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DESIGN OF A TRIPLE-LAYER DOUBLE-DISK TABLET CONFIGURATION FOR PHASE-CONTROLLED DRUG DELIVERY

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science in Medicine (Pharmaceutical Affairs).

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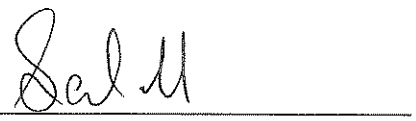
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2009

DECLARATION

I, Seshni Sewlall declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine (Pharmaceutical Affairs) in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



Signed on this 14th day of September 2009

RESEARCH OUTPUTS, PATENT AND ACCOLADES

Poster presentations

1. 'Design and development of a multi-layered polymeric device for use in chronotherapeutics' Seshni Sewlall, Viness Pillay and Yahya Choonara, presented at the University of the Witwatersrand Faculty of Health Sciences Research Day, Johannesburg, South Africa, August 2006. ***Awarded first place in best poster category by the School of Therapeutic Sciences at the Faculty of Health Sciences Research Day.***

2. 'Design of a heterogeneously-layered polymeric device for phase-controlled drug delivery' Seshni Sewlall, Viness Pillay and Yahya Choonara, presented at the Annual Meeting of the American Association of Pharmaceutical Scientists (AAPS), Texas, USA, November 2006.

Patent application

A multi-layered device for drug delivery, Seshni Sewlall , Viness Pillay , Yahya E. Choonara and Zaheeda Khan, SA Provisional Patent Application 2007/10998 filed on December 19, 2007.

Accolades

1. Adcock Ingram's *AdComment* newsletter highlighted Seshni Sewlall's research: 'Research into New Drug Delivery Systems: Industry Help Spearhead Drug Delivery Research in Pharmacy and Pharmacology', 2006 (Issue 2).

2. *Student Choice C magazine, Achiever's issue* highlighted Seshni Sewlall in an interview as: *'Achiever of the Year'*, Vol. 7, Oct/Nov 2006.

SUMMARY

The existence of circadian rhythms in nature and their influence on biological systems in man have given rise to the concept of chronotherapy; the science of delivering drugs in synchrony with the rhythm-dependent circadian variation inherent in the human body. Circadian rhythms vary amongst organ systems which influences the time at which the most severe symptoms of chronic medical conditions are experienced. Some of these chronic diseases that have been studied are asthma, rheumatoid arthritis, cardiovascular disease and cancer.

Asthma, for instance, is characterized by the worsening of symptoms at night. The mechanisms of nocturnal asthma are further elaborated on in this study. Sustained release theophylline has been the treatment of choice for asthma and nocturnal asthma due to its anti-inflammatory properties and its ability to decrease the late-phase response to allergens seen in asthmatic patients.

Current oral chronopharmaceutical drug delivery technologies are usually multiple-unit non-monolithic systems that often comprise several process steps and involve a large degree of complexity in their development, involving the application of multiple-unit technology. The present study set out to develop a novel oral triple-layer double-disk tablet configuration with potential use in chronotherapy and with fewer process variables. This was achieved with the use of compression and polymer technology.

Using theophylline as a model drug, a triple-layer double-disk polymeric tablet configuration was developed. The polymers employed in this study were HPMC, PEO, HEC and PLGA. An Extreme Vertices Design was used in a statistical approach to develop possible formulations for the configuration. *In vitro* drug release studies were performed on the experimental formulations to assess the drug release kinetics. This data was then used to obtain optimal desirability parameters for an optimized formulation. The drug release profiles from *in vitro* dissolution studies on the experimental and optimized formulations resulted in triphasic release kinetics. All profiles were characterized by an initial lag phase of 2-3 hours, followed by an upcurving steady drug release phase, which was then followed by an accelerated drug release phase. Further assessment of the physicochemical, swelling/gelation and fluid uptake/erosion properties of the tablet configuration correlated with the *in vitro* dissolution results. Drug release was characterized by swelling-controlled and diffusion-controlled kinetics.

A triple-layer double-disk tablet configuration was successfully prepared using a combination of hydrophobic and hydrophilic polymers and simple, cost-effective direct compression methods. The resulting configuration shows potential application in the field of chronotherapy.

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To my family, mum Saroj who is not with us anymore, dad Harry and brother Nivesh, thank you for your support and sacrifices to afford me the educational opportunities I have been privileged to have. Also to extended family and friends, thank you for the kind words of encouragement. To my mum especially, for teaching me that nothing is impossible and that true strength lies within.

DEDICATION

To my mum, my inspiration.

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SECTION 1

LITERATURE REVIEW AND MOTIVATION FOR STUDY

1.1. Introduction

Biological rhythms exist in all living organisms as genetic responses to cyclical changes in time in the external environment. These rhythms can be categorized into hour-glass timers (for example lifespan, embryonic development, reaching sexual maturity and the evolutionary clock) and oscillator timers. Oscillators can be further classified into ultradian rhythms (shorter than 24 hours), circadian rhythms (about 24 hours) and infradian rhythms (greater than 24 hours). Examples of ultradian oscillations include the heartbeat (seconds) and yeast respiration (≈40 minutes) while examples of infradian oscillators include the female oestrus cycle (≈days to years) and circannual (yearly) rhythms. In mammals, the most important influence on physiological processes is the circadian rhythm (Knapp and Pownall, 1984; Smolensky and D'Alonzo, 1988; Schibler, 2005).

The term 'circadian' was first used by Franz Halberg in 1959 and was coined from the Latin words '*circa*' meaning about and '*dies*' meaning day (Aronson, 1991; Youan, 2004). Although recognition of the importance of the circadian rhythm and its influence on biological rhythms has only increased in the past few decades, the evidence of its acknowledgment dates back to the early centuries when Soranus of Ephesus described the nocturnal occurrence of asthma in the 2nd century AD (Lemmer, 2007).

Modern technology has facilitated genetic studies to confirm the occurrence of circadian rhythms. Studies on the *Drosophila* species demonstrated the first evidence that genes control circadian rhythms (Lemmer, 2000). In studies on the pineal gland, cancer and peripheral blood cells, some of these circadian clock genes (e.g. Per1, Per2, Per3, Cry1

and Bmal1) have been identified (Green, 2005; Maronde and Stehle, 2007; Kusanagi et al., 2008). Their gene expression is described as a feedback loop that originates in the mammalian brain.

From its literal meaning, the circadian rhythm lasts 'about a day'. The actual circadian clock in humans lasts about 25 hours. This endogenous clock is further influenced by the Zeitgeber (derived from the German 'time giver') phenomenon which refers to the environmental signals such as light, temperature and feeding conditions that tailor the circadian rhythm into a predictable 24-hour pattern. The most important Zeitgeber is the light-dark cycle of day and night or wakefulness and sleep (Aronson, 1991; Smolensky and Portaluppi., 1999; Lemmer, 2000; Schibler, 2005).

These light cues form part of a complex feedback system in the human brain that results in the gene expression to power the body's circadian rhythm. This system is the photoneuroendocrine system (PNS) and consists of the following (Figure 1.1):

(i) The neuroretinae which act as receivers of the photoreceptor cues;

(ii) The retinohypothalamic tract which transmits the signals to the suprachiasmatic nuclei (SCN) in the hypothalamus and pineal gland;

(iii) The pineal gland which synthesizes melatonin to relay a message of darkness in a feedback loop to the SCN; and

(iv) The SCN which acts as the master clock and encodes genes that result in the 24-hour circadian rhythm (Aronson et al., 1993; Maronde and Stehle, 2007). This gene expression is communicated to peripheral body tissues.

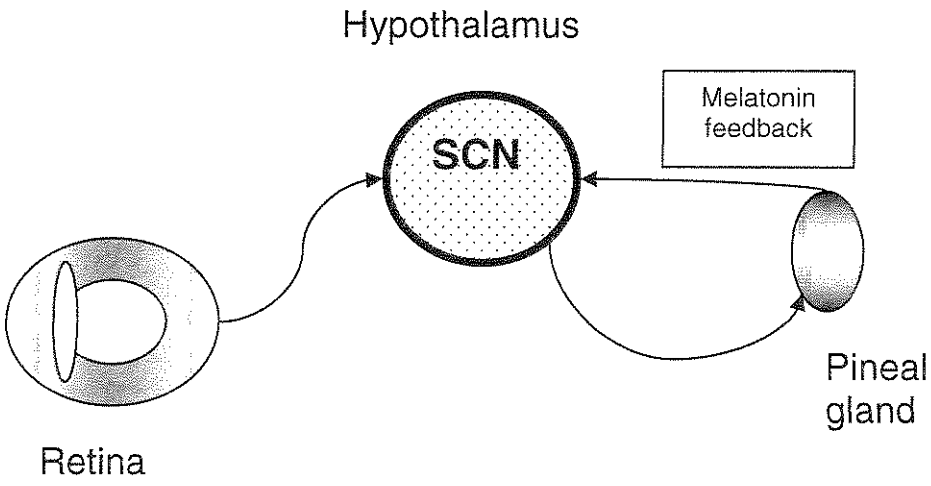
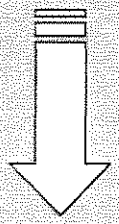
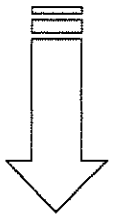
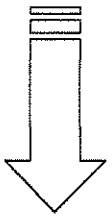
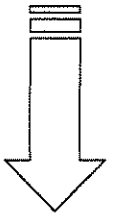


Figure 1.1. *Schematic showing the photoneuroendocrine (PNS) system (adapted from Aronson et al., 1993; Maronde and Stehle, 2007).*

Biological rhythms are characterized by periods (the duration of time to complete a single cycle), levels (the baseline around which the rhythmic variation occurs), amplitudes (a measure of the magnitude of the predictable variability), and phases (peaks and troughs to the corresponding time scale) (Smolensky and Peppas, 2007). Some circadian rhythms like heart rate have small amplitudes whereas others like blood cortisol concentrations have high amplitudes (Smolensky and D'Alonzo, 1988). These characteristics determine the effect of a circadian rhythm on the body's physiological systems. The peak times of some of these functions can help us to understand the physiology of some disease states and can display the corresponding relevance in the field of medicine. Table 1.1 elaborates on some of these peak times.

Table 1.1. *Peak times of function of some physiological processes in the body (Adapted from Smolensky and Peppas, 2007).*

TIME	PEAK TIME OF FUNCTIONS*
Midnight 	Thyroid stimulating hormone Growth hormone Melatonin, Prolactin Lymphocytes Atrial natriuretic peptide Eosinophils Adrenocortical tropic hormone Follicle stimulating hormone, Luteinizing hormone
6am 	Cortisol, Testosterone Plasma renin activity Aldosterone, Angiotensin Catecholamines Blood pressure/Heart rate Arterial compliance/Vascular resistance Platelet adhesiveness Blood viscosity
Noon 	Haemoglobin, Serum iron Serum total proteins Airway patency (Peak expiratory flow, forced expiratory volume in 1s) Insulin Respiratory rate Body temperature Triglycerides
6pm 	Cholesterol Diuresis Blood flow (forearm) Neutrophils Basal gastric acid secretion Calcitonin gene-related peptide White blood cells

**The separations and groupings of function are depicted to illustrate their time-relatedness.*

Traditional medicine and biology are based on the concept of homeostasis, that is, the body's internal environment remains constant (Smolensky and D'Alonzo, 1988). Drug therapies and disease management have also been geared around this theory.

Knowledge of circadian rhythms challenges the theory of homeostasis and places emphasis on chronobiology, a term used to describe the science of biological rhythms. Chronobiology has thus given rise to a whole new collection of other terms used in disease management according to the rhythmic changes in physiological function (Table 1.2).

Table 1.2. *Terms and definitions used in the science of biological rhythms (Adapted from Smolensky and D'Alonzo, 1988; Youan, 2004; Smolensky and Peppas, 2007).*

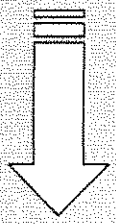
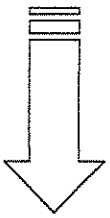
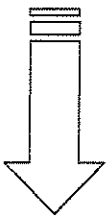
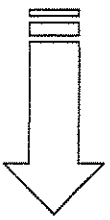
Term	Definition
Chronobiology	The study of biological rhythms and their mechanisms.
Chronopharmaceutics	The branch of pharmaceutics devoted to the design and evaluation of drug delivery systems that release drug to match the biological requirement of a disease therapy.
Chronotherapy	The delivery of drugs in synchrony with the rhythm-dependent circadian variation inherent in the human body.
Chronopharmacology	The study of the manner and extent to which the kinetics and dynamics of drugs are affected by biological rhythms and the effect of the drugs on biological rhythms.
Chronokinetics/ Chronopharmacokinetics	The rhythm-dependent differences in the absorption, distribution, metabolism and elimination of drugs.
Chronodynamics/ Chronopharmacodynamics	The rhythm-dependent differences in the effects of drugs.
Chronotoxicology	The rhythm-dependent differences in the manifestation and severity of adverse effects and thus intolerance of patients to drugs.

1.2. Circadian rhythms in disease pathophysiology: Implications for chronotherapy

The idea of homeostasis is based in the history of early medical research when methods, technology and diagnostic tools had not yet reached the stage of development to adequately highlight the importance of inter- and intra-individual variability. Circadian rhythms are not homeostatic in nature and vary amongst organ systems. As a result, day-night patterns influence the occurrence and severity of many chronic diseases (Smolensky and Portaluppi, 1999), which may influence the manner in which they are

managed. Table 1.3 highlights the approximate time during the day when symptoms of chronic medical conditions or life-threatening events are most likely to occur in a normal sleep-wake routine. Following this, a discussion will proceed to detail some model disease states, namely asthma, rheumatoid arthritis, cardiovascular disorders and cancer which follow the circadian phenomenon.

Table 1.3. *Approximate times during the day when symptoms of chronic medical conditions or life-threatening events are most likely to occur (adapted from Smolensky and Portaluppi, 1999).*

TIME	SYMPTOMS*
Midnight 	Peptic ulcer disease exacerbation Congestive heart failure Apnea Prinzmetal's angina Asthma
6am 	Epilepsy Bronchitis/Emphysema Epistaxis Rheumatoid arthritis Allergic rhinitis/Migraine Angina/Myocardial infarction Sudden cardiac death Thrombotic/haemorrhagic stroke
Noon 	Sickle cell anaemia crises Haemorrhagic and perforated ulcer hospitalization
6pm 	Epistaxis Osteoarthritis Cutaneous reactivity Intractable pain Epilepsy

**The separations and groupings of function are depicted to illustrate their time-relatedness.*

1.2.1. Model disease states that follow the circadian phenomenon

1.2.1.1. Asthma

Asthma is a chronic inflammatory disease of the respiratory tract. It is characterized by the hyper-responsiveness of the lower airways to several stimuli. Symptoms experienced by patients include difficulty in breathing, bronchospasm and excessive secretion of mucus (Smolensky et al., 2007).

1.2.1.1.1. Mechanisms of nocturnal asthma

The day-night circadian-related pattern of asthma was first reported in the 5th century AD by Caelius Aurelianus who documented the worsening of asthmatic symptoms at night. Since then, there have been several studies that have established the night-time exacerbation of symptoms. Lung function (measured by instituting peak expiratory flow rate [PEFR] and forced expiratory volume in 1 second [FEV_1]) fluctuates over a 24-hour period in healthy individuals but it is the abnormally high amplitude fluctuations in asthmatic patients that relate to the symptoms experienced in nocturnal asthma (Skloot, 2002; Smolensky et al., 2007). A study in 1988 by Turner-Warwick showed that a significant percentage of asthmatic patients experienced awakenings and sleep disturbances. These nocturnal symptoms are associated with respiratory arrests and sudden deaths between midnight and 8am (Sutherland, 2005).

Asthmatics experience a late phase response to allergens. It is suggested that this may be related to the increased inflammatory cells and mediators present in the lungs at night. A study involving intravenous atropine administration at 4am and 4pm also suggested that increased bronchial tone at night may affect patients with nocturnal asthma (Skloot, 2002). The number and physiological response of β_2-adrenergic receptors decreases between 4pm and 4am. Glucocorticoid receptor binding is also significantly lower at 4am compared to at 4pm. This receptor binding function may alter the effect of anti-asthmatic drugs and may contribute to the exacerbation of nocturnal

asthma as a result of a decreased response to these drugs (Sutherland, 2005). High-amplitude circadian rhythms in cortisol and adrenalin release may also be significant since both reach their lowest concentrations at night – cortisol has an anti-inflammatory effect and enhances β-receptor function while adrenalin induces bronchodilation and has a stabilizing effect on mast cells (Smolensky et al., 2007).

1.2.1.1.2. Asthma treatment and implications for chronotherapy

Asthma can be viewed as a reversible airways disease for which several bronchodilators and anti-inflammatory drugs are available to restore low airflow rates. Table 1.4 describes a possible chronotherapeutic approach to nocturnal asthma.

Table 1.4. *A proposed chronotherapeutic approach to nocturnal asthma (Skloot, 2002).*

AM	3pm	6-7pm	At bedtime
Long acting β-agonist	Oral corticosteroid	Sustained release theophylline	Leukotriene modifier/ Long acting β-agonist/ Anti-cholinergic

In a study which examined the effect of a single dose of the oral corticosteroid prednisone at 8am, 3pm or 8pm in patients with nocturnal asthma, it was found that only the 3pm dose resulted in an improvement of symptoms (Beam et al., 1992). Studies by Pincus and co-workers (1995, 1997) were carried out to assess the chronotherapy of asthma with inhaled corticosteroids. By using inhaled triamcinolone acetonide once daily, they were able to demonstrate that a single dose at 3pm was as effective as the standard four times a day regimen.

A study using the long acting β-agonist salmeterol displayed significantly improved lung function as measured by morning PEFR and FEV_1. The percentage of night awakenings as a result of asthma symptoms was also significantly decreased (Lockey et al, 1999). Leukotriene modifiers have been shown to improve lung function; an example is montelukast which is administered at bedtime with this application in mind. In addition,

since vagal tone is increased at night (increased bronchial tone), anti-cholinergic therapy would be effective as night-time therapy (Skloot, 2002).

Sustained release (SR) theophylline has been the treatment of choice for asthma and nocturnal asthma for many years. It has anti-inflammatory properties and decreases the late-phase response to allergens (Smolensky et al., 2007). The goal with these sustained release preparations is to produce a high blood level of theophylline that would last throughout the night. Studies have shown that there is a significant difference in serum theophylline concentration when sustained release preparations of theophylline are administered during the day as opposed to at night. In two studies by Smolensky et al. (both 1986) which tested the administration of equal doses of SR theophylline at either 7am and 7pm or 8am and 8pm, the results displayed the day-night differences in theophylline bioavailability. Higher concentrations of serum theophylline were consistently found after evening dosage times. A different study exhibited a change in the bathyphase (period of decreased function) of peak expiratory flow which has displayed a circadian trough that occurs between midnight and morning (Burioka et al., 2001). Patients showed an improvement in nocturnal symptoms after an evening administration of theophylline. It is important to note that all of the studies were conducted on diurnally active patients i.e. sleep-wake cycles according to the rhythms of night and day. The pharmacokinetics of theophylline can be altered and the expected reduction of its metabolism can be changed in patients that work at night (Decourt et al., 1982). Therefore, the patient's period of wakefulness should be a factor when considering the chronotherapeutic approach.

1.2.1.2. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic immune/inflammatory condition that presents with joint pain, joint stiffness and a functional disability in the early hours of the morning. The close relationship between neuroendocrine pathways and the immune/inflammatory

response may explain this circadian rhythm in symptom presentation. The hormone cortisol, the synthesis of which is regulated by the hypothalamic-pituitary-adrenal axis in the brain, is the most potent endogenous anti-inflammatory substance. Cortisol levels in the body are higher during the day than at night. The hormones prolactin and melatonin may contribute to proinflammatory conditions since their levels are higher at night. Proinflammatory cytokines may also play a role since they have displayed elevated levels in the early morning (Cutolo and Straub, 2007). Given these proposed mechanisms of RA, a practical approach to disease management would be the chronotherapeutic use of immunosuppressive/anti-inflammatory drugs like corticosteroids to increase their efficacy and decrease the likelihood of adverse events.

Buttgereit et al. (2008) carried out a randomized double-blind trial over twelve weeks in which the chronotherapeutic application of prednisone was assessed (N=288). The evening administration of a modified-release prednisone tablet (with a 4-hour lag time) was compared to the morning administration of an immediate-release prednisone tablet. The evening administration was intended to be aligned with the circadian rhythms outlined above (Cutolo and Straub, 2007). The results showed a reduction in morning stiffness with the use of the modified-release preparation in the evening over the immediate-release preparation in the morning (Buttgereit et al., 2008). This study highlighted the clinical relevance of the chronotherapeutic use of low dose glucocorticoid therapy in suppressing RA symptoms as well as slowing the progression of joint destruction.

1.2.1.3. Cardiovascular disorders

Several functions of the cardiovascular system like blood pressure (BP), heart rate, stroke volume, cardiac output, and blood flow exhibit circadian rhythms (Youan, 2004).

The development of monitoring devices has facilitated the characterization of circadian patterns in hypertension, as well as in the different forms of hypertension – those that are normotensive or have primary hypertension generally display a drop in BP at night (dippers), while those that have secondary hypertension (where the primary cause may be renal disease, Cushing's disease etc.) generally display an increase in BP at night (non-dippers) (Lemmer, 2000). A number of trials exploring the chronotherapy of hypertension have established the fact that changing the time of treatment rather than the combination of treatment may be a more practical approach to BP control (White, 1996; Calvo et al., 2005; Hermida et al., 2005, 2005, 2007). A study in non-dipper patients with chronic kidney disease showed that changing the time of antihypertensive therapy decreased nocturnal BP (Minutolo et al., 2007).

Cardiac disorders also display circadian variation in which there is a general increased incidence of myocardial infarction, myocardial ischaemia, stroke and sudden cardiac death in the early hours of the morning (Cohen et al., 1997; Youan, 2004). The mechanisms of these events may lie in the circadian patterns of sympathetic activity, catecholamine levels, BP, platelet aggregation and heart rate. Cardiac arrhythmias in diurnally active patients peak between 6am and midday (Portaluppi and Hermida, 2007). One of the studies regarding chronotherapy in cardiac events assessed the treatment of vasospastic angina pectoris with long-acting calcium channel blockers (Mori et al., 2002). The high amplitude of basal total vascular tone and occurrence of attacks both decreased.

1.2.1.4. Cancer

The role of chronotherapy in cancer chemotherapy has been established and studied extensively (Pogue-Geile et al., 2006; Takimoto, 2006; Generali et al., 2007; Ishida, 2007; Geddes, 2008). The Chronotherapy Group of the European Organization for Research and Treatment of Cancer (EORTC), formed in 1996, embarks on several

studies to further explore the timed administration of chemotherapy (Coudert et al., 2003). A currently used circadian-timed therapy resulted from a study in which 6-mercaptopurine and methotrexate were either administered in the morning or the evening to children with acute lymphoblastic leukemia (ALL) – survival rates were significantly higher in the group that was dosed in the evening (Levi et al., 2007).

1.3. Current chronopharmaceutical formulations

From the above, the established existence and importance of circadian rhythms in the chronopharmacology of organ systems in man is clearly evident. This generates the potential for chronotherapy. In theory, one could change the timing and dosing regimens of current pharmaceutical formulations to match the relevant chronopharmacology of the different disease conditions. Practically however, this may not be a suitable solution since it may require complex dosage regimens that may even result in patients waking up to adhere to administration times. Alternatively, patients fail to awake at night and this leads to non-compliance. This then gives rise to the opportunity to further advance drug delivery systems in the science of chronotherapy, which has led to the development and research into chronopharmaceutical technologies. This approach also sheds new light on how one may view existing treatments and seemingly 'older' or first line treatments that are normally considered outdated.

Table 1.5 describes the current chronopharmaceutical formulations and their application on the market.

Standard controlled release systems promote drug release at an ongoing rate (0 order, 1st order and 2nd order). In contrast, chronopharmaceutical formulations like those in Table 1.5 aim to release drug at the time of need. The body is not exposed to excess quantities of drug and thus the side effect profile is decreased while efficacy is increased.

Table 1.5. Current chronopharmaceutical formulations.

Technology	*Properties*	*Applications*	*References*
CONTIN® technology (Purdue Frederick, Norfolk, CT, USA)	Drug blended with hydrophilic cellulose, then hydrated with polar solvent and fixed with a higher aliphatic alcohol to produce a semi-permeable matrix with uniform porosity.	Uniphyl® once daily theophylline. MS Contin® and Oxycontin® for use in pain management.	Leslie, 1986; Arkinstall, 1988; FDA: Electronic Orange Book, 2008.
CODAS® technology (Elan Drug Technologies, San Francisco, CA, USA)	Chronotherapeutical Oral Drug Absorption System consisting of drug loaded beads that are coated with release-controlling polymer. Polymer consists of water-soluble and water-insoluble polymers to induce a lag time.	Verelan® PM containing verapamil for use in hypertension.	Smith, 2001; Weber, 2002; www.elan.com, Feb 2009.
CEFORM® technology (Fuisz Technologies, Chantilly, VA, USA)	Biodegradable polymers/bioactives are subjected to varying temperatures, thermal gradients and flow processes to produce microspheres of uniform size and shape (150 – 180 μm in diameter).	Cardizem® LA containing diltiazem for use in hypertension.	Verma and Garg, 2001; www.biovail.com, Feb 2009.
DIFFUCAPS® technology (Eurand Pharmaceuticals LTD, Dayton, Ohio, USA)	A multi-particulate system consisting of an inactive core, coated with an active pharmaceutical ingredient mixed with a water-soluble composition. This may be in the form of beads, pellets or granules.	Innopran® XL containing Propranolol for use in hypertension.	Patel et al., 2007; www.eurand.com, Feb 2009.
TIMERx® technology (Penwest Pharmaceuticals, Danbury, CT, USA)	A novel polysaccharide system that adopts the use of xanthan gum and locust bean gum in the presence of secondary and tertiary components, to form water-soluble granules.	Possible use in pain management. Technology has been expanded to a 'tablet within a tablet' to obtain different chronotherapeutic profiles. Geminex® is an improvement which provides the potential for dual therapy.	Kelly et al, 1996; Baichwal and Neville, 2002); www.penwest.com, Feb 2009.

Table 1.5 continued.

OROS® technology (Alza Corporation, Mountainview, CA, USA)	An osmotic pump system comprising a central drug reservoir surrounded by a semi-permeable membrane, which is surrounded by osmotically active agents in a tablet with a strategically laser-drilled orifice.	Covera® HS containing verapamil for use in hypertension.	Zentner, 1991; Santus and Baker, 1995; Smolensky and Portaluppi, 1999; Verma et al, 2002; Makhija and Vavia, 2003; Okimoto et al, 2004; Prabakaran et al., 2004.
Pulsincap® technology (R. P. Scherer International Corporation, Troy, MI, USA)	Consists of a drug reservoir housed within a water-soluble capsule body. The open end is plugged with swellable polymers that are pushed out when in contact with fluid, releasing drug from the reservoir.	A versatile system that can create lag times as well as allowing tablets/minitablets, solutions or beads to be housed within the capsule body.	Mastiholimath et al., 2007; Patel et al., 2007.
PULSYS™ technology (Middlebrook Pharmaceuticals, Westlake, Texas, USA)	A novel pulsatile release technology that consists of one immediate-release and two delayed-release components with the use of soluble and insoluble coatings.	Moxatag™ containing amoxicillin for use in antibiotic therapy.	Roy and Shahiwala, 2008; www.advancispharm.com, Feb 2009.
Infusion pumps	Programmable systems that can allow the release of medication from a reservoir according to required target concentrations.	Diprifusor® containing propofol for use in pain management.	Bruguerolle and Labrecque, 2007.
Computer-based technology	Computer-based systems that simplify the processing of novel systems such as oral dosage forms and implantable devices.	Theriform™ employs three dimensional printing to develop complex dosage forms.	www.therics.com, Feb 2009

1.4. Hydrogel technology employed in chronotherapy

The application of bioerodible polymer systems is popular in the development of chronotherapeutic dosage forms (Freiberg and Zhu, 2004). A bioerodible polymer device was shown to exhibit pulsatile release of simvastatin (Jeon et al., 2007). Hydrogels consist of hydrophilic water-soluble polymers that absorb water and swell, forming gel matrices across which the rate of drug release can be controlled. This rate may also be regulated by the use of hydrophobic components and the degree of cross-linking, since both control swelling (Smolensky and Peppas, 2007). Surface cross-linking from poly (2-hydroxyethyl methacrylate) (PHMEA) resulted in delayed release of proxyphylline in a study by Wu and Brazel (2008). Hydrogels may be ionically-sensitive (Berger et al, 2004), pH-sensitive (Zhang et al, 2007) or temperature-sensitive (Birgersson et al, 2008), creating the potential to further control their behaviour. Eudragit® (Rohm Pharma, Darmstadt, Germany) is an example of a pH-sensitive hydrogel. It has displayed its potential use in sustained release and pulsatile drug delivery systems (Cui et al., 2008; Schellekens et al., 2008). Copolymerization has been used to manipulate the physicomechanical properties of hydrogels in order to achieve desired controlled drug release (Saboktakin et al, 2009).

1.5. Consideration of the complexities involved in oral multiparticulate drug delivery systems in chronotherapy

One of the common factors found in the development of oral chronotherapeutic drug delivery systems is that they comprise multiple-units for pulsatile or phasic drug delivery in the form of capsules or compressed beads. In some cases, these multiple-units consist of beads or microspheres that undergo several process steps involving coating technologies, and in other instances the delivery system is subjected to various forms of manipulation to produce a final dosage form. All of these systems deviate from the conventional simple monolithic oral drug delivery system because of the complexities of their design. These complexities introduce many process variables into

the production process, thus making cost of manufacture, human or mechanical error and irreproducibility some of the factors to be considered. A practical approach would be to reduce the complexity of design and limit the process variables while still introducing novelty to the formulation. A comprehensive explanation of the advantages and disadvantages of oral multiparticulate delivery systems can be found elsewhere (Ishida et al., 2008; Roy and Shahiwala, 2008).

1.6. Consideration of compression techniques in drug delivery systems for chronotherapy

Direct compression techniques are known to reduce labour, time and the associated costs in the production of tablets. High cost is a major factor which sometimes limits the availability of advanced technology to the wider public. These cost implications and the advantages of compression techniques have been acknowledged by researchers such as Pather et al. (1998) who developed a sustained release theophylline tablet by direct compression.

However, novel compression-coating has also been developed and researched to pave the way for tablet designs. Several studies have shown that drug cores which are compression-coated with one or more polymers result in successful timed-release tablet formulations (Turkoglu and Ugurlu, 2002; Sawada et al., 2004, 2004; Sundy and Danckwerts, 2004). When developing a design using compression techniques, the compression properties of the formulation components are the main consideration.

1.7. Properties of an ideal chronotherapeutic drug delivery system

In the preparation of a potential chronotherapeutic drug delivery system (DDS), one would aim to achieve the following three critical properties (Kraft and Martin, 1995):

1. A DDS that targets the intended organ system according to its circadian pathophysiology;

2. It should have a feedback control system; and
3. It should be convenient to administer to patients to enhance compliance.

1.8. Rationale and motivation for the present study

The field of chronotherapeutics paves the way for various advances and complexities in drug delivery technology. The ultimate goal is to formulate a system that will incorporate the properties of an ideal chronotherapeutic DDS as outlined in Section 1.7. A major drawback of the currently available technologies for chronotherapy on the market is that they comprise complex designs, which has an impact on the number of process variables. The resulting cost implications then limit the accessibility of these technologies to a minority of the population, particularly in developing countries such as South Africa. The use of compression techniques in the manufacture of a chronotherapeutic system is a practical approach, and the basis of which has been well established (Pather et al., 1998; Turkoglu and Ugurlu, 2002; Sawada et al., 2004, 2004; Sundy and Danckwerts, 2004). The present study considered all of the above points in order to develop a novel formulation as outlined in the aim and objectives below.

1.9. Aim and objectives of this study

The aim of the present study was to design, develop and evaluate an oral drug delivery system that provides phase-controlled drug release and a possible lag time as a potential device for chronotherapeutic drug delivery. This was achieved by the use of different grades of polymers (hydrogels) and with the exploitation of the direct compression tableting technique, which promotes ease of manufacture and decreases the number of processing variables that are present in currently researched and marketed technologies. This study proposes a triple-layer double-disk tablet configuration for oral drug delivery that will exhibit drug release in either a biphasic or

triphasic manner for possible application in the chronotherapeutic management of diseases such as asthma and cardiovascular disorders.

In order to achieve this aim, the following objectives have been outlined:

1. Selection of appropriate polymers and a model drug following a thorough review of the literature;
2. Assessment of the compression properties of the various polymers;
3. Formulation of triple-layer double-disk tablet configurations with the selected polymers and model drug in the proportions determined by an Extreme Vertices design, based on statistical design parameters;
4. Investigation of the drug release potential of the triple-layer double-disk tablet configurations employing *in vitro* dissolution methods;
5. Based on the dissolution results obtained in Objective 4, the development of an optimized formulation using statistical optimization technology will follow;
6. Determination of the *in vitro* drug release behaviour from the optimized formulation for establishment of the chronotherapeutic potential of the configuration;
7. The assessment of physicomechanical changes and swelling/gelation characteristics of the polymers as a result of fluid uptake; and
8. The establishment of a correlation between the textural and swelling/gelation transitions induced within the configuration, to the drug release rate for elucidation of the release mechanism from the triple-layer tablet configuration.

1.10. An overview of this research report

Section 1 outlines a literature review with regard to the rhythmic patterns inherent in nature and the subsequent need for drug delivery systems to respond to these episodic changes. The different circadian-dependent disease pathophysiologies are illustrated and the varied current technologies and researched applications are described. This

section also delineates the desired properties of a chronotherapeutic drug delivery system. The rationale and motivation for this study is outlined as well as its aim and objectives.

Section 2 comprises the formulation and development of the triple-layer double-disk tablet configuration for oral drug delivery having application in chronotherapy. A motivation for the selection of the polymers and model drug theophylline is provided for in the introduction. The materials and methods for the manufacture of the configuration are described. The optimization of the formulation is detailed and the studies on this optimized formulation are elucidated. The resulting changes in textural and swelling/gelation characteristics in the polymers are discussed with regard to the *in vitro* drug release profiles.

Section 3 outlines the conclusions of this research into a potential chronotherapeutic system, and recommendations for its improvement and/or potential for further studies.

1.11. Concluding remarks

The circadian rhythm is a biological rhythm that influences the body's physiological processes in a 24-hour cyclical pattern. The manner in which this predictable patterned behaviour is displayed in chronic diseases such as asthma, rheumatoid arthritis, cardiovascular disorders and cancer has many implications for chronotherapy. Acknowledgment of the impact of this phenomenon is evident in the establishment of scientific journals such as *Journal of Circadian Rhythms*, *International Journal of Chronobiology* and *Chronobiology International: The Journal of Biological and Medical Rhythm Research*, that are dedicated to this subject. The science of chronotherapy paves the way for chronopharmaceutical novel drug delivery systems. Although the background is adequately understood, the pharmaceutical applications of chronotherapeutic delivery systems are often complex and impractical for wide-scale

production and accessibility. The ensuing section will explore the concept of a simple tablet-like configuration with a wide scope for chronotherapeutic application.

SECTION 2

FORMULATION AND DEVELOPMENT OF THE TRIPLE-LAYER DOUBLE-DISK TABLET CONFIGURATION

2.1. Introduction

The use of hydrophilic, swellable polymers in controlled release formulations is widely accepted (Rokhade et al., 2007; Bajpai et al., 2008). Drug is principally released from the polymer matrix by diffusion-controlled and swelling-controlled mechanisms or by degradation of the polymer (Colombo et al., 2000). The rate of drug release is determined by the structure of the matrix and the physicochemical and physicomechanical properties of the polymer and drug (Pillay and Fassihi, 2000). Modulation of drug release rate can be effected by manipulating factors such as polymer molecular weight, drug distribution and polymer blending (Freiberg and Zhu, 2004). These modifications influence the dynamics of the rate and extent of polymer relaxation and swelling, hence impacting the kinetics of drug release. Various polymeric systems were thus considered for the design of the proposed drug delivery device.

Efentakis and co-workers (2006) found that when compared to sodium alginate and sodium carboxymethylcellulose, the hydrophilic hydrogel polyethylene oxide (PEO) exhibited intermediate behaviour with regard to swelling and erosion, where those polymers form a top layer in a core-in-cup delivery system. On contact with fluid, the top layer immediately absorbed the fluid resulting in swelling and expansion, and subsequent diffusion of drug. PEO in combinations with lactose/polyethylene glycol (PEG) has shown potential application in oral time-controlled delivery systems in that its use may induce a lag phase before drug release (Sawada et al., 2003, 2004). It has also been postulated that PEO acts as a pore-former and compatibilizer (Brook et al., 2008).

Hydroxypropylmethylcellulose (HPMC) is a non-ionic, non-toxic, hydrophilic polymer that is easy to handle and has minimal influence on processing variables (Ishida et al., 2008). It forms a swellable and erodible barrier and has also been shown to create a lag phase in time-controlled preparations using HPMC dispersions in ethanol/water cosolvents (Cao et al., 2004) or HPMC/polyvinylpyrrolidone (PVP) blends (Karavas et al., 2006) as tablet-core coating materials. Hydroxyethylcellulose (HEC), a hydrophilic, non-ionic cellulose polymer, exhibits water uptake and swelling. When compared to another cellulose, hydroxypropylcellulose (HPC), studies revealed that HEC exhibited greater swelling and higher erosion rates. Drug release was modulated via a combination of polymer swelling and solute diffusion (Roy and Rohera, 2002).

Poly(lactic-co-glycolic acid) (PLGA) is a water-insoluble block copolymer that has displayed potential in various controlled release and time-controlled formulations (Mallarde et al., 2003). Although documented durations of drug release can vary from days to months (Setterstrom et al., 2005), the addition of hydrophilic components (Mallarde et al., 2003) and the presence of drug crystals at the particle surface (Wischke and Schwendeman, 2008) can alter the drug release rate kinetics.

In the present study, the well-known model drug theophylline has been chosen for its potential in chronotherapeutics, as previously discussed in Section 1. Direct compression and compression-coating methods are simple and relatively cost-effective since the number of processing variables in production is decreased. Previous research studies in other areas employing direct compression/compression-coating techniques have displayed stable compressibility characteristics of theophylline (Pather et al., 1998), HPMC (Turkoglu and Urgulu, 2002; Sundy and Danckwerts, 2004), HEC (Roy and Rohera, 2002) and PEO (Sawada et al., 2003 and 2004). This study exploits

the above characteristics of HPMC, PEO, HEC and PLGA to design a novel chronotherapeutic drug delivery tablet configuration as depicted in Figure 2.1.

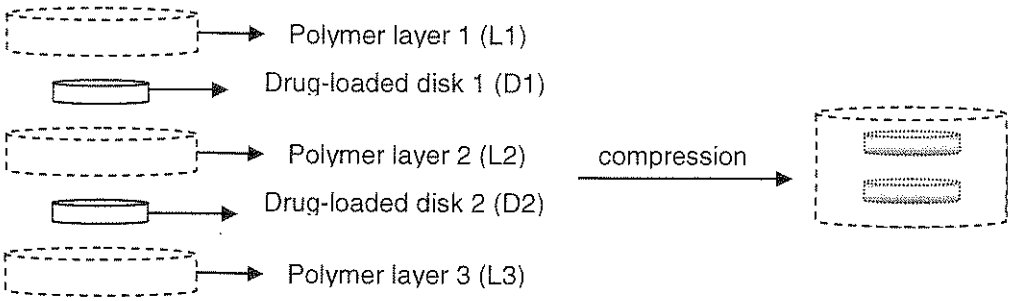


Figure 2.1. *Schematic of the triple-layer double-disk tablet configuration.*

2.2. Materials and Methods

2.2.1. Materials

The polymers that were purchased and employed in this study included: hydroxyethylcellulose (HEC Type 250 G-Pharm, Hercules Ltd, Salford, Lancashire, UK), polyethylene oxide (PEO WSR 303, molecular weight 7,000000, Union Carbide Corporation, Danbury, CT, USA), hydroxypropylmethylcellulose (HPMC, Sigma Chemical Company, St Louis, MO, USA) and poly(lactic-co-glycolic acid) (PLGA, Resomer® grade RG504, Boehringer Ingelheim, Ingelheim, Germany). The model drug theophylline was purchased from Fluka Biochemika (Industriestrasse, Buchs, Switzerland). All other reagents used were of analytical grade.

2.2.2. Methods

2.2.2.1. Statistical approach to tablet configuration formulation

An Extreme Vertices Design which incorporates lower and upper constraints, was built using 5 factors and 3 centre points on Minitab® V15 software (Minitab® Inc., PA, USA) which generated 20 randomized standard run orders. Preliminary experimentation demonstrated that this design was found to be the best-fitting model to the data set to

provide reliable estimates that can be used for precise, stable mathematical and statistical predictions. The 5 independent variables used were the Drug Percentage (%) in Disk 1 (D1), Drug % in Disk 2 (D2), Type of Polymer in Layer 3 (L3), Type of Polymer in Disk 1 (D1) and Type of Polymer in Disk 2 (D2). The randomized proportions and types of the independent variables which were used to prepare the design formulations are illustrated in Table 2.1. The dependent variables were the rate and % of drug release.

Table 2.1. *Extreme Vertices Design for the formulation of different tablet configurations.*

Standard Run Order	INDEPENDENT VARIABLES				
	Drug % D1	Drug % D2	Polymer Type L3	Polymer Type D1	Polymer Type D2
1	30	70	PEO	PLGA	HEC
2	70	30	PEO	PLGA	HEC
3	50	50	PEO	PLGA	HEC
4	40	60	PEO	PLGA	HEC
5	60	40	PEO	PLGA	HEC
6	30	70	HPMC	PLGA	PLGA
7	70	30	HPMC	PLGA	PLGA
8	50	50	HPMC	PLGA	PLGA
9	40	60	HPMC	PLGA	PLGA
10	60	40	HPMC	PLGA	PLGA
11	30	70	PEO	HEC	PLGA
12	70	30	PEO	HEC	PLGA
13	50	50	PEO	HEC	PLGA
14	40	60	PEO	HEC	PLGA
15	60	40	PEO	HEC	PLGA
16	30	70	HPMC	HEC	HEC
17	70	30	HPMC	HEC	HEC
18	50	50	HPMC	HEC	HEC
19	40	60	HPMC	HEC	HEC
20	60	40	HPMC	HEC	HEC

2.2.2.2. Formulation of the tablet configuration

The experimental formulations in Table 2.1 were conceived as triple-layer double-disk tablet configurations as shown in Figure 2.1. The drug-loaded disks (D1 and D2), composed of blended drug and polymer in a 1:1 ratio were compressed at 1 ton using the Beckmann Hydraulic Press (Brakel, North Rhine Westfalia, Germany). A flat-faced punch and die set with a diameter of 5mm was used to produce uniform circular disks of 5mm in diameter. The thickness varied from 0.1 to 1 mm, depending on the ratios of drug: polymer. The final tablet configuration was formulated using a flat-faced punch

and die set of 10mm and a method of alternating polymer combinations with manually-centred disks. A final compression force of 5 tons was used to compression-coat the disks. The quantity of polymer used in the layers was kept constant (L1: L2: L3 = 1.5:1:2) such that the weights of all 20 experimental tablet formulations remained constant. The type of polymer (PEO) was also kept constant in L1 and L2.

2.2.2.3. Construction of calibration curve

Analysis of drug release samples was carried out on an ultraviolet (UV) spectrophotometer (Specord 40, Analytik Jena, Jena, Germany) at a maximum wavelength (λ_{max}) of 270nm for the absorbance of theophylline. Standard solutions were prepared by dissolving accurately weighed quantities of theophylline in 100mL of phosphate buffer solution (PBS, pH 6.8) to yield the following concentrations: 0.004mg/mL, 0.008mg/mL, 0.011mg/mL, 0.015mg/mL and 0.019mg/mL. Using buffer (PBS, pH 6.8) as a blank, the absorbance of each solution was measured at 270nm and a calibration curve with a correlation coefficient of R^2 = 0.9937 was constructed (Figure 2.2).

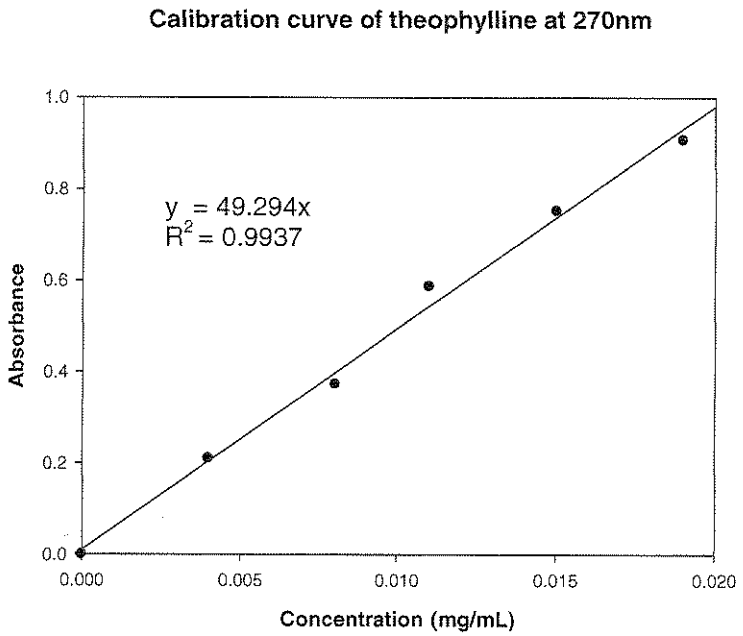


Figure 2.2. *Calibration curve of theophylline in phosphate buffer of pH 6.8 (simulated intestinal fluid) (N = 3; in all cases SD<0.02).*

2.2.2.4. *In vitro* drug release studies

Dissolution studies were performed on all 20 experimental design formulations and the optimized formulation over 24 hours in triplicate. The assessment was performed in a six station dissolution test apparatus (Caleva 7ST, GB Caleva Ltd, Sturminster, Newton, Dorset, UK) using a modified version of the USP 32 Apparatus 2 paddle method in 900mL of simulated small intestinal fluid (pH 6.8; 37°C; 50rpm). The simulated intestinal fluid was prepared as a phosphate buffer solution (PBS without enzymes) of pH 6.8 as per USP recommendations. Due to the stickiness of the PEO in the outer layers of the tablet configuration, a ring-mesh assembly was utilized (Pillay and Fassihi, 2000). Each formulation was placed on top of the assembly which was fitted on the bottom of the dissolution vessel. This modification allowed for all surfaces of the formulation to be exposed to the circulating simulated intestinal fluid.

2.2.2.5. Statistical optimization of the design formulations

After *in vitro* dissolution studies were conducted on the 20 design formulations outlined in Table 2.1, a summary of the drug release profile results was used to determine an optimized formulation. Cube plots, Interaction plots, Main effects plots and Cox response trace plots were generated to test parameters for the development of an optimized formulation. Minitab® V15 software (Minitab® Inc.,PA, USA) was then used to generate a Response Optimizer plot to obtain the optimal desirability parameters for the optimized formulation.

2.2.2.6. Assessment of the physicochemical properties of the tablet configuration

A measure of hardness was investigated using a ball probe (2mm diameter) on a calibrated Texture Analyzer (TA.*XTplus*, Stable Micro Systems, Godalming, Surrey, UK). 50mg each of PLGA and HEC, and 200mg each of PEO and HPMC were compressed into flat 10mm disks using a flat-faced punch and die set on the Beckmann Hydraulic Press (Brakel, North Rhine Westfalia, Germany). These disks as

well as the optimized formulation were tested in triplicate. The hardness parameters and settings utilized are illustrated in Table 2.2. Hardness was measured as the force (N) required to indent the matrices to a set distance (mm). Figure 2.3 depicts a typical textural profile for matrix hardness.

Table 2.2. *Parameters and settings used in textural profiling to determine matrix hardness.*

Parameter	Settings
Test mode	Compression
Pre-test speed	1.00 mm/sec
Test speed	0.50 mm/sec
Post-test speed	1.00 mm/sec
Target mode	Force
Force (optimized)	40 N
Trigger type	Auto
Trigger force	0.01 N

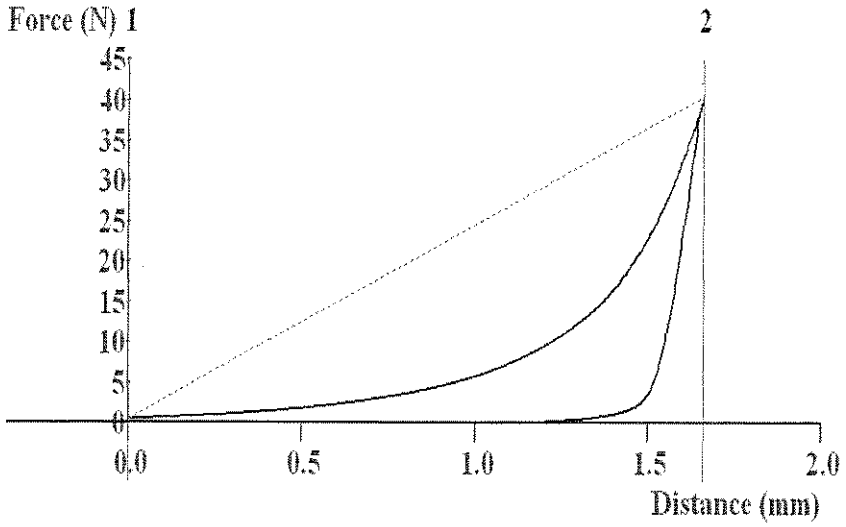


Figure 2.3. *Typical textural profile for the determination of matrix hardness (N=3).*

2.2.2.7. Assessment of gelation and swelling properties of the tablet configuration

A determination of the gel strength as a result of swelling was conducted on the optimized formulation using a flat-tipped probe (2mm diameter) on a calibrated Texture Analyzer (TA.*XTplus*, Stable Micro Systems, Godalming, Surrey, UK). The

formulations were each placed in separate 150mL glass jars containing 100mL of buffer (PBS, pH 6.8). The sealed jars were then placed in a shaker bath (Orbital Shaker Incubator, Model LM-530, MRC Ltd, Hahistadrut, Holon, Israel) set at 37°C and 50rpm and removed at one-hourly time intervals to test the gel strength of both sides of the tablet configurations in triplicate. The gel strength transition test parameters and a typical textural profile are illustrated in Table 2.3 and Figure 2.4 respectively. The determination of the swelling characteristics was assessed by measuring the degree of fluid uptake into the tablet configuration (Section 2.2.2.8).

Table 2.3. *Parameters used in textural profiling to determine gel strength transitions of the formulations.*

Parameter	Settings
Test mode	Compression
Pre-test speed	1.00 mm/sec
Test speed	0.50 mm/sec
Post-test speed	1.00 mm/sec
Target mode	Force
Force (optimized)	1.15 N
Trigger type	Force
Trigger force	0.01 N

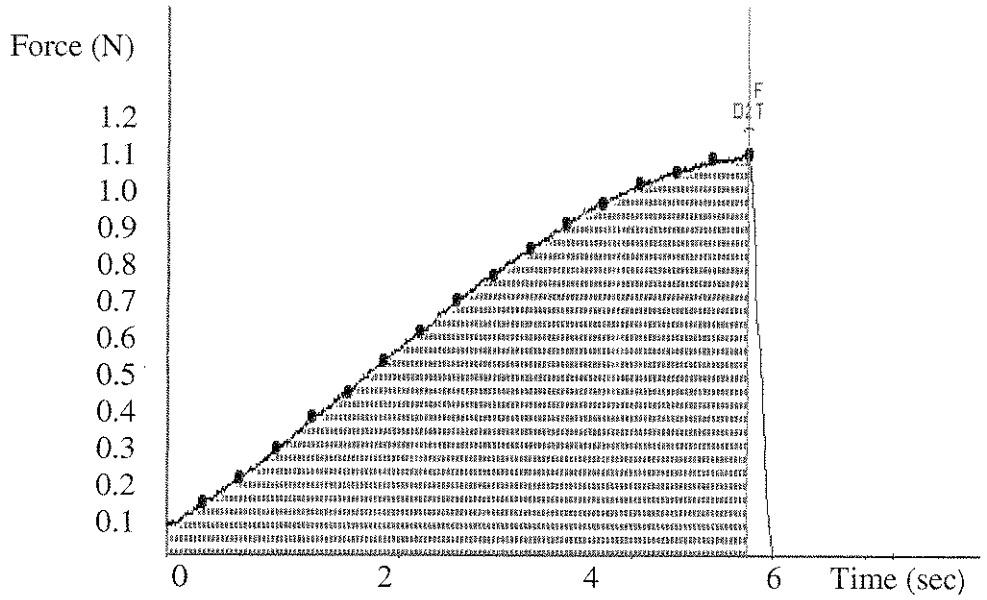


Figure 2.4. *Typical textural profile for the determination of gel strength transitions (N=3).*

2.2.2.8. Determination of fluid uptake and erosion characteristics of the tablet configuration

To assess the fluid-uptake and/or erosion characteristics of the formulation, 72 of the optimized formulations were each weighed and placed in 150mL sealed glass jars, each containing 100mL of buffer (PBS, pH 6.8). The jars were then placed in a shaker bath (Orbital Shaker Incubator, Model LM-530, MRC Ltd, Hahistadrut, Holon, Israel) set at 37°C and 50rpm and 3 tablets were removed at one-hourly intervals. Each formulation removed, was then blotted lightly with filter paper to remove excess buffer and weighed immediately. All weighing operations were carried out on a mass balance (Mettler Toledo, Greifensee, Switzerland) to three decimal places. To determine the percentage (%) of fluid uptake and/or erosion of the matrix over the 24-hour time period, Equation 1 was employed (Sungthongjeen et al., 2004):

$$Fluid Uptake\,(\%) = \frac{Wt - Wo}{Wo} \times 100 \qquad (1)$$

Where Wt is the weight of the wet tablet and Wo is the weight of the dry tablet.

The fluid uptake determined the swelling dynamics of the tablet configuration.

2.3. Results and Discussion

2.3.1. *In vitro* drug release from tablet formulations

Figure 2.5 (a) and (b) illustrates the drug release profiles of the 20 experimental design formulations for the triple-layer double-disk tablet configurations which encompassed compression-coated protection for the 2 drug disks and induced predictable lag times of 2–3 hours before drug release. All release profiles displayed triphasic behaviour with an initial lag time, an upcurving release phase of theophylline and thirdly, an inflection point in the profiles at 12–17 hours, followed by an accelerated release

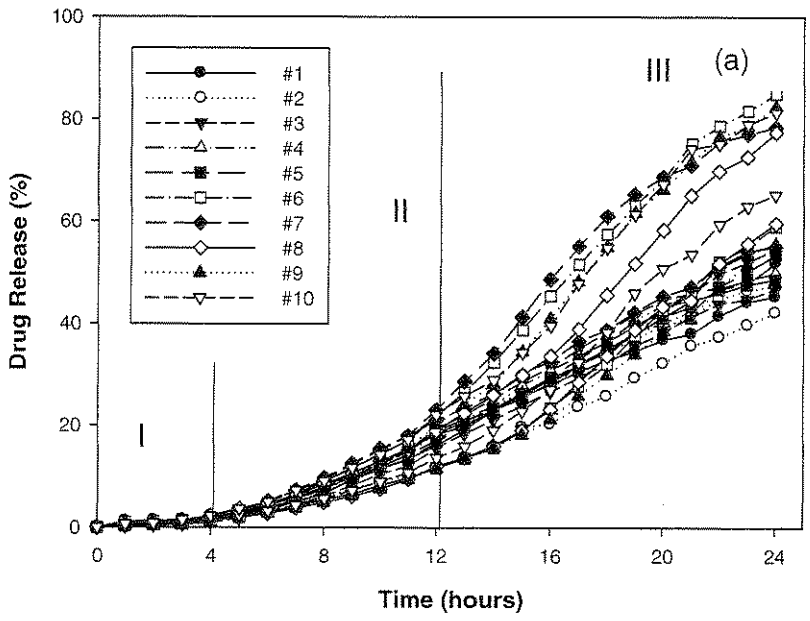
phase. The lag phase allows the possibility of early administration of the delivery system without exposing the patient to undue drug levels. This phase also helps to reserve the drug reservoir for later release phases.

In order to further characterize the dissolution profiles, the mean dissolution times (MDTs) were calculated at the end of the 24-hour period and at 40% and 50% drug release (MDT_{24h}, $MDT_{40\%}$ and $MDT_{50\%}$). A maximum MDT refers to the fastest drug release achievable. Equation 2 was employed (Pillay and Fassihi, 1998):

$$MDT = \sum_{t=1}^{n} ti \frac{M_t}{M\infty} \qquad (2)$$

Where M_t is the fraction of dose released in time ti = (ti + ti-1)/2 and $M\infty$ corresponds to the loading dose.

A summary of the dissolution profile characteristics is illustrated in Table 2.4.



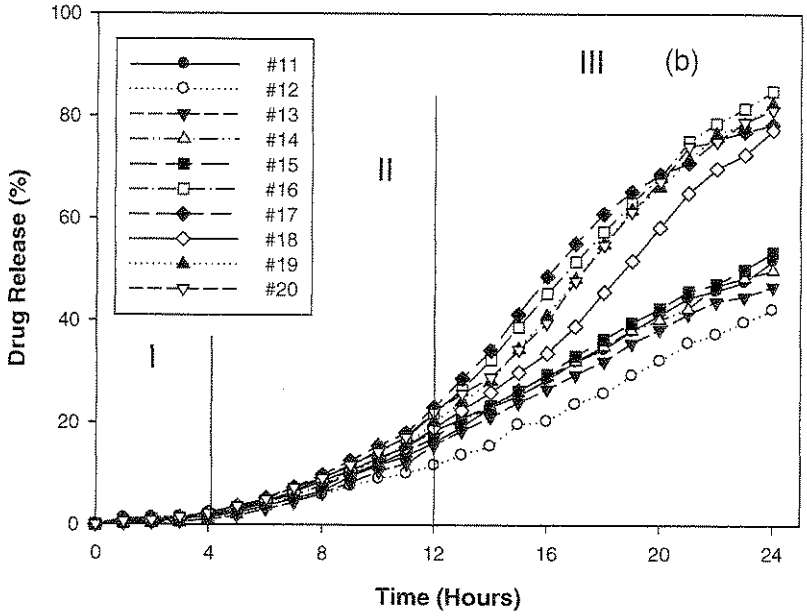


Figure 2.5. *In vitro drug release profiles for (a) Formulations # 1-10 and (b) Formulations # 11-20 (N=3; in all cases SD<0.24).*

Table 2.4. *Summary of the dissolution profile characteristics for formulations generated by the Extreme Vertices Design.*

Formulation	Lag Phase (hours)	Upcurving phase (hours)	Inflection point (hours)	MDT_{24h}	$MDT_{40\%}$	$MDT_{50\%}$
1	3	3 - 13	13	6.274	5.078	7.423*
2	3	3 - 12	12	6.627	5.026	7.286*
3	3	3 - 12	12	7.875	4.799	6.833
4	2	2 - 12	12	8.189	4.880	6.931
5	3	3 - 12	12	6.884	5.061	7.238*
6	3	3 - 14	14	9.646	5.543	7.601
7	3	3 - 14	14	7.701	4.566	6.573
8	3	3 - 15	15	9.796	5.432	7.080
9	3	3 - 15	15	9.059	5.713	7.893
10	2	2 - 17	17	10.438	5.245	7.139
11	3	3 - 14	14	7.658	5.067	7.222
12	3	3 - 15	15	6.353	5.846	7.397*
13	3	3 - 12	12	6.792	5.307	7.596*
14	3	3 - 12	12	7.057	4.909	7.082*
15	3	3 - 13	13	7.846	4.931	7.029
16	2	2 - 12	12	12.845	4.305	5.903
17	2	2 - 14	14	11.179	4.183	5.735
18	2	2 - 16	16	12.189	4.632	6.426
19	3	3 - 12	12	12.725	4.536	6.189
20	3	3 - 12	12	12.259	4.362	6.028

**Extrapolated time points at 50% release*

Table 2.5 illustrates the release rates during the three phases of drug release which confirms the characteristics of an initial lag phase (I), which is followed by an upcurving phase (II) and finally an accelerated release phase (III).

Table 2.5. *Release rates during the three phases of drug release.*

	Computed Release Rates (mg/hr)		
Formulation	**Phase I**	**Phase II**	**Phase III**
1	0.175	0.939	1.158
2	0.114	0.995	1.197
3	0.130	1.035	1.462
4	0.135	0.915	1.529
5	0.114	0.891	1.326
6	0.230	0.648	2.168
7	0.189	1.144	1.436
8	0.171	0.751	2.251
9	0.177	0.712	2.051
10	0.131	1.063	2.339
11	0.200	0.989	1.444
12	0.243	0.759	1.249
13	0.086	0.836	1.299
14	0.226	0.944	1.314
15	0.179	0.987	1.485
16	0.106	1.060	2.628
17	0.116	1.405	2.211
18	0.126	1.174	2.737
19	0.053	1.102	6.030
20	0.240	1.146	2.465

2.3.2. Optimization of the dissolution data

In order to build a design for an optimized formulation, all 5 independent variables in the Extreme Vertices Design (drug concentration in D1 and D2, polymer type in L3, D1 and D2) as well as the dissolution profile characteristics (lag phase (LP), upcurving phase (UP) and MDT_{24h}) were employed for the modeling of the ensuing statistical plots.

2.3.2.1. Cube plots of response data means for modeling optimization

Three-factor cube plots displaying response data means for MDT_{24h}, the LP and UP were built to determine the best fits. The mean data values represent the means of the raw response variable data for the 3 factor levels. Figure 2.6 shows the data means at the points on the cube plot where responses were analyzed.

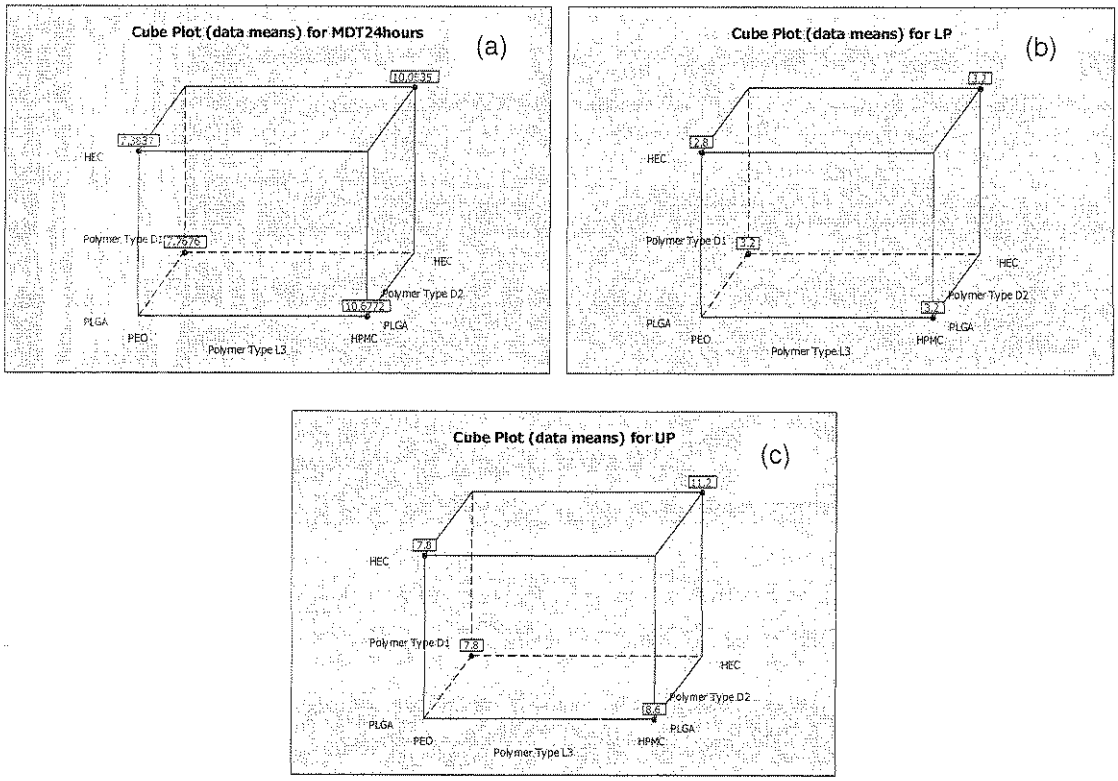


Figure 2.6. *Cube plots displaying data means for (a) MDT_{24h}, (b) the lag phase (LP) and (c) the upcurving phase (UP).*

2.3.2.2. Main effects plots of response data means for modeling optimization

A main effects plot (or dex mean plot) allows us to plot the mean value of each condition so that we can see the effect of the different conditions on the dependent variables. This can show us the strength of the effect across the factors. Figure 2.7 illustrates the effects of the different types of polymer on the data means of MDT_{24h}, LP and UP. All 3 plots display a significantly increased effect on all 3 variables when the polymer type in L3 is changed from PEO to HPMC. Changing the polymer type from PLGA to HEC in D1 has a smaller effect on (a) MDT_{24h}, in comparison to (b) the greater decreased effect on LP and (c) the increased effect on UP. When the polymer type in

D2 was changed from PLGA to HEC, there was virtually no effect ($p>0.05$) on (a) MDT_{24h}, but significantly increased ($p<0.05$) effects on (b) LP and (c) UP. Of particular importance from these plots, is that the effect of the different polymers on the MDT was displayed. Figure 2.7 (a) illustrates that utilizing HPMC as the polymer in L3 can potentially result in maximum MDT. The use of PLGA in D1 also lends itself to a greater MDT. Although the use of PLGA in D2 may show a slightly increased MDT, the use of HEC instead displays significantly better ($p<0.05$) means for the LP and UP.

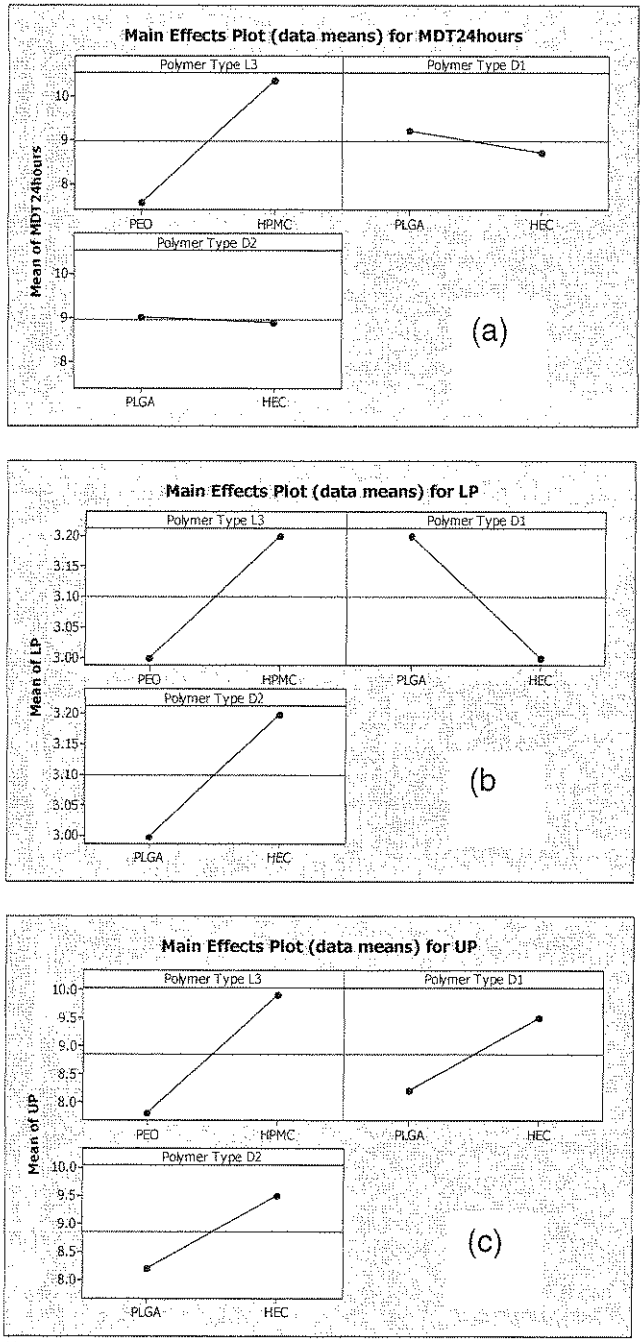


Figure 2.7. *Main effects plots displaying data means for (a) MDT_{24h}, (b) the lag phase (LP) and (c) the upcurving phase (UP).*

2.3.2.3. Interaction effects plots of response data means for modeling optimization

The interaction effects plot is an extension of the main effects/dex mean plot and also compares the strength of the effects across factors, by plotting the interaction between factors. The means of the response variables were plotted (Figure 2.8). All plots displayed large interactions between selection of the polymer types for L3, D1 and D2. The significant effects of these interactions on MDT_{24h}, LP and UP were noted.

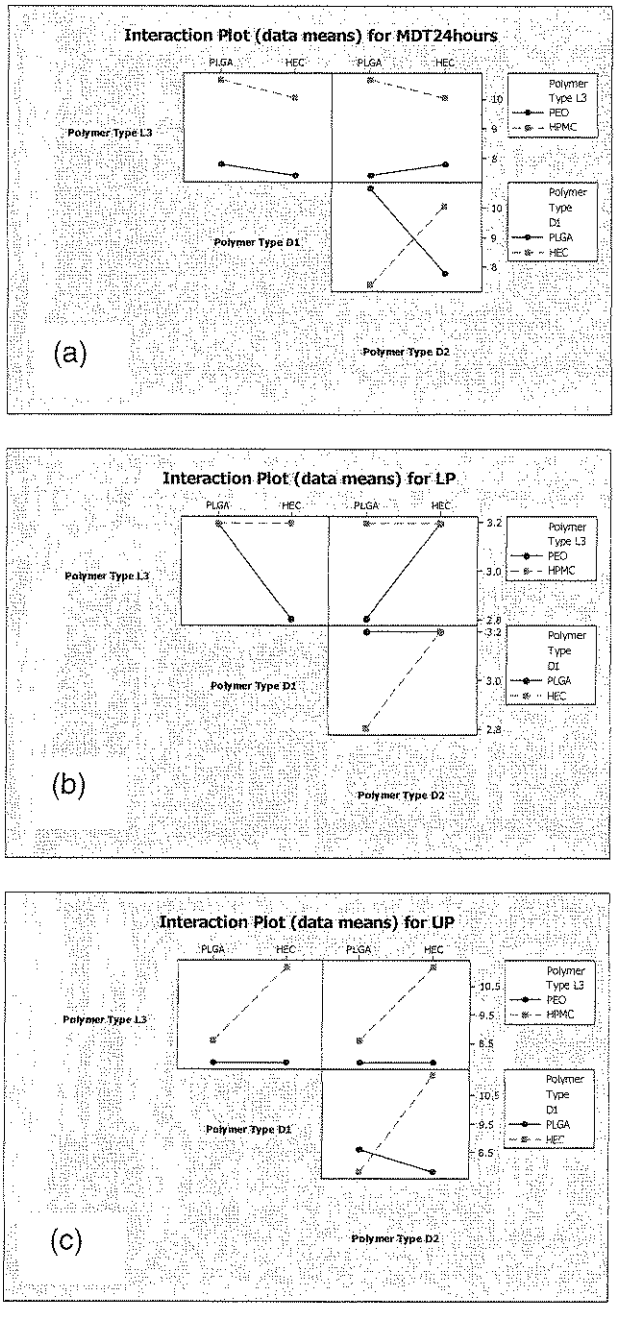


Figure 2.8. *Interaction effects plots displaying data means for (a) MDT_{24h}, (b) the lag phase (LP) and (c) the upcurving phase (UP).*

2.3.2.4. Cox response trace plots of response data means for modeling optimization

These can display surface responses employing various design points (Figure 2.9). Response trace plots (also called component effects plots) depict curves that display the effect of the components on the response. Two scenarios have been plotted using the polymer type variables, namely:

1. Polymer Type L3 = PEO; Polymer type D1 = PLGA; Polymer type D2 = PLGA, and
2. Polymer Type L3 = HPMC; Polymer type D1 = HEC; Polymer type D2 = HEC.

All plots for scenario 2 (d, e and f) showed greater effects on the MDT_{24h}, LP and UP than scenario 1 (a, b and c). The use of HPMC in L3 and HEC in the disks forms a complex hydrogel system in which L1 and L2 consist of PEO. The contrasting effect of a different polymer in L3 was exhibited. The advantage of incorporating the more hydrophilic HEC in the disks rather than only PLGA was also apparent.

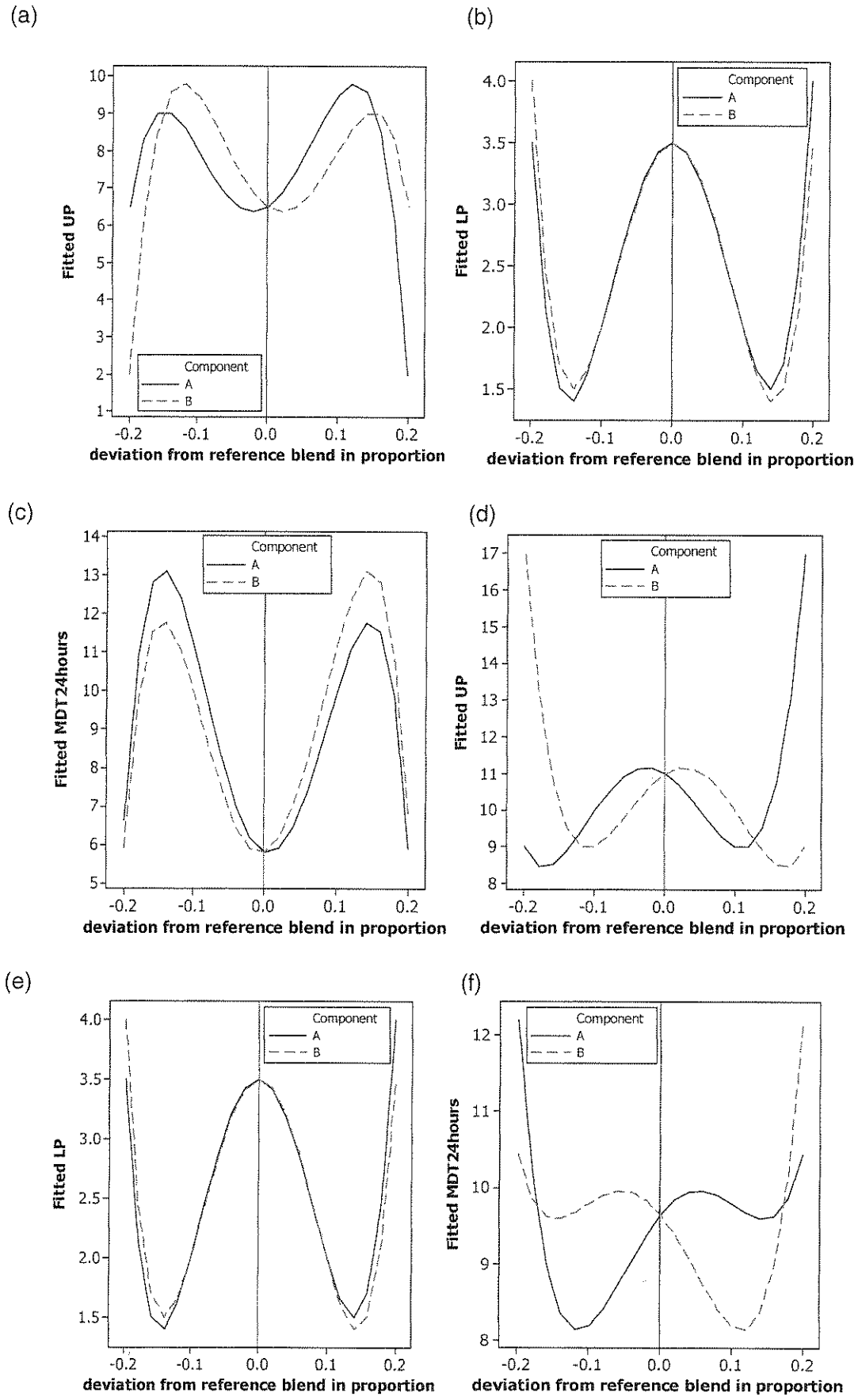


Figure 2.9. *Cox response traces plots for scenarios 1 (a - c) and 2 (d - f).*

2.3.2.5. Design of the optimized triple-layer double-disk tablet configuration

The Response Optimizer was used to maximize the responses for the UP and MDT_{24h} while targeting the response for LP at 3 hours. The experimental design measured the individual desirabilities (d) as well as the composite desirability (D) for the responses (Figure 2.10).

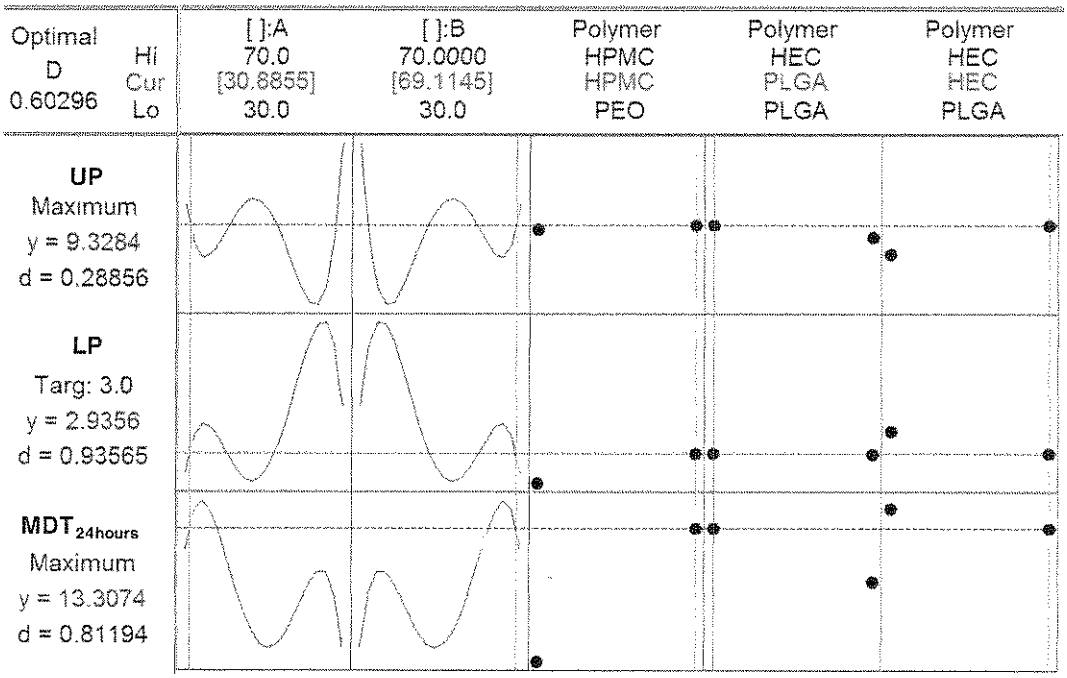


Figure 2.10. *Statistical formulation parameters for the optimized formulation.*

The parameters for the optimized formulation are displayed in Table 2.6.

Table 2.6. *Composition of the optimized formulation for the triple-layer double-disk tablet configuration.*

	Drug % D1	Drug % D2	Polymer Type L3	Polymer Type D1	Polymer Type D2
Optimized formulation	**30.89**	**69.11**	**HPMC**	**PLGA**	**HEC**

2.3.3. *In vitro* drug release from the optimized triple-layer double-disk tablet configuration

In agreement with the design dissolutions, the optimized formulation dissolution profile displayed ideal triphasic drug release. A lag phase of about 2 hours was followed by

steady drug release until the 16-17 hour time period at which point ±30% of theophylline had been released, after which there is an increase in rate of drug release which is characterized by a slightly convex-like shape of the profile (Figure 2.11). At the 24-hour time point, 70% of theophylline had been released into the surrounding medium. It is calculated that theophylline release during the first 16-17 hours occurred from the PEO-surrounded PLGA disk (D1 = 30.89% drug), while the third phase of the profile is theophylline being released from the second HEC disk (D2 = 69.11%).

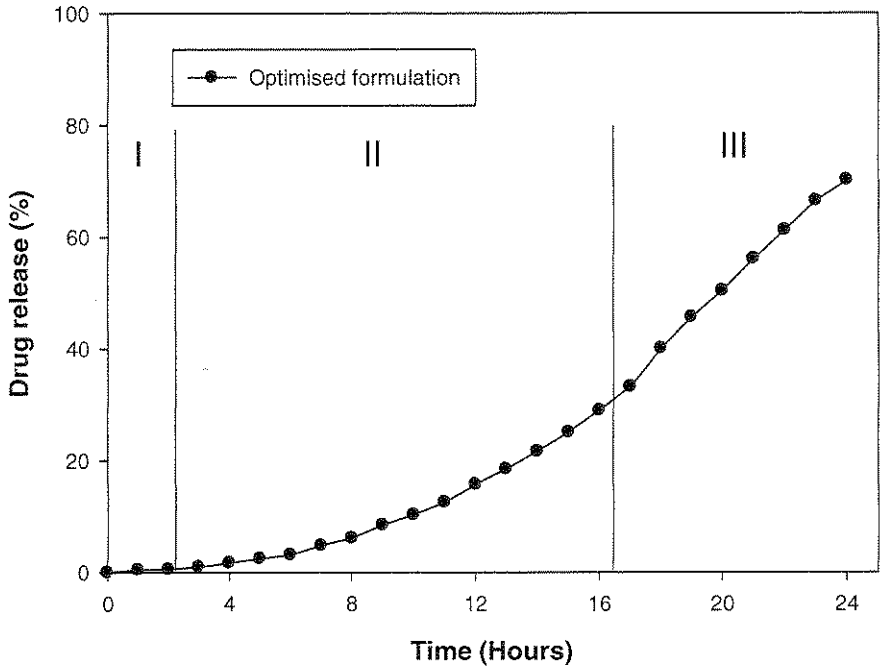


Figure 2.11. *In vitro drug release profile for the optimized formulation (N=3; in all cases SD<0.08).*

2.3.4. Textural profiling and swelling characteristics of the optimized formulation

Swellable matrices or gel-forming matrices are characterized by their ability to imbibe fluid, causing the polymer to undergo glassy to rubbery transitions. When swellable glassy polymers like PEO or HPMC in this formulation come into contact with solvent,

there is a change from glassy to rubbery state in which the polymer chains relax (Colombo et al., 2000). This allows drug transport across the gelled matrix. This transition in the polymers can be observed visually as well as by determining the % weight gain when compared to the original matrix. Figure 2.12 depicts the % weight increase over the 24-hour period. A constant increase in % weight is seen in the first 4-5 hours, during which the matrices are imbibing the solvent medium thus creating the initial lag time of 2 hours. After this period we observe slight fluctuations in the tablet configuration weight which correspond to the dissolution drug release profile (Figure 2.11). These fluctuations may not only depict the loss of drug but also the erosion of polymer. In Figure 2.13, the macroscopic swelling of the device is observed.

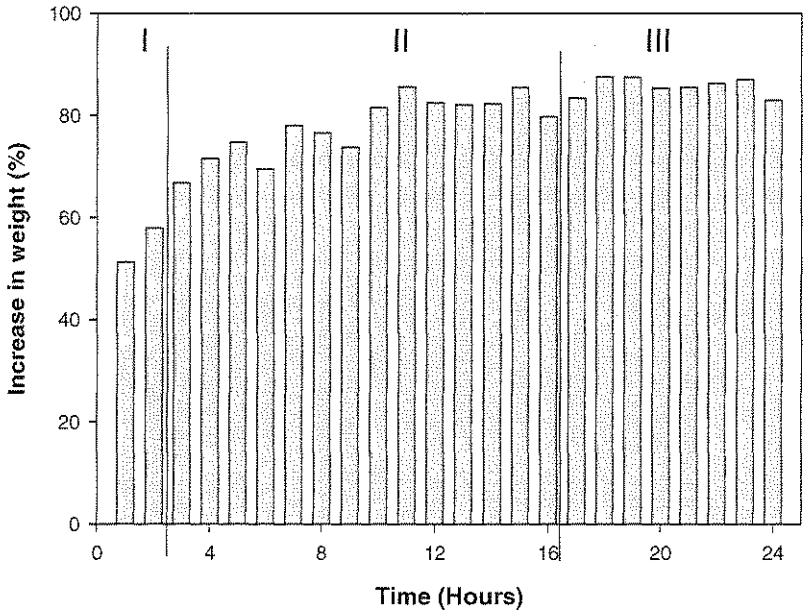


Figure 2.12. *Percentage weight gain in the formulation over 24 hours (N=3; in all cases SD < 10).*

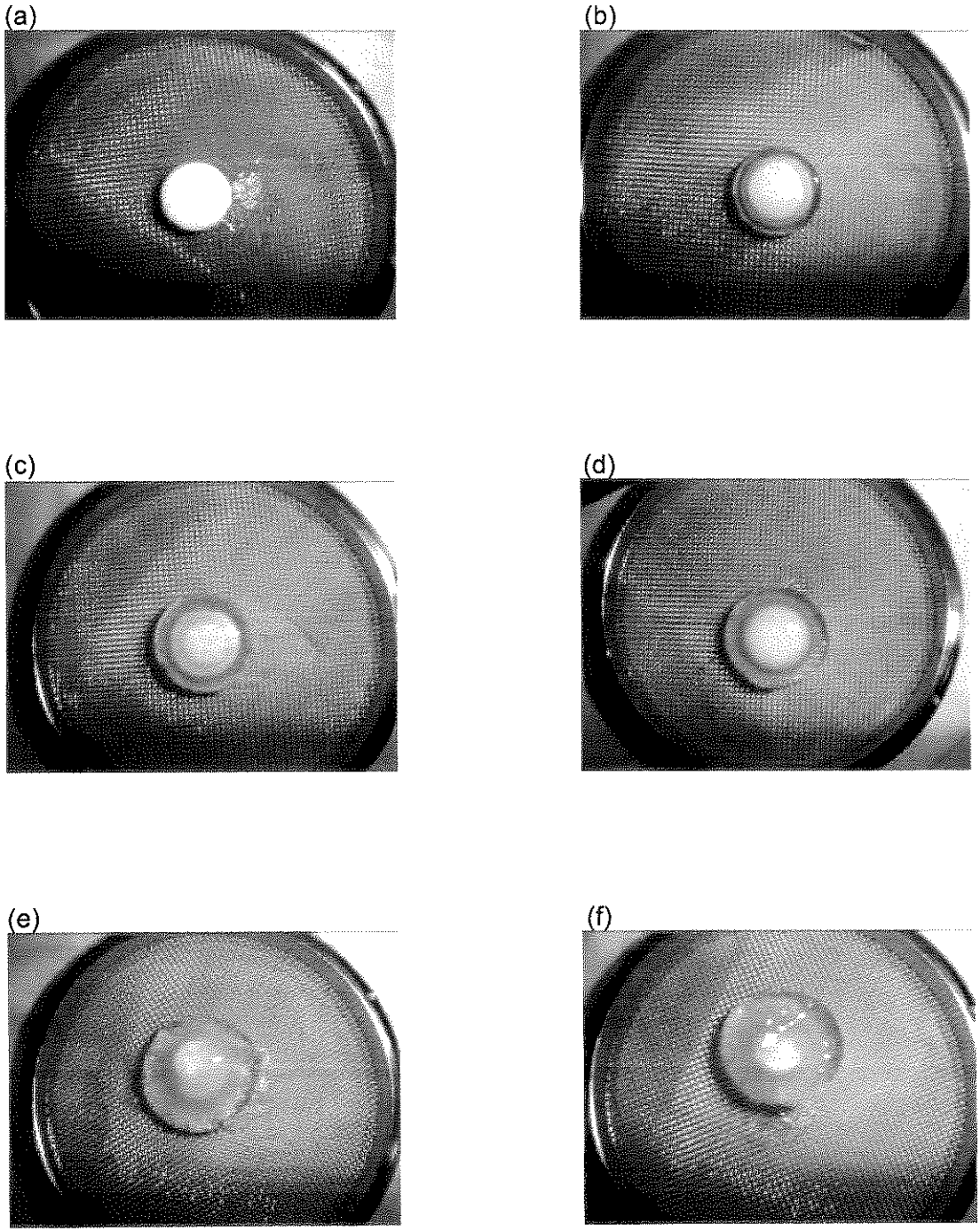


Figure 2.13. *Observed swelling of the tablet configuration at (a) 0 hours, (b) 1 hour, (c) 3 hours, (d) 7 hours, (e) 14 hours and (f) 22 hours.*

A measure of hardness of the polymer can allow one to predict the swelling and gelation characteristics when the polymers are exposed to a solvent medium. When compressed individually (Figure 2.14 (a)), only PLGA displayed a markedly higher value while the differences in hardness between HEC and PEO were negligible. HPMC showed a slightly higher value than PEO. In the final compressed drug delivery system (Figure 2.14 (b)), the HPMC still maintained this characteristic of greater hardness when compared to PEO.

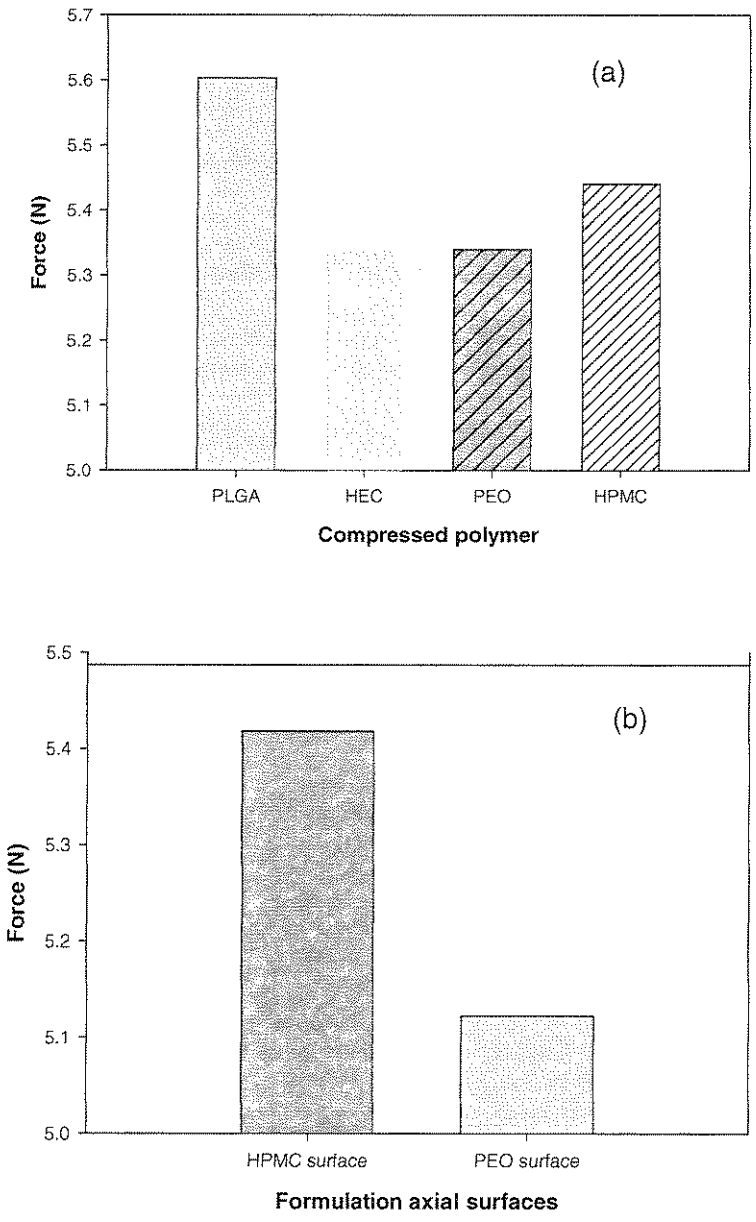


Figure 2.14. *Hardness tests on a) compressed polymer and b) the axial planes of the formulation (N=3; in all cases SD<0.2).*

A measure of gel strength can be used to further characterize drug release kinetics from swellable matrices. In Figure 2.15, a greater propensity for fluid uptake is displayed by PEO when compared to HPMC. The greater relaxation of PEO polymer chains is typified by the lower gel strength in the first 8 hours of contact with the solvent. PEO would have formed pores within its matrix, allowing solvent to reach disk D1 first. The drug in D1 was suspended in a PLGA matrix, which is hydrophobic. However, the 1:1 ratio of polymer to drug allowed some drug particles to reside on the surfaces of the disk. These drug particles would have dissolved out into the dissolution medium, creating pores in the PLGA matrix, which allowed further leaching-out of drug, leaving a pore-filled PLGA matrix behind. This process coincides with the dissolution profile (Figure 2.11) within the first 16-17 hours of upcurving drug release phase.

HPMC's glassy-rubbery transition on initial contact with solvent creates a gel layer that acts as a barrier to water transport. In this study, HPMC exhibited a decreased gel strength after the 16-17 hour time period, after which we assume that the polymer chains had sufficiently relaxed to allow polymer erosion. In the formulated tablet configuration, the HPMC layer acted as a protective layer for D2 which had drug suspended in HEC in a 1:1 ratio. However, one side of this disk was also in contact with a PEO layer which separated D2 from D1. It is possible that the accelerated release kinetics after 16-17 hours are a combination of drug leaching out through the PEO pores on one side of D2 (in contact with PEO) as well as the slow erosion and growth of the gel front created by HPMC, allowing drug to diffuse out from the other side of D2 (in contact with HPMC).

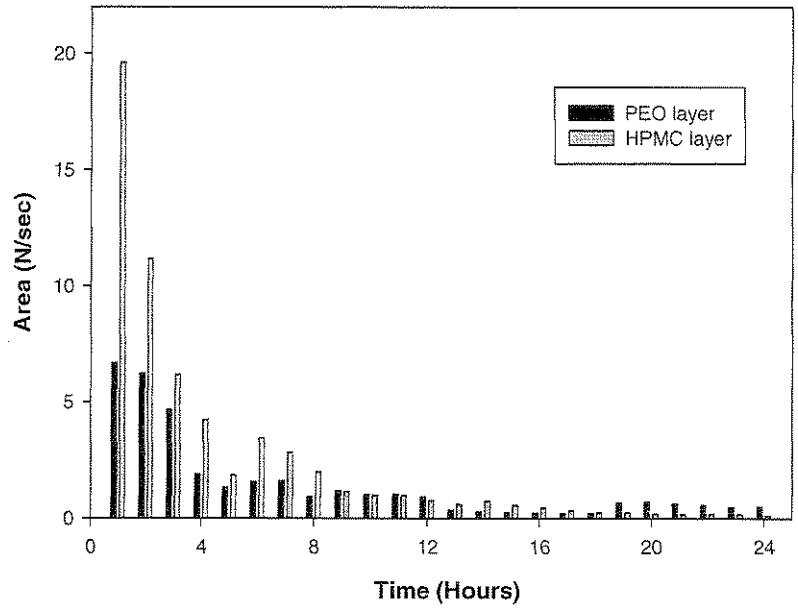


Figure 2.15. *Gel strength transitions measured on both axial planes of the tablet configuration (PEO layer and HPMC layer), (N=3; in all cases SD<0.5).*

In Figure 2.15, it is interesting to note that after a steady decrease, the gel strength transitions of PEO displays a slight increase in the last few hours of the investigation period. Interpenetrating polymer networks (IPNs) are a class of blended polymers that are usually crosslinked. In this way, properties from one polymer may be imparted to another (Bajpai et al., 2008). It is postulated that after drug had leached out from D1, the dissolution medium moving between the pores formed in the PLGA disk and the PEO, as well as the study temperature conditions (37°C) may have initiated a complex reaction between the two polymers, causing the PEO to take on some of the hydrophobic properties of PLGA, resulting in matrix stiffening and increased gel strength. Another possibility is that as a result of prolonged exposure to the ionic buffer medium, electrolyte interactions within the PEO matrix may have caused matrix stiffening (Pillay and Fassihi, 2000).

2.4. Concluding remarks

In this section, a triple-layer double-disk tablet configuration for oral drug delivery was successfully developed. The technique of suspending two drug-loaded disks within a polymeric configuration matrix of varying hydrophobic and hydrophilic properties resulted in triphasic drug release. These phases consisted of an initial lag phase preceding drug release, a second phase of steadily increasing drug release and thirdly, a phase of accelerated drug release. An analysis of the drug release kinetics from this configuration included *in vitro* dissolution as well as measures of hardness, swelling and textural profiling. The resulting drug release profiles that displayed different phases of drug release have implications for the potential chronotherapeutic application of this configuration. The use of compression techniques also made the manufacturing process simple and practical.

This report outlines an approach to formulating a heterogeneous dosage form based on an intricate blend of polymers to obtain appropriate release kinetics. With most products that are either in the research phase or on the market, there is a significant lack in terms of obtaining the required release phases necessary in synchrony with the body clock. Moreover, these products are complex and employ several formulating techniques and technology to affect appropriate kinetics.

SECTION 3

CONCLUSIONS AND RECOMMENDATIONS

3.1. Conclusions

This study set out to create a novel triple-layer double-disk tablet configuration for oral administration that will release drug in a phasic manner, which can have application as a chronotherapeutic drug delivery system. This polymeric system was successfully prepared using simple, cost-effective direct compression methods. The use of a combination of hydrophobic and hydrophilic polymers resulted in a complex, composite hydrogel system from which the mechanisms of drug release were swelling-controlled and diffusion-controlled. All formulations from the Extreme Vertices Design and optimization resulted in triphasic release kinetics. A lag phase of 2-3 hours was followed by upcurving drug release, which was then followed by an accelerated release phase that was characterised by a convex-like profile in dissolution studies. Investigations into polymer matrix hardness, swelling/erosion characteristics by the extent of degree of fluid uptake and gel strength were used to further define the drug release kinetics. The resulting formulation has potential in the field of chronopharmaceutical delivery systems, where patterned drug release is critical to successful application. Given the fact that pharmaceutical research into new active pharmaceutical ingredients (APIs) is a costly exercise, novel delivery systems such as this one can provide new applications for 'older' drugs like theophylline, and can enhance their use by applying different approaches to timed-therapy. This device also displayed reproducibility which, along with the cost-effective direct compression methods, makes this formulation suitable for scale-up to production batches.

3.2. Recommendations

Further studies can assess the composition of the polymeric configuration design and potential formulation changes can be made to enhance drug bioavailability. The

remaining drug was present in D2, around which HPMC acted as a protective polymer layer. The possibility of changing the quantity or varying grades of HPMC may be considered, as well as selection of an entirely different polymer. Core erosion ratios have been shown to increase when hydrophilic components in a compression-coated core are increased. In light of this, the polymer (HEC) suspending the drug in D2 can be investigated. Blending HEC with a different hydrophilic polymer is a potential alternative. A study using a similar design concept but using one drug disk employed electrolyte interactions within the polymer matrices to enhance release patterns. In some disease pathophysiologies like hypertension, previous research into the benefits of dual drug therapy has been established. This presents further potential for the present research since the design consists of two drug disks and further studies using two different drugs may be explored. Animal studies using the proposed novel delivery system in this study may also be explored as a pre-clinical component to this work.

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