In Vitro and *In Vivo* Evaluation of an Oral Multi-Layered Multi-Disk Tablet for Specialized Chronotherapeutic Drug Delivery

Zaheeda Khan [1], Mershen Govender [1], Sunaina Indermun [1], Pradeep Kumar [1], Yahya E. Choonara [1], Lisa C. du Toit [1], Leith C.R. Meyer [2] and Viness Pillay [1,*]

[1] Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 7 York Road, Parktown, 2193, South Africa

[2] Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

***Corresponding Author:**
Professor Viness Pillay
Tel: +27-11-717-2274
Fax: +27-11-642-4355
Fax2Email: +27-86-553-4733
Email: viness.pillay@wits.ac.za

ABSTRACT

Chronotherapeutic disorders such as hypertension, cardiovascular disease and asthma commonly involve the use of controlled zero-order release formulations. Ideally, the required drug should be released at predetermined rates with two or more pulses released from the dosage form. This ultimately exposes the patient to drug only when required, reducing the number of dosages, reducing side-effects and increasing patient compliance. The aim of this research was to evaluate two Multi-Layered Multi-Disk Tablet (MLMDT) systems incorporating drug-loaded disks enveloped by three polymeric layers. The model drugs used for each system were theophylline and diltiazem hydrochloride. The proposed chronotherapeutic system was designed to provide a lag phase and then two pulses of drug release separated by a 'switch-off' phase. *In vitro* drug release analysis revealed that the MLMDT generated a lag phase or a 'switch-off' phase followed by two pulses of drug release over the evaluated 24 h period. *In vivo* testing was undertaken using a Large White Pig Model, where concentration analysis from the evaluated conventional products revealed increasing plasma concentrations up to 2 h followed by a steady decline in concentration while the developed MLMDT displayed two pulse prolonged drug release profile separated by a switch-off phase.

Keywords: Chronotherapeutics; Multi-Layered Multi-Disk Tablet; Drug Delivery; Switch-off phase.

1. INTRODUCTION

Chronotherapeutics is a branch of drug therapy that involves synchronizing drug application in a manner that matches circadian rhythms in order to achieve optimal therapeutic success [1]. A major objective of chronotherapy is to deliver drug in higher concentrations during the times of greatest need [2]. Conventional zero-order controlled release drug delivery systems used in the treatment of chronotherapeutic conditions are traditionally 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 the drug release within a 24 h period is in synchrony with the biological determinants of the disease [3]. Therefore, to meet these requirements, pulsatile drug release is beneficial so that drug may be released after a lag-phase at predetermined time intervals [4-6]. 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.

This study therefore evaluates a Multi-Layered Multi-Disk Tablet (MLMDT) for use in chronotherapeutic disorders. The MLMDT system incorporates two drug-loaded disks enveloped by three polymeric layers and 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 the switch-off phase no drug is released from the system. In this manner, the patient is exposed to drug only when required resulting in increased, more effective therapeutic outcomes for the patient. The timing of drug administration is such that drug is only released when required in a 24 h period, implies that the patient is not continually exposed to drug thereby reducing side-effects. Two MLMDT systems, incorporating different actives, have been prepared and evaluated in this study to determine the versatility of the MLMDT system for chronotherapeutic drug delivery.

Magnetic Resonance Imaging was used to characterize the MLMDT *in vitro* while *in vivo* drug release studies were performed using a Large White Pig Model. It is well established that humans share a similar gastrointestinal anatomy and physiology to pigs, allowing for a suitable drug release correlation between pigs and humans to be developed [7-8]. These physiological properties include similarities in metabolism, biotransformation, feeding patterns, dietary habits, kidney function and structure [9]. A correlation between the MLMDT and currently available commercial products has also been performed to ascertain the benefit of using the MLMDT over the conventional unmodified products. Pharmacokinetic modelling as well as Level A IVIVC analysis has also been undertaken to further

characterize the release the actives from the MLMDT in addition to determining its accuracy and reproducibility under physiological conditions.

2. MATERIALS AND METHODS

2.1. Materials

Hydroxypropylmethylcellulose (HPMC), Surelease® aqueous dispersion and Sureteric® aqueous dispersion were purchased from (Colorcon Limited, Kent, United Kingdom). Hydroxypropylcellulose was purchased from EF Pharm Hercules Inc. (North Carolina, USA). Hydroxyethylcellulose (HEC, viscosity 4000 mPas) was purchased from Merck (Schuchardt, Hohenbrunn, Germany). Polyethylene oxide (Polyox N12K®; M_w= $1x10^6$ g/moL) and sodium bicarbonate was purchased from Dow Chem. Corp. (Michigan, USA). Poly (lactic-co-glycolic acid) (PLGA, Resomer RG504; M_w=55,000 g/moL) was purchased from Boehringer Ingelheim GmbH (Ingelheim, Germany). Pectin was purchased from Herbstreith and Fox (Neuenburg Germany). Ethylcellulose (48-49.5 % ethoxy content), Polyvinyl alcohol (PVA, M_W=49,000 g/moL), Theophylline (TPH) and Diltiazem hydrochloride (DTZ) was purchased from Sigma-Aldrich (Missouri, USA). All other reagents used were of analytical grade and used as received.

2.2. Preparation of the MLMDT system

The MLMDT was prepared as outlined previously by Khan et al. [10]. Briefly, 2 drug discs (D1 and D2) and 3 polymeric layers (L1, L2 and L3) were compressed together to form the MLMDT as depicted in Fig. 1. D1 constituted of lactose (160 mg) and 30 % of the total drug content compressed at 5 tons using a Karnavati Mini Press II (Rimek Products, Gujarat, India) equipped with a punch and die of 10 mm diameter. D2 consisted of a blend of hydroxyethylcellulose (HEC), ethylcellulose (EC) and the remaining 70 % of the drug prepared with a cube blender (Erweka Apparatebau, Heusenstamm, Germany), which was thereafter wet granulated using Surelease® and Sureteric® solutions as the granulating fluid. The formulated mass was passed through a 2 μm aperture sieve, collected and placed in an oven at 37˚C until dry. The dried granules were thereafter compressed at 5 tons as described previously. Pectin (150 mg) was used in L1 with hydroxypropylmethylcellulose (HPMC) used in L2 and L3. The Theophylline (TPH) MLMDT consisted of HEC and EC at a ratio of 1:3 (HEC: EC) in D2. The Diltiazem hydrochloride (DTZ) MLMDT consisted of HEC and EC at a ratio of 1:3 (HEC: EC) with sodium bicarbonate (50 mg) included in D2, L1 and L2. Sodium bicarbonate was utilized to confer a matrix-stiffening effect [10] on the dosage form and control the release rate of DTZ as demonstrated previously by Pillay and Fassihi [11]. The MLMDT was prepared using a Beckman hydraulic tablet press (Beckman

Instruments, Inc., Fullerton, CA, USA), fitted with a flat-faced punch and die of 13 mm diameter. The MLMDT was prepared in the orientation as depicted in Fig. 1 by adding the polymeric powder layer for L3 to the punch and die prior to centrally placing D2. The polymeric powder of L2 was thereafter added prior to D1 being centrally placed. The polymeric powder of L1 was finally added prior to the compression of the discs and layers to form the MLMDT system.

INSERT FIGURE 1 HERE

2.3. *In vitro* drug release analysis of the MLMDT

The *in vitro* drug release analyses were carried out on the MLMDT using USP dissolution apparatus II (Erweka, Heusenstamm, Germany) rotating at 50 rpm at a temperature of 37 °C. Dissolution was performed in simulated human gastric fluid (SHGF; pH 1.2) for the first 2 h. The SHGF was prepared as per USP guidelines. Briefly, 2 g NaCl was added to 3.2g pepsin and 7 mL hydrochloric acid diluted to 1 L of autoclaved distilled water. The final solution was adjusted using hydrochloric acid to a pH of 1.2. Thereafter the *in vitro* drug release analysis was carried out in simulated human intestinal fluid (SHIF; 900 mL) at pH 6.8 for the remainder of the study. Sampling of 5 mL was carried out every 1 h for 12 h and thereafter at 24 h. Fresh buffer (5 mL) was replaced after each sampling point to maintain physiological sink conditions. All drug release samples were analyzed using an Ultra Violet spectrophotometer (Hewlett Packard 8453, Boeblingen, Germany) at λ280nm for TPH [12] and λ238 nm for DTZ [13] with the drug concentration in solution calculated utilizing pre-constructed calibration curves (ε = 48.79 and 51.99 for TPH and DTZ respectively). This analysis was repeated using the commercial TPH and DTZ products prior to *in vivo* studies. The commercial TPH-containing product is a controlled-release tablet whereas the commercial DTZ-containing product is a gelatin capsule loaded with controlled release beads.

2.4. Magnetic Resonance Imaging of the MLMDT

A magnetic resonance imaging system (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 and swelling behaviors of the respective components of the MLMDT. Each component was placed in position within the cell, which was thereafter positioned within the magnetic bore of the system. Magnetic resonance images were acquired every 30 min over a 13 h period with MARAN-iP Version 1.0 software.

2.5. *In vivo* studies

2.5.1. Surgical insertion of the intra-jugular catheter

Large White pigs (30-35 kg; n=5) were each anaesthetized with ketamine (11 mg/kg) and midazolam (0.3 mg/kg) prior to surgery and maintained under 2% isoflurane in 100% oxygen for the duration of the procedure. Briefly, under aseptic conditions, a 7 French gauge double lumen 35 cm catheter (CS-28702) (Arrow Deutschland GmdH, Erding, Germany) was surgically inserted into the left jugular vein by making an incision into the vein dorsal to the jugular groove on the left lateral aspect of the neck [14-15]. The lumen of the catheter was fastened to the wall of the vein using a purse suture technique. The remaining length of the catheter was tunneled subcutaneously to an exit point cranial to the dorsal aspect of the scapular. Blood was removed via the catheter and the catheter was flushed and thereafter maintained with heparinized saline (1000 IU in 1 L 0.9% saline) [14-15]. Buprenorphine (0.05 mg/kg) and carpofen (4 mg/kg) were administered for analgesia and inflammation. Each pig was allowed 10 days to recover from the surgical procedure prior to *in vivo* analysis of the MLMDT.

2.5.2. Oral administration of MLMDT and comparative commercial systems

Large White Pigs (n=5) were administered with the TPH-loaded MLMDT via a gastric tube. Prior to insertion of the gastric tube, each pig was sedated with intramuscular midazolam (0.3 mg/kg) and anesthetized with ketamine (4 mg/kg). The animal was then maintained under anesthesia by gaseous administration of 2% isoflurane in 100% oxygen via a face mark. The pigs were then placed in an upright position and intubated with a bore gastric tube through the esophagus and into the stomach. The MLMDT was inserted through the tube and then flushed down to the stomach with ±25 mL of water. All pigs were fasted 12 h prior to administration of the MLMDT. This dosing procedure was repeated on the same 5 Large White pigs using the DTZ-loaded MLMDT, the TPH commercial product and the DTZ commercial product. The crystalline forms of the TPH and DTZ in the commercial products were kept consistent with that of the MLMDTs. As a control, a placebo MLMDT containing no active drug was administered as per the above dosing procedure. Adequate time (based on the plasma half-life of the TPH and DTZ) was given between each dosing to allow for complete ‘washout’ of the respective drug from the pig prior to the next dosing.

2.5.3. Collection of blood samples

Blood samples (5mL) were collected via the intra-jugular catheter at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20 and 24 h for the respective MLMDTs and at 2, 4, 6, 8, 10, 12, 16, 20 and 24 h from pigs dosed with commercial formulations. At each sampling point, the blood samples were immediately transferred into heparinized vacutainers (BD Vacutainer®, New

Jersey, USA) and centrifuged (Nison Instrument Limited, Shanghai, China) at 10 0000 rpm (5957 G) for 15 min. The clear plasma supernatant was pipetted using an adjustable volume micropipette (Boeco GmbH, Hamburg, Germany) and stored at −70 °C until further analysis.

2.5.4. *In vivo* release sample analysis

2.5.4.1. UPLC 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 all blood samples. Separation was achieved on an Acquity UPLC BEH RP 18 column (100 mm x 2.1 mm i.d., 1.7 µm particle size) maintained at 25 °C. Water and Acetonitrile at a ratio of 55:45 were used as Mobile Phase A and Mobile Phase B respectively with methylparabens (MP; 5 µg/mL) as the internal standard. An injection volume of 2.5 µL was injected in the mobile phase at a flow rate of 0.500mL/min. A total run time of 1.5 min was required at a wavelength of 238nm for DTZ and 280nm in the case of TPH. Plasma TPH and DTZ were thereafter calculated using pre-constructed calibration where ε = 0.0278 and 0.21 for TPH and DTZ respectively. Fig. 2 depicts a typical UPLC chromatograph for TPH and DTZ. All samples were prepared prior to analysis using a validated Liquid-Liquid plasma extraction procedure.

2.6. Pharmacokinetic Modeling and Establishment of an *In Vitro-In Vivo* Correlation

Pharmacokinetic modeling of the generated *in vivo* data was achieved using PKSolver to undertake non-compartmental and compartmental analysis of the *in vivo* plasma DTZ and TPH release data [16]. The ideal model for prediction of the *in vivo* release data was determined through use of AIC and Schwarz's Bayesian Criterion (SBC). R software (V3.4.1 with an IVIVC package, R Foundation for Statistical Computing, Vienna, Austria) was employed for the establishment of a Level A *in vitro-in vivo* correlation (IVIVC) for DTZ and TPH release. Input data for the IVIVC included *in vitro* DTZ and TPH release data in addition to the relevant pharmacokinetic data. Deconvolution and convolution of the release data was thereafter undertaken for the development and evaluation of an IVIVC model.

3. RESULTS AND DISCUSSION

3.1. *In vitro* drug release from the MLMDT and commercial products

In vitro drug release studies were undertaken on the MLMDTs containing TPH and DTZ as well as the currently available TPH and DTZ commercial products to form a suitable correlation prior to *in vivo* studies. The commercial TPH product presents itself as a controlled-release tablet whereas the commercial DTZ product is a gelatin capsule loaded with controlled release beads. A comparison of the rate of drug release between the drug-

loaded MLMDT and the comparator products for both TPH and DTZ are outlined in Fig. 2. Observation of the TPH drug release profiles (Fig. 2a) 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 h and remains constant for the remainder of the 24 h study indicating that the patient requires more than one dose in a day to maintain an adequate therapeutic response. The TPH-loaded MLMDT exhibits the desired release profile and provides a lag phase and first pulse of drug release (Fig. 2a). This first pulse continued for duration of 3 h followed by a 'switch-off' phase of approximately 6 h. Thereafter, a second pulse of controlled drug release was achieved for the remainder of the 24 h study with a FDR of 0.28, 0.40, 0.61, 0.68 and 0.72 at 8 h, 12 h, 16 h, 20 h and 24 h observed respectively. 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 and increases patient compliance.

Drug release from the DTZ conventional produced a controlled release profile over the test period with a similar fractional drug release after 24 h when compared to MLMDT containing DTZ (Fig. 2b). Evaluation of the commercial DTZ formulation depicted a controlled release of drug for the first 12 h. Thereafter, a 5% increase in FDR between Hour 12 and Hour 24 is observed. This also, as determined with the commercial TPH product, signifies that the patient may require more than one dose within a 24 h 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 h. This was followed by a 'switch-off' phase of 8 h and subsequently, a second pulse of drug release between 12 h and 24 h where a FDR of 0.36, 0.56, 0.81 and 0.93 was observed at 12 h, 16 h, 20 h and 24 h, respectively. The results of the *in vitro* drug release therefore determined that the MLMDT effectively delivered both TPH and DTZ at effective concentrations over a 24 h period when compared to its commercially available controlled-release comparator products.

INSERT FIGURE 2 HERE

3.2. Magnetic Resonance Imaging of MLMDT's performance

Magnetic Resonance Imaging (MRI), which covers the distribution and dynamics of a species within a material based on their nuclear magnetic resonance effects, was employed to observe and confirm the chronotherapeutic behaviors and performance of the MLMDTs during dissolution studies [17-18]. Fig. 3 exhibits the images obtained for the TPH-loaded MLMDT under continuous solvent flow conditions while Fig. 4 displays the images obtained for DTZ-loaded MLMDT.

Analyses of the MRI images of the TPH-loaded MLMDT (Fig. 3a) depicted Layer 1 of the gelling faster than Layers 2 and 3. As the MLMDT gelled, no drug release was observed (Fig. 3a-c). After 2.5 h, media penetrated the MLMDT (Fig. 3d) hydrating the inner layers. Based on the dissolution data achieved (Fig. 2a) complete drug release from Disk 1 occurred after 3 h (Fig. 3e) while complete erosion of Disk 1 occurred after 6 h (Fig. 3f). 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 Disk 1 across the swollen outer layer into the dissolution media. Disk 2 was protected from the surrounding media due to the highly viscous nature of HPMC contained in Layers 2 and 3 that prevented buffer penetration at the layer-disk interface. Disk 2 was fully visible after 11 h (Fig. 3g) due to the ingress of buffer into the MLMDT. Drug release from Disk 2 occurred thereafter due to the decrease in the viscosity of the surrounding gel after 12 h (Fig. 3h), thus facilitating drug movement from the core into the media. The erosion and swelling properties of the MLMDTs have been reported previously [10].

INSERT FIGURE 3 HERE

The DTZ-loaded MLMDT system generated CO_2 due to the inclusion of sodium bicarbonate in the system. This liberation of CO_2 resulted in floatation of the system (Fig. 4a-d). Layer 1 swelled more than the other layers upon contact with the media (Fig. 4b). Penetration of buffer into the system happened after 1 h (Fig. 4b-c). Disintegration of the PEC layer (Layer 1) occurred after 3.5 h (Fig. 4d-e) with complete hydration of Layers 2 and 3 occurring after 8 h (Fig. 4f). Although the matrix around Disk 2 was hydrated no drug release occurred due to a ‘matrix-stiffening’ effect generated by sodium bicarbonate resulting in a ‘switch-off’ phase (as seen in drug release profile Fig. 2b). Electrolytes have been determined previously to exert a rate-controlling effect on polymeric drug delivery systems [19]. This effect diminished after 11 h (Fig. 4g-h) and drug release commenced for the remainder of the 24 h.

INSERT FIGURE 4 HERE

3.3. *In vivo* animal studies

3.3.1. *In vivo* drug release analysis

In vivo drug release profiles of both TPH-loaded and DTZ-loaded MLMDT’s displayed immediate drug release during the first hour followed by a decrease in the rate of drug release after 2 h due to elimination of the respective drug (Fig. 5). This initial burst of drug release corresponds to the first pulse of release emanating from Disk 1, though no initial lag

phase was observed as seen during the *in vitro* release analysis. The decrease in drug concentration after 2 h corresponds to the 'switch-off' phase separating the two pulses. The 'switch-off' phase was, however, not as pronounced as demonstrated from the *in vitro* release data. This could be attributed to the concurrent metabolism of the drug in the pig's body and even though no drug is released into the blood during the 'switch-off' phase, drug is nonetheless present in the blood and undergoing elimination, thereby resulting in a distinct, pronounced 'switch-off' phase being absent. Thereafter, the second pulse is observed with the TPH-loaded MLMDT reaching peak plasma concentration (T_{max}) after 6 hand DTZ-loaded formulations attaining a maximum concentration of 887.46ng/mL at a T_{max} of 5 h. In both MLMDT formulations, release of the respective drug was constant with plasma drug concentrations maintained with an acceptable range. Drug plasma concentrations after 24 h were determined to be ~22 mg/mL and ~620 ng/mL for TPH and DTZ respectively. In both *in vivo* release analyses, drug-loaded MLMDT's displayed a two-phase release although the 'switch-off' phase was not as distinct when compared to the *in vitro* release data obtained. A decrease in release rate is however seen between hour 1 and hour 2 for both the TPH-loaded and DTZ-loaded MLMDT systems, which can be attributed to the switch-off phase, however a horizontal profile is not achieved at this interval, which could be due to a delay in the absorption of the actives from the gastrointestinal system.

In vivo release analysis of the commercial comparative TPH and DTZ products detailed significant differences when compared to the MLMDT. Evaluation of the commercial TPH product (Fig. 5a) detailed a significant spike in plasma TPH concentration with T_{max} occurring after 2 h. TPH plasma concentration then drops to ~32 mg/mL at Hour 12 and is maintained to ~ 29.5 mg/mL after 24 h. Evaluation of the commercial DTZ product noted significantly different results (Fig. 5b). This formulation, as with the commercial TPH product, provided a large spike in plasma DTZ concentration with T_{max} occurring after 2 h. Plasma DTZ concentrations then drop rapidly due to elimination, with the plasma DTZ concentration being ~375 ng/mL and ~160 ng/mL after 12 and 24 h respectively. Analysis of the commercial products therefore detailed the decreased ability of these formulations to control the release of drug and maintain drug plasma concentrations over a 24 h period after administration when compared to the MLMDT system. This was significantly seen in the DTZ commercial product where limited release control was determined (drug plasma range of 280-1360 ng/mL compared to the drug plasma range of 630-890 ng/mL for the MLMDT system). The large initial spike noted in both formulations is also of concern due to the active ingredient and therapeutic indications of these products. Drug plasma concentrations were seen to be within a smaller range when delivered through the MLMDT system therefore detailing the ability of the system to deliver drugs with a narrow therapeutic window. The

drug plasma range for the TPH-loaded MLMDT system was determined to be 20-37.5 mg/mL compared to the conventional product which had a drug plasma range of 31.5-49.5 mg/mL. The MLMDT could therefore be more beneficial when compared to these 2 products for not only for its chronotherapeutic benefits but also for its controlled release nature as well. All animals were healthy prior to the study, variation in the delivery system to treat specific chronotherapeutic disorders can be achieved using the statistical design undertaken in Khan et al. [10].

INSERT FIGURE 5 HERE

3.4. Pharmacokinetic Modelling of the MLMDT

Pharmacokinetic modelling of the generated *in vivo* release data revealed that both the DTZ and TPH MLMDTs conformed best to a one compartmental pharmacokinetic model without lag where the most favorable AIC and SBC values were determined (Table 1). The pharmacokinetic parameters for this model were noted to have a 1st order absorption rate constant of 0.721 1/h and 0.57 1/h, an elimination rate constant of 0.023 1/h and 0.034 1/h and an elimination half-life of 30.36 h and 20.54 h for DTZ and TPH respectively. The results of this analysis revealed significant increases in the plasma half-life of DTZ and TPH respectively detailing the controlled release of these actives under physiological conditions.

INSERT TABLE 1 HERE

3.4.1. Establishment of a Level A IVIVC

Level A IVIVC analysis yielded levy plots with a R^2 value of 0.863 and 0.881 for the DTZ and TPH MLMDTs, respectively. Thus *in vitro* data was predictive of *in vivo* data with an 86.3% and 88.1% accuracy for DTZ and TPH delivery respectively. The prediction errors (PE) for C_{max} of the DTZ MLMDT was determined to be 10.53%, the only prediction error within the 15% limit for a good correlation. The large prediction errors however can be attributed to variations in the modified release and absorptions of DTZ and TPH as well as variations in physiological conditions that could not be simulated during *in vitro* release studies.

4. CONCLUSION

The proposed MLMDT consists of two drug loaded disks enveloped by drug-free polymeric layers comprising of PEC/AVC, HPMC, EC and HEC was proven to be effective in the providing 2 pulses of drug separated by a ‘switch-off’ phase. Thermal analysis of the MLMDT determined thermal stability of its respective components with MRI analysis detailing the controlled hydration of the various portions of the formulation. In vivo evaluation of the

MLMDTs also detailed the ability of the formulation to provided controlled drug release after an initial spike in plasma concentration and a 'switch-off' phase over a 24 h period. In comparison with commercial TPH and DTZ products, the MLMDT was noted have a smaller initial spike in drug plasma concentration with a greater control of drug release over the test period. Pharmacokinetic modelling of the *in vivo* release data detailed that the release of DTZ and TPH was best described by a one compartmental model without lag with IVIVC analysis detailing a 86.3% and 88.1% accuracy between *in vitro* and *in vivo* release of DTZ and TPH respectively. This study therefore provides for an effective drug release system that can respond to the chronotherapeutic requirements of a patient providing drug release in a controlled manner after a 'switch-off' phase has been achieved.

Declaration of interest

The Authors confirm that there are no conflicts of interest.

Acknowledgements

This work was funded by the National Research Foundation (NRF) of South Africa.

Animal Ethics

The protocol of the study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand, South Africa (Ethics clearance number: 2007/56/04). The European Community guidelines as accepted principles for the use of experimental animals, are incorporated in the guidelines of the University of the Witwatersrand for use of animals in research. The guidelines were adhered to.

References

[1] I. Landau, A. Chabaud, G. Cambie, H. Ginsburg, Chronotherapy of malaria: an approach to malaria chronotherapy, Parasitol. Today. 7 (1991) 350-355.

[2] S. Sewlall, V. Pillay, M.P. Danckwerts, Y.E. Choonara, V.M.K. Ndesendo, L.C. du Toit, A timely review of state-of-the-art chronopharmaceuticals synchronized with biological rhythms, Curr. Drug Deliv. 7 (2010) 370-388.

[3] V.K. Pawar, R. Awasthi,Chronotherapy: an approach to synchronize drug delivery with circadian rhythm, J. Chronother. Drug Deliv. 1 (2010) 1-8.

[4] L.E. Kalantzi, E.Karavas, E.X. Koutris, D.N. Bikiaris, Recent advances in oral pulsatile drug delivery, Recent Pat. Drug Deliv. Formul. 3 (2009) 49-63.

[5] V.D. Kadam, S.G. Gattani, Development of colon targeted multiparticulate pulsatile drug delivery system for treating nocturnal asthma, Drug Deliv. 17 (2010) 343-351.

[6] K.P.S. Kumar, D. Bhowmik, C.M. Chandira, K.K. Tripathi, Innovations in sustained release drug delivery system and its market opportunities, J. Chem. Pharm. Res. 2 (2010) 349-360.

[7] R.L. Oberle, H. Das, S.L. Wong, K.K. Chan, R.J. Sawchuk, Pharmacokinetics and metabolism of diclofenac sodium in Yucatan miniature pigs, Pharm. Res. 11 (1994) 698-703.

[8] D.L. Anderson, F.D. Bartholomeusz, I.D. Kirkwood, B.E. Chatterton, G. Summersides, S. Penglis, T. Kuchel, L. Sansom, Liquid Gastric Emptying in the Pig: Effect of Concentration of Inhaled Isoflurane, J. Nucl. Med. 43 (2002) 968-971.

[9] B. Brunet, C. Doucet, N. Venisse, T. Hauet, W. Hébrard, Y. Papet, G. Mauco, P. Mura, 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 Sci. Int. 161 (2006) 169-174.

[10] Z. Khan, Y.E. Choonara, P. Kumar, L.C. du Toit, V.M.K. Ndesendo, V. Pillay, A Novel Multilayered Multidisk Oral Tablet for Chronotherapeutic Drug Delivery. BioMed Research International, Article ID 569470 (2013) 16 pages.

[11] V. Pillay, R. Fassihi, Evaluation and comparison of dissolution data derived from different modified release dosage forms: an alternative method, J. Control. Release. 55 (1998) 45-55.

[12] J.C. Reijenga, A. Gaykema, F.E.P. Mikkers, Determination of theophylline binding to human serum proteins by isotachophoresis, J. Chromatogr. A. 287 (1984) 365-370.

[13] V. Ascalone, M. Locatelli, B. Malavasi, Determination of diltiazem and its main metabolites in human plasma by automated solid-phase extraction and high-performance liquid chromatography: a new method overcoming instability of the compounds and interference problems, J. Chromatogr. B. 657 (1994) 133-140.

[14] M. Fudge, R.E. Coleman, S.B. Parker, A Minimally Invasive Percutaneous Technique for Jugular Vein Catheterization in Pigs, J. Am. Assoc. Lab. Anim. Sci. 41 (2002) 38-42.

[15] M. Štukelj, D. Mihelčič, J. Butinar, A. Nemec, J. Pečar, Surgical Intravenous Catheterisation of Pig, Slov. Vet Res. 42 (2005) 43-48.

[16] Y. Zhang, M. Huo, J. Zhou, S. Xie, PKSolver: An add-in programfor pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel, Comput. Methods Prog. Biomed. 99 (2010) 306-314.

[17] J.C. Richardson, J.W. Bowtell, K. Mäder, C.D. Melia, Pharmaceutical applications of magnetic resonance imaging (MRI), Adv. Drug Deliv. Rev. 57 (2005) 1191-1209.

[18] P.R. Laity, M.D. Mantle, L.F. Gladden, R.E. Cameron, Magnetic resonance imaging and X-ray microtomography studies of a gel-forming tablet formulation, Eur. J. Pharm. Biopharm. 74 (2010) 109-119.

[19] S. Vidyadhara, P.R. Rao, J.A. Prasad, Formulation and valuations of Propranolol hydrochloride oral controlled release matrix tablets, Indian J. Pharm. Sci. 66 (2004) 188-192.

FIGURES

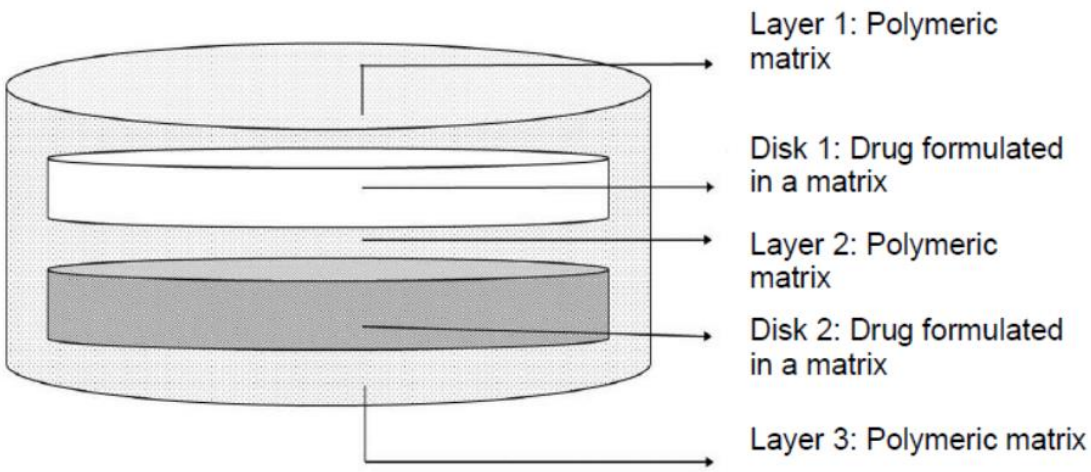

Fig. 1. A schematic representation of the formulated MLMDT.

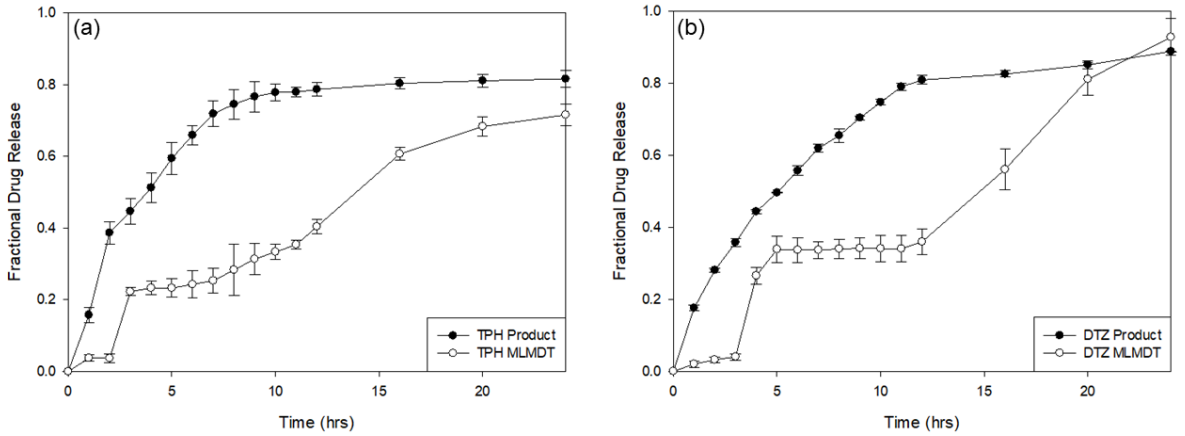

Fig. 2. *In vitro* drug release profiles of the drug-loaded MLMDT and commercial comparator product for (a) TPH and (b) DTZ (N=3; SD≤0.071 in all cases).

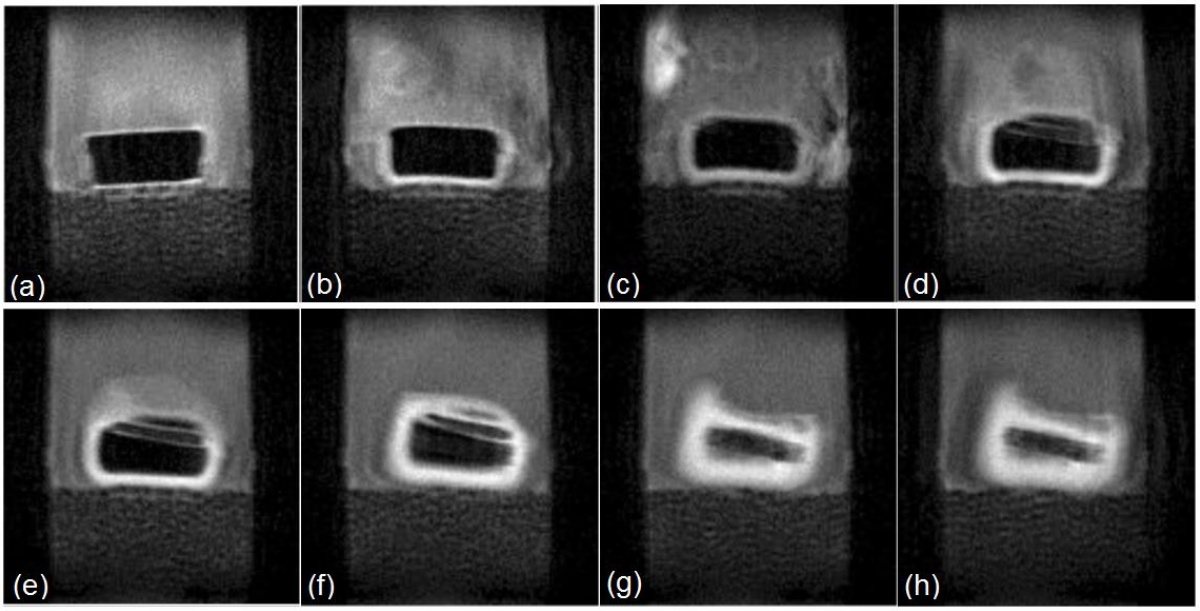

Fig. 3. MRI depicting the progression of hydration of a TPH-loaded MLMDT at (a) 0 h, (b) 1 h, (c) 2 h, (d) 2.5 h, (e) 3 h, (f) 6 h, (g) 11 h and (h) 12 h.

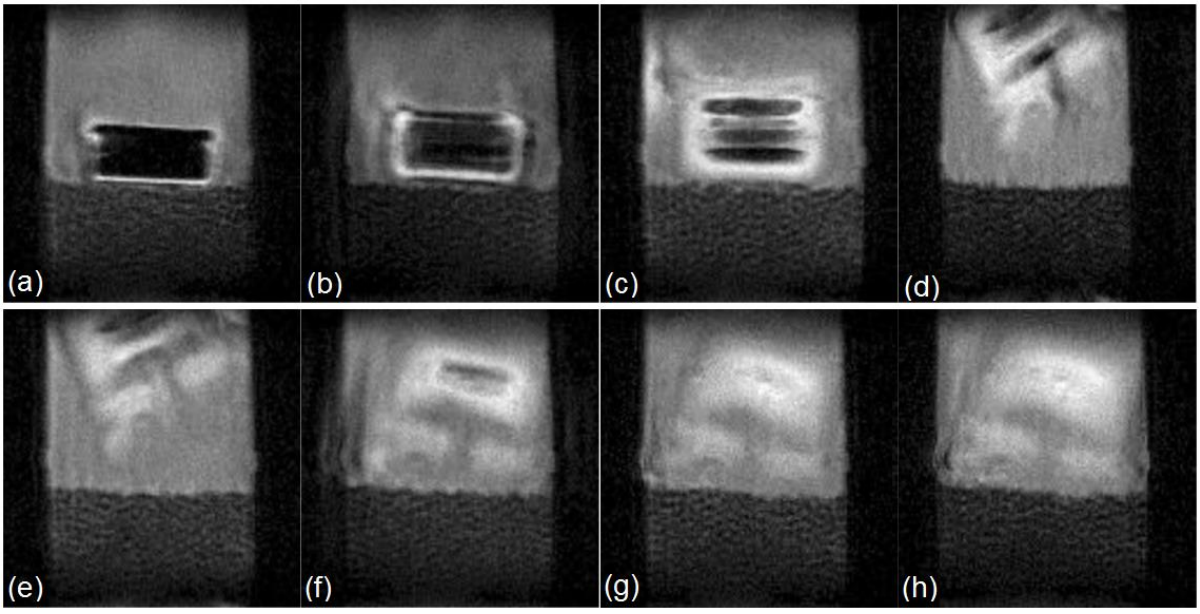

Fig. 4. MRI depicting the progression of hydration of a DTZ-loaded MLMDT at (a) 0 h, (b) 1 h, (c) 2 h, (d) 4 h, (e) 6 h, (f) 8 h, (g) 11 h and (h) 12 h.

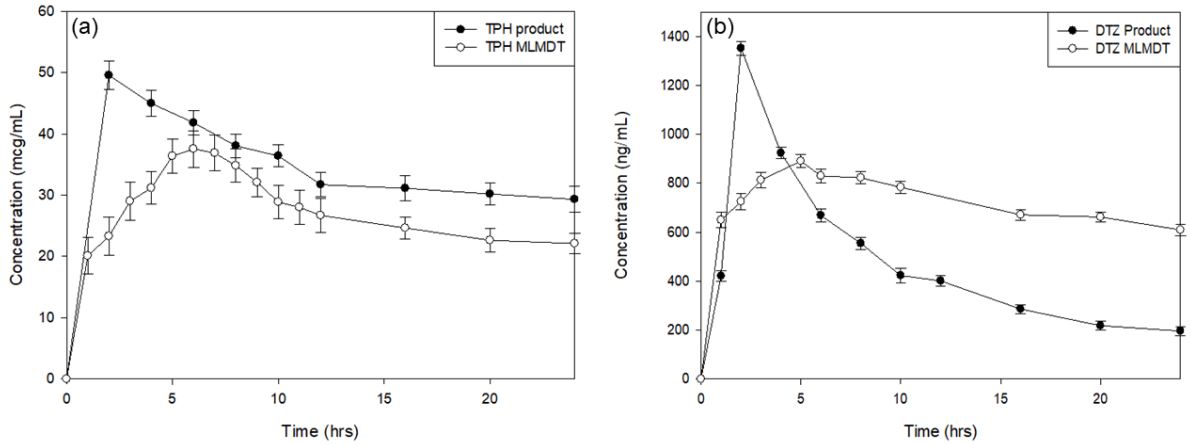

Fig. 5. *In vivo* plasma concentration-time profiles for a) the TPH MLMDT and the commercial TPH product (N=5; SD≤3.13 in both cases) and b) the DTZ MLMDT and the commercial DTZ product (N=5; SD≤34.14 in both cases).

TABLE

Table 1. Pharmacokinetic modelling of the *in vivo* DTZ and TPH release data

Model	DTZ		TPH	
	AIC	SBC	AIC	SBC
One Compartment without lag	146.52	147.97	78.23	80.55
One Compartment with lag	148.08	150.02	80.23	83.32
Two Compartment without lag	150.10	152.52	78.84	82.70
Two Compartment with lag	151.74	154.65	80.84	85.48

Graphical Abstract