incomplete drug release (34% SD ± 0.12). This is due to the hydrophobic nature of EC. FTIR analyses displayed no change in the structure of both the polymer and the drug suggesting that no interactions are present. FTIR analyses also showed no structural variation between compressed and uncompressed forms of the system. Textural analyses data performed on drug disks displayed BHN values ranging from 1.8-2.2 N/mm² with EC formulations displaying the highest value.

Conclusion:

Formulations displayed biphasic release with a lag phase thus demonstrating desirable release kinetics for the treatment of chronotherapeutic formulations.

A novel layered tablet matrix for application in chronotherapeutic drug delivery

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Keywords: chronotherapy, pulsatile drug release, drug discs

Introduction:

Research suggests that chronotherapeutic disorders such as asthma and hypertension would benefit from timed-pulsatile drug release. Therefore, drug should be released at times when it is most needed to minimize adverse effects.

Purpose:

To develop a novel multi-layered disc tablet with a crown disc and a base disc that produces two-phase release.

Methods:

The base disc was produced by granulating theophylline with HEC and EC using an EC aqueous dispersion as a granulating fluid. The crown disc comprised theophylline with lactose as a bulking agent. These discs were suspended between layers of Pectin and HPMC. Drug release studies were performed using a USP apparatus II (pH 6.8; $\pm 37^{\circ}\text{C}$) at 50rpm. FTIR and DSC analyses were performed to assess any structural changes as a result of interactions between the polymer and drug.

Results:

Drug release studies show two phases of drug release, with an initial burst occurring between $T=2_{\text{hours}}$ and $T=4_{\text{hours}}$, a drug free period of approximately 5 hours and thereafter controlled release over the remaining 24 hours. FTIR analyses and DSC analyses showed no changes in the structure, T_g and melting points of the drug and polymers.

Conclusion:

A novel layered chronotherapeutic system has been developed.

Funding has been provided by BioPad.

A Discoidal Multi-Layered Compressed Matrix for Use in Chronotherapeutic Disorders

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***University of the Witwatersrand, Department of Pharmacy and Pharmacology, 7
York Road, Parktown 2193, Johannesburg
Correspondence: viness.pillay@wits.ac.za***

Introduction:

Chronopharmaceutics is a branch of pharmaceutics that is devoted to the design and evaluation of drug delivery systems that release a bioactive agent at a rhythm that ideally matches the biological requirements of a given disease therapy (Youan, 2004). By using the concept of chronotherapy, it would be ideal to time drug application within the 24 hours of a day such that drug is released when it is required. Furthermore, by timing drug application, the pharmacokinetics and/or drugs side effects may be modified (Lemmer, 1996; Mastiholimath, 2007).

The present study aims to develop a multi-layered multi-disc tablet (MLMDT) with a two-phase release and lag phase for use in chronotherapeutic disorders. Conventional controlled release preparations release the same amount of drug throughout the day. Accordingly, drug is released even at times when it may not necessarily be required, exposing the patient to unnecessary quantities of drug. This device should produce a two-phase release which is achieved through the use of two drug loaded disks, with the crown disk providing the first phase of drug release and the base disk providing the second phase of drug release.

Methods:

Preparation of the device: To produce a base disk, various ratios of the polymers HEC and EC were granulated with model drug theophylline using an aqueous dispersion of EC as granulating fluid. The granules were then compressed using a Beckman Hydraulic Press into drug disks. The crown disk comprised pure theophylline, with lactose used as a bulking agent. The disks were suspended between layers of HPMC and Pectin using a flat faced punch and die set.

Drug release studies: Drug release studies were performed using USP dissolution apparatus II comprising 900mls PBS (pH 6.8; $\pm 37^{\circ}\text{C}$). Matrices were subjected to dissolution for 24 hours at 50rpm. Samples were removed at regular intervals and tested using a UV spectrophotometer at a wavelength of 280nm.

FTIR: FTIR analyses was undertaken to assess any possible structural variations in the polymeric backbone, as a result of any interactions of the drugs, excipients, or polymers themselves, in the formulation. Analyses was carried out on the native polymers, the granulation blend and the compressed formulations.

DSC: Differential scanning calorimetry was undertaken to analyse changes, such as glass transition temperature of the polymer, melting point and any interaction between the polymers, excipients or drug during formulation. Samples were tested from 0-300°C at a heating rate of 10°C per minute. Analyses were carried out on the native polymers, the granulation blend and the compressed formulations.

In vivo studies: *In vivo* animal studies were carried out using healthy female pigs weighing between 35 and 45 kg. The pigs were dosed with a conventional theophylline tablet (Theoplus[®]), a placebo and the novel system via an intra-gastric tube. Blood samples were collected over a period of 24 hours and analysed via UPLC.

Results:

In vitro drug release studies show that formulations with a 2:1 ratio of EC to HEC show optimal drug release. Drug is released in a phasic manner after a lag phase of approximately 2 hours. The first phase of drug release occurs between $t=2$ and $t=4$ hours followed by a drug free period of approximately 5 hours during which no drug release is observed. Thereafter drug release resumes and is maintained over the remaining 24 hours. *In vivo* animal studies support this data with placebo studies showing no adverse effects on the pigs. In addition, when compared to the conventional theophylline formulation (Theoplus[®]), the novel MLMDT is able to produce phasic drug release proving that the MLMDT is superior to the conventional formulation in terms of producing a two phase release. FTIR analyses show no significant changes in both the polymeric and drug structure as a result of granulation and compression. This is further motivated by DSC analyses which show no change in the glass transition temperature and melting points of the various polymer and drug.

Conclusions:

A novel multi-layered multi-disc tablet capable of producing two-phase release to be used in the treatment of chronotherapeutic disorders has been developed.

APPENDIX D1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 December 2009 (23.12.2009)



(10) International Publication Number
WO 2009/153635 A1

(51) International Patent Classification:
A61K 9/28 (2006.01)

(21) International Application Number:
PCT/IB2009/005832

(22) International Filing Date:
3 June 2009 (03.06.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2007/1099 19 June 2008 (19.06.2008) ZA

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(74) Agent: **BOWMAN GILFILLAN INC. (JOHN & KERNICK)**; 165 West Street, Sandton, Johannesburg (ZA).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: A CHRONOTHERAPEUTIC PHARMACEUTICAL DOSAGE FORM

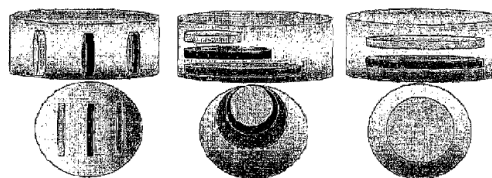


Figure 1

(57) Abstract: This invention relates to a pharmaceutical dosage form for the phase-controlled and chronotherapeutic delivery of at least one and, preferably, several pharmaceutically active ingredients. The dosage form has a carrier platform which, preferably, is a polymer having known biodegradable characteristics. The platform may include a pharmaceutically active ingredient which is released over a predetermined period of time as the platform polymer degrades. At least one pharmaceutically active ingredient in the form of a disc is embedded in the platform and, once the polymer of the platform has degraded, the disc is released and releases its ingredient in the same location as that of the platform or it travels to another region of the body where it releases its ingredient.

APPENDIX E1

AESC 3

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2007/56/04

APPLICANT: Professor V Pillay
SCHOOL: Pharmacy and Pharmacology
DEPARTMENT:
LOCATION: Medical School

PROJECT TITLE: An in vivo assessment of novel biocompatible polymeric drug delivery system in pigs

Number and Species

30 male/female pigs

Conditions:

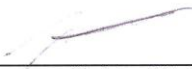
- i. The applicant must negotiate with CAS on the logistics and times
- ii. Should blood sampling be done by the applicant or co-workers, they must prove that they are capable of performing this.

Approval was given for the use of animals for the project described above at an AESC meeting held on 20070626. This approval remains valid until 20090626

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:

Signed:  Date: 12/07/07
(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: 18/07/07
(Registered Veterinarian)

cc: Supervisor: #
Director: CAS

Works 2000/1ain0015/AESCcert.wps

APPENDIX E2



CENTRAL ANIMAL SERVICE

Tel: 011 717-1312

Fax: 011 643-4318

DEAR ANIMAL USERS

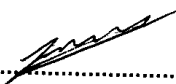
It is with great satisfaction that we can relay to users, the positive outcome of their respective in vivo research.

Central Animal Service acquired a batch of 30 pigs for in vivo research with the Department of Pharmacy and Pharmacology, for disease screening.

An ongoing animal surveillance program pre and post studies continued on a daily basis to assess the health and well being of the animals. We are confident to say that no unforeseen or fatal circumstance due to researchers or the research material itself had occurred. All pigs maintained an acceptable health status during and post studies.

Kind regards

Dr Leith Meyer (Director of the Central Animal Services) and the CAS team
06th September 2010.


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