THE DEVELOPMENT AND ASSESSMENT OF BOTH A SEPARATE, ONCE-DAILY MODIFIED RELEASE MATRIX FORMULATION OF METOPROLOL TARTRATE AND A COMBINATION FORMULATION WITH HYDROCHLOROTHIAZIDE

A Thesis Submitted to Rhodes University in

Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

By

Jessica Arjun

December 2001

Faculty of Pharmacy
Rhodes University
Grahamstown

ABSTRACT

The use of controlled release dosage forms has increased significantly in recent years as they result in increased patient compliance and higher therapeutic efficiency. This research focused on the development of a once daily dosage form that could be used for the treatment of hypertension. Both a separate sustained release dosage of metoprolol tartrate and a combination dosage form that included both an immediate release hydrochlorothiazide and a sustained release metoprolol component, were developed and evaluated. A matrix tablet, consisting of an ethylcellulose granulation of metoprolol tartrate compressed into a hydrophilic hydroxypropyl methylcellulose polymer matrix, effectively sustained metoprolol release over a 22-hour experimental period. A multiparticulate combination dosage form that consisted of six coated mini matrix tablets of metoprolol and a powder blend of hydrochlorothiazide packed into a gelatin capsule, displayed zero order release kinetics for metoprolol release over 22 hours (r^2=0.9946). The release of hydrochlorothiazide was found to be comparable to that of a commercially available product tested.

Differential Scanning Calorimetry was used to identify possible incompatibilities between MPTA and excipients initially, and long term stability testing was used to assess to behaviour of the dosage form. Dissolution testing of the dosage forms was performed using USP Apparatus III, which was found to be more discriminating between the batches assessed. Dissolution curves were evaluated for similarity and difference using f1 and f2 fit factors. Samples were analyzed using a high performance liquid chromatographic method that was developed and validated for the simultaneous determination of the compounds of interest.

Various factors influencing drug release from the developed dosage forms were assessed and recommendations for further optimization of the formulation are made. Factors evaluated included

the quantity of granulating fluid, matrix polymer content, drug load and process variables, including drying time and compression force. The influence of various coating levels on drug release was assessed and none of the levels assessed were found to adequately retarded drug release over a 22-hour period. Combinations of tablets coated to different levels allowed for the successful development of a sustained release metoprolol component, which could be included into the combination dosage form.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to the following people:

My supervisor, Prof R.B.Walker, for his guidance, patience and assistance throughout the course of my studies.

The Dean and Head, Professor I. Kanfer and the staff of the Faculty of Pharmacy for the use of departmental facilities.

Mr Andy Soper, for his assistance in the laboratory.

The National Research Foundation and the Joint research Council (Rhodes University) for financial support.

The Dow Chemical Company (Michigan, USA), and Colorcon (Kent, UK) for their donation of certain excipients.

My colleagues in the laboratory for their support, in particular Jennifer Edmonstone (nee Verner) and Leith Kieser.

Dr Bharathi Avula, for her support, motivation and assistance in various aspects of my work.

Ms Victoria Longshaw, for her support and friendship and willingness to assist when needed.

My parents for their support, particularly my mother whose understanding and encouragement are greatly appreciated.

Vickesh Dhookie, for his support, encouragement, assistance and particularly, for his patience throughout this period.

STUDY OBJECTIVES

Metoprolol is a beta-blocker that is commonly prescribed for the treatment of hypertension, angina pectoris and more recently for the treatment of congestive heart failure. Hydrochlorthiazide is a thiazide diuretic that is indicated as first line therapy for the treatment of hypertension in many countries, including South Africa, where it has been included in the Essential Drugs List. A combination of these drugs has been reported to be more beneficial than increased doses of either drug used alone. Metoprolol and hydrochlorothiazide exhibit a synergistic relationship, in which hydrochlorothiazide acts to decrease sodium and water retention caused by metoprolol, and the beta-blocker serves to counter-act the increased renin levels caused by the diuretic. This combination is therefore particularly advantageous in elderly or black patients that have low renin levels, and may therefore be particularly advantageous in a country such as South Africa where the majority of hypertensive patients fall into this category. Furthermore, a rapid decrease in blood pressure caused by an immediate release hydrochlorothiazide component followed by sustained release of metoprolol over a twenty-four period may be even more advantageous. A sustained release product of metoprolol only may also be advantageous for patients on beta-blocker monotherapy, and metoprolol sustained release products have been found to lower blood pressure more effectively than conventional dosage forms.

The objectives of this study were therefore:

1. To develop and validate a suitable High Performance Liquid Chromatographic (HPLC) method for the simultaneous determination of both metoprolol and hydrochlorothiazide in aqueous solutions
2. To develop a novel sustained release dosage form of metoprolol (100mg) that could be used as a stand-alone product or in a combination dosage form with hydrochlorothiazide (12.5mg).
3. To investigate the feasibility of using a matrix formulation developed in-house for sustaining the release of metoprolol tartrate over a 22-hour period.
4. To evaluate the release of both drug components from the dosage form developed, using an appropriate dissolution method.
5. To identify key aspects of the dosage form for further study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE
DRUG MONOGRAPHS

1.1 METOPROLOL TARTRATE

1.1.1 INTRODUCTION

Metoprolol is a synthetic beta$_1$-selective adrenoceptor antagonist [1,2]. The FDA approved metoprolol for the treatment of hypertension, in 1978 [3], and since then it has conventionally been used in hypertensives and in patients with ischaemic heart disease [4]. In recent years, however, the use of metoprolol has been extended to the treatment of stable angina and acute myocardial infarction. Metoprolol free base is highly lipophilic, therefore it is available either as succinate, fumarate or tartrate salts, which vary in solubility [1]. Only the tartrate salt will be discussed in further detail as this was selected for experimental work.

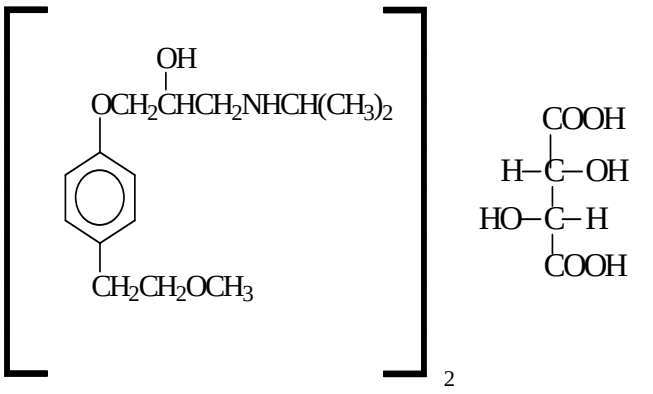
1.1.2 PHYSICO-CHEMICAL PROPERTIES

1.1.2.1 Description

Metoprolol tartrate (MPTA) is a white, practically odourless crystalline powder [1,5]. Metoprolol tartrate is a salt consisting of a racemic mixture of optical isomers of the base and *dextro*-tartaric acid in a 2:1 ratio [1].

MPTA may be described by several chemical names, which include [1]:

1. 2-propanol, 1-[4-(2-methoxyethyl) phenoxyl]-3-[(1-methylethyl) amino]-, (±)-, [R-(R*, R*)]-2,3-dihydroxybutanedioate (2:1) (salt)
2. (±)-1-(Isopropylamino)-3-[- (2-methoxyethyl)- phenoxy]-2-propanol L-(+)-tartrate (2:1) (salt)
3. 1-(isopropylamino)-3-[-(2-methoxyethyl)-phenoxy]-2-propanol (2:1) *dextro*-tartrate salt



Metoprolol Tartaric Acid

Figure 1.1: Structure of Metoprolol Tartrate $[(C_{15}H_{25}NO_3)_2.C_4H_6O_6]$ (MW = 684.82)

1.1.2.2. Dissociation Constant and Distribution ratio

MPTA has only one ionisable group, that is, a secondary amine. The dissociation constant (pKa) in water (ionic strength = 0.1) at 25°C has been reported by three separate sources to range between 8.9 –9.70 [1], however a pKa of 9.7 is quoted most often [6,7,8]. The pKa values for tartaric acid are 2.93 and 4.23 at 25°C [1]. In comparison to other β-blockers, metoprolol is more lipophilic than most other β-blockers, except for propranolol, which exhibits the largest degree of lipophilicity [4], as indicated by the permeability coefficient (Log Kp) in Table 1.1. Consequently, the route of elimination and potential for central nervous system (CNS) side effects differ from the more hydrophilic β-blocking agents [3], as will be discussed in §1.1.4.4.

Table 1.1 Log Kp values for commonly used beta-blockers

Compound	Kp value
Metoprolol	2.15
Propranolol	3.65
Atenolol	0.23
Acebutolol	1.90
Nadolol	0.70
Timolol	2.10
Pindolol	1.75

1.1.2.3 Solubility and water absorption isotherm of metoprolol

Metoprolol itself is highly insoluble in aqueous media [9]; however using different salt forms of the drug may increase the solubility. Of these, MPTA shows the greatest solubility in water. The approximate solubility of MPTA in several solvents at 25°C is listed in Table 1.2. MPTA is hygroscopic at humidities greater than 70% and is desorbed as the relative humidity decreases. The state of hydration and crystal form has been observed to be stable upon drying and re-analysis of MPTA [1].

Table 1.2 Solubility of MPTA in various solvents

Solvent	Solubility (mg/ml)	Reference
Water	> 1000	1,5,10
Methanol	> 500	1,10
Chloroform	496	1,10
Acetone	1.1	1,10
Acetonitrile	0.89	1,10
Hexane	0.001	1,10
Ether	insoluble	5,10

1.1.2.4 Optical rotation

MPTA contains three asymmetric carbon atoms; two of these are in the tartaric acid portion of the molecule, and one occurs at the 2-propanol position of the racemic base. The compound exhibits optical rotation in solution due to the optically active *dextro*-tartaric acid used in the preparation of the compound. The specific rotation for a typical sample of a 2% MPTA aqueous solution at 20°C, using the sodium D line, is +8.5°. The optical rotation range lies between +6.5° and +10.5°[1].

1.1.2.5 Ultraviolet absorption spectrum

The UV absorption wavelength maxima (λ_{max}) and molar absorptivities (ε) of MPTA in various solvents are listed in Table 1.3. A typical UV absorption spectrum of metoprolol tartrate in 0.1M hydrochloric acid (HCl) is depicted in Figure 1.2.

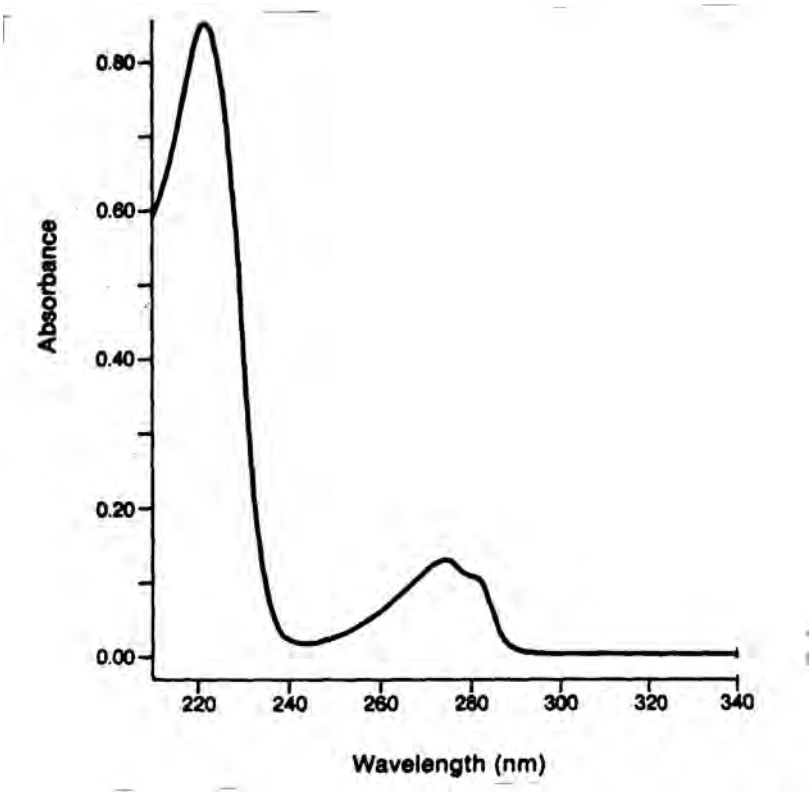


Figure 1.2: UV absorption spectrum of MPTA in 0.1M HCl

Table 1.3: Absorption maxima and molar absorptivities of MPTA

Solvent	λ_{max} (nm)	$\varepsilon \times 10^{-3}$
0.1N HCl	221 274 281(shoulder)	19.5 2.83 2.31
Water	223 274 280(sh)	23.4 3.60 2.94
0.01N NaOH	223 274 280(sh)	24.0 3.66 3.00
Methanol	223 276 282	21.5 3.11 2.62
Chloroform	277 283	3.36 2.86

1.1.2.6. Infrared spectra (IR)

The infrared spectrum reveals (Figure 1.3) that the frequencies and functional group assignments of the major absorption bands, as listed in Table 1.4, are consistent with the structure of MPTA [1].

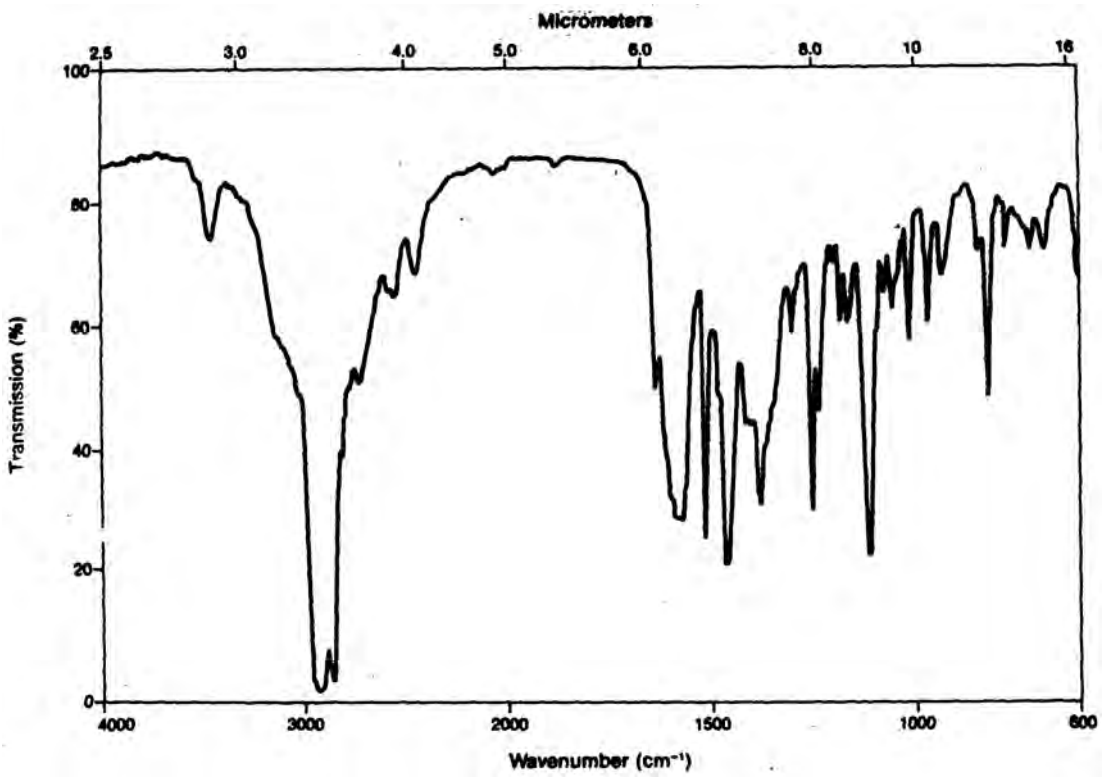


Figure 1.3: IR spectrum for MPTA

Table 1.4: Frequency and functional group assignment for MPTA

Wavenumber (cm)	Assignment (s)
3600-2300	NH2, -OH, Aliphatic and aromatic CH
1580	Carboxylic acid salt
1580, 1515	Aromatic ring
1250, 1015	Aromatic ether
1180	Isopropyl group
1100	Aliphatic ether, Secondary alcohol
820	1,4-disubstituted benzene

1.1.2.7 Melting range

MPTA melts over a 1-2° range between 120-123°C [1].

1.1.3 STABILITY

1.1.3.1.Solid state stability

MPTA stored at room temperature and at 35°C for five years have been found to be both physically and chemically stable. No degradation is seen after storage at 50°C for 30 months, however a slight change in colour, from white to off-white, occurs. MPTA is found to retain its chemical and physical integrity upon drying [1].

1.1.3.2 Stability of solutions of MPTA

Solutions of MPTA at pH 4, 7 and 9 stored at 60°C for 10 days show no chemical changes [1]. In another study of a prepared MPTA extemporaneous paediatric liquid, MPTA showed on 3% loss in potency when stored at 5°C and 25°C in the absence of light, for 60 days [8].

1.1.4 CLINICAL PHARMACOLOGY

Metoprolol is a competitive β_1-selective adrenergic antagonist, and therefore has been found to exhibit cardioselectivity. It acts as a partial agonist, as it is devoid of intrinsic sympathomimetic activity and therefore reduces exercise heart rate to a greater extent than β-blockers with intrinsic sympathomimetic activity. Metoprolol does not exhibit membrane-stabilizing activities at the dosage levels required for β-blockade [2,3,11,12]. The pharmacological properties of metoprolol compared with those of some other beta-blockers are listed in Table 1.5 [12] and it is evident that comparatively metoprolol is as potent as propranolol and atenolol.

Table 1.5: Comparison of the pharmacological properties of certain β-blockers

Drug	Beta-blockade potency*	$Beta_1$-selectivity	Partial agonist activity	Membrane stabilizing activity
Acebutolol	0.3	±	+	+
Atenolol	1	+	0	0
Metoprolol	1	+	0	±
Pindolol	6	0	+++	+
Propranolol	1	0	0	++

***** Relative to propranolol, where 1 = equivalent potency

0 Indicates no selectivity/activity

± Indicates that selectivity/activity depends on the dose

+, ++, +++ Indicates increasing levels of selectivity/activity

1.1.4.1. Mechanism of action

Metoprolol, in similarity to other β-antagonists competes with adrenergic neurotransmitters such as the catecholamines for binding at the sympathetic receptor sites. In low doses, metoprolol selectively blocks β_1-adrenergic receptors in the heart and vascular smooth muscle. The β_1-receptor blockade results in a decrease in both resting and exercise heart rate and cardiac output, and thus a decrease in both systolic and diastolic blood pressure [3,5]. Inhibition of isoproterenol-induced tachycardia and reduction of reflex orthostatic tachycardia also occurs [5]. At high doses in excess of 400mg per day MPTA β_2-adrenoreceptors are also inhibited [3,5]. The pharmacodynamic effects of metoprolol have been categorized and include haemodynamic, electrophysiological, pulmonary, endocrine and metabolic effects.

1.1.4.1.1 Haemodynamic, Electrophysiological and Pulmonary Effects

Metoprolol results in a negative chronotropic effect on the heart; hence decreasing cardiac output and systolic blood pressure. In patients with mild to moderate hypertension, systolic blood pressure falls rapidly, however due to an increase in total peripheral resistance, diastolic pressure is maintained and a full anti-hypertensive effect may only be seen after several weeks of repetitive dosing. Patients with angina pectoris show a decrease in systemic arterial pressure and a mild reduction in contractility, resulting in a reduction in the myocardial oxygen demand. In myocardial ischaemia, the reduction in heart rate results in prolonged diastole which facilitates blood flow through poorly perfused regions of the myocardium [12]. The effect of metoprolol on human myocardial electrical activity results in a prolonged repolarisation time after 5 weeks of oral administration [12].

The β-receptors in the lung are primarily of the β_2 type [13], therefore the β_1-selectivity of metoprolol suggests that it would have fewer effects on lung function than the non-selective β-blockers. However, this varies according to the type and status of pulmonary disease [4,12], and increased airway resistance caused by β-blockers when used in asthmatics and patients with chronic obstructive pulmonary disease may lead to fatal bronchoconstriction [4].

1.1.4.1.2 Endocrine and Metabolic Effects

Slight or no increase in plasma catecholamine has been reported with metoprolol treatment and plasma dopamine levels are unaffected after 4 weeks therapy, however such levels have been increased by 10-12 fold after 15 months of treatment [12].

Plasma renin activity in both healthy and hypertensive patients are decreased after short and long-term administration of metoprolol; the exercise induced increase in plasma renin activity is not consistently reduced [12]. Plasma renin levels are also important with respect to β-blocker treatment, where patients with low plasma renin levels are identified as poor responders to β-blockers. This group includes blacks and elderly patients, who respond better to diuretic and calcium antagonist therapy, whereas whites and young patients who have higher renin levels respond better to the drugs which act on the renin-angiotensin system, that is the β-blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin-II antagonists [14-17]

Blood glucose regulation mechanisms are unaffected by β-blockers and in moderately hypertensive patients with or without non-insulin dependent diabetes. The glucose response to glucagon challenge in patients on haemodialysis has also been reported to be unaffected by metoprolol [12]. In a study by Lager *et al*, it was found that neither the non-selective nor the cardioselective β-blockers potentiated the effects of insulin on glucose-uptake in vitro, however propranolol results in an impaired rate of recovery of blood glucose levels in comparison to metoprolol [18]

Acute effects of metoprolol on lipid metabolism has shown a reduction in the uptake of free fatty acids by normal or occluded myocardium in dogs [14], however this effect was found to be less pronounced than with use of non-selective β-blockers [18]. Similar studies with rat and rabbit aorta have shown that metoprolol may have important effects against the formation of atherosclerotic tissue. In the long term, metoprolol has the tendency to elevate plasma triglyceride and very low-density lipoprotein triglyceride concentrations, hence elevating total serum cholesterol, whilst high-density cholesterol is decreased [14]. Although various studies have reported different results, there is as yet no evidence to support increased risk of ischaemic heart disease with prolonged metoprolol use [12].

1.1.4.1.4.3 Effects on Physical performance

β-blockers may also adversely affect the extent and quality of physical performance. However, metoprolol being a selective antagonist has fewer negative effects on performance than non-selective β-blockers. Heavy short-term exercise and prolonged exercise are both negatively affected to a larger extent by the non-selective beta-blockers, and anaerobic exercise may be affected by both the selective and non-selective β-blockers similarly [12].

1.1.4.2 Uses and indications

Metoprolol has been used in the treatment of mild to moderate hypertension, and can be used alone or in combination with other antihypertensive agents [5]. Evidence from both primary and secondary prevention trials indicate that the use of cardioselective β-blockers such as metoprolol have a better preventive effect on total mortality, cardiovascular mortality and atherosclerotic complications than non-selective beta-blockers or diuretics alone [19,20]. Metoprolol has been found to be useful in this capacity due to its negative chronotropic effects, negative inotropic effects that decrease cardiac output, reduction of sympathetic outflow from the central nervous system and suppression of renin release from the kidneys. Thus, metoprolol affects blood pressure via multiple mechanisms that have been previously discussed in §1.5.1. Metoprolol is also used for the treatment of congestive heart failure (CHF), and has also been found to significantly decrease the morbidity and mortality associated with chronic CHF [21].

High efficiency and tolerability of metoprolol tartrate or metoprolol in combination with hydrochlorothiazide, in patients older than sixty-five years has been reported [12,22]. Metoprolol in pregnant hypertensives reduces diastolic blood pressure slightly, but does not prevent the ocurrence of toxaemia in these patients [12]. In asthmatic patients the risk of bronchospasm is lower during acute and chronic metoprolol treatment as compared to the use of the non-selective β-blockers, however, it is probably safer to use other classes of antihypertensives such as the calcium channel blockers or the ACE inhibitors. Metoprolol has also been found to be safe and effective for use in diabetics for whom blood pressure was reduced but no significant changes in blood glucose or glucose excretion were noted [3,12].

Metoprolol has also been used in the management of chronic stable angina [3,11], where it reduces myocardial oxygen demand, and hence the frequency of anginal attacks and nitrate requirements, with a corresponding increase in exercise tolerance [3]. It may also be used in the prevention of myocardial infarction, atrial fibrillation, atrial flutter and for the symptomatic treatment of hypertrophic subaortic stenosis [3,11]. In the management of hereditary or familial essential tremor its use resulted in the reduction in tremor amplitude but not tremor frequency. The unlabeled uses of metoprolol include ventricular arrhythmias, atrial ectopy, migraine prophylaxis, aggressive behavior and essential and familial tremor [11].

1.1.4.3. Contraindications and Precautions

Metoprolol is contraindicated in sinus bradycardia, heart block greater than first degree, cardiogenic shock and overt cardiac failure. It should also not be used for myocardial infarction in patients with a heart rate of less than 45 beats per minute [5].

In the treatment of hypertension and angina patients who have congestive heart failure, ß-blockers should be used with caution as myocardial contractility can be further depressed, precipitating more severe failure. Furthermore, metoprolol should not be used in patients on digitalis treatment as atrio-ventricular (AV) conduction is made slower by both these compounds [5]. Patients without a history of cardiac failure should not use β-blockers for prolonged periods as continual depression of the myocardium may ultimately lead to cardiac failure [5]. Furthermore, abrupt cessation of therapy could exacerbate angina pectoris and very rarely may lead to myocardial infarction. If chronically administered metoprolol needs to be discontinued, it should be gradually reduced over a 1-2 week period, and the patient carefully monitored during the titration period [5].

Although adrenergic selective β-blockers are preferred in patients with asthma or pulmonary conditions in which bronchospasm could put them at risk, all β-blockers should be used with caution [3,5]. Since β_1-selectivity is not absolute, the concomitant administration of a β_2-stimulating agent may be required, whilst using the lowest possible dose of metoprolol [5].

Metoprolol should also be used cautiously in patients with diabetes mellitus as it may mask symptoms of hypoglycaemia. β-blockers can also precipitate hypoglycaemia via inhibition of glycogenolysis, therefore should not be used in brittle diabetes but can be used cautiously in patients with stable diabetes [3,5]. When used in patients with hyperthyroidism or thyrotoxicosis, metoprolol can mask certain clinical signs such as tachycardia, and should therefore be used cautiously, and abrupt withdrawal of the drug in patients with hyperthyroidism can precipitate a thyroid storm [3,9]. The use of metoprolol before or during surgery is also controversial as the impaired ability of the heart to respond to reflex adrenergic stimuli may increase the risks of inotropic general anaesthetics and surgical procedures [3,5].

Caution should be exercised in patients with severe hepatic disease as dosage adjustment, may be required due to decreased drug clearance [3]. Metoprolol is only relatively

contraindicated in Raynaud's disease and peripheral vascular disease [23], and should be used cautiously in patients with depression [5]. β-blockers may also exacerbate conditions such as psoriasis and may potentiate the symptoms of myasthenia gravis [5].

Metoprolol is classified by the FDA as a pregnancy category C drug and therefore should only be used in pregnant women if it is clearly indicated and consideration of the risks and benefits are essential before it is used. Metoprolol is also contraindicated in breast-feeding mothers as it is excreted into breast milk. Whilst studies in rats have revealed no evidence of impaired fertility or teratogenicity, there are no well-controlled studies in pregnant women [3], and therefore its use should be avoided if possible.

The use of β-blockers in geriatrics may increase their risk to certain conditions such as hypoglycaemia due to decreased gastrointestinal, hepatic and renal functioning in these patients [25]. Safety and efficacy in children has not been established [3].

1.1.4.4 Adverse reactions

The adverse reactions of metoprolol are usually mild and transient and usually occur at the onset of therapy, diminishing with prolonged therapy [3,5].

Adverse central nervous system (CNS) effects include dizziness, fatigue and depression, and less commonly, CNS depression results in headaches, nightmares and insomnia [3,5]. Bronchospasm and dyspnoea may occur with doses greater that 400mg/day [3,5]. The most predominantly reported gastro-intestinal adverse effects include diarrhoea, nausea and vomiting, and flatulence has also been reported in about 1% of patients [3,5]. Hypersensitivity reactions such as pruritus, rash, exfoliative dermatitis and xenosis have been reported in about 5 in 100 patients [5]. Musculo-skeletal pain, myalgias and tinnitus are less frequently reported. Sexual dysfunction, impotence and decreased libido are less frequent adverse effects with a less than 2% occurrence in patients [3].

Both hypoglycaemia and hyperglycaemia can occur on metoprolol therapy [3]. Congestive cardiac failure may occur but it is more likely in patients with pre-existing left ventricular dysfunction. Sinus bradycardia and hypotension may also occur however this can be easily reversed with intravenous atropine, if necessary [3].

1.1.4.5 Drug interactions

The anthypertensive effects of metoprolol are additive with other antihypertensive agents and therefore combinations may be used more effectively to reduce blood pressure [3]. The use of metoprolol should be avoided with catecholamine depleting drugs such as reserpine, guanethidine and rauwolfia alkaloids, as they may result in hypotension, marked bradycardia with resultant vertigo, syncope and postural hypotension [3,5]. When used with anti-arrhythymics such as amiodarone, cardiac glycosides, diltiazem and verampamil it may result in complete atrio-ventricular block [3]. Care must be taken when clonidine therapy is stopped so as to avoid the possibility of rebound hypertension. The concurrent use of metoprolol with other sympathomimetic agents may result in antagonism of the desired therapeutic effects [3].

The effects of metoprolol may be enhanced or reduced with the concomittant use of certain drugs, which may affect bioavailiblity and hence, plasma levels of metoprolol. Metoprolol has been reported to interact with food and alcohol. Alcohol exacerbates drowsiness, dizziness, lightheadedness and blurred vision that may be caused by metoprolol, and should therefore be avoided [24].

1.1.5 PHARMACOKINETICS OF METOPROLOL

1.1.5.1 Dosage and administration

The dosage of metoprolol should be individualized when used for hypertension; however, the initial dosage for adults is usually 100mg, in single or divided doses. Metoprolol may be used alone or in combination with diuretics at the same dosages. The dosage may be increased weekly until the optimal blood pressure reduction is achieved [2,5]. The effective dosage range is 100-450mg per day [2,5,9,25]. Once daily dosing is effective and can reduce blood pressure throughout the day but the full effect may not be maintained at the end of the 24-hour period therefore blood pressure should be carefully monitored initially. In elderly patients an initial daily dose of 25mg is recommended but may be increased up to 300mg per day [9]. In patients with hepatic disease a reduced dose may be necessary [9].

In the treatment of angina pectoris the usual dosage is 100mg daily in two divided doses but the dose may be increased at weekly intervals up to 400mg/day. If the drug is to be discontinued, it should be reduced over a period of 1-2 weeks [9]. When used for myocardial infarction, IV administration is required for the early treatment, thereafter 100mg twice daily is recommended as maintenance treatment for 1-2 years [5,9]. The usual dosage for children

is 1-5mg per kilogram per day, twice daily in divided doses. Dose adjustments should be performed every three days to optimize therapy [5].

Oral dosage forms must be taken continuously and regularly with or immediately following meals and should not be discontinued without consulting a physician. Missed doses must not be taken with the following scheduled dose [9].

1.1.5.2 Overdose

Several cases of overdose with metoprolol have been reported. Symptoms of overdosing include hypotension, cardiac failure, bronchospasm and bradycardia. The oral LD_{50} in mice has been found to be 1158-2460 mg/kg, and is 3090-4670 mg/kg in rats [11]. There is as yet no specific antidote for the treatment of metoprolol overdose [11] therefore treatment should be symptomatic.

1.1.5.3 Absorption

The absorption of metoprolol is both rapid and complete and after administration about 95% of the drug is quickly and completely absorbed from the gastro-intestinal tract [3,5,27]. However, the oral bioavailability is low as only 40-50% of an oral dose reaches the systemic circulation as unchanged drug due to the effects of hepatic first-pass metabolism [25,28,29]. The onset of action for orally administered metoprolol is 20-30 minutes, and for IV administration is approximately 5 minutes [5]. Peak antihypertensive effects (t_{max}) are reached within 1.5-4 hours after administration and within 20 minutes after IV administration [3,5,27]. Peak serum concentrations have ranged from 0.035-0.125µg/ml after a 50mg dose and 0.046-0.270µg/ml for a 100mg dose [30]. Following administration of extended release products, peak serum concentrations are approximately one third of those achieved with conventional release products, hence the peak concentration occurs about 7 hours after dosing [3]. Food increases the bioavailability of metoprolol, hence increasing absorption; therefore metoprolol doses should be taken at the same time each day [30]. Gastric motility may also have an impact on metoprolol absorption, and is especially important when sustained release products such as hydrophilic matrix tablets are administered, as it has been found that strong contractions may lead to crushing of the dosage form with subsequent increase in drug release [31]. It has also been found that the AUC during multiple dosing was increased, possibly due to decreased systemic clearance as a result of potential reduction in hepatic blood flow or due to decreased presystemic clearance as a result of saturation of the first-pass effect [32].

Certain drugs also affect the bioavailability of metoprolol and examples of which are listed in Table 1.6 [3,11].

Metoprolol pharmacokinetics are best described by a two-compartment model following IV administration of the drug and a one-compartment model after oral dosing [27,30].

Table 1.6: Some drugs that alter the bioavailability (F) of metoprolol

Drugs which increase F	Drugs which decrease F
contraceptives flecainide mono-amine oxidase inhibitors hydralazine propafenone thyroid hormones H_2-antagonists ciprofloxacin, calcium blockers, such as diltiazem, felodipine, nicardipine	aluminium salts barbiturates calcium salts cholestyramine colestipol non-steroidal anti-inflammatories rifampin salicyclates sulfinpyrazone

1.1.5.4. Distribution

The distribution of metoprolol is typical of a moderately lipophilic, basic drug [1]. Following absorption, metoprolol is rapidly and widely distributed to the peripheral tissue [27]. It crosses the blood brain and the placenta and can concentrate in breast milk [3,5]. Only a small fraction of the drug (about 12%) is bound to human serum albumin [1,3,5], however the hypotensive effects can last up to one month after discontinuation of the drug, possibly due to extensive tissue binding [3]. Due to minimal protein binding, metoprolol has a high apparent volume of distribution (V_d) of 5.6L/kg body weight [1,30]. The volume of distribution has been reported to be in the range of 1.2 to 10.4L/kg body weight [27,28], however the V_d has been reported to be much larger when drug is administered orally in comparison to IV administration [28].

1.1.5.5 Metabolism

Metoprolol is extensively metabolized by the hepatic mono-oxygenase system in the liver [28]. The rate of drug metabolism is subject to genetic polymorphism [33] hence the extent of metabolism of β-blockers ranges from very little to almost complete [27]. The rate of hepatic hydroxylation by the cytochrome P-450 isozyme system is determined by the genetic polymorph of the patient [3]. Hydroxylation generally occurs relatively rapidly, resulting in a

half-life ($t_{½}$) of about 3-4 hours, but in slow hydroxylators the ($t_{½}$) may be increased up to 7 hours [3]. Metoprolol has three major metabolites, the major hydroxylated metabolite being α-hydroxy metoprolol [34,35], which exhibit no therapeutic effect [3,36].

1.1.6.6 Elimination

Elimination of orally administered metoprolol is mainly by biotransformation in the liver [1,7,30], and does not induce its own metabolism [1,30]. Approximately 3-10% of unchanged drug is recovered in the urine and the rest is excreted as metabolites via the kidney [36]. The three major metabolites, account for approximately 85% of the total urinary excretion. The excretion of these metabolites occurs primarily via glomerular filtration within 72 hours of administration [3].

The elimination half-life ($t_{½}$) of metoprolol is 3-4 hours but can range from 2.5 to 7.5 hours [1,5], where patients with a longer $t_{½}$ value may be classified as poor metabolisers [28]. Half-life is independent of the dose and duration of therapy, however, the duration of action of orally administered metoprolol is dose related [1,5]. This is demonstrated by the fact that a 50% reduction of the maximum registered effect for single doses of 20mg, 50mg and 100mg occurs at 3.3, 5.0 and 6.4 hours, respectively [5,30]. This suggests that drug elimination from the plasma follows first-order kinetics, whilst the decline in response follows zero-order kinetics [30]. The systemic availability and $t_{½}$ of metoprolol in patients with renal failure is not significantly different from normal subjects, therefore no reduction in dose is required in patients with chronic renal failure [5].

IV doses of 5mg and 15mg yield a maximal reduction in exercise-induced heart rate of approximately 10% and 15%, respectively. The effect on exercise heart rate decreases linearly with time at the same rate for both oral and IV doses. There was no effect after 5 hours and 8 hours for the 5mg and 15mg doses, respectively. Maximal beta-blocking effect after oral and IV administration is achieved with a ratio of the doses being about 2.5:1 [5].

1.2: HYDROCHLOROTHIAZIDE

1.2.1 INTRODUCTION

Hydrochlorothiazide (HCTZ) is a widely used saluretic agent, which exhibits both diuretic and antihypertensive activity [37,38]. HCTZ belongs to the thiazide group of drug compounds It was which were discovered during research in sulfonamide chemistry [37]. It has been included into the South African Essential Drugs List for the treatment of hypertension and it is often used in combination with other antihypertensive agents such as beta-blockers [39].

1.2.2 PHYSICO-CHEMICAL PROPERTIES

1.2.2.1 Description

HCTZ appears as a white or practically white, practically odourless crystalline powder, which has a slightly bitter taste [37]. It has a moleculer weight of 297.73 [37,40].

HCTZ may be described by several chemical names, which include [37]:

1. 6-chloro-3, 4-dihydro-7-sulfamoyl-2H-1, 2, 4-bezothiadiazine 1,1-dioxide
2. 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1,1-dioxide
3. 6-chloro-7-sulfamyl-3, 4-dihydro-1, 2, 4 - benzothiadiazine 1,1-dioxide
4. 2H-1, 2, 4-Benzothiadiazine-7-sulfonamide, 6-chloro-3, 4-dihydro-1, 1-dioxide

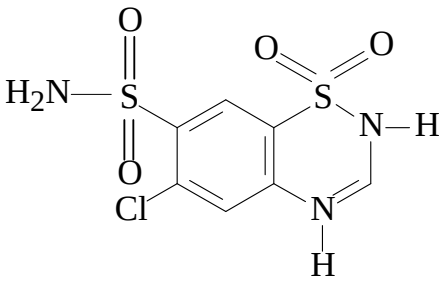


Figure 1.4 Structure of Hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) MW = 297.73

1.2.2.2 Dissociation Constant and Partition Coefficient

The ionization constants (pKa) quoted in the literature range from 8.60-8.81 (Table 1.7), depending on the method by which the value was identified [37,41]. The apparent n-octanol: aqueous phase partition coefficients (P) are listed in Table 1.8.

Table 1.7: pKa values for HCTZ

pKa-Value	Method
8.81 ± 0.05	Photometric titration
8.6	Potentiometric titration
8.7	Spectrophotometry

Table 1.8: Partition coefficients for HCTZ

Aqeous phase	P
0.1M HCl pH1.06	1.94
0.1 M glycine buffer pH 3.0	0.866
0.067M phosphate buffer pH 7.4	0.855

1.2.2.3 Solubility

HCTZ is only very slightly soluble in water and therefore has the potential for poor gastrointestinal absorption [37,42]. It is soluble in aqueous solutions of organic bases, for example, n-butylamine, and inorganic bases such as sodium hydroxide. The solubility of HCTZ in aqueous solutions and certain commonly used inorganic solvents are listed in Table 1.9A-B. The solubility of HCTZ increases upon the addition of non-ionic surface-active agents [37]. The surface tension of the saturated aqueous solutions at 23°C was found to be 724µN.cm-1 [43].

1.2.2.4. Thermal Analysis

The melting point of HCTZ has been reported to be 268°C [44], but may vary within a range of 263-275°C. The melting point is strongly dependent on the heating conditions and the effect responsible for this melting behavior is not clearly understood [37].
HCTZ is reported to contain < 0.1% volatile impurities up to a temperature of 280°C [37], and overall loss on drying at 105°C for one hour is not more than 0.5% of the weight of HCTZ [40]. Thermogravimetry results also indicate that decomposition of HCTZ starts at 307°C [37].

1.2.2.5 Density and crystal structure

HCTZ is a monolithic crystal system [37], with a density of 1.68 ± 0.01 $g.cm^{-3}$ [37,43].

Table 1.9A: Solubility of HCTZ in aqueous solvents

Solvent	Temp (°C)	pH of solution	Solubility (g/100ml)
Water	37	7.2	108×10^{-3}
0.1M HCl	25	1.0	60.8×10^{-3}
0.067M phosphate buffer	25	7.4	61.6×10^{-3}
0.05M borate buffer	25	9.0	103×10^{-3}
1.0M ammonia	25	11.6	2.2
0.1M NaOH	25	10.2	1.79
Simulated gastric fluid pH 1.1	37	1.1	108×10^{-3}
simulated intestinal fluid	37	7.5	109×10^{-3}

Table 1.9B: Solubility of HCTZ in non-aqueous solvents

Solvent	Temp (°C)	Solubility (g/100ml)
acetone	25	13.7
acetic acid	25	0.15
acetonitrile	25	2.0
ethylacetate	25	0.59
chloroform	23	0.1
ethanol(96%)	23	1.3 - 1.4
methanol	23	3.9 - 4.1
dichloromethane	23	< 0.02

1.2.2.6 UV Absorption

UV absorption data of HCTZ in various solvents are listed in Table 1.10 and a typical UV spectrum of HCTZ is shown in Figure 1.5 [37].

Table 1.10: UV Absorption data for HCTZ [35]

Solvent	λmax (nm)	log ε
ethanol	225	4.576
	269	4.307
	316	3.505
methanol	226	4.513
	271	4.279
	317	3.471
water	270	4.286
	315	3.495
0.01N HCl	270	4.290
	315	3.500
0.01N NaOH	272	4.193
	323	3.435

1.2.2.7 Infrared Spectra

The IR spectrum for HCTZ is shown in Figure 1.6. Assignments for the characteristic bands

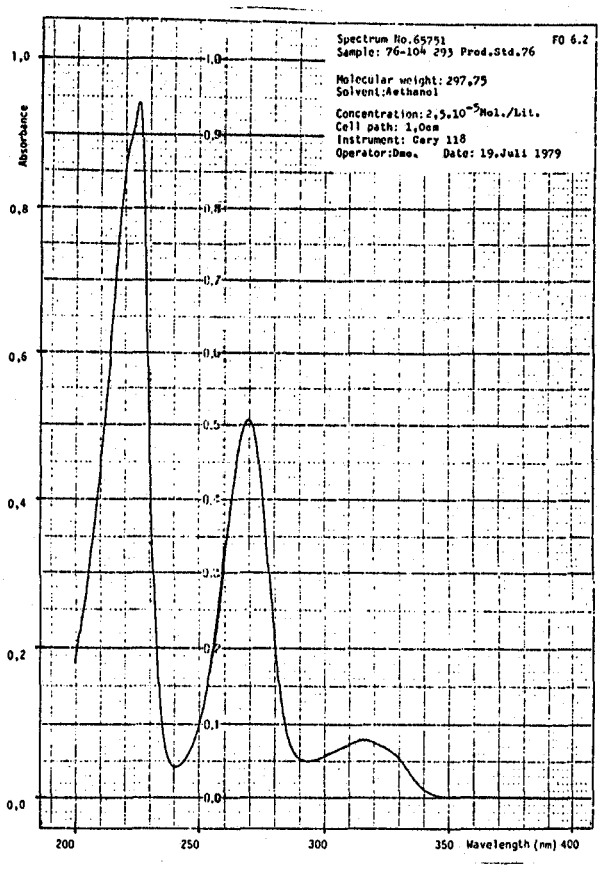


Figure 1.5: UV scan of HCTZ in ethanol

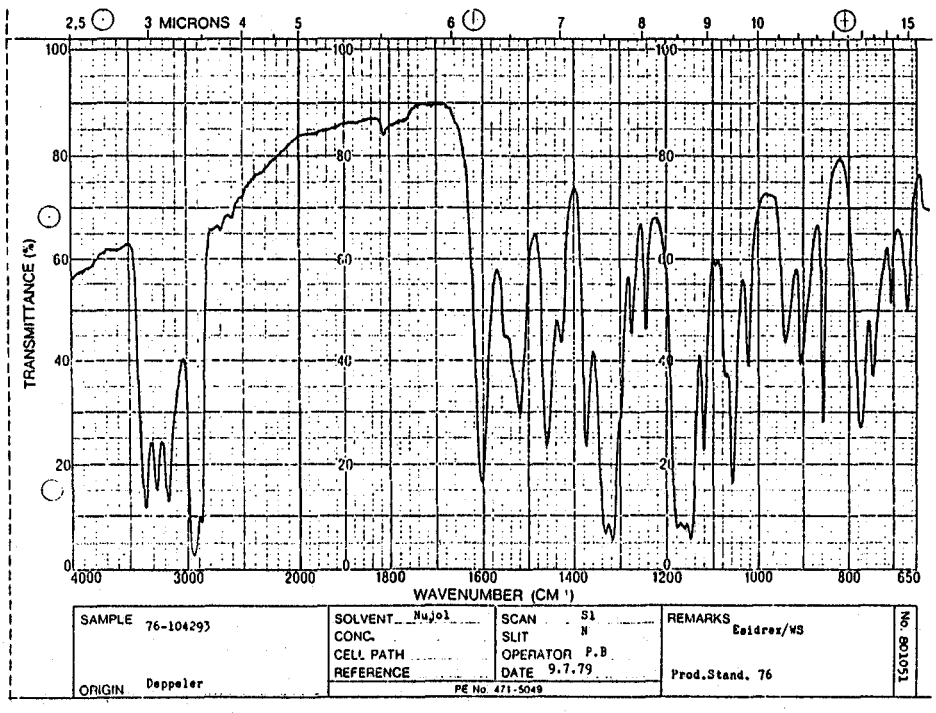


Figure 1.6: Infrared spectrum of HCTZ

in the spectrum are listed in Table 1.11, and are consistent with the structure [37].

Table 1.11: IR assignments for HCTZ

Frequency cm^{-1}	Assignments
3380, 3280, 3180	NH stretching
3080, 3020	aromatic CH stretching
2960, 2900	CH2 stretching
1600, 1525, 1460	C=C stretching
1335, 1320	SO2 asym. stretching
1165, 1155	SO2 sym. stretching
940, 900	S-N stretching + NH deformation
710, 675	ring deformations

1.2.3 STABILITY

1.2.3.1 Bulk stability and solid-solid interactions

When stored at room temperature for five years, HCTZ shows no degradation. Heat affects it very slowly and treatment for 2 hours at 230°C results in slight discolouration but no change in physical properties [37]. In humid conditions, HCTZ reacts with excipients containing metal compounds, however, under normal manufacturing and storage conditions there are no indications of degradation of the drug when in contact with Aerosil® 2000, calcium stearate and talc [37].

1.2.3.2 Aqueous stability

In aqueous solutions, HCTZ undergoes hydrolysis to give formaldehyde and 4-amino-6-chloro-1,3-benzenedisulfonamide, via an equilibrium process [37,45,46]. This reaction is specific-acid and specific-base-catalyzed [47]. However, the equilibrium constant is independent of pH from 1.5 to 8.2 [46,47], and in this range the equilibrium favors HCTZ. In very alkaline solutions (pH > 12) or in the presence of reagents that react with formaldehyde, complete hydrolysis can occur [37,46,47]. The pH-Rate profile for the hydrolysis of HCTZ is shown in Figure 1.7.

The slopes of the linear portions are -1.0 and +1.0 at the low and high pHs, respectively. This indicates first order dependence on both the hydrogen and hydroxide ions. The degradation rate constant (*k)* is representative of the pseudo-first order rate constant for the forward reaction. The intermediate portion is a bell-shaped curve. It has been established that this curve is a reflection of the kinetics of the reaction rather than the ionization constants of the

reactants. The portion of the profile from pH 7 to 11.5 probably results from the dissociation equilibra of HCTZ. The pKa values of HCTZ have been reported as 8.6 and 9.9. The decreasing degradation rate from pH 7 to 3 can be accounted for in terms of the degree of ionization of the substrate since there are no pKa values in this range, indicating an absence of the ionized species. This drop-off in rate may be due to a change in the rate-determining step of the reaction, and the occurrence of one or more intermediates resulting from the reaction, which is of two or more steps. The most common proposal is the formation of an imine intermediate, which results from ring opening of the HCTZ molecule [37,46,47].

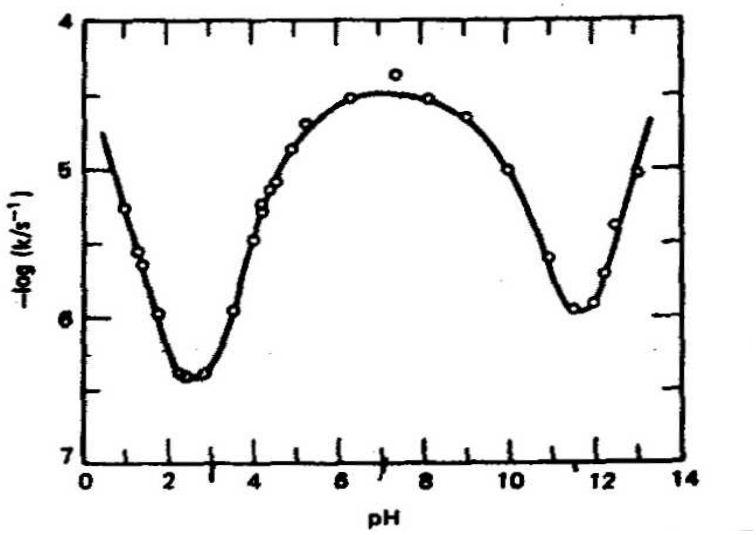


Figure 1.7: pH-Rate profile for the hydrolysis of HCTZ

1.2.3.3 Photolytic decomposition of HCTZ

HCTZ decomposes upon irradiation with near-UV light (λ >310nm) in both methanol and aqueous solutions therefore these solutions should be protected from light [45,48].

It is capable of acting as a skin photosensitizer by both free radical and excited single molecular oxygen mechanisms [49]. The chlorine substituent has been found to be photolabile, as seen from the photolytic pathway of HCTZ, as depicted in Figure 1.7 [45,48]

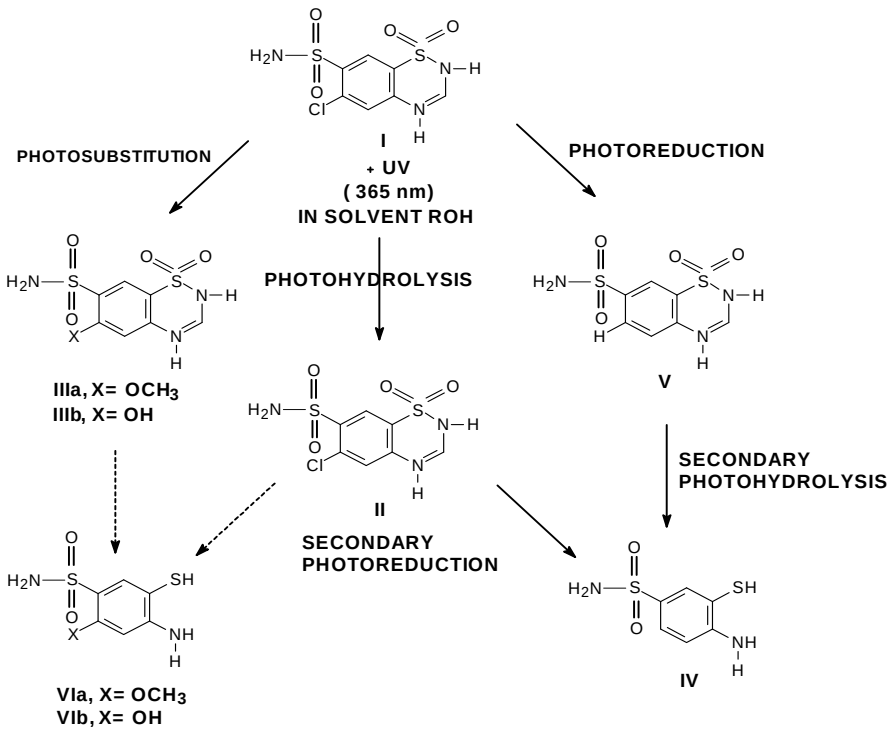


Figure 1.8: Photolytic pathway of HCTZ

1.2.4 CLINICAL PHARMACOLOGY

1.2.4.1. Mechanism of action

HCTZ does not usually affect the normal blood pressure, but it does affect the distal renal tubular mechanism of electrolyte absorption. The diuretic effect is achieved by interfering with the transport of sodium ions across the distal renal tubes, hence increasing excretion of water and sodium and chloride in equal amounts [50]. Diuresis usually begins within two hours of an oral dose, peaks at about 4 hours and lasts for between 6-12 hours [51].

1.2.4.2 Uses and Indications

HCTZ is indicated in the management of hypertension, either as the sole therapeutic agent or to enhance the effect of other antihypertensive agents. It is also indicated as adjunctive therapy in oedema associated with congestive heart failure, corticosteroid and oestrogen therapy and hepatic cirrhosis. It may also be useful in oedema due to various forms of renal dysfunction, such as chronic renal failure and acute glomerulonephritis. A short course of thiazides can also be used if a generalized oedema resulting from hypovolaemia occurs [51].

Routine use of diuretics during pregnancy is not usually recommended; hence HCTZ should not be used unless it is essential to improve the well being of the patient. Thiazides are also indicated as treatment for oedema resulting from pathological causes during pregnancy [51].

1.2.4.3 Contraindications and Precautions

HCTZ is contraindicated in patients with anuria and those who exhibit hypersensitivity to the drug and other sulfonamide derivatives. It should also be used with caution in patients with severe renal disease as thiazides may precipitate azotaemia, and in patients with impaired hepatic function or progressive liver disease. Sensitivity reactions may occur in patients with a history of allergy or bronchial asthma. HCTZ may also activate or exacerbate systemic lupus erythematosus. Thiazides may increase cholesterol and triglyceride levels [14,51]. Hyperuricemia or acute gout may also be precipitated in susceptible patients as plasma urates are increased with HCTZ use [30,52].

Generally, patients on diuretic therapy should be monitored for evidence of fluid and electrolyte imbalance, namely, hyponatremia, hypokalaemia and hypochloremic alkalosis. Signs of such imbalance include dry mouth, drowsiness, muscle pain and fatigue, tachycardia, gastro-intestinal disturbances, weakness and restlessness. Hypokalaemia is more likely to occur with prolonged therapy, in patients with severe cirrhosis or where the patient does not have an adequate oral electrolyte intake. This may be avoided by using potassium-sparing diuretics or potassium supplements. Thiazides may also induce hypomagnesemia and hypercalcaemia [14,51].

Caution should be exercised in diabetic patients and dosage adjustments of insulin or oral hypoglycemic agents may be required. Thiazide diuretics may also cause hyperglycaemia and the manifestation of latent diabetes mellitus [51].

Thiazides have been found to cross the placental barrier; hence there is a risk of neonatal jaundice and thrombocytopaenia. It is also excreted into breast milk, therefore either nursing or HCTZ have to be discontinued in such instances [51]. The safety and efficacy of HCTZ use in paediatrics has not yet been established [51].

1.2.4.4 Adverse reactions

The most common adverse reaction experienced includes weakness, hypotension and various effects on the digestive system including pancreatitis, jaundice, diarrhoea, vomiting, constipation and gastric irritation [51]. Another commonly reported side effect is hypokalaemia, which in cases may be particularly dangerous as it may lead to cardiac complications [53]. Less common effects include haematological effects such as aplastic anaemia, agranulocytosis, hypersensitivity reactions such as anaphylactic reactions, respiratory distress, photosensitivity, fever and rash. Other effects include electrolyte imbalance, hyperglycaemia, glycosuria, hyperuricaemia, and muscle spasm, vertigo, dizziness, headache, renal failure and renal dysfunction. Alopecia, exfoliative dermatitis erythema multiformae, xanthopsia, transient blurred vision and impotence occur even less frequently [14]. More severe side effects may include the HCTZ-induced pulmonary oedema triggered by an autoimmune mechanism, in woman [54].

1.2.4.5 Drug Interactions

The adverse effects caused by HCTZ may be potentiated by the simultaneous determination of certain drugs including alcohol, barbiturates, narcotics, corticosteroids and ACTH. Drugs such as cholestyramine and colestipol resins and the non-steroidal anti-inflammatory drugs decrease the absorption or the antihypertensive effect of HCTZ. When used with non-depolarizing skeletal muscle relaxants, there may be a possible increased responsiveness to the muscle relaxant. Concurrent use of lithium and diuretics should be avoided because the renal clearance of lithium is decreased, and this may increase the risk of lithium toxicity [51].

1.2.5 PHARMACOKINETICS OF HYDROCHLOROTHIAZIDE

1.2.5.1 Dosage and Administration

As with most drugs, the dose of HCTZ used should be individualized according to patient response, and generally the smallest dose possible should be used, as the dose response curves of thiazide diuretics are flat, therefore increased dose does not correspond with a proportional increase in clinical effect [55].

To treat oedema in adults the usual dosage is 25-100mg daily in single or divided doses. In such patients intermittent therapy may be used, that is, on alternate or three to five days per week. This therapy reduces the risk of undesirable electrolyte imbalances. When used to treat hypertension, the initial adult dose is 25mg given daily as a single dose. Doses above 50mg

are associated with marked reductions in serum potassium and should be avoided. The usual paediatric dose is 0.5 to 1.0 mg /pound/day in two doses not exceeding 37.5mg per day. Infants under 6 months may require 1.5 mg/pound/day in two doses. Infants up to 2 years may be given 12.5-37.5 mg daily in two divided doses. Children over 12 years may be given 37.5-100 mg per day, however, pediatrics with hypertension rarely benefit from doses exceeding 50 mg/day [51].

1.2.7.2 Overdose

The oral LD_{50} of HCTZ in the mouse and rat is greater than 10g/kg. The most common signs of overdosage are those associated with electrolyte depletion and dehydration resulting from excessive diuresis. In the event of overdosage, symptomatic and supportive measures should be used. Emesis should be induced and gastric lavage performed. Electrolyte imbalances and dehydration must also be corrected by established procedures [51].

1.2.5.3 Absorption

After administration of IV and oral doses of HCTZ to human volunteers, 90-93% and 58-63%, respectively, were excreted into the urine. The bioavailability of oral doses is in the range of 60-80% of the dose, however this is reduced in patients with congestive heart failure, renal and hepatic diseases and only about 40% of the dose is excreted. The existence of an absorption window of HCTZ in the gastrointestinal tract has been reported, as HCTZ is mainly absorbed in the duodenum and the first part of the jejenum, whilst absorption occurs in the stomach to a very small extent [54]. The onset of action is approximately 2 hours, with the peak plasma effect being achieved in about 4-6 hours. The AUC correlates linearly with the dose [37]. An AUC value of 1193.9ng.h/mL from a 25mg dose of HCTZ has been reported, where this value was significantly increased with co-administration of a calcium channel antagonist [50]. The duration of action for hydrochlorothiazide ranges between 6-12 hours [37].

HCTZ shows enhanced bioavailability when administered with food [43]. The bioavailability, however, remains unaffected by the concomitant administration of drugs such as metoprolol, sotalol or hydralazine. Redalieu *et al* studied the kinetics of HCTZ absorption in humans. The plasma level data was fitted to a two-compartment body model and tests for first-order and zero-order absorption were performed. It was concluded that the kinetics of HCTZ absorption is a zero-order process as opposed to the commonly implied first-order process [43,50]. It has

not yet been established why this is a zero-order process, however it has been suggested that the zero order process occurs either as a result of the concentration of drug at the site being constant or due to the presence of saturated carrier-mediated transport systems such as those involved in the gastro-intestinal absorption of riboflavine and thiamine [43]. Mal-absorption of HCTZ may occur in patients with congestive heart failure and after intestinal shunt surgery [37].

1.2.5.5 Metabolism

HCTZ undergoes only minimal hepatic metabolism, but is eliminated rapidly by the kidney [56]

1.2.5.5 Distribution

The distribution pattern in rats obtained after a single oral dose of HCTZ (5mg) showed that the highest concentrations of HCTZ were in the liver (27.8µg/ml) and in the gastro-intestinal tract (36.0µg/ml) within an hour after dosing. At this time point the concentration in the plasma was found to be 1.53µg/ml. HCTZ does not bind significantly to serum protein and only a low degree of binding to bovine serum albumin was obtained with only one binding site class [37]. HCTZ displays a total protein binding of about 40% [57].

1.2.5.6 Elimination

The percent of dose of HCTZ excreted unchanged in the urine is approximately 60% [57]. It is eliminated from the plasma in a biphasic manner with plasma half-lives of 5.6-14.8 hours. There is a correlation between urinary excretion and the dose administered. At oral doses of 12.5, 25, 50 and 75mg, the urinary excretion was 8.5, 17.9, 33.4 and 48.9mg, respectively, the cumulative urinary recovery being 65-72% of the dose. Renal clearance was found to be independent of dose [37]. HCTZ has a renal clearance value of about 300-335mL/minute [50,57]. In patients with congestive heart failure high peak plasma concentrations are achieved and the terminal $t_{1/2}$ in plasma is 8.9-28.9 hours, implying that urinary excretion of the intact drug may be reduced. A study has shown that an average urinary recovery of 40.7% was achieved for a person with congestive heart failure [37]. Patients after intestinal shunt surgery also show decreased urinary excretion with an average urinary recovery of 30.7% of the dose [37].

1.3 RATIONALE FOR DOSAGE FORM DESIGN

Metoprolol has a long been established as a relatively safe and effective antihypertensive agent and consequently it has become one of the more commonly prescribed β-blocking agents. Furthermore, metoprolol has a relatively short half-life, is completely absorbed from the gastrointestinal tract and exhibits a degree of β-blockade that corresponds well with plasma concentrations and this is particularly well suited to be formulated as a controlled release dosage form. A number of controlled release metoprolol formulations are currently available on the international market [58]. Such formulations include, Seloken® Durules, Betaloc® SA, Betaloc® Durules (Astra Cardiovascular), Lopressor® SR (Novartis) and Toprol XL®(Astra). Whilst a large number of these are matrix-based products, newer developments include the use of osmotic type systems and divisible tablets where pellets are embedded in an inert tablet mass. These dosage forms, which allow for once daily dosing of metoprolol, have increase in use substantially as they offer an opportunity for more convenient dosage regimens, and thus the potential for greater patient compliance. Furthermore, studies have shown that the use of controlled release, once daily formulations of metoprolol were equally or more effective than twice daily conventional metoprolol tablets [59,60].
Both HCTZ and metoprolol have been found to be equally effective for the treatment of hypertension in certain populations [61], however in the black and elderly population, metoprolol does not result in effective blood pressure reduction due to lower renin levels in such patients. HCTZ has been found to be effective in such patients and therefore a combination of the two would be beneficial in such populations.

A combination of both metoprolol and HCTZ has also been found to be more effective in reducing blood pressure than increasing doses of metoprolol used alone [62-69]. The two drugs exhibit a synergistic relationship with respect to lowering of blood pressure.

Combination dosage forms of MPTA and HCTZ have therefore been developed, an example of which, is Selekomb® (100mg metoprolol, 12.5mg HCTZ). Combination dosage forms of these compounds are usually formulated to contain 100mg metoprolol and 12.5mg HCTZ [63,64]. These dosage forms deliver metoprolol at a controlled rate, and the HCTZ is immediately released [63,64], so that there is an initial immediate reduction in blood pressure, which is maintained throughout the day by sustained metoprolol release. A dosage form with these characteristics was selected as the target for development.

Although, a number of products already are in existence on the global market, they are comparatively more expensive than immediate release or single drug dosage forms. This makes such products inaccessible to developing countries in both public and private sectors, hence the development of cheaper, yet effective generic products would allow for more widespread use of such products in the developing world. This would be particularly advantageous in a country such as South Africa, where the majority of hypertensives are either black or elderly patients.

CHAPTER TWO

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF METOPROLOL TARTRATE AND HYDROCHLOROTHIAZIDE

2.1 METHOD DEVELOPMENT

2.1.1 INTRODUCTION

The quantitative analysis of metoprolol in plasma has been accomplished using a variety of methods including electron-capture gas-liquid chromatography after derivatization [70,71], gas chromatography-mass spectroscopy [71], high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [71,72], and reversed phase ion-pair HPLC with fluorescence detection [71]. Analytical methods for the determination of metoprolol in pharmaceutical preparations include HPLC with UV detection [73-75], non-suppressed ion chromatography [76], HPLC with spectroflourimetric detection [76] HPLC with UV spectrophotometry [70,77]. Analytical methods for the analysis of HCTZ include first derivative UV spectrophotometry [78], and HPLC [72,73,78-81]. Metoprolol and HCTZ in a multi-component dosage form have been simultaneously analyzed using HPLC with UV detection [73,74] and electron-capture gas-liquid chromatography [82].

HPLC with UV detection was selected as the preferred method of analysis for these components. This decision was based on the existing methods described in the literature and on availability of equipment in our laboratory. Furthermore, HPLC is a widely used technique that has proven to be efficient and in most cases a superior method for the analysis of ionic species [83]. A number of published HPLC methods for the determination of metoprolol and HCTZ, either alone or in combination in pharmaceutical dosage forms, are listed in Table 2.1. These existing methods were used as the starting point for the development of an HPLC method for the simultaneous determination of MPTA and HCTZ.

2.1.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Chromatography, in general, uses a range of techniques to separate a mixture of solutes by using a mobile phase, which pushes the mixture along an immobile, immiscible stationary phase, hence allowing solutes to separate out based on their different mobilities in that system [73,83,84]. Prior to the 1970's there were few reliable chromatographic methods available for

use in the laboratory, however, during this period, pressure liquid chromatography was increasingly used. This method, however, did not offer consistent flow rates and HPLC was developed in the mid-1970s. HPLC technology has developed rapidly and since the 1980's it has become a method commonly used for the separation of pharmaceutical and chemical compounds [86].

The HPLC method selected for an appropriate separation is dependent on the properties of the compound to be analyzed. These include liquid-liquid chromatography (LLC), liquid-solid chromatography (LSC), size exclusion chromatography, and normal or reversed-phase (RP) chromatography, ion exchange and affinity chromatography. Each of these operates on a different basis, whereas LLC and LSC operate on the basis of polarity, normal and RP chromatography operates on the basis of hydrophilicity and lipophilicity of compounds. Ion-exchange chromatography is used for the separation of charged molecules and operates on the basis of selective exchange of the ions in the sample with counterions in the stationary phase [87], size-exclusion chromatography depends on the molecular size of compounds and affinity chromatography uses immobilized bio-chemicals that have a specific receptor for the compound of interest [86].

2.1.3 EXPERIMENTAL

2.1.3.1 Reagents

All chemicals used were at least of analytical reagent quality. Acetonitrile (HPLC grade, distilled in glass) was purchased from Burdick and Jackson (Michigan, USA). Sodium hydroxide pellets and ortho-phosphoric acid (85%, analytical grade) were obtained from BDH Chemicals (UK) and PAL Chemicals, respectively. Metoprolol tartrate was obtained from Genpharm (Toronto, Canada) and hydrochlorothiazide was purchased from Aspen-Pharmacare (Port Elizabeth, SA). HPLC grade water was purified using a Milli-Ro®-15 Water Purification System (Millipore, Bedford,MA,USA), which consists of a Super-C® carbon cartridge, two Ion-X® ion-exchange cartridges and an Organex-Q® cartridge. The water was filtered through a 0.22µm Millipak® stack filter prior to use.

. **Table 2.1 HPLC Methods used for the analysis of Metoprolol Tartrate and Hydrochlorothiazide**

Compounds	Stationary Phase	Mobile Phase	λ (nm)	Internal Standard	Reference
MPTA	C-18	Methanol (550ml): Water (470ml) with 961mg pentane sulfonic acid, 82mg anhydrous sodium acetate and 0.57ml glacial acetic acid	254	Oxprenolol HCl	73
	C-18	30%v/v acetonitrile in monobasic potassium phosphate aqueous solution (pH 5)	274	Propranolol HCl	75
MPTA & other β-blockers	non-suppressed ion chromatography	50mM nitric acid in aqueous solution of 4% v/v acetonitrile	270	-	76
HCTZ	C-18	0.067M phosphate buffer (pH 3.0), with 0.2% triethylamine: 25%v/v acetonitrile	227	-	88
	C-18	MeCN: H2O: acetic acid 10:88:2 v/v, 2.5% THF organic modifier	271	Hydroflu-methiazide	79
	C-18	5%v/v MeOH in double distilled water (pH adjusted to 4.5 with 0.1M acetic acid)	254	Sulfadiazine	80
	Anion exchange	0.005Na2SO4 in pH 9.2 borate buffer: MeOH, 35:5		-	37
	Lichrosorb® SI60	n-hexane:2-propanol :chloroform: diethylamine, 77:18:5:0.01		-	37
	C-18	0.1M monobasic sodium phosphate (adjusted to pH 3.0): MeCN, 9:1	254	-	73
HCTZ + captopril	Phenyl column RP	Gradient elution using different concentrations of MeOH: phosphoric acid in water at various flow rates	210	-	72
HCTZ and amiloride	C-18	Water: MeOH 60:40 v/v pH3.2	280	Caffeine	89
HCTZ and MPTA	C-18	0.02M monobasic K_2PO_4 aq.solution (pH5.9): MeCN, 30%/v/v	274	Propranolol HCl	75
	C-18	0.02M monobasic K_2PO_4 aq.solution (pH5): MeCN, 30%/v/v. Diluted 4:1 with water	274	Propranolol HCl	75
	Nucleosil® 5 SA	0.015M $KMeSO_3$, 0.025M tetramethylammonium chloride, 50% MeCN	212	-	74

2.1.3.2 Preparation of stock solutions

The stock solutions were prepared as follows:

Approximately 0.017g of HCTZ was accurately weighed and transferred into a 1L A-grade volumetric flask. This was dissolved in less than 10%v/v acetonitrile and 100mL of HPLC grade water was added. Approximately 0.130g of MPTA was accurately weighed and added to the volumetric flask. The solution was then made up to volume using HPLC grade water. Standards ranging in concentration from 0.28-17μg/mL of HCTZ and 2.25-130μg/mL of MPTA were prepared by serial dilution of the stock solution using A-grade glassware.

2.1.3.3 Preparation of buffers

Buffer solutions were prepared by pipetting the appropriate volumes of 85% phosphoric acid into an A-grade volumetric flask and making up to volume with HPLC grade water. The pH was then adjusted as required using sodium hydroxide pellets. The pH was measured using a Crison pH meter (Crison, LASEC, South Africa)

2.1.3.4 HPLC system

Two modular systems were used for the in-vitro analysis of the compounds of interest. System A was used in the initial stages of method development and analysis of the MPTA cores developed, whilst System B was used in the latter stages of formulation development.

System A

The modular HPLC system was comprised of a Waters Model 6000A solvent delivery system, with a Waters Intelligent Sample Processor (WISP) Model 710B autosampler (Waters Associates, Milford, USA), a Spectrochrom UV-100 UV detector (Linear instruments Corporation) and a Perkin Elmer 561 strip chart recorder (Hitachi, Japan). The separation was achieved on an Inertsil® (250 x 4.6mm i.d) 5μm column (MetaChem Technologies Inc, USA).

System B

The modular system differed from system A in that it consisted of a P100 pump (Thermo-Separation Products, USA) and a second WISP 710B autosampler. All other components were the same as used in System A.

2.1.3.5 Column selection

Reversed-phase HPLC was identified as the preferred method for the analysis of the compounds of interest, as is evident from the methods described in Table 2.1. In contrast to normal phase chromatography, RP-chromatography uses a non-polar stationary phase and a polar mobile phase. The more hydrophobic the matrix, the greater the tendency of the analytical column to retain hydrophobic moieties whilst hydrophilic compounds elute more quickly, hence separation of compounds is achieved [86]. Normal phase HPLC is especially useful for highly non-polar compounds, such as the high molecular weight hydrocarbons, whereas polar compounds may be readily analyzed by RP-HPLC [90,91]. As MPTA is a polar weak base, either ion-suppression or ion pairing with RP-high performance liquid chromatographic methods have traditionally been used for its analysis, however newer methods that exclude the use of an ion-pairing agent, have since been developed [75].

Column packings for HPLC may be either porous or superficially porous. Normally columns are usually packed with uniform porous silica particles either with spherical or irregular shape. Different chemical groups are bonded to the surface of the silica particles to produce the bonded phase. The most commonly used bonded phase consists of C-18 alkyl groups that are attached to the surface of the silica particles, and are called octadecasilane (ODS) bonded phases. C-8 and phenyl columns may also be used. The efficiency of a column can be improved by reduction of the particle size of the stationary phase material, hence, stationary phase particle sizes have becomes progressively smaller, and particles with 3, 5 and 10µm diameter have been used. Typical column lengths range between 10-25cm long and 4.6mm internal diameter and they are usually made of stainless steel [83]. The use of small bore/ microbore columns (2mm diameter or less) has also increased considerably. Microbore columns are advantageous as they are operated at slower flow rates than larger columns, therefore solvent consumption is reduced. Furthermore, the efficiency of the column is dependent on the velocity of the mobile phase and not column diameter, and smaller peak widths are achievable with these columns [83].

C-18 columns have been most frequently used for the analysis of MPTA. Consequently RP-HPLC with a C-18 column was chosen for the analysis of the compounds. An Inertsil ® 250 x 4.6mm-i.d column with 5µm-particle packing was used for initial work. This column offered adequate

separation of compounds and resulted in well-resolved peaks, as will be discussed in latter sections, and it was therefore selected for use in this method of analysis.

2.1.3.6 Mobile phase selection

2.1.3.6.1 Factors influencing the choice of mobile phase

The mobile phase or eluent not only moves the sample components through the chromatographic column but also interacts with the solute molecules and the stationary phase itself. The strength of these interactions determines the resolution obtained and hence the efficacy and efficiency of the separation, therefore the selection of an appropriate mobile phase is imperative [83,91]. The mobile phase selected must not alter the characteristics of, or be miscible with the stationary phase, and those that could be potentially detrimental to the life span of a column, should be avoided. For silica based RP columns mobile phases to be avoided are those with pH < 3 or > 9, as these may lead to hydrolysis of the bonded phase or dissolution of the silica [83,92]. The mobile phase must also be compatible with all analytical system components.

Solutions for use in HPLC systems must be filtered to remove particulate matter that could interfere with the pumping action of the solvent delivery device or cause damage to the seals and valves, or collect on the top of the column causing irregular behavior and subsequent column blockages [83]. Solutions must also be degassed to remove dissolved or suspended air bubbles so that they do not collect in the pump or the detector cell and cause erratic behavior of the detector or an irregular pumping action. The viscosity of the mobile phase is also an important consideration and should not exceed 0.5cps [83] as high viscosity solvents reduce solvent diffusion coefficients, hence decreasing column efficiency [91].

When UV detection is used, the solvents selected must exhibit very low absorbance at the operating wavelength; for example, if a wavelength of 254nm is selected, aromatic solvents cannot be used as all such solvents absorb UV light to some extent at this wavelength [91]. In general, however, wavelengths above 210nm can be used with limited interference but it is important that the UV cut-off values for all solvents to be used are considered.

The choice of a mobile phase for a specific analyte is critical and the greater the solubility of the analyte in the mobile phase, the shorter the resultant retention time (R_T). Conversely, if the analyte displays limited solubility in the mobile phase, it is likely to partition readily onto the stationary phase, resulting in longer retention times. The retention times of weak acids and bases analyzed by RP-HPLC may also be altered by changing the pH of the mobile phase or by including suitable organic modifiers into predominantly aqueous phases [83]. Organic modifiers in predominantly aqueous mobile phases will alter the retention characteristics of the compounds to be analyzed. The choice and amount of the organic modifier required depends on the properties of both the analyte and the stationary phase [83,91].

Ion-pairing agents may also be added to the aqueous phase. When the solute is a non-absorbing ion, the inclusion of a UV absorbing agent of opposite charge results in a detector response. Ion pairs may also be used for the detection of samples of the same charge as the ion-pair and uncharged particles [93].

2.1.3.6.2 Selection of a mobile phase for MPTA and HCTZ

The selection of the mobile phase was based on methods published in the literature and on the USP methods for the individual analysis of MPTA and HCTZ. The USP methods for the individual drugs resulted in broad peaks with long retention times that was possibly a function of the column selected.

A method described by Das Gupta *et al* was then considered in preference to the USP and other methods [75]. Monobasic potassium phosphate buffer (0.02M), pH 5.9 with acetonitrile (30%v/v) at a flow rate of 2.3 mL/minute was used, however due to differences in column length and diameter, the method was optimized for the analytical system used in our laboratory.

The affects of buffer molarity and pH and the type and amount of organic modifier used, were assessed and considered in the selection of the mobile phase. For the assessment of the effect of these variables, compounds were detected at a wavelength of 274nm and 0.1 AUFS at 1.0mL.minute [75].

2.3.1.6.2.1 Buffer molarity

An assessment of 0.01M-0.05M buffers showed that increasing the buffer molarity led to an increased $R_{T.}$ The 0.02M (Figure 2.1) buffer resulted in the shortest R_T for both compounds, with 2.2 minutes for MPTA and 4.6 minutes for HCTZ, respectively. The peaks of interest were sharp and well resolved and exhibited no tailing for all buffers assessed, except for the 0.03M buffer, which resulted in poorly separated peaks. The 0.02M buffer, which displayed both good resolution and short $R_{T,}$ was selected for further use.

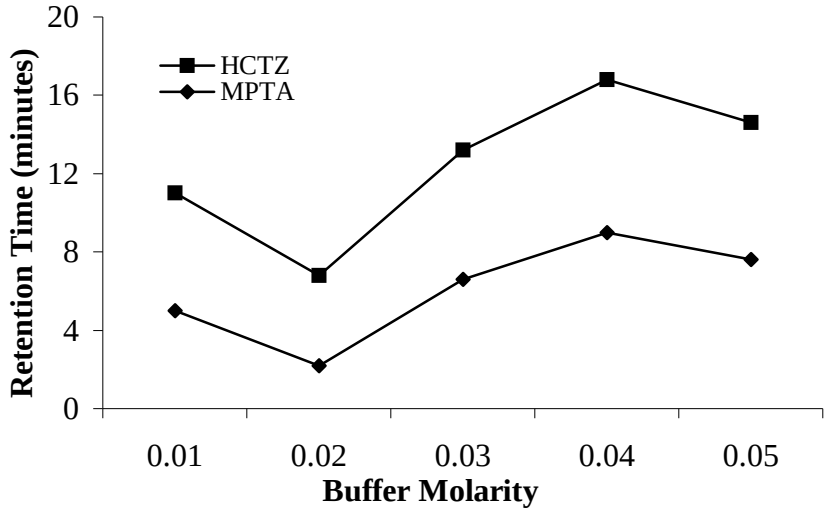


Figure 2.1: The effect of buffer molarity on retention time of MPTA and HCTZ

2.1.3.6.2.2 Buffer pH

A change in buffer pH influenced both the R_T and resolution of the compounds. pH values less than 2.5 resulted in well resolved peaks, however the MPTA peak eluted too close to the solvent front making quantitation difficult. The resolution and peak shapes were acceptable between the pH range of 2.5-5.0 and the R_T in this region also did not vary significantly (Figure 2.2). The peaks that eluted from a mobile phase prepared using pH 5.0 buffer showed good resolution and shape but the absorbance was lower than at pH 2.5-3.65. A buffer that allowed for higher absorbance by the compounds is likely to be advantageous in allowing for the development of a more sensitive method.

A pH of 3.1 was selected as it exhibited similar retention and resolution characteristics to those observed for pH 2.5 with only a minimal increase in R_T. pH 2.5 was not selected as buffers with a pH of less than 3.0 are known to cause damage to columns [92]. Furthermore, it has been reported that compounds with a pKa > 8 provide better peak shape at pH 3.0 [92], therefore the selection of pH 3.1 would have a short R_T, good peak shape and would minimize damage to the column.

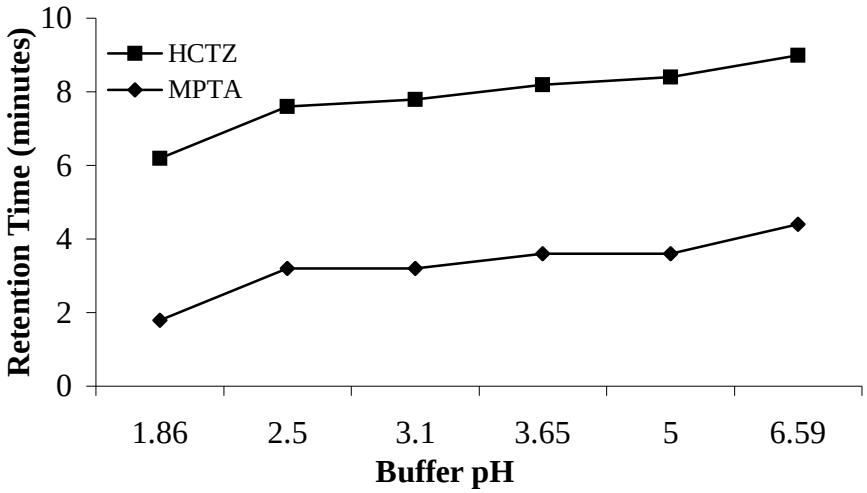


Figure 2.2: The effect of buffer pH on retention time of MPTA and HCTZ

2.1.3.6.2.3 Organic modifier

The initial organic modifier used was acetonitrile (MeCN). The ratio of aqueous phase: organic phase is of importance particularly with respect to R_T and optimal proportions of acetonitrile were determined by assessing the impact of varying the organic content of the mobile phase. An increase of the organic modifier composition resulted in sharper, well-resolved peaks and decreased R_T for both compounds, however, the inclusion of methanol resulted in broader peaks. Acetonitrile was selected as the organic modifier for use as good resolution and sharp peaks were obtained when it was included in the mobile phase. The proportion of acteonitrile: buffer selected as optimal was 72:28 %v/v. Furthermore, the possibility of an incompatibility, which may result between the organic modifier in the mobile phase and acetonitrile used in the preparation of HCTZ solutions, would be minimized.

2.1.3.7 FLOW RATE SELECTION

Mobile phases are usually pumped through the column at a rate or 1-5 cm^3/min [83]. The flow rates used by Das Gupta *et al* were 2.3mL/min and 2.5mL/min, depending on the mobile phase being used [75]. Although this flow rate is within the acceptable range, it was thought that a slower flow would also be advantageous in terms if maximizing the life span of the pump, the column and would also be more economical. Consequently a flow rate of 1.0mL/min was selected for use in method development.

Table 2.2 The effects of altering the organic modifier ratio and the addition of other organic modifiers on retention time, peak shape and resolution.

Mobile Phase*	R_T (minutes)		Resolution	Peak Shape
	MPTA	HCTZ		
buffer (pH 2.5):MeCN, 50:50	11.0	3.0	Compounds are well resolved	MPTA peak is broad with tailing. HCTZ peak is sharp
buffer (pH 2.5):MeCN, 90:10	11.2	9.0	Peaks were well resolved	Peaks were sharp, no tailing (broader than 70:30)
buffer (pH 2.5):MeCN, 80:20	7.2	6.2	Peaks were well resolved	Peaks were sharp, no tailing
buffer (pH 2.5):MeCN, 75:25	4.4	5.5	Peaks were well resolved (possible degradant of HCTZ not resolved)	Peaks were sharp, no tailing
buffer (pH 3.1):MeCN, 72:28	3.2	4.5	Peaks were well resolved, separated from solvent front	Peaks were sharp, no tailing
buffer: MeCN:MeOH, 70:15:15	5.4	5.0	Not well resolved	Sharp peaks, no tailing
buffer: MeCN: H_2O, 70:15:15	8.2	9.0	Well resolved peaks	No tailing, peaks are broader

*(0.02M PO_4 buffer was used for all mobile phases tested)

2.1.3.8 Detection

2.1.3.8.1 Method of Detection

Detectors based on UV or visible light absorption, refractive index, flame ionization, electrical conductivity and heat of adsorption have been used with HPLC systems [91]. Perusal of the literature revealed that commonly used methods of detection for HCTZ and MPTA include spectroflourimetric and UV detection, with UV detection being the more commonly used option. UV detectors measure the ability of the sample to absorb light and can be used at one or more wavelengths. Detectors that may be used include fixed wavelength detectors, variable wavelength detectors that measure at one wavelength but can detect over a wide range of wavelengths, and

diode array detectors, that measure a spectrum of wavelengths simultaneously [83,91]. UV detection with a variable wavelength detector was used in this case.

2.1.3.8.2 Detection Wavelength (λ)

The λ_{max} for MPTA in water occurs at 223 and 274nm with a shoulder at 280nm (Table 1.3). HCTZ exhibits a λ_{max} at 270 and 315nm in water (Table 1.10). To select a wavelength for the simultaneous detection of both compounds, the effect of varying wavelengths on the absorbance of the compounds was investigated (Figure 2.4). λ_{max} values and wavelengths frequently reported in the literature for these compounds were assessed.

Based on the results depicted in Figure 2.3, a wavelength of 227nm was chosen for the detection of MPTA and HCTZ. This wavelength was found to produce reproducible, well-resolved peaks and allowed for the detection of low concentrations of the drugs without the need to increase the sensitivity such that concentrations within the range of study would be off scale and require dilution prior to analysis.

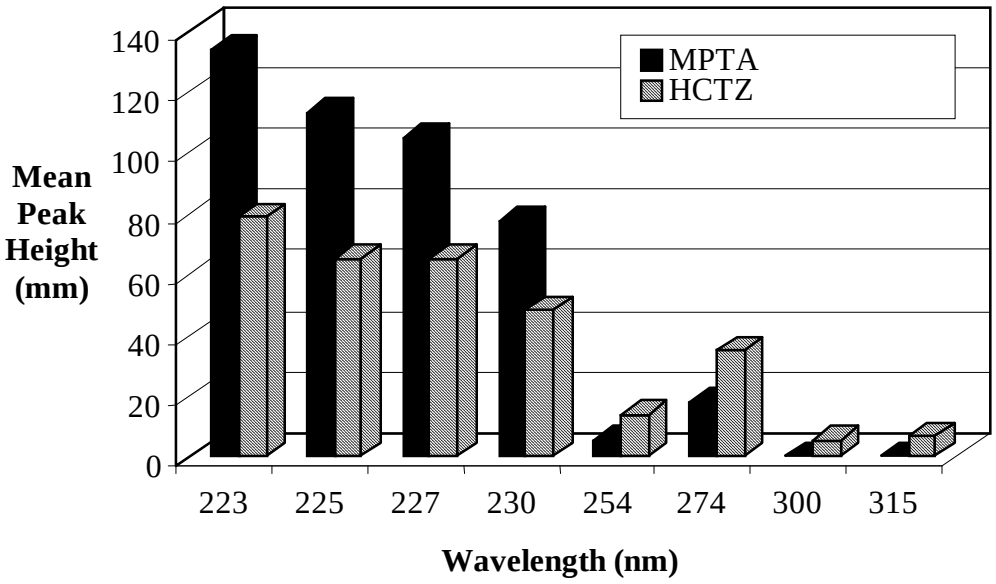


Figure 2.3 Peak height comparison obtained at different wavelengths for MPTA and HCTZ

An attenuation (AUFS) of 0.05AUFS was selected as it offered adequate sensitivity for the detection of both compounds and early detection of degradation products.

2.1.4 CHROMATOGRAPHIC CONDITIONS SELECTED

The optimal chromatographic conditions that were established are described below.

Mobile Phase	0.02M phosphate buffer (pH 3.1): acetonitrile, 72:28
Flow Rate	1.0mL/minute
Detection Wavelength	227nm
AUFS	0.05
Injection volume	3μL
Temperature	Ambient
Retention Time	MPTA: 4.0 minutes
	HCTZ: 5.2 minutes

A typical chromatogram obtained for the two compounds is shown in Figure 2.4.

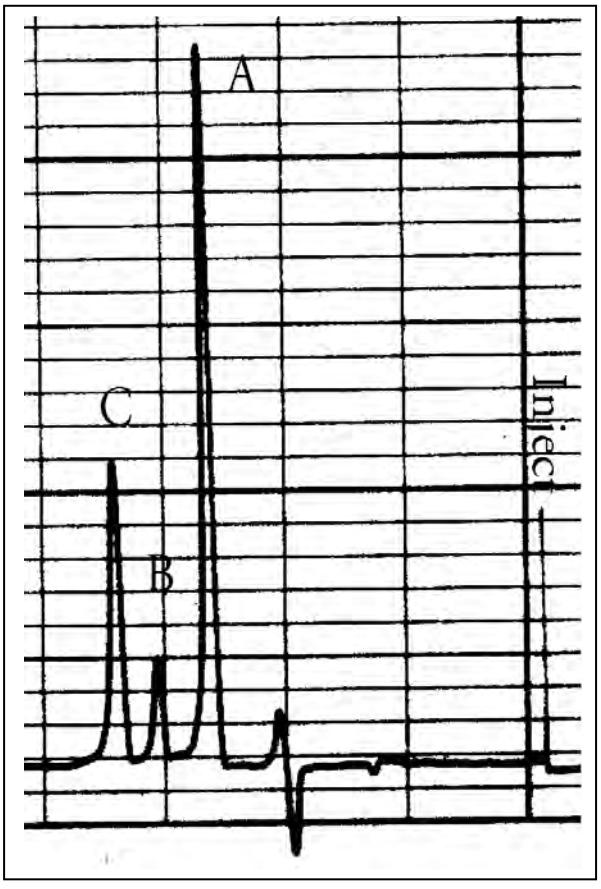


Figure 2.4: A typical chromatogram of the separation of HCTZ (A), possible degradant (B) and MPTA (C)

2.1.5 STABILITY INDICATING ASSAY

A stability-indicating assay is one in which the active drugs can be quantified in the presence of any of its degradation products; however, such an assay may not always be optimum, especially if the degradant increases from < 0.1% to 0.3% [94]. Both impurities and degradants must be detected and quantified by the developed method and minimum validation data must be provided as proof of efficacy of the method. The method selected was able to identify the presence of degradants however these were not quantified. The appearance of these degradants was merely used as an indication that new standard solutions were required and to identify the storage time for samples to be stored prior to analysis being completed.

2.1.6 CONCLUSIONS

The variables investigated indicate that buffer pH, molarity and organic modifiers all have a significant effect on peak shape and retention times of the compounds of interest. The choice of a suitable wavelength is also important, in terms of method precision, selectivity and sensitivity. The chromatographic conditions were optimized to yield well-resolved peaks with reasonable retention times.

2.2 METHOD VALIDATION

2.2.1 INTRODUCTION

Validation is the process whereby the performance characteristics of an analytical method are established and the analytical method meets the requirements for its intended purpose [73,95,96]. A validation process is essential as it serves to ensure that the quality of analytical data generated is both reliable and accurate, and it is also capable of identifying potential problems with the method. Validation may not be able to rule out all the potential problems of a particular method, however the most common problems may be identified during the validation process. Such problems include column degeneration, changes in column behavior due to changes in the manufacturing process, and the co-elution of impurities, derived from the synthesis of the active, with the analyte of interest [96].

For pharmaceutical preparations, guidelines which form the framework for validation of analytical methods have been published in the United States Pharmacopoeia (USP), the FDA, regulatory

bodies in Europe and Canada and more recently a validation guideline has been published by the International Conference on Harmonization (ICH) [97]. Whilst these sources are in general agreement about the type of studies to be conducted and they provide definition of terms useful for carrying out the validation procedure, there is little agreement as to how these studies should be conducted [95,97] and few published strategies for determining validity of methods are available [98].

The validation parameters outlined by the various organizations vary slightly. The USP and ICH includes eight parameters for evaluation, namely, accuracy, precision (intermediate precision and repeatability/reproducibility), specificity, limit of detection (LOD) and limit of quantitation (LOQ), linearity and range. However, the ICH guidelines also include robustness and system suitability, and suggest that precision be assessed at three levels, namely repeatability, intermediate precision and reproducibility [73,99]. Most validation methods for HPLC analyses include some or all of the parameters described above [97, 100-102]. It is usually unnecessary to perform all the validation studies in the very early stages of drug development and many researchers focus on specificity, linearity, accuracy and precision studies. Other studies are performed when the drug reaches the efficacy stage of development and have a greater chance of becoming a marketed product [95]. The parameters described above are specific to the validation of a developed method, and forms a part of an overall validation process, which includes validation of hardware and software used and verification of system suitability and performance [99].

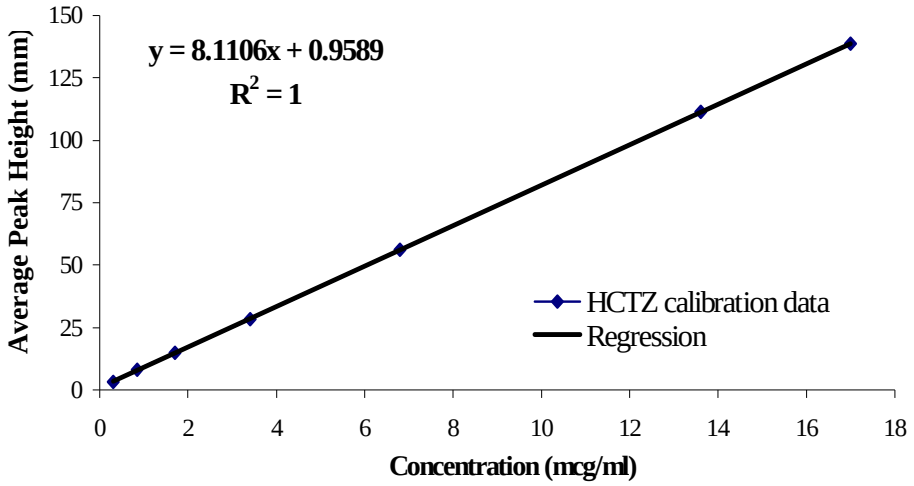
2.2.2 SPECIFICITY

Specificity is defined as the ability to measure the analyte in the presence of other components that may be expected in the sample matrix, accurately and specifically. This may include degradation products, impurities and placebo ingredients [73,95,99,103,104]. Specificity was assessed by comparing chromatograms obtained from analysis of a standard solution containing the analyte only with a sample mixture obtained by dissolving commercial tablets of MPTA and HCTZ in the dissolution medium. The resultant peaks were well resolved from the solvent front and each other and no interference was observed, indicating that the method is specific for HCTZ and MPTA. A possible hydrolysis product of HCTZ was also identified as a well-resolved peak, as shown in Figure 2.4.

2.2.3 LINEARITY

A linearity study is used to verify that the sample solutions are in a concentration range where the analyte response is linearly proportional to concentration [73,95]. The linearity of the range of detectability is dependent on Beer's Law, such that the absorbance of a solute is directly proportional to its concentration in the solution [105]. Linearity in this range is dependent on both the compound analyzed and the detector used [103].

The ICH guidelines recommend that five concentrations spanning the concentration range to be studied are used [99], and it is necessary that a minimum of twenty assays be performed for statistical validity [106]. Linearity was assessed by repeated measurements (n=5) of seven concentration levels spanning the concentration range of 0.28-17μg/mL for HCTZ and 2.25-130μg/mL for MPTA. Acceptability of linearity data was judged by examining the correlation coefficient (r^2) on the regression line for the response versus concentration plot. An r^2 value of >0.990 was considered to be sufficient to demonstrate linearity of the method. The calibration curve was linear over the concentration range studied, with $r^2 = 1.0$ for both MPTA and HCTZ. The equations of the regression lines were y = 8.1111x + 0.9514 and y = 2.1078x + 1.6424 for HCTZ and MPTA, respectively. Typical calibration curves for HCTZ and MPTA are shown in Figure 2.5.



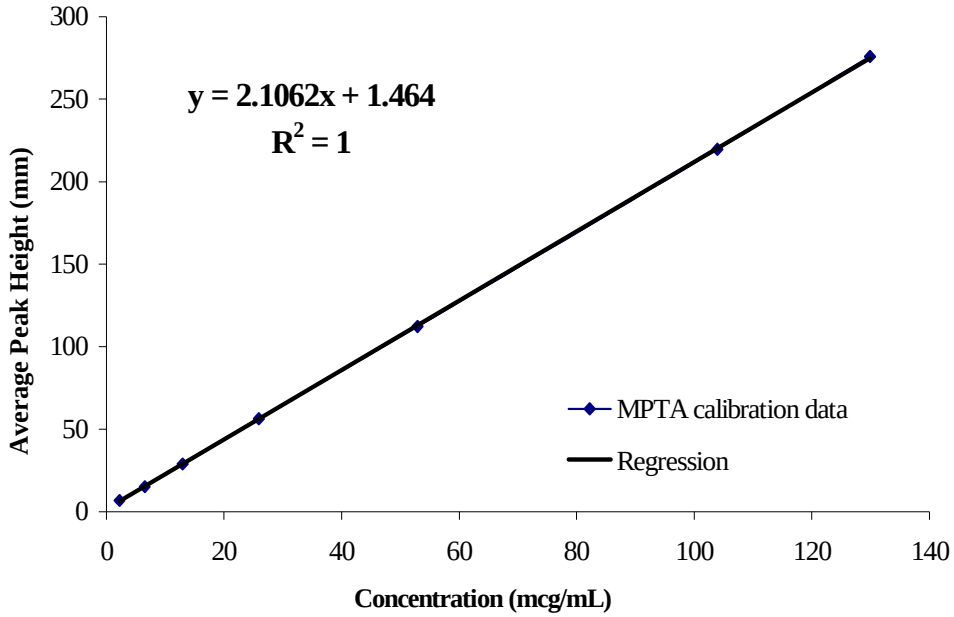


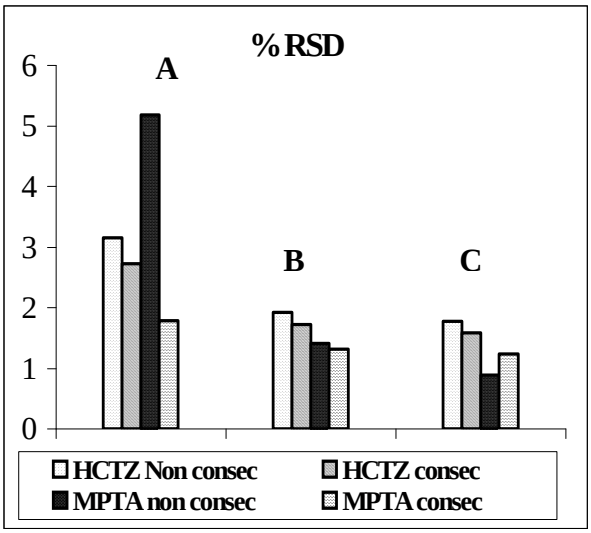
Figure 2.5: Typical calibration curves for MPTA and HCTZ

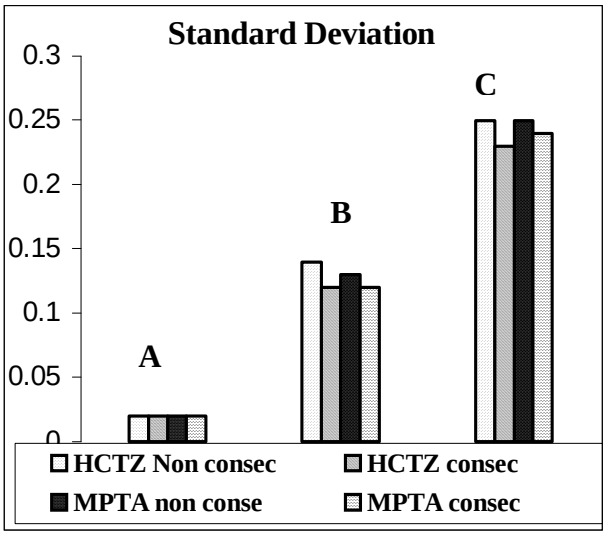
2.2.4 PRECISION

Precision is a measure of the closeness of data values to one other when a number of measurements are taken under the same analytical conditions. The ICH defines precision as having three components, namely: repeatability, intermediate precision and reproducibility [95,99]. Precision is usually expressed as the percentage relative standard deviation (%RSD) [73,95,99] and the tolerance for %RSD was set at ± 10% in our laboratory.

2.2.4.1 Repeatability

Repeatability refers to inter-assay precision and is expressed as the degree of variation arising from consecutive and non-consecutive injections run on the same day. It should be determined using a minimum of nine determinations covering the specified analytical range, for example three determinations at three concentration levels [99]. Consecutive measurements were obtained by repeated injections of the same concentration taken from different vials in succession. Interspersing vials of the same concentration with those of different concentrations was the method used to obtain non-consecutive measurements. Repeatability of this system was assessed by repeat measurements (n=6) of calibration solutions containing 5µg/mL, 50µg/mL and 100µg/mL of MPTA and 0.74µg/mL, 7.43µg/mL and 14.86µg/mL of HCTZ, respectively. Samples were run in both consecutive and non-consecutive sequence. Standard deviations and %RSD values obtained were < 6% (Figure 2.6), which was the limit set in our laboratory, indicating that the method displayed adequate repeatability





*A, B, C represent High, Middle and Low concentrations, respectively

Figure 2.6: Precision data (repeatability) for the analytical method developed

2.2.4.2 Intermediate Precision

Intermediate precision is used to evaluate the reliability of the method in different environments other than that used for the development of the method, and depending on time and res C , the method may be tested on different days, using different analysts and instruments [103,107]. Inter-day precision was determined using calibrators of the same concentrations as described in § 2.3.4.1.Samples were run over a six-day period. Results (Table 2.3) show that all standard deviations values were within the acceptable range, resulting in %RSD values less than C ual to 5% which is within the limits set in our laboratory.

Table 2.3: Intermediate precision data for MPTA and HCTZ

Concentration (µg/mL)	Mean concentration determined (µg/mL)	Standard deviation	Precision (%RSD)
MPTA			
5.0	4.59	0.16	3.40
50.04	49.89	0.82	1.64
100.08	98.96	0.97	0.98
HCTZ			
0.74	0.82	0.32	5.00
7.43	7.26	0.17	2.37
14.86	14.76	0.32	2.19

2.2.4.3 Reproducibility

Reproducibility is used to express the precision of an analytical method when used in different laboratories, however this is not always possible, therefore tests for reproducibility are not normally expected if intermediate precision is accomplished [103], thus no tests were performed.

2.2.5 Accuracy and bias

Accuracy is a measure of the closeness between the true and measured values of a sample [73, 95,99,103]. A tolerance of 2% was set for % RSD for this parameter. This complies with the limits set by a number of pharmaceutical industries [108]. Bias assesses the influence of the analyst on the performance of the method. Accuracy and bias were evaluated by making repeat measurements of three samples of varying concentration. Accuracy studies for drug products are recommended to be performed at 80, 100 and 120% of the target concentration [95,103]. These targets may be specific to the apparatus used for the in vitro tests, where USP Apparatus II may result in larger concentrations from the same dose of drug than in USP Apparatus III. Both Apparatus II and III were used for dosage form assessment, thus accuracy studies were performed in triplicate on samples representative of high, medium and low concentrations. Measurements were performed in triplicate. The results are shown in Table 2.4 and shows that largest value obtained for % bias was 6.16%, indicating that no value obtained deviated by greater than approximately 6% of the stated value. % RSD values all complied with the 2% tolerance set, indicating that the method was accurate for the determination of both MPTA and HCTZ.

Table 2.4 Percent error obtained during determination of blinded samples in accuracy testing

Theoretical concentration (µg/mL)	Mean concentration determined	SD	% RSD	% Bias
	METOPROLOL TARTRATE			
5.00	4.71	0.04	0.19	-6.16
50.04	50.03	0.26	0.52	-0.02
100.08	98.97	0.19	0.83	-1.12
	HYDROCHLOROTHIAZIDE			
0.74	0.70	0.00	0.00	-5.71
7.43	7.1	0.12	1.74	-4.65
14.86	14.44	0.24	1.63	-2.41

2.2.6 RANGE

The range of an analytical method is the interval between and including the upper and lower levels of the analyte that have been quantitated with the necessary accuracy, precision and linearity. The range was determined to be precise and linear over the 0.30-17.00μg/mL range for HCTZ and over a 2.25-130.00μg/mL range for MPTA. Although dissolution studies would be performed in both USP Apparatus II and III, the concentration of the highest calibrator was determined as 120% of that obtained in Apparatus II.

2.2.7 LOQ and LOD

The LOQ is a measure of the level of analyte that can be measured with the required accuracy and precision, and LOD is the lowest analyte concentration that is detectable above the baseline noise of the system [73,95,99,103]. There are several methods for the determination of these parameters, the first of which suggests that the LOQ is the lowest concentration measurable resulting in a %RSD value of <10% upon multiple injections of the sample. The LOD would then be 30% for the concentration obtained for the LOQ [103]. The ICH recommends that LOQ and LOD are determined according to the Equations 2.1 and 2.2, only the calibration curve and replicate blank injections are considered in this case [109, 110].

$$LOD = 3.3 \times \sigma/S \qquad \text{Equation 2.1}$$

$$LOQ = 10 \times \sigma/S \qquad \text{Equation 2.2}$$

where, σ = noise estimate/ standard deviation of ten blank injections

S = slope of calibration curve determined in linearity studies

The USP describes the LOQ as having a signal to noise ratio of 10:1, and the LOD as having a signal to noise ratio of 2:1 or 3:1. Although this concept is widely used, it must be noted that these values are likely to vary with changes in the detector, which may the include deterioration of the detector lamp on prolonged use [103].

The USP method was selected for the determination of the requisite validation parameters. Repeat measurements (n=6) of blank injections were used to establish the baseline noise. Repeat injections of decreasing sample concentrations (n=6) yielded LOD values of 0.15μg/mL and 1.25μg/mL for

HCTZ and MPTA, respectively. LOQ values of 0.30µg/mL (%RSD = 6.93) for HCTZ and 2.27µg/mL (%RSD = 5.67) for MPTA were obtained. Although the %RSD values obtained were higher than for samples of higher concentration, all %RSD values were less than 7%.

2.2.8 RUGGEDNESS

This is expressed as the lack of influence of environmental and instrumental conditions on the analytical method. It is usually determined by analyzing samples under a variety of test conditions such as running the analysis on different days, with different analysts, and using different temperatures and reagents. Results from different day analysis have been described for intermediate precision and thus no further tests were conducted.

2.2.9 ROBUSTNESS

The robustness of an analytical method may be described as its capacity to remain unaffected by small but deliberate changes in the method parameters. This will provide an indication of its performance during normal use [73]. The effect of varying the organic modifier content was assessed in § 2.3.1.5.2. Tests for robustness were continued in the latter stages of development and are discussed in § 2.5.7.

2.2.10 STABILITY OF ANALYTE

MPTA and HCTZ are both known to be photolabile [1,45] and HCTZ undergoes hydrolysis in aqueous solutions. Consequently detection of possible degradants was thought to be advantageous, as this would allow for identification of acceptable storage periods for aqueous solutions of the compounds before they can be analyzed.

Mixtures of MPTA and HCTZ (100µg/mL MPTA, 15µg/mL HCTZ) were prepared and stored under various conditions as described in Table 2.5. These were stored over a twelve-week period, with fresh calibration curves prepared on each day of analysis. Samples were stored to determine the effects of light and temperature on the stability of MPTA and HCTZ in solution.

Table 2.5: Conditions for the assessment of the stability of MPTA and HCTZ in aqueous solutions

CONDITION	ASSESSMENT POINTS
Room Temperature (22°C), No foil cover	0, 6, 12, 24, 48 hours 1, 2, 3, 4, 8, 12 weeks
Room Temperature, Foil covered	1,2,3,4,8,12 weeks
Refrigerator (8°C), No foil cover	
Refrigerator, Foil covered	

Samples were initially analyzed over a 48-hour period in order to determine whether samples could be analyzed after storage for this period of time prior to analysis. Thereafter they were assessed weekly for a month, and then after two and three months of storage, as described.

The statistical method developed by Timm *et al* was used to determine whether the resultant differences in concentrations constituted a relevant or significant change [111]. The method involves the calculation of the 90% confidence intervals for the difference between two data sets, where a change is considered significant if the confidence intervals for the difference between the two do, does not include zero change. However, a change may only be considered relevant if both the upper and lower limits of the confidence interval are either > 10% or < -10% [111]. This concept is graphically represented in Figure 2.7.

The observed differences for the 48-hour assessment of samples stored at room temperature and at 8°C showed neither significant nor relevant changes in MPTA concentration (Figures 2.8 and 2.9). This result was expected as MPTA was found to be stable in solution at 60°C for 10 days (§ 1.4.2). It was expected that MPTA solutions exposed to light would show a significant decrease in analyte concentration, due its instability upon exposure to light, however it was found that all solutions stored at room temperature over the three month period showed both significant and relevant increases in concentration. With the exception of the analysis of one of the samples stored at 8°C, changes in sample concentration were found to be significant and possibly relevant. Samples protected from light and stored at 8°C displayed less variability and resulted in less significant changes in comparison to uncovered samples and those stored at room temperature. Subsequently all samples were stored in foil covered glassware at 8°C if they were to be stored for longer than 48 hours prior to analysis.

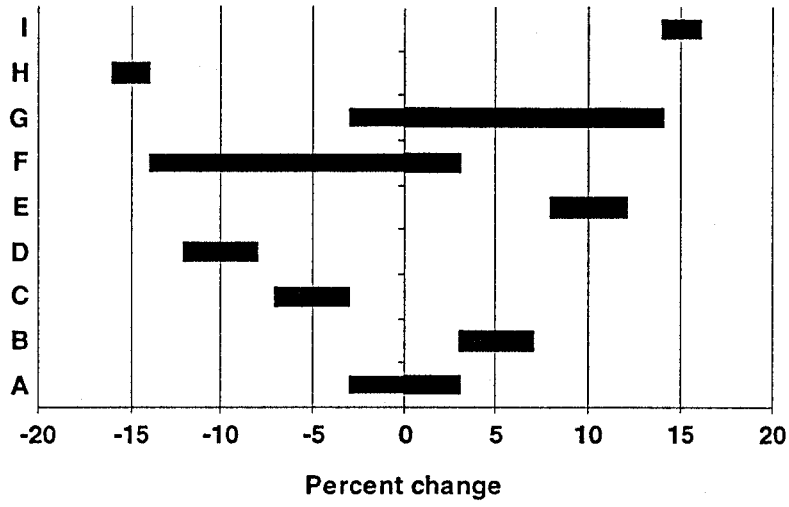


Figure 2.7: Determination of significant and relevant changes in response (Timm analysis)

The bars represent confidence intervals for possible scenarios:

A: The change us neither significant or relevant

B: There is a significant, but not relevant increase in response

C: There is a significant but not relevant decrease in response

D: There is a significant and possibly relevant decrease in response

E: There is a significant and possibly relevant increase in response

F: There is a possibly relevant, but not significant decrease in response

G: There is a possibly relevant, but not significant increase in response

H: There is a significant and relevant decrease in response

I: There is a significant and relevant increase in response

With respect to HCTZ, a significant and relevant decrease in drug concentration occurred after two weeks storage at room temperature (Figures 2.10 and 2.11) and about 90% of the drug appeared to be degraded at the end of the three-month period. Comparatively, a change in drug concentration that was both significant and relevant occurred after four weeks storage at 8°C and this decrease did not exceed 20% of the original drug concentration. These results indicate that HCTZ undergoes rapid degradation, probably via a hydrolytic reaction, in solution and consequently samples must be analyzed with a 48-hour period, after which time drug solutions show both a significant and relevant decrease in concentration. Furthermore, samples stored in the refrigerator show significantly lower degradation rates than those stored at room temperature, over an extended period, however there appeared to be minimal difference in the decrease in drug concentrations of

any of the samples over the first 48 hours.

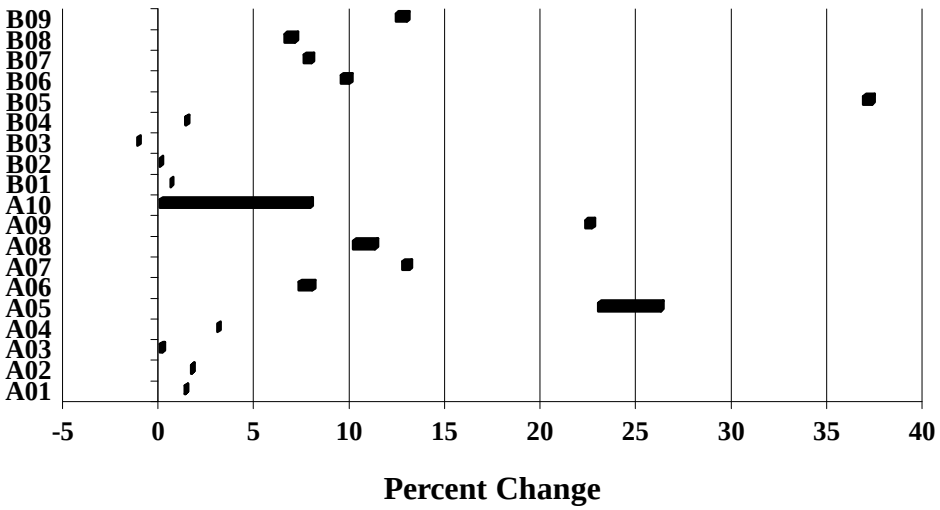


Figure 2.8: 90% Confidence Intervals for differences in response for MPTA solutions stored at room temperature
(A01-A04 and B01-B04 represents uncovered and covered solutions stored for 6,12,24,48 hours, respectively. A05-A10 and B05-B09 represents uncovered and covered solutions stored for 1,2,3,4,8,12 weeks, respectively.)

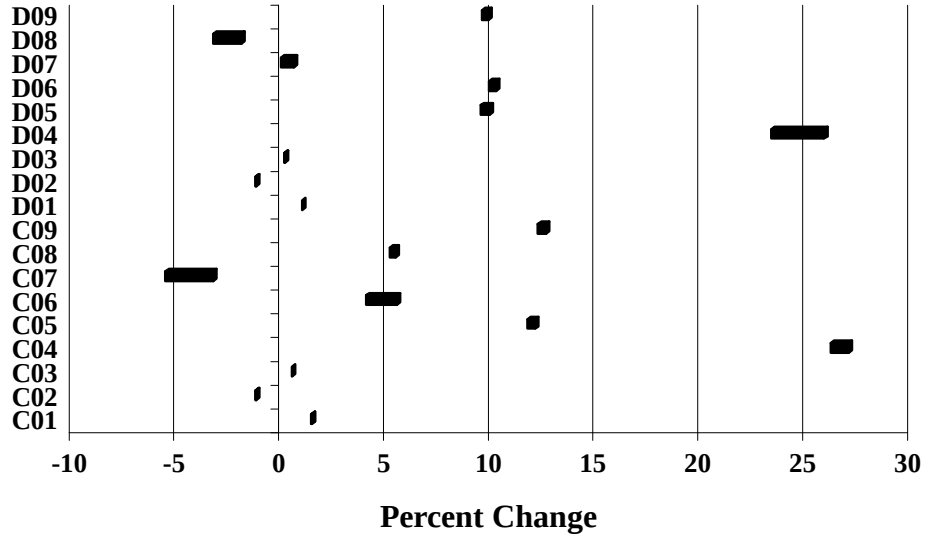


Figure 2.9: 90% Confidence Intervals for differences in response for MPTA solutions stored at 8°C
(C01-C03 and D01-D03 represents uncovered and covered solutions stored for 12,24,48 hours, respectively. C04-C09 and D04-D09 represents uncovered and covered solutions stored for 1,2,3,3,8,12 weeks, respectively).

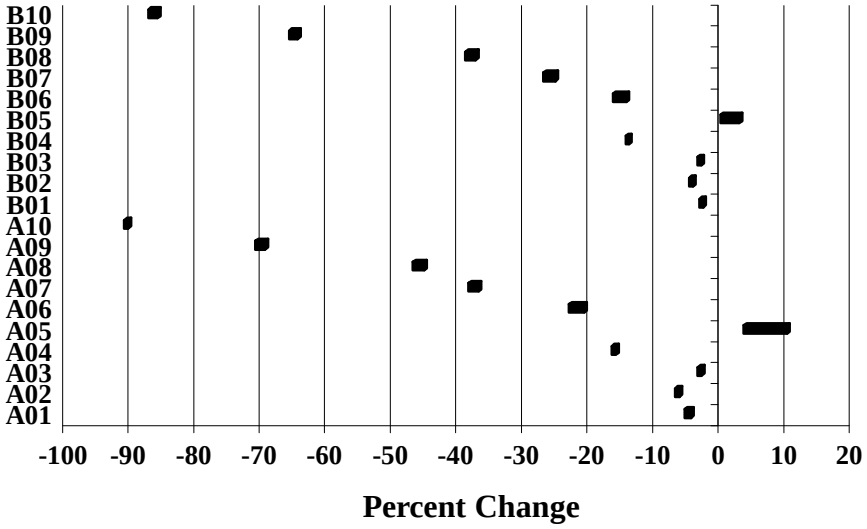


Figure 2.10: 90% Confidence Intervals for differences in response for HCTZ solutions stored at room temperature
(A01-A04 and B01-B04 represents uncovered and covered solutions stored for 6,12,24,48 hours, respectively.
A05-A10 and B05-B09 represents uncovered and covered solutions stored for 1,2,3,4,8,12 weeks, respectively.)

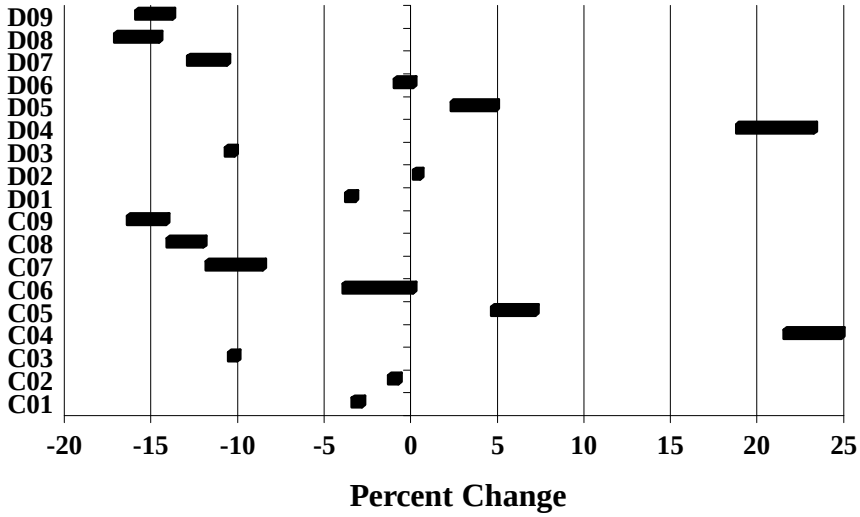


Figure 2.11: 90% Confidence Intervals for differences in response for HCTZ solutions stored at 8°C
(C01-C03 and D01-D03 represents uncovered and covered solutions stored for 12,24,48 hours, respectively.
C04-C09 and D04-D09 represents uncovered and covered solutions stored for 1,2,3,4,8,12 weeks, respectively.)

Over this extended period, a possible product from the hydrolysis of HCTZ was found to increase in peak height as HCTZ showed a corresponding decrease, as shown in Figures 2.12 and 2.13. This degradant occurred as a well-resolved peak that did not interfere with the other compounds being analyzed as shown in the chromatogram in Figure 2.4. Graphs were plotted using the ratios of drug and degradant with the internal standard for comparison as the degradants was not identified and therefore quantitative analysis was not feasible.

It is likely that the degradant is 4-amino-6-chloro-m-benzene disulfonamide, which is one of the hydrolysis products of HCTZ. This reaction of HCTZ is first-order, however at constant pH the reaction kinetics are pseudo first-order (§1.2.3.2). The rate constants for the degradation of HCTZ under each of the conditions studied were calculated by linear regression of a semi-log plot of the ratio of drug/degradant to the IS versus time. These are depicted in Table 2.6. As expected the reaction rate was reduced significantly with a decrease in temperature, as was noted for samples stored in the refrigerator.

Table 2.6: Calculated degradation rate constants for HCTZ

	Rate constant (k)
Room Temperature, No foil covering	-0.0441 hr^{-1}
Room Temperature, Foil covered	-0.0461 hr^{-1}
Refrigerator, No foil covering	-0.0138 hr^{-1}
Refrigerator, Foil covered	-0.0128 hr^{-1}

This study was conducted to determine whether the method was capable of detecting possible degradation products and to determine time limits for the storage of samples before analysis. In terms of storage conditions, it may be concluded that whilst MPTA may be stored for extended periods before analysis, HCTZ samples should be analyzed within 48 hours of collection. The method developed was also found to be capable of detecting possible degradants, although these were not identified.

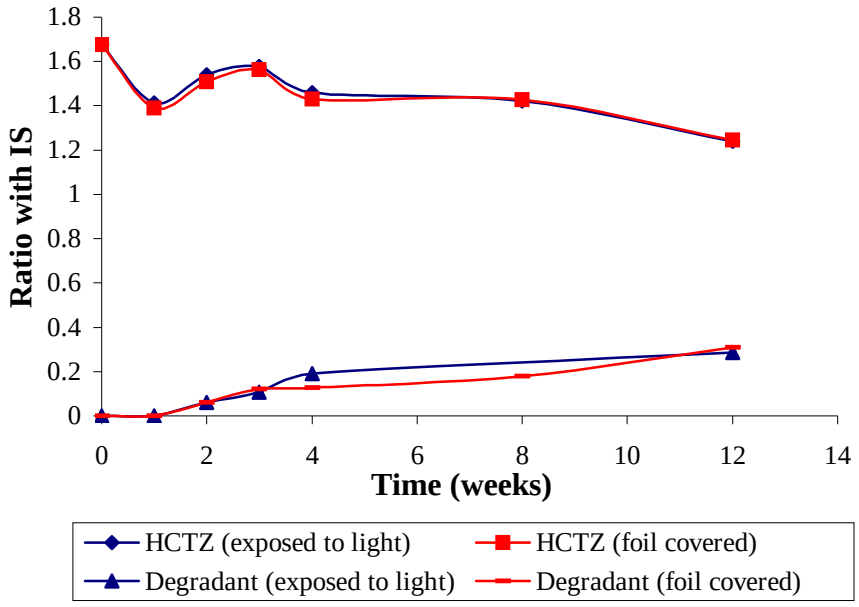


Figure 2.12: Plot depicting HCTZ and degradant ratios with IS after storage at 8°C

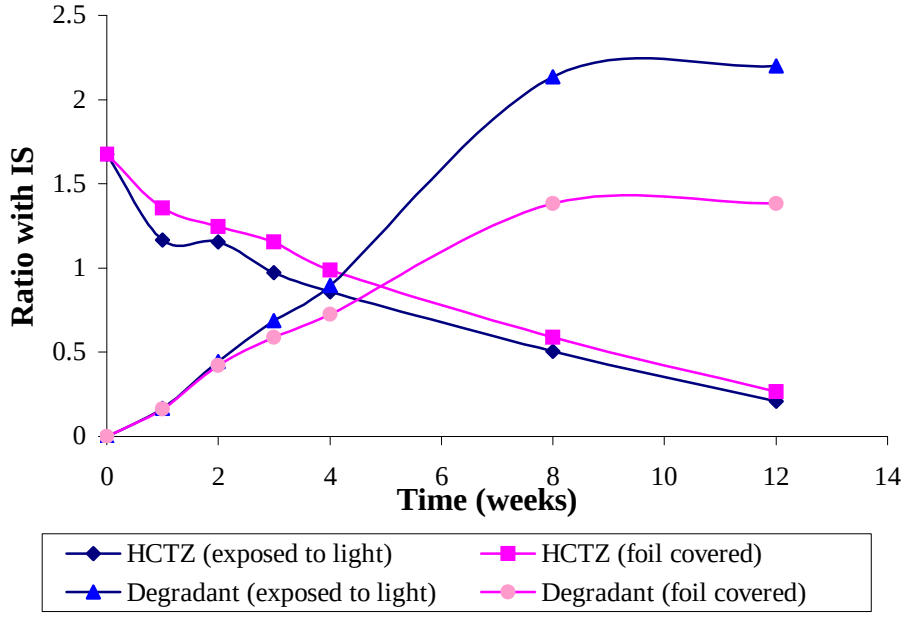


Figure 2.13: Plot depicting HCTZ and degradant ratios with IS after storage at room temperature

2.3 METHOD RE-VALIDATION

2.3.1 INTRODUCTION

Revalidation of a method is usually necessary when changes to the chromatographic conditions are effected [106]. However, revalidation may be necessary when changes in instrumentation, analyst and the product are made [112]. Although no changes to the method were made, the system was changed to System B (§ 2.3.1.2). Furthermore, results obtained were observed to show greater deviations than were previously recorded. It was therefore deemed necessary to perform a re-validation of the method. It was thought that this would also serve as a ruggedness test. Linearity, precision and accuracy were assessed.

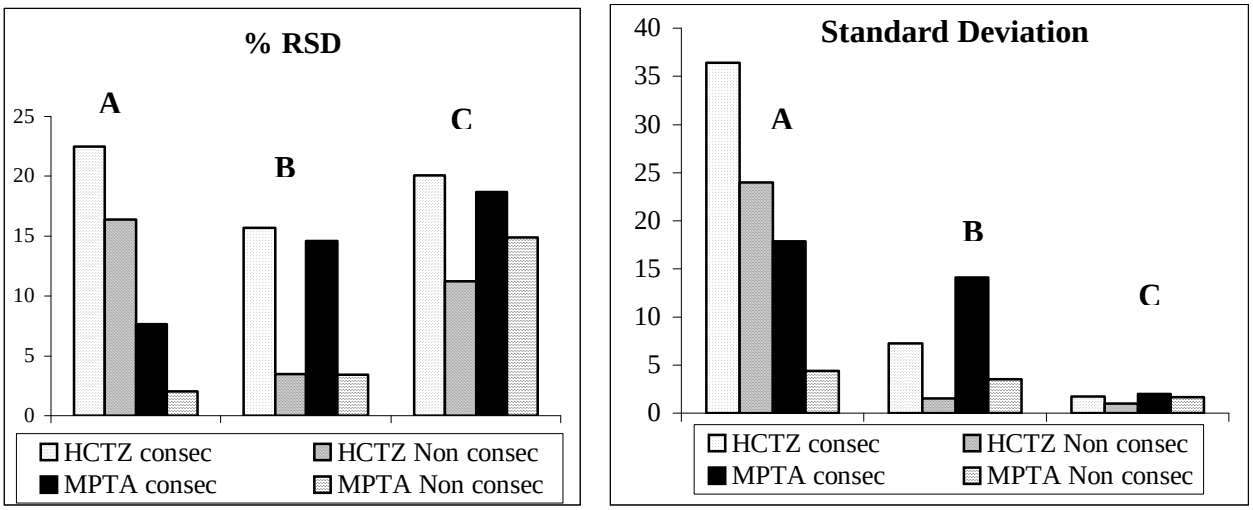
2.3.2 LINEARITY

A calibration curve with the equation $y = 2.1161x – 0.2759$ and a correlation coefficient of 0.9994 for MPTA, and an equation $y = 7.5041x – 2.1586$ and $r^2 = 0.9983$ for HCTZ were obtained. It was concluded that linearity was maintained over the range studied.

2.3.3 PRECISION

2.3.3.1 Repeatability

The data obtained for % RSD and standard deviations for both consecutive and non-consecutive samples of the same concentrations as listed in Table 2.7 are shown in Figure 2.14. The values obtained for both parameters far exceed the limits (6 % for RSD and 5.0 for SD) set for each parameter. Values for %RSD ranged from 2.54 –22.46. Poor repeatability was considered to be a possible function of varying samples volumes being injected.



A, B, C represent High, Middle and Low concentrations, respectively

Figure 2.14: %RSD and Standard deviation values obtained from re-validation of the method

2.3.3.2 Intermediate Precision

Freshly prepared calibrators were run on six consecutive days to determine intermediate precision. Results are shown in Table 2.7. None of the values obtained for both % RSD and SD fell within the range of acceptable limits set, therefore the method was no longer considered to be precise.

Table 2.7: %RSD and SD values of samples run over six days on system B

Concentration (µg/mL)	Mean Peak Height (mm)	Standard deviation	Precision (%RSD)
MPTA			
5.0	13.42	2.87	21.40
50.00	115.03	21.16	18.40
100.00	220.67	18.91	9.23
HCTZ			
1.25	9.92	1.80	18.16
6.25	43.25	8.59	19.86
20.00	177.33	15.25	11.04

2.3.4 LOQ and LOD

No evaluation of LOQ and LOD was performed. However it was noted that the sensitivity of the system had decreased therefore previously determined values would no longer be applicable. The most likely reason for the decrease in sensitivity was the ageing of the detector lamp.

2.3.5 OUTCOMES

It was concluded that the method could no longer be considered to be reliable; therefore changes were required in order to ensure that the method could still be used for the accurate and precise determination of the analytes of interest. The injector was presumed to be the most likely source of the inaccuracies and a decision was made to include an internal standard in the sample mixtures. The internal standard would be used to compensate for variable injections volumes, as the results would be independent of that specific variable. Furthermore, the decrease in the sensitivity of the system may require re-assessment of linearity over the range selected, LOQ and LOD values.

2.3.6 INTERNAL STANDARD (IS)

An internal standard is usually added to ensure accuracy of a method [94]. The IS chosen should be completely resolved from all other peaks and it should elute close to the solute to be quantified. Generally, it should also have similar chemical and physical properties to the analyte and should not be a degradation product of the analyte. An internal standard was not incorporated in the method initially, as it is stated that they should be avoided in stability indicating assays due to possible co-elution with unknown degradation products [94]. The method was not used specifically as a stability-indicating assay; however, the presence of the degradation product of HCTZ precluded the initial inclusion of an internal standard.

Other β-blockers, such as propanolol HCL and oxprenolol HCL are commonly used as internal standards in HPLC either for MPTA only or MPTA in combination with HCTZ, as indicated in Table 2.1. Other β-blockers are considered as suitable candidates as they have similar chemical and physical properties. Although the use of propranolol HCL was commonly reported in literature (Table 2.1), retention time obtained with this candidate on this system was greater than 15 minutes. Other β-blockers were then tested and of these labetalol HCL was selected as the internal standard as it resulted in a R_T of 8.0 minutes only. Furthermore, it displayed a sharp well-resolved peak and no interference with degradants or other analytes was observed.

2.4 METHOD VALIDATION WITH AN INTERNAL STANDARD

The addition of an internal standard does not require a complete re-validation, and some parameters including precision, accuracy, specificity and recovery need to be re-assessed [106].

For this phase of the validation, all parameters excluding ruggedness were re-evaluated. Tests for robustness were also continued using the internal standard.

2.4.1 SPECIFICITY

Labetolol hydrochloride eluted after both MPTA and HCTZ, and the degradation product, therefore minimal interference was incurred by the inclusion of the internal standard. Peaks for all compounds were well resolved and no changes in R_T were noted. A representative chromatogram of the compounds with IS is shown in Figure 2.15.

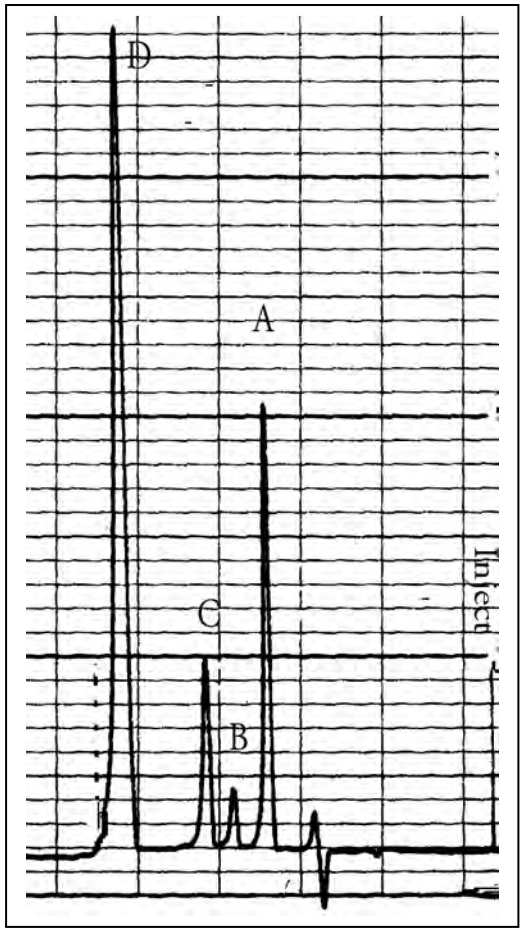


Figure 2.15: A typical chromatogram of HCTZ (A), possible degradant (B), MPTA (C) and labetalol hydrochloride (D)

2.4.2 LOQ and LOD

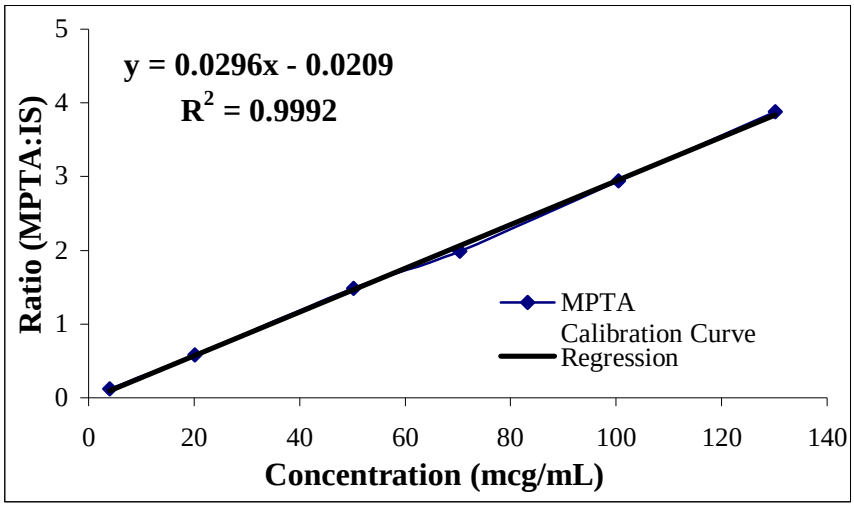
The LOQ and LOD were determined in the same manner as before, using six samples for each. The LOD was found to be 0.19µg/mL and 1.25µg/mL for HCTZ and MPTA, respectively. The LOQ was determined to be 1.22µg/mL (%RSD = 4.56) and 4.00µg/mL (% RSD = 5.20) for HCTZ and MPTA, respectively. Whereas the LOD values did not alter significantly, LOQ values were significantly changed, possibly as a function of a decrease in the sensitivity of the detector.

2.4.3 RANGE

The change in LOQ resulted in a subsequent alteration of the range over which the analytes could be accurately and precisely determined. The range over which adequate linearity was demonstrated was therefore 1.22 -17.00µg/mL for HCTZ and 4.00-30.20µg/mL for MPTA.

2.4.4 LINEARITY

The equations for calibration curves of the compounds were now representative of the concentration and the ratio of drug to internal standard. Representative equations were y = 0.0296x-0.0209 for MPTA (r^2 =0.9992) and y = 0.0113x-0.0098 for HCTZ (r^2 =0.9997). The linearity of the method over the new range was comparable to that obtained during the method development stages, and showed a definite improvement over the results obtained in the method re-validation without the inclusion of the internal standard. Typical calibration curves are depicted in Figure 2.16.



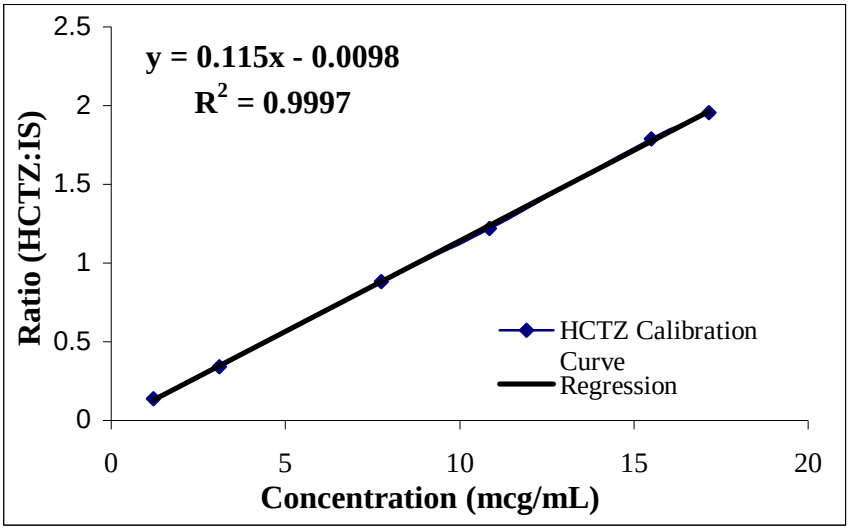


Figure 2.16: Typical calibration curves for MPTA and HCTZ obtained using an internal standard

2.4.5 PRECISION

2.4.5.1 Repeatability

Precision data was obtained at high, intermediate and low concentrations as described in § 2.2.4.1. The % RSD and standard deviation data are depicted in Figure 2.17. The % RSD values obtained all fell within the range of acceptable limits set (< 10%). The repeatability of the system was therefore improved with the inclusion of an internal standard, indicating that the earlier assumption that poor repeatability was a function of variable injection volume may be a probability.

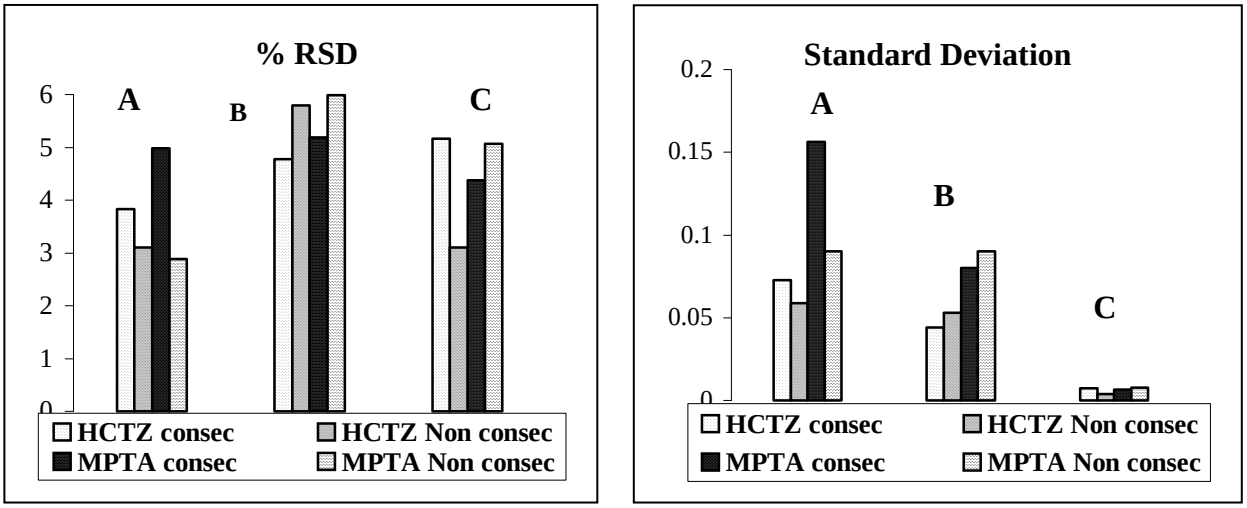


Figure 2.17: % RSD and standard deviations of drug: IS ratio from validation with an IS

2.4.5.2 Intermediate Precision

Intermediate precision was determined over a six-day period. The concentrations used are listed in Table 2.8, and although they differed slightly from those used for the previous validation studies, they are representative of the range that was considered. %RSD and standard deviation are listed in Table 2.8. Both parameters were significantly improved over data generated from method from the re-validation of the method without an IS, as all values obtained were with in the limits for acceptance set in our laboratory.

Table 2.8: Intermediate Precision results from validation studies with an IS

Concentration (µg/mL)	Average Ratio Obtained	Standard deviation	Precision (%RSD)
MPTA			
4.0	0.1064	0.0058	4.98
50.00	1.4441	0.9649	4.19
100.00	2.8197	0.1364	4.63
HCTZ			
1.20	0.1345	0.0067	5.44
7.60	0.8755	0.0368	4.49
15.2	1.6916	0.0783	4.84

2.4.6 ACCURACY

Accuracy was determined using blinded samples of three concentrations (n=6), as was previously described (§2.2.5). The results (Table 2.9) revealed that the percent error was less than 5% for all samples analyzed, which compared favourably to the results obtained previously in § 2.2.4.2.

Table 2.9: Percent error obtained during determination of blinded samples

Theoretical conc (µg/mL)	Mean conc determined (µg/mL)	SD	% RSD	% Bias
METOPROLOL TARTRATE				
4.00	3.94	0.0028	2.19	-1.50
50.40	48.76	0.0724	5.17	-3.26
90.72	90.41	0.1152	4.49	-0.34
HYDROCHLOROTHIAZIDE				
1.20	1.15	0.0034	2.73	-4.17
7.50	7.46	0.0429	5.08	-1.34
13.50	13.68	0.0665	4.32	0.48

2.4.7 ROBUSTNESS

The compounds were dissolved in a range of buffers that would possibly be used as dissolution media for dissolution testing in USP Apparatus II and III. Results are shown in Table 2.10. Results are depicted for solutions containing 100.00µg/mL MPTA, 15.00µg/mL HCTZ and 50.00µg/mL labetalol HCL. The %RSD calculated for average ratios from the dissolution assessed are below 5% for both compounds, indicating that the absorption is not significantly affected. The same set of calibration solutions may therefore be used for the analysis of all samples in dissolution studies.

Table 2.10: Assessment of compound abosrbance in different dissolution media

Dissolution Medium	HCTZ			MPTA		
	Average Ratio	SD	% RSD	Average Ratio	SD	% RSD
Water	1.48	0.06	4.14	2.63	0.13	4.80
0.1M PO_4 Buffer pH 1.6	1.62	0.04	2.56	2.49	0	0
0.1M PO_4 Buffer pH 3.4	1.50	0.02	1.35	2.60	0.13	4.91
0.1M PO_4 Buffer pH 4.7	1.49	0.13	8.60	2.84	0.09	3.25
0.1M PO_4 Buffer pH 6.8	1.52	0.0054	1.79	2.73	0.0059	0.22
0.1M PO_4 Buffer pH 7.2	1.55	0.02	1.22	2.76	0.05	1.88
Mobile Phase	1.52	0.03	2.27	2.64	0.07	2.71
Average for all media ± SD	1.52 ± 0.05			2.67 ± 0.11		
% RSD	3.02			4. 25		

2.5 CONCLUSIONS

A linear, precise and accurate method has been developed for the simultaneous determination of MPTA and HCTZ over the range studied. The method is also suitable for the determination of drug release from both immediate and modified release dosage forms.

CHAPTER THREE
FORMULATION, DEVELOPMENT AND ASSESSMENT OF A CONTROLLED RELEASE MPTA TABLET CORE

3.1 CONTROLLED RELEASE SYSTEMS

3.1.1 INTRODUCTION

In contrast to conventional dosage forms, controlled release products have a dual functionality, that is to deliver the drug to that particular part of the body that it is needed, and to control the rate at which the drug is made available to its site of delivery [113]. There is often confusion surrounding the terms ‘controlled’ and ‘sustained’ release. The term ‘controlled release’ refers to a dosage form, which releases the drug in vitro by predicted physicochemical mechanisms that are believed to be operating in physiological in vitro test conditions [114]. As such, it describes the delivery of a drug specifically to the needs of a condition such that an optimal amount of drug, delivered at an optimal rate, is used to control the condition. Sustained drug delivery becomes necessary when the needs of the condition are such that drug release over a prolonged period is vital to therapeutic success [114].

Research into and clinical applications of controlled drug delivery have increased rapidly in the past few decades [114]. Such systems are considered advantageous as they can be used to ensure safety or enhance efficacy of drugs. The benefits are primarily achieved by better control of plasma drug levels and less frequent dosing. The decreased frequency of dosing also leads to increased patient compliance [115]. The use of controlled release dosage forms also leads to improved safety and minimization of side effects by reducing the fluctuations in blood plasma levels that are usually observed on frequent dosing of immediate release products [115]. In addition, there may also be economic advantages, despite the cost per dosage being greater than conventional dosage forms; the efficiency of the dosage regimen is improved in the long term [116].

3.1.2 ORAL CONTROLLED RELEASE SYSTEMS

The interest in controlled drug delivery systems has not been limited to the oral route, however, this route of administration is by far the most popular as it allows for greater flexibility in dosage form design than for parenteral or transdermal routes of delivery [115]. The following discussion

will focus primarily on oral delivery systems, which may be broadly categorized as membrane, matrix or hybrid type systems [117].

3.1.2.1 Membrane systems

A drug core surrounded by a rate controlling membrane characterizes such systems [117] and these may also be described as reservoir systems [114]. Membrane systems are generally non-disintegrating technologies that release drug either by osmotic pumping or a solution diffusion mechanism [117]. Examples of commercial products that represent such systems include Adalat XL® (Bayer, SA) which is an osmotic pump system, and Erythrocin® (Abbott Laboratories, SA), which consists of coated beads in a rapidly disintegrating caplet. Membrane systems will be discussed in greater detail in Chapter Four.

3.1.2.2 Matrix systems

Matrix systems, in general consist of drug that is either dissolved or dispersed in a carrier matrix consisting of one or more polymers [114,117], and may include beads, pellets or tablets where the drug is uniformly dispersed or dissolved. Matrix systems have become increasingly utilized as they are relatively cheap to manufacture when compared to other types of controlled delivery systems. They are also easier to manufacture and they lack dose dumping potential of immediate release products [117].

3.1.2.3 Hybrid systems

Such systems may also be referred to as sandwich systems, and are usually a combination of both matrix and membrane systems [114,117]. They combine a number of drug release mechanisms to achieve constant drug release and an example of a system in use is Theo-Dur® (Astra, SA). Theo-Dur® tablets are a multiple unit system that consists of coated beads embedded in a tablet matrix. These systems will be discussed in greater detail in Chapter Four.

3.1.3 MATRIX SYSTEMS

Matrix systems are prepared with an excess of uniformly dispersed drug occurring either as discrete crystals or as solid particles within the matrix system. A matrix system that contains only one rate-controlling polymer is referred to as a monolithic device.

The choice of the matrix-forming polymer is critical and it should the proper ratio of ionisable groups and should exhibit gradual hydration and swelling, to allow for constant drug release throughout the gastro-intestinal tract [118]. Cellulose ethers have been increasingly used in matrix formulations in recent years [119]. Certain polymers such as glyceryl monostearate may be selected for use in matrix systems on the basis of their lipophilic character, where its lipophilic nature plays a greater role in retarding the release than do other factors such as the porosity and tortuosity of the matrix [120]. Polyvinylchloride, ethylcellulose, polyethylene and methacrylate polymers and copolymers have also been used as polymers for hydrophobic matrices [121].

Ethylcellulose may be used as a matrix-forming agent in both direct compression and wet granulation formulations, however selection of the appropriate viscosity grade is essential [122]. Furthermore, interactions between drug to be delivered and polymer may lead to altered release mechanisms. Salicylic acid interacts advantageously with high viscosity chitosan to achieve a sustained release effect [123], whereas pseudoephedrine sulphate has been found to alter the hydration and erosion characteristics of HPMC in combination with PVP [124].

Drug release from monolithic matrix systems may be controlled by the gelled matrix and the matrix-bulk medium interface [125]. The drug release from such systems is also in part dependent on the solubility of the drug in the matrix, however in a porous matrix, release depends primarily on the solubility of drug in the sink conditions within the particle pore network in addition to the tortuosity of that network [116]. Matrix formulations are seldom used for water-insoluble drugs due to difficulties in achieving one hundred percent drug release from these dosage forms [126]. Mehta *et al* found that for a highly water-soluble drug, the rate of release from matrix pellets with a higher drug loading was less than for lower levels of drug loading which may have resulted in differences in pore diameter within the matrix, and shape and surface area of the pellets [127]. The continual evolution of matrix technology had led to the development of innovative systems, which may utilize a combination of polymers to achieve the desired level of control over drug delivery. Such systems include the matrix forming polymers used in combination with channeling agents to achieve the required release rates [128] or systems where the drug is separated from the matrix by compression into a core that is surrounded by the matrix material [128]. Streubel *et al* described a multi-layered matrix tablets that may also be used to achieve bimodal drug release such

that rapid drug release occurs both initially to provide rapid onset of action and at a later time to compensate for poor absorption from the intestinal regions [129]. The inclusion of acidic excipients into a dosage form that contains a basic drug has also been reported to result in sustained release of the drug candidate [130]. It has also been reported that a hydrophilic polymer and an enteric polymer used in combination may be used to prevent the release of basic drugs in acidic media. In acidic media the enteric polymer contributes towards retarding drug release however in alkali media, the polymer dissolves, increasing the porosity of the dosage form, thereby increasing drug release from the dosage form [130]. Biodegradable matrices using lactide-glycolide copolymers have also been used and release from such systems has been found to be coherent with the Higuchi model [131]. Pillay and Fassihi have also proposed that the inclusion of appropriate electrolytes into a matrix dosage form may be capable of sustaining the release of a highly water-soluble drug such as MPTA over an extended period in a pH-independent manner [132].

3.1.3.1 Mechanisms of drug release from matrix systems

3.1.3.1.1 DIFFUSION CONTROL

Drug release from polymers that do not absorb large amounts of water, for example, the polyamides and polyethylene is primarily controlled by Fickian diffusion. Control from hydrogel matrices that absorb water and simultaneously release the enclosed drug occurs via a different mechanism [133]. The dissolution medium has to penetrate into the matrix to dissolve the drug, which then diffuses through the matrix into the external medium. This is the primary mechanism of release for highly water-soluble drugs from these types of systems [117,134]. However, diffusion often occurs together with other mechanisms of release, including erosion, swelling or a dissolution-controlled mechanism [117]. In contrast, hydrophobic matrices exhibit counter-current diffusion as a primary mechanism of release [135]. Zero order release is not easily achieved in matrix systems, as the rate of drug release continually decreases with the increasing diffusional path length within the matrix [133,136,137]. In general release from matrix systems obeys square root of time dependent kinetics such that drug release continuously decreases with time at a rate that is proportional to the amount of drug remaining in the matrix at a specific time [113,133].

Diffusion-controlled release is described by Fick's Second Law (Equation 3.1). The simultaneous influx of solvent and efflux of drug from the polymeric dosage from must be taken into account in order for the relationship to hold [134].

$$\frac{\delta C}{\delta t} = D \frac{\delta^2 C}{\delta X^2} \qquad \text{Equation 3.1}$$

Where,

D = diffusion coefficient of the drug
C = drug concentration in the reservoir
X = the perpendicular distance traveled by the drug

The Higuchi model (Equation 3.2) best describes the square root of time dependent release kinetics usually exhibited by most matrix systems [117].

$$Q = [D (2A - C_s) C_s t]^{½} \qquad \text{Equation 3.2}$$

Where,

Q = weight in grams of drug released per unit surface area
D = diffusion coefficient of drug in the matrix
A = concentration of the drug in the tablet (g/mL)
Cs = solubility of drug in the release medium
t = time

In deriving Equation 3.2, Higuchi assumed that an excess solute was present in the matrix (A >>Cs) and that psuedo steady state is maintained during drug release. It was also assumed that perfect sink conditions were maintained and that the diffusion coefficient was constant throughout the test period. In addition drug particles should also be significantly smaller than those of the matrix material and no chemical or physical interactions should occur between the drug and the matrix material [138].

This model was initially developed for non-eroding matrices and was later modified to describe release from eroding matrices, and therefore includes terms for porosity and tortuosity (Equation 3.3).

$$Q = \left[\frac{D\varepsilon}{\tau}(2A - \varepsilon C_s) C_s t\right]^{1/2} \qquad \text{Equation 3.3}$$

Where,

ε = porosoity of the matrix, which may be approximated by calculating the volume of drug (A) as a percentage of the matrix volume.
τ = tortuosity of the matrix, which refers to the degree of convolution of the channels.
D = diffusion coefficient of the drug in the fluid filled channels, which may be modified by τ, hence the effective diffusion coefficient is expressed as D/τ.
For eroding matrices, the diffusion coefficient differs from Fick's Law as it refers to diffusion through the fluid filled pores and not through the matrix.

3.1.3.1.2 DISSOLUTION CONTROL

Dissolution controlled release occurs mainly in the case of poorly water soluble drug particles within a matrix or in cases where a water soluble drug is present at a high drug loading [117]. In such cases drug dissolution becomes the rate-limiting step in drug release, in this instance where the depletion layer is small, the release profile will be linear over time.

3.1.3.1.3 SWELLING AND EROSION CONTROL

In general matrix systems commonly include a hydrophilic polymer and thus drug release may be controlled by both swelling and erosion dependent mechanisms [117]. Swelling is a complex process that is dependent on swelling inhomogeneity, polymer stress relaxation and solute diffusion which in turn is related to the structure and composition of the gelled matrix [125]. On contact with an aqueous medium, the polymer rapidly forms a viscous gel layer, as depicted by region A in Figure 3.1. At this stage there are two distinct fronts established around the matrix, namely, the penetrating swelling front and the erosion-dissolution front as depicted by the schematic diagram of a swelling hydrophilic matrix tablet in Figure 3.2. [117,125]. Swelling continues (region B in Figure 3.1), until the polymer concentration near the gel surface falls to below the 'disentanglement' concentration for the specific polymer. This is a region of synchronization (B) of both the swelling and erosion/dissolution fronts, and is characterized by a constant gel layer thickness. In this region almost constant rate of drug release occurs. In region C both erosion and dissolution occur and the gel layer recedes (Figure 3.2) becomes depleted.

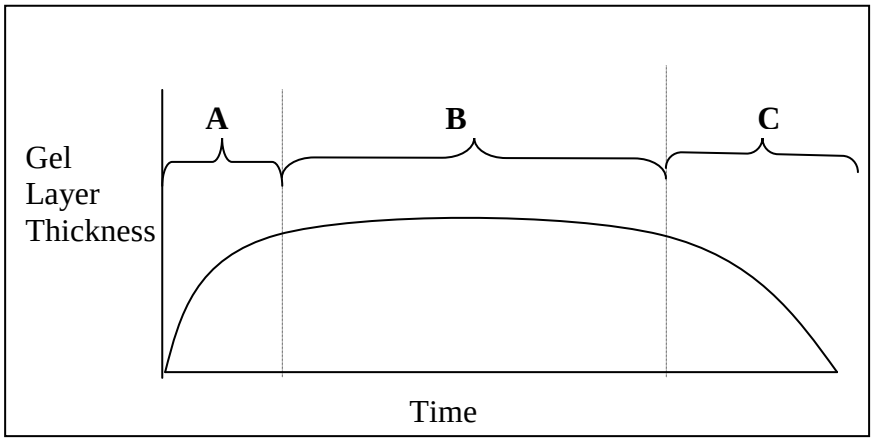


Figure 3.1: Development of gel layer thickness in a matrix tablet over time

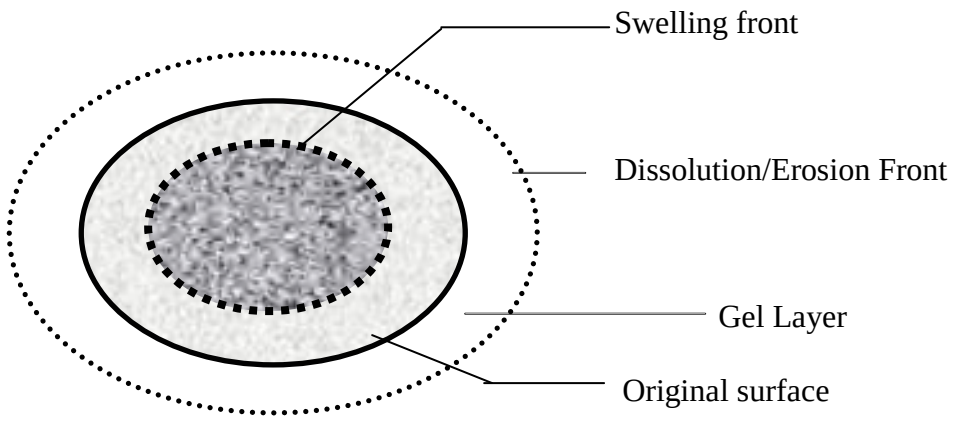


Figure 3.2: Schematic showing the moving fronts during swelling and dissolution of a matrix tablet

The release for certain drugs from a matrix system may be predominantly controlled by erosion of the matrix [139]. For example, it has been found that theophylline release from a *cissus populnea* polymer matrix is primarily erosion controlled [139]. Certain matrices may control drug release via polymeric degradation at a specified location in the body. Degradation may occur by breaking of cross-links, hydrolysis or ionization of polymer side chains or cleavage of the insoluble polymers into soluble monomers [113]. However, due to the possibility of bulk degradation and subsequent dose dumping degradation systems are not often used for controlled drug delivery.

3.1.3.1.4 NONUNIFORM DRUG DISTRIBUTION

In systems in which drug load increases from the surface to the core of a matrix tablet, drug release is a function of its non-inform distribution within the dosage form, which compensates for the increasing diffusional resistance and decreasing area of the diffusion front. Drug release via this mechanism may approach zero-order and systems that have been based on this mechanism include pulsatile drug release systems and multi-layered tablets [117].

3.1.3.1.5 GEOMETRY AND AREA CHANGES

Drug release for a particular drug may be optimized by changing the area and geometry of a dosage form. Changes in area and geometry compensate for the decreasing release rate displayed by most matrix systems and have been found to display linear release [117]. Ainaoui *et al* suggested that by calculation of the dimensions of dosage forms of different shapes, the required sustained release profile might be achieved [140].

3.1.3.2 Hydroxypropyl methylcellulose (HPMC) as a matrix-forming agent

HPMC has become one of the most commonly used polymers for matrix tablet formulations [117,141,142], as a result of its nontoxic nature, ease of handling and the elimination of uncomplicated methods of tablet fabrication [126].

HPMC matrices exhibit a continuous increase in gel layer thickness during the synchronization phase of polymer swelling, however polymer erosion and dissolution occur simultaneously but at a significantly slower rate than the rate of swelling. Dissolution and erosion occur in all viscosity grades of HPMC but have a minimal impact on the rate of release of water-soluble drugs [117]. For water-soluble drugs release occurs predominantly by a swelling controlled diffusion process, as is the case for most other polymeric matrices whereas release of water insoluble drugs occurs via an erosion-dissolution controlled processes [117,143]. The release of high doses of water insoluble drugs may be both erosion and diffusion controlled [118]. This effect is enhanced by increasing the percentages of polymer in the dosage form and leads to the formation of a thicker gel layer [144].

For certain drugs such as indomethacin which is water-insoluble, HPMC used in direct compression formulations, resulted in lower drug release than from a wet granulation of the same composition, suggesting the possibility that drug release from this particular dosage form is controlled by mechanisms other than erosion or diffusion [141].

Drug diffusion through HPMC matrices may be influenced by various factors including swelling ratio, which refers to the amount of water contained within the hydrogel at equilibrium and specific pore size, which refers to the space available for drug transport through the matrix [145]. Characteristics of the drug candidate under investigation that are important include particle size and shape and the degree of ionization of the drug [145].

3.2 DOSAGE FORM DEVELOPMENT

3.2.1 PROPOSED DESIGN

Existing systems for the sustained release of metoprolol include matrix-based, OROS® and multi-particulate systems. Although it has been reported that the matrix-based products are not ideal as they release almost the entire dose within 10 hours [141], matrix systems are still frequently used, as discussed in § 3.1.2.2, as they tend to be cheap and easy to manufacture.

3.2.2 PRELIMINARY STUDIES

A matrix formulation that was developed ‘in-house’ [146] was thought to be capable of retarding the release of highly water-soluble drugs, and was chosen as the basis for the development of a sustained release metoprolol tablet core. The tablet was manufactured by granulation with an ethylcellulose aqueous dispersion, and compression of the granules into a hydrophilic matrix tablet. HPMC was used as the matrix-forming polymer with dibasic calcium phosphate (DCP) and microcrystalline cellulose (MCC) included as additional excipients into the formulation. Magnesium stearate was added as a lubricant.

Preformulation studies included screening for compatibility of MPTA with the chosen excipients and evaluation of the previously developed formulation using MPTA as the active. Differential Scanning Calorimetry (DSC) was used as a rapid method to determine potential incompatibilities.

3.2.2.1 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

3.2.2.1.1 Introduction

The solid-state stability of a drug may be altered in the presence of excipients either directly in the form of a chemical interaction or indirectly as a result of adsorption of moisture or catalysis of a reaction [147,148]. Such information is usually obtained during preformulation studies and offers valuable information about potential physical or chemical incompatibilities between the active pharmaceutical ingredient and potential formulation excipients [149]. Techniques, which are commonly employed to detect incompatibilities between drug and excipients, include isothermal stress testing and thermal analysis using DSC or differential thermal analysis (DTA) [150].

DSC has a number of useful applications including the determination of drug purity, detection of polymorphic forms, melting and boiling point determination and the determination of glass transitions [151]. More recently its application has been extended to the determination of solid-state interactions such as drug-excipient incompatibility in preformulation studies. Its applicability in this area has increased due to the relative speed and convenience of this method of analysis [147,152], which makes it particularly useful in early stages of drug testing or product development where time lines are critical. Furthermore, long-term storage and subsequent chromatographic analyses that are required for isothermal stress tests are eliminated [150,153]. DSC is also particularly advantageous when there is a limited availability of the drug, as only small samples (2-5mg) are required for this analytical technique [150,153,154]. It may also be used as a screening tool and as a complimentary technique to the proposed multivariate methods for the selection of excipients for a tablet formulation [155].

Although some industries routinely use DSC as a means of detecting possible incompatibilities between drug and excipients, this method remains controversial, as it is not considered to be fully reliable [154]. Therefore it should not replace long term stability testing and other tests should be performed in conjunction with DSC or DTA methods of analysis [147-150,154,156]. DSC has other pharmaceutical applications and may for example be used to characterize the effects of manufacturing processes such as powder grinding on drug release from a dosage from [157], and to identify the effects moisture on the drug release from microspheres [158].

DSC is a thermal analytical technique of thermal analysis that measures differences in energy inputs between a substance and a reference material as a function of temperature. The substance under investigation and the reference material are both subjected to a controlled temperature-heating programme [156]. The appearance of, or shifts or disappearance of melting endotherms/exotherms, or variations in the enthalpy are indications of possible drug-excipient interactions [147]. Generally, a decrease in the melting peak area and heat of melting is an indication of a potential incompatibility. The greater the decrease in the melting point the more likely the potential for an interaction being the materials being heated.

3.2.2.1.2 Experimental

3.2.2.1.2.1 REAGENTS

The excipients tested included DCP (Emompress®), MCC (Emcocel® 90M), magnesium stearate and HPMC (Methocel® K4M), which were utilized for the initial tablet batches. HCTZ was also included for screening.

3.2.2.1.2.2 INSTRUMENTATION

A Perkin-Elmer DSC-7 Differential Scanning Calorimeter (Norwalk, Conneticut, USA) was used for all thermogram scans. A Sartorius® 4305 microbalance was used for weighing of samples.

3.2.2.1.3 METHOD

Samples (2-5mg) were weighed into standard aluminum pans and covered with lids, and samples were heated in an atmosphere of nitrogen, at a constant heating rate of 10°C per minute. Thermograms of individual compounds, as well as, 1:1 mixtures of drug: excipient or drug:drug were used. The physical mixtures were prepared by grinding equal quantities of each component in a mortar and pestle. Physical mixtures (1:1) were used to maximize the likelihood of interaction and to avoid any endotherm masking phenomena resulting from higher ratios of either component [152]. Thermograms obtained from the individual compounds and of 1:1 mixtures were superimposed for comparison.

3.2.2.1.4 RESULTS AND DISCUSSION

A trace of metoprolol tartrate heated alone is shown in Figure 3.3. The melting point temperature of metoprolol occurred at 120.33°C and was confirmed by values reported in the literature (§ 1.1.2.5). The tartrate salt resulted in a broad melting peak at 215.67°C that also corresponded with values reported in literature [159].

DSC scans of HPMC, MPTA 1:1 physical mixtures are shown in Figure 3.4. HPMC is a polymer therefore no endotherm was expected. Glass transitions of compounds such as polymers may lead to changes in the DSC curve rather than reveal a distinct peak [151]. The glass transition temperature of HPMC has been reported to lie between 170-180° C [159]. The trace for the mixture shows differences in the 190°C and 240°C region. This may possibly be attributed to the glass transition of the polymer. Browning of HPMC at 190-200°C and subsequent charring at 225-230°C [159] may have also been contributors to the altered thermal activity observed.
Typically a scan of a glassy polymer shows the glass, recrystallization and melting transitions as can be seen in the schematic in Figure 3.5 [151]. The scan does not show these events, however, the observed exotherms may correspond to recrystallization of the polymer.

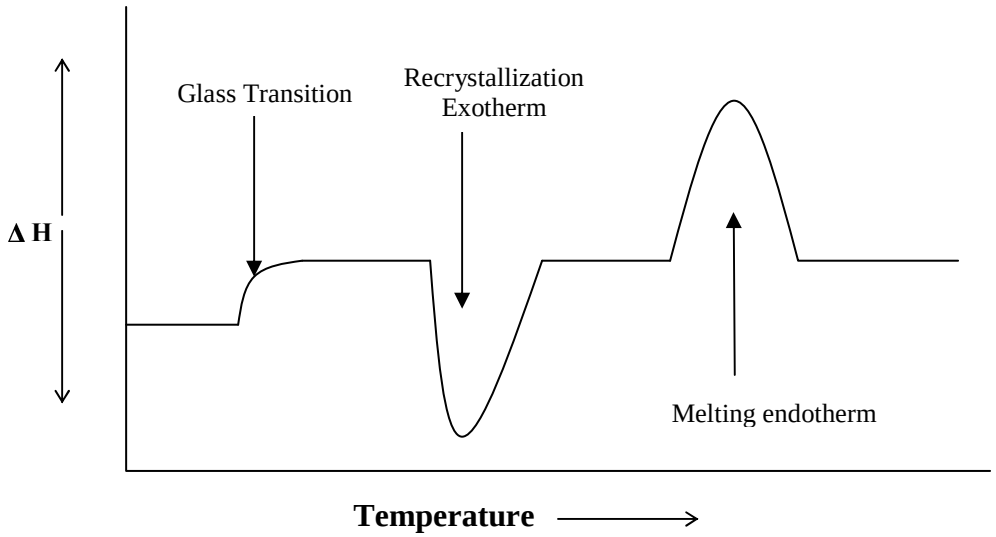


Figure 3.5: A schematic DSC scan of a glassy polymer

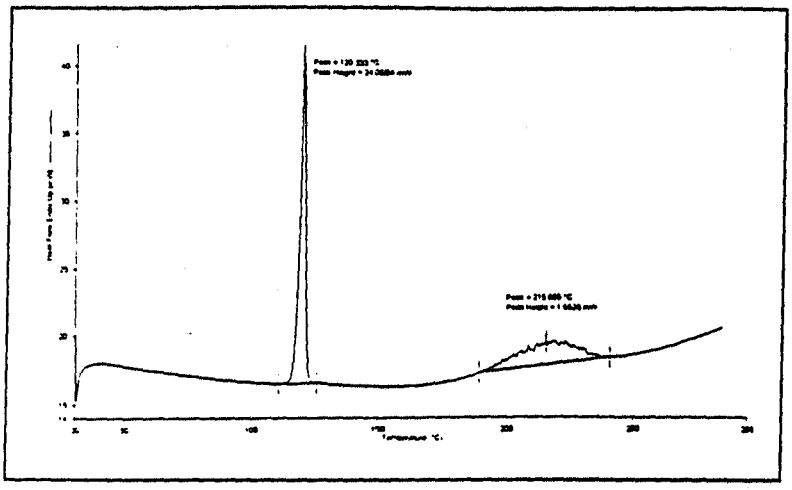


Figure 3.3: DSC scan of metoprolol tartrate

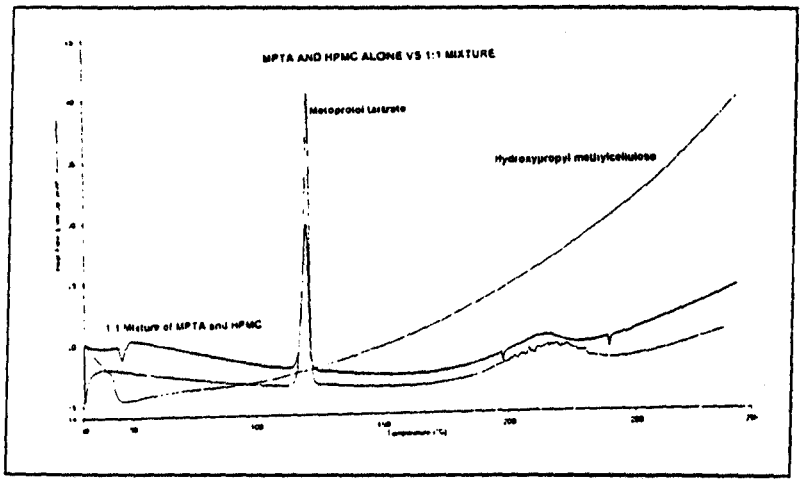


Figure 3.4: DSC scans depicting the melting behaviour of MPTA, HPMC and a mixture thereof

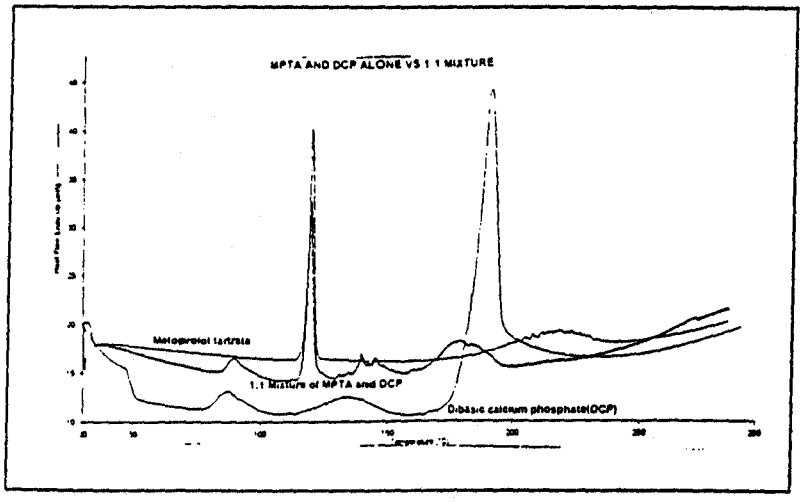


Figure 3.6: DSC scans depicting the melting behaviour of MPTA, DCP and a mixture thereof

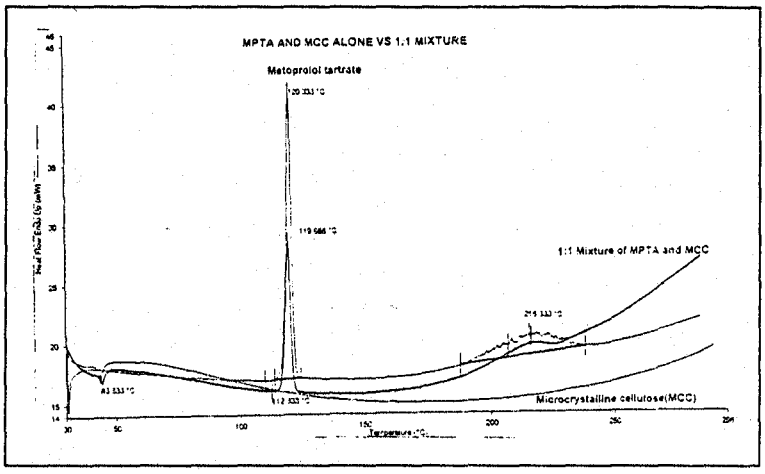


Figure 3.7: DSC scans depicting the melting behaviour of MPTA, MCC and a mixture thereof

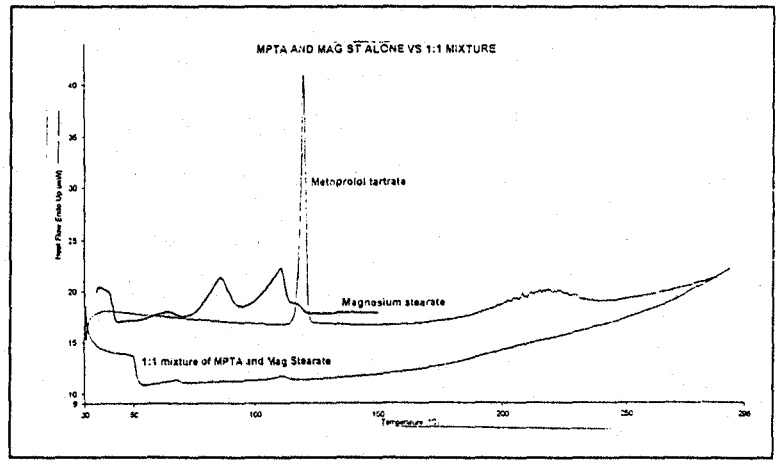


Figure 3.8: DSC scans depicting the melting behaviour of MPTA, magnesium stearate and a mixture thereof

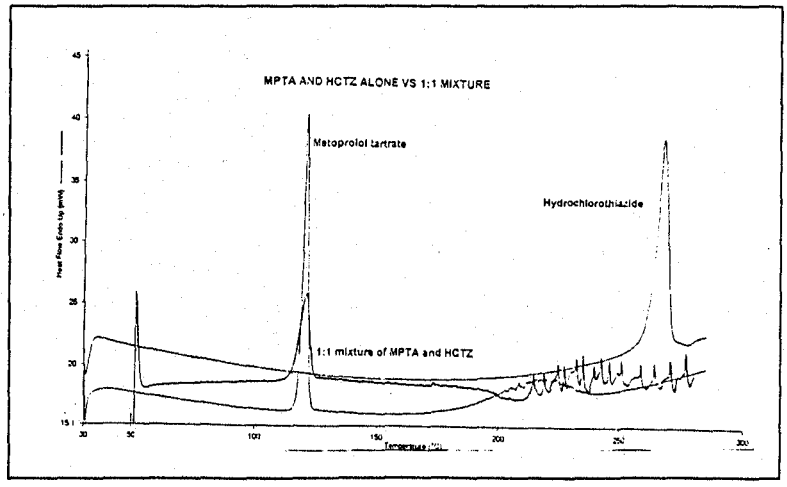


Figure 3.9: DSC scans depicting the melting behaviour of MPTA, HCTZ and a mixture thereof

Figure 3.6 shows the DSC scans of DCP, MPTA and the mixture. DCP shows both considerable and complex endothermic activity, with potential interference with the MPTA curve. DCP is observed to decompose at temperatures below 100°C with a corresponding loss in water [159]. The scan of the mixture shows that although the metoprolol base peak is not displaced there is a definite change in the thermal behavior of DCP, with a disappearance of the peak at 192°C and displacement of the tartrate peak. This may indicate that although there is some form of interaction, the stability of metoprolol may remain unaffected.

The scan of MPTA, MCC and the mixture (Figure 3.7) shows no significant displacements of the melting endotherm of the drug. MCC is depolymerized cellulose, which chars at 270°C and therefore no significant endothermal events were expected or observed [159].

The DSC thermogram of magnesium stearate, MPTA and a mixture are shown in Figure 3.8. Magnesium stearate has a melting point of 88.5°C [159], and shows two broad melting endotherms in the 80-120°C region. Magnesium stearate consists of several fatty acids of varying chain length [151] and several pseudopolymorphs and hydrates of magnesium stearate have been characterized [150,151]. It is likely that the observed endotherms correspond to any of the pseudopolymorphs or hydrates of magnesium stearate. The scan of the mixture reveals a total disappearance of all endotherms, including that of MPTA. This is an indication of a definite interaction between MPTA and magnesium stearate, which is known to interact with a large number of acidic and alkaline substances. [159,160].

Thermograms of MPTA, HCTZ and the mixture are shown in Figure 3.9. A DSC scan of the mixture shows definite changes, however it is likely that the early melting of MPTA resulted in the release of water that subsequently led to the hydrolysis of HCTZ, resulting in the endothermal activity in the 220-280°C region. This may suggest that MPTA and HCTZ in combination dosage forms may be susceptible to interaction, especially under conditions of elevated temperature and humidity.

3.2.2.1.5 CONCLUSIONS

There were no potential interactions detected between MPTA and MCC and HPMC, however there are potential interactions between MPTA and HCTZ, DCP and magnesium stearate. Excipients that undergo thermal events at temperatures less than the melting point of the drug are more likely to result in incompatibilities, as seen with DCP and magnesium stearate. Whilst the detection of the interactions between each of the compounds provides valuable information, these conditions represent the worst case scenario and it is unlikely the dosage form would be subjected to the harsh conditions during normal manufacture and storage as those used for the purposes of DSC screening. Furthermore, DSC is unlikely to replace real time stability studies of each of the individual components or mixtures thereof.

Therefore, although potential interactions were detected they did not seem to warrant exclusion of any of the compounds from the formulation. The interactions with magnesium stearate may result in softening of the dosage form, however as MPTA would mainly be incorporated into granules, the interaction between these agents would be minimized. Consequently all of the excipients originally considered for the formulation were used in the development of the core tablet.

3.2.2.2 EVALUATION OF A MATRIX FORMULATION FOR THE SUSTAINED RELEASE OF MPTA

The formulation, which was developed in-house for the sustained release of highly water-soluble drugs, is described in Table 3.1. MPTA was used in the formulation and drug release from the formulation was assessed over a 22-hour period, as described in § 4.5.

Feasibility batches M0022005 and M011508 using MPTA as the model drug were manufactured to produce a dosage form with a 50 mg drug load. A significant retardation of drug release was observed as 50% drug was released in 4 hours when compared to an immediate release tablet (Lopressor®) from which all drug was released in less than 1 hour (Figure 3.10). All other relevant batch data are included in Appendix I.

Table 3.1: Formula for matrix tablet core

Excipient	Percent (w/w)
Water-soluble drug (MPTA)	15
HPMC	10
MCC	40
DCP	35
Surelease®	± 0.18g
(g suspension/ g powder blend)	
Granules	**67**
HPMC	15
MCC	7
DCP	10
Magnesium stearate	1

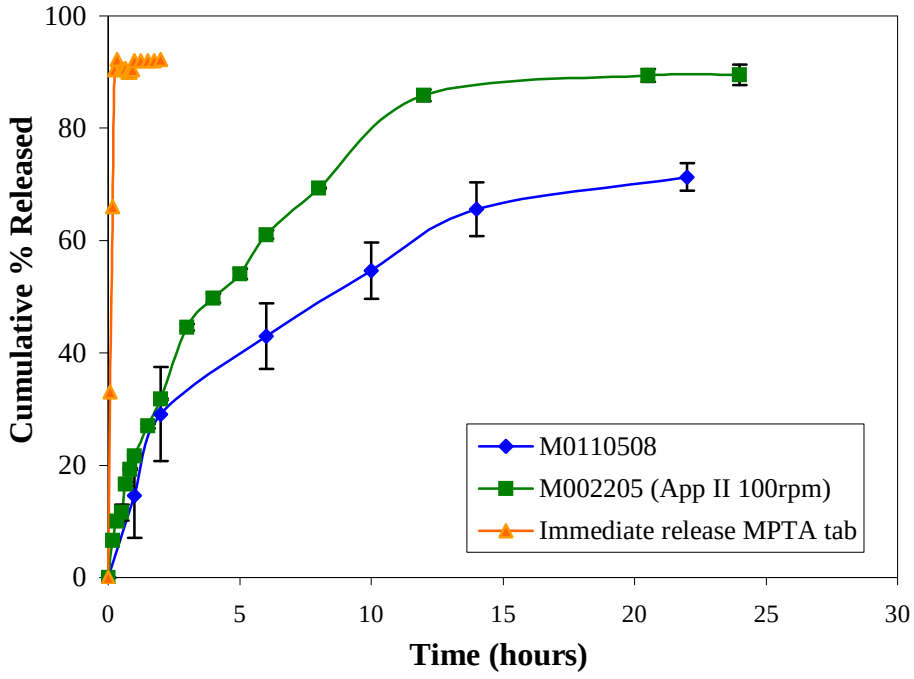


Figure 3.10: Drug release profiles of feasibility batches (M002205 and M0110508) compared to an immediate release tablet (Lopressor®) (n=6)

To obtain a minimum drug load of 100mg required for the envisaged sustained release MPTA dosage form, an increased tablet weight and size were required. The calculated theoretical weight was found to be greater than the capacity of the tooling available in our laboratory. Furthermore,

tablets with a weight of greater than 800mg are often difficult for patients to swallow, and are recommended to be manufactured in an oblong shape so that they can be taken along the longitudinal axis to facilitate swallowing [161]. For our purposes a spherical concave or deep concave tablet was required to facilitate a coating procedure if required in the later stages of product development. The results from the preliminary batches indicated that the formulation was capable of retarding the release of the highly water-soluble MPTA over an extended period, and therefore further optimization of the formulation was considered feasible.

3.2.3 OPTIMIZATION OF THE PROTOTYPE FORMULATION

3.2.3.1 MATERIALS USED

3.2.3.1.1 Drug Used

Metoprolol tartrate (Genpharm, Canada) was used for initial studies. MPTA was later obtained from K.A.Malle Pharmaceuticals Ltd (India) and used for all further studies.

3.2.3.1.2 Excipients

3.2.3.1.2.1 Microcrystalline cellulose (MCC)

MCC is a partially depolymerised cellulose which has been widely used as a pharmaceutical excipient [159,162,163]. A number of different MCC products including Emcocel® and Avicel® are currently available. Emcocel® 90M (Mendell, NY, USA) was used for all experimental formulations. Emcocel® 90M has similar properties to that of Avicel® PH 101 in terms of specific surface area, particle density, moisture content and flow and binding properties [164]. Different product grades may be used for specific applications [159]. MCC may be used as a binder or diluent in tablet formulations [165]. MCC powders exhibit large structural variations and it is this characteristic that has been thought to be responsible for its diverse and useful functional properties [163,166]. MCC is widely used due to properties that include good compaction, low friability and inherent lubricity [165]. Although it is most suited to direct compression formulations, MCC has been used in both wet and dry granulations [159,163,165] and in extrusion and spheronisation [162,167-170]. Due to its water absorbing capability MCC may also be used in a new moist granulation technique where the drying step is eliminated as excess water is absorbed by MCC [171]. Tablets that include MCC show good compaction with minimum compression force, and

due to plastic deformation [164], the bonds formed under pressure will remain intact even after pressure is released, hence resulting in dense tablets with minimal capping potential.

3.2.3.1.2.2 Dibasic Calcium Phosphate (DCP)

DCP is another commonly used diluent in tablet formulations due to its inherent flow properties and good compression characteristics, and is therefore commonly used in direct compression formulations. It is also non-hygroscopic at 20°C and at relative humidities up to 90% [159]. Unlike MCC, compaction takes place mainly by brittle fracture, that is, an applied stress initiates crack propagation rather than plastic deformation of the material [172]. The brittle fracture index can be determined as the stress concentration around a hole under the conditions of a tensile strength test, which is approximately three times the nominal applied stress, therefore the tensile strength in the presence of a hole should be one-third that of the strength without a hole [172]. The brittle fracture index may therefore be useful in assessing the potential for capping of a tablet formulation Emcompress® (Mendell, NY, USA) was used in all experimental formulations.

3.2.3.1.2.3 Hydroxypropyl methylcellulose (HPMC)

HPMC is a non-ionic long chained cellulose polymer. A number of HPMC products are available, such as Methocel® (Colorcon, Kent, UK) and Metolose® (Shin-Etsu) products. Such products are available in varying grades and with different nominal viscosities and as such may be selected for a particular purpose based on these specific properties [173-175]. HPMC with a lower nominal viscosity (4000cp) was used in the granules and that of a higher viscosity (100 000cp) was used in the tablet matrix. Methocel® K4M and K100M Premium grades were selected as they exhibit fast hydration rates which would lead to rapid gel formation, therefore providing optimal retardation of release of the highly water soluble drug from this formulation [175].

3.2.3.1.2.4 Granulating fluid

Surelease® grade E-7-19010 (Colorcon, Kent, UK) was used as the granulating fluid. Surelease® is a complete pre-plasticised and stabilised aqueous polymeric dispersion of colloidal ethylcellulose [176]. It is mainly used in barrier coating but may be used as a granulation binder [176,177]. Ethylcellulose has also been used as hydrophobic polymer in matrix systems to retard drug release [122].

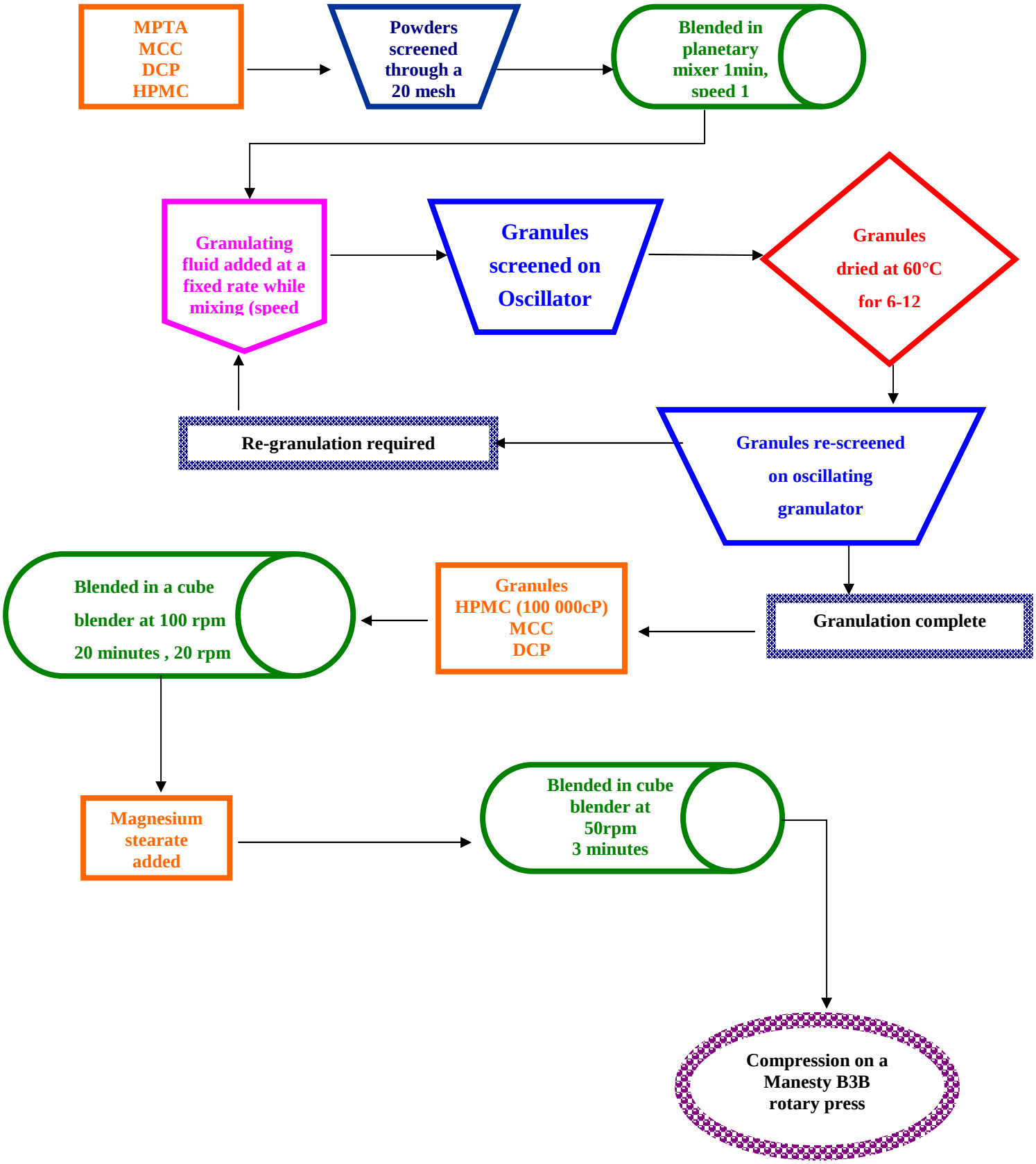
3.2.3.2 METHODS

The process described below and illustrated in Figure 3.11 was used for manufacture of the tablets. All powders for granulation were individually weighed, screened through a 20-mesh sieve and then granulated in a Kenwood Major planetary mixer (Kenwood, UK) set on speed 1. Powders were blended for 1 minute prior to the addition of granulating fluid. The granulating fluid was added at a constant rate using a peristaltic pump (Masterflex Easyload, Cole-Palmer Instrument Company, IL, USA). The wet powder mass was then screened through a 10-mesh using an oscillating granulator (Erweka, Germany) at 50rpm. Granules were dried in an oven at 60°C for 12 hours, and then re-screened. They were re-screened using a 20-mesh size on the oscillating granulator. Granulation was a single step procedure and no batches were re-worked. The dried granules were stored in an airtight container and blended with the accurately weighed and pre-screened matrix excipients in a cube blender for 20 minutes at 20 rpm prior to compression. Magnesium stearate was then added to the blend and the combination was mixed for a further 3 minutes at 20rpm. Tablets were compressed on a Manesty B3B rotary press using either 2 or 4 concave punches, to a target hardness of 13-17 kiloponds (kp).

Drug release from the formulation was assessed after modifications to the proportions of excipients, the matrix polymer, drug load and the granulating fluid used in the formulation, were made. Drug release was assessed using dissolution testing as described in § 4.5. Physical characteristics of the tablets, including weight uniformity, hardness and friability were also assessed and are included in Appendix I for each batch, respectively.

Content uniformity of representative batches was also assessed. Ten tablets from each batch were individually weighed and crushed separately using a mortar and pestle. The powder for each tablet was quantitatively transferred to a volumetric flask and made up to 100mL volume with methanol, and sonicated for ten minutes. An appropriate aliquot was removed with the aid of a swinney filter with 0.45µm filter paper, and subsequently diluted with HPLC grade water. Samples were analyzed using the validated HPLC method described in § 2.2.3.

Figure 3.11: Flow diagram of the Manufacturing Process



3.2.3.3 RESULTS AND DISCUSSION

A brief summary of the batches manufactured, modifications made and the time taken to release 50 and 100% of MPTA is included in Table 3.2.

3.2.3.3.1 Effect of larger drug load in granules

An increased percentage of drug in the granules (Batches M012208, M023009), which contained 26.09% drug, with a corresponding decrease in percentages of granulation excipients, resulted in retarded drug release with 50% of drug being released in 3.5 hours and 100% in 14 hours (Table 3.2, Figure 3.12). Drug release was found to be faster from these batches than from the batch originally manufactured (M011508). The similarity (f1) and difference factors (f2) were used to determine the similarity between two batches of tablets. These are discussed in greater detail in § 4.3, however f1 values < 15 and f2 values > 50 indicate that there are no significant differences between the profiles compared. These values verified that there was no significant difference between the batches with increased drug load, however both batches are significantly different from the feasibility batch M011508, where f1=52.7, f2=29.7 values were obtained from comparison of M011508 to M023009. Although the differences in the release profile may have been a function of larger amount of granulating fluid, it is also likely that the amount of polymer (15%w/w) may have been insufficient to maintain sustained drug release over a 24-hour period. In addition it may be possible that as the amount of drug in the granules was increased greater amounts of drug were present at the granule surface and ultimately the tablet surface, resulting in more rapid dissolution from this formulation. It was found that increases in the amount of drug might be compensated for by increases in the amount of the HPMC K100M, the rate-controlling polymer used.

Table 3.2. Summary of formula modifications and time for 50% and 100% drug release from experimental batches

Batch No.	Modifications	50%	100%
M0022005	None (USP Apparatus II)	4 hrs	14 hrs
M001270502	↑ HPMC K100M to (20%)	5 hrs	22 hrs (80%)
M011508	↑ Surelease®	8 hrs	22 hrs (70%)
M012208	↑ drug (26%), ↓ granulation excipients	3.5 hrs	22 hrs
M013009 *M011711*	As for M012208 *17 hour drying time*	3 hrs *2.5 hrs*	22 hrs *14 hrs*
M023009	↑ drug (30%), ↑ HPMC K100M (20%)	3 hrs	22 hrs
M010711	Drug (23%), HPMC K100M (18%)	2.8 hrs	22 hrs
M011210 *M021711*	Drug (20%), HPMC K100M (20%) *As for M011210*	6 hrs (53%) *5 hrs*	22 hrs (83%) *22 hrs (85%)*
M001080301	As for M011210, ↓ granulating fluid	3 hrs	22 hrs
M001080302	40% drug, ↓ % of granulation excipients, 20% HPMC K100M	3.2 hrs	22 hrs
M001270501	As for M001080302	4.4 hrs	22 hrs (90%)
M001270503	20% drug, 20% HPMC, ↑ MCC, ↓ DCP, ↑ Surelease®	4.5 hrs	22 hrs
M001270504	As for M011210, 1 kg batch	3.5 hrs	22 hrs
M00050601	As for M011210, small mesh size used in both screening steps	5 hrs	22 hrs (90%)

- All batches were assessed in USP Apparatus III unless otherwise indicated
- ↑ / ↓ arrows indicate increases or decreases in the amounts of excipients or drug used

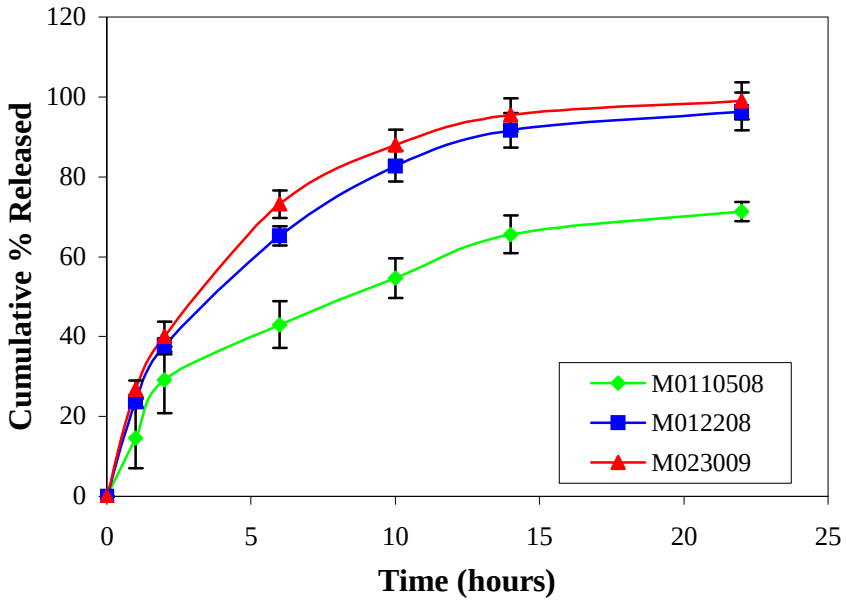


Figure 3.12: The effect of altered drug load on the MPTA release profiles from experimental formulations (n=6)

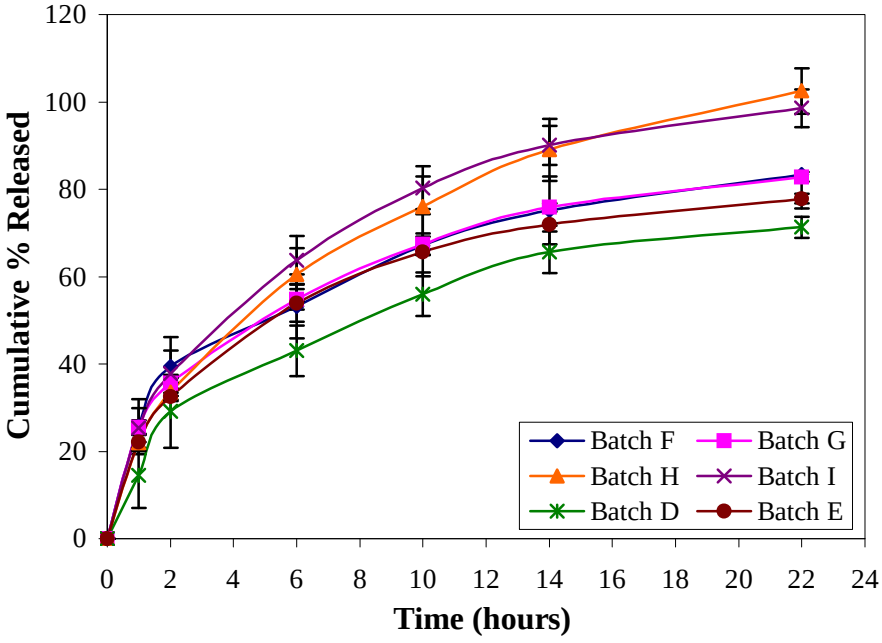


Figure 3.13: The effect of altering the amount of granulation fluid on MPTA release profiles (n=6)

3.2.3.3.2 The effect of Surelease® on the drug release profile

Drug release from the original batches (M0022005, M011508) manufactured with the same formulation occurred at different rates, and this was thought to be a function of a higher amount of Surelease® used in the granulation of one of the batches. The amount of Surelease® added was found to have a significant effect on drug release characteristics. This may be expected as Surelease® contains the hydrophobic polymer, ethylcellulose which may also have a rate-controlling effect on drug release. An assessment of six batches (Table 3.3) with varying amounts of Surelease® was found to exhibit different release profiles. Deviations in the amount of Surelease® that were greater than approximately 15% from a target amount of 0.21g/g of Surelease® added to the granules resulted in altered drug release profiles whereas batches that contained amounts of Surelease® that did not deviate by greater than 15% from the target amount resulted in similar release profiles (Table 3.1, Figure 3.13).

Table 3.3: Surelease® composition of batches manufactured to assess the effect of granulating fluid on drug release rates

	M011508 (D)	**M01270502 (E)**	**M011210 (F)**	**M021711 (G)**	**M001080301 (H)**	**M00270504 (I)**
Amount of Surelease® (g/g of powder)	0.36	0.18	0.19	0.21	0.16	0.14
% Deviation from target amount	71.43	14.29	9.52	0.00	23.81	33.33

Table 3.4: f1 and f2 for batches used to assess the impact of the amount of Surelease® added on drug release

	f1	**f2**
Batch G vs D	18.3	48.7
Batch G vs E	5.3	73.2
Batch G vs F	2.1	85.0
Batch G vs H	15.4	48.5
Batch G vs I	15.8	48.2

Batches containing the higher amounts of Surelease® appeared to control the release of MPTA from the matrix more effectively than batches which contained less than 0.16g/g of Surelease®. This may indicate that increasing the amount of Surelease® added would possibly aid in achieving more controlled drug release from this formulation, however, increasing the amount of granulating

fluid to greater than 0.21grams/gram of powder blend resulted in an unworkable mass and granules obtained were spaghetti-like in nature. This was possibly due to over-granulation and the possible premature hydration and swelling of HPMC K4M incorporated into the granules, as a result of the excessive amounts of aqueous liquid from the granulation fluid. Klinger *et al* suggested that the controlled release effects exerted by Surelease® may be enhanced by the use of a double granulation procedure as it was found that the double granulation resulted in sustained release characteristics for three drugs namely, theophylline, chlorpheniramine maleate and acetaminophen [177].

The amount of Surelease® added to each batch could not be standardized precisely as the granulations may be influenced somewhat by environmental conditions such as temperature and humidity. Manufacturing this formulation in an environmentally controlled room where all conditions can be optimized may eliminate these effects. It was found that a deviation of >15% from the target value resulted in altered sustained release characteristics over a 22-hour period. Furthermore, the addition of larger amounts of granulating fluid would result in low batch yields, as a large amount of granules were lost on the screening mesh and as a result of large granule size.

3.2.3.3.3 Effect of altering polymer content in the matrix

The amount of polymer was then increased to compensate for the larger percentage of drug in the total formulation. The amount of polymer added was increased from 15 to 18 and then 20% of the total formulation (M011711, M010711, M021711, respectively) of the total formulation and the release profiles are depicted in Figure 3.14. It was found that increasing the content of Methocel® K100M added in the matrix even by a very small percentage of the total formulation resulted in significant retardation (f1, f2 in Table 3.5) of drug release. Although the f1 value obtained for a comparison of batches containing 15% and 18% polymer was <15, the f2 value was also < 50 indicating that the profiles were significantly different. This was expected as increasing amount of polymer would lead to increased gel layer thickness and hence a more tortuous path for drug diffusion to occur, thus slowing down the release rate of drug from the tablets with 20% HPMC.

Table 3.5: f1 and f2 for batches used to compare the effect of varying polymer content

Comparison	f1	f2
A vs B	13.1	41.4
A vs C	39.7	30.2
B vs C	23.6	42.6

(A, B, C represent 15%, 18%, 20% respectively)

The influence of polymer content on drug release is well documented [178], however drug release may also be affected by other polymer variables such as substitution type, viscosity and particle size [178], which were not evaluated. Furthermore, the ratio of drug: polymer is a critical consideration in the development of matrix systems, where increasing polymer content in relation to the drug load, results in decreased drug release [179]. The mechanisms of in-vitro drug release from this formulation are discussed in further detail in Chapter Four.

3.2.3.3.4 Effect of increasing MCC content in granules

The effect of increasing the content of MCC in the granules was assessed in batch M01270503 and compared to batch M011210. M01270503 resulted in slower initial release of the drug from the matrix, although f1 and f2 values (f1=15.4, f2=51.9) indicate that there was a significant difference between the release profiles. This may have been a result of increased binding capability due to the larger amount of MCC used in the wet granulation [165]. Furthermore, due to its hygroscopic nature, the larger amount of MCC may have resulted in greater swelling, which aided in retarding drug release to a greater extent in the early phases of the experiment.

MCC has also been found to have less brittle properties and to be more plastically deformable than other excipients such as lactose [180] and it was expected that this might have had an impact on the physical and compression properties of tablets, particularly with respect to hardness [180,181]. However, the average hardness for this batch of tablets was found to resemble values obtained for other batches (Appendix I), and this may have been due to the fact that only the MCC content in the granules was varied. This represents only a small percentage of the total formulation and therefore there was minimal impact on drug release from this formulation

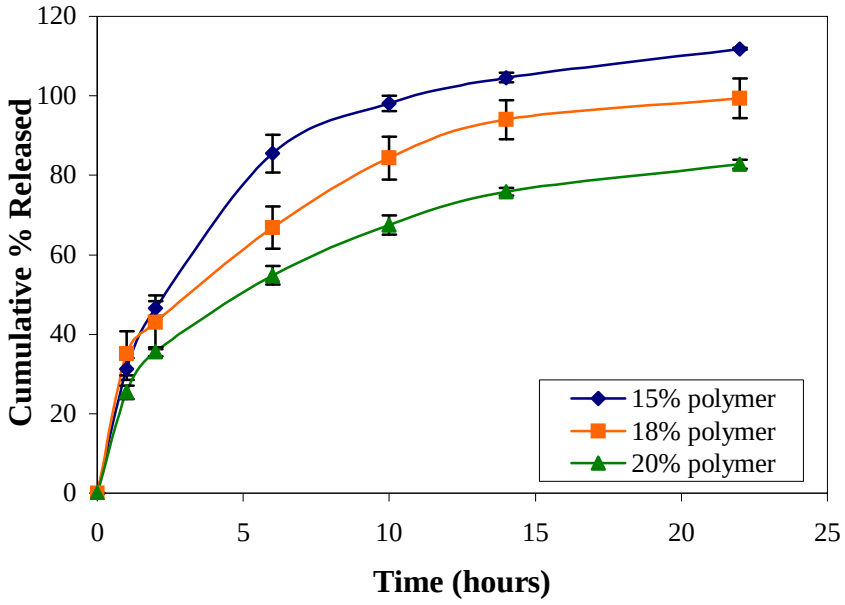


Figure 3.14: The effect of altered polymer (HPMC K100M) content on the drug release profile (n=6)

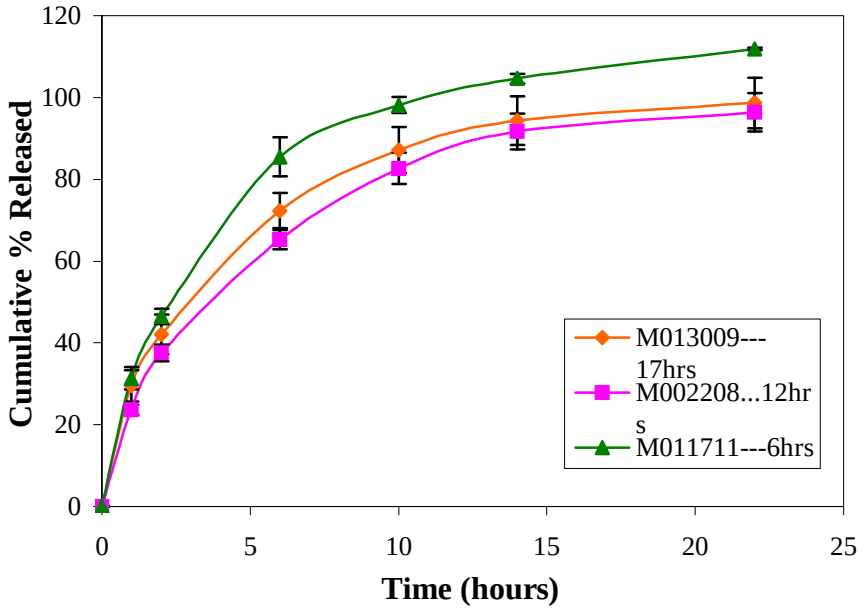


Figure 3.15: The effect of granule drying time on MPTA release (n=6)

3.2.3.3.5 Effect of certain process variables

It is important to evaluate the effects of both formulation and process variables on drug release rates from tablets. Therefore the effects of granule drying time and mesh sized used for granule screening and compression force (§ 3.2.3.3.6) were evaluated.

3.2.3.3.5.1 Drying Time

The duration that the granules were dried had an impact on drug release rate from the formulation. A drying time of 6 hours resulted in a faster rate of drug release than from batches in which granules were dried for 12 and 17 hours (Figure 3.15). A fit factor comparison of M011711 and M002208, which were dried for 6 and 12 hours, respectively, resulted in an f1=6.9 and f2=42.5, indicating that there is a significant difference between the release rate profiles of MPTA from these batches.

As there appeared to be little difference between 12 and 17 hours drying time (f1=6.4, f2=65.5), granules for all batches were dried for 12 hours. The excess water present from incomplete drying in the case of 6 hours exposure, may have led to the formation of large pores within the tablet, hence facilitating water penetration into the dosage form [182], which may have resulted in a subsequent increased drug release rate.

3.2.3.3.5.2 Mesh size for granule screening

In order to assess the impact of granule size on drug release batch M00050601 was formulated to be similar to batch M011210. Batch M00050601 was screened through the 20-mesh both before and after drying, whereas granules for M011210 were screened through a 10mesh before drying, and the 20-mesh subsequent to drying. The release profile (Figure 3.16) was found to be unaffected (f1=7.5, f2=66.6) by the size of the granules to be incorporated in to the matrix formulation.

3.2.3.3.6 Physical Evaluation of Tablets

Tablet weight

The weight of some batches manufactured was not adjusted to achieve the100mg dose exactly (690mg tablet weight), however as these were preliminary batches and data was to be presented as a percentage of drug released versus time, these batches were still tested. Variations between tablet

weights of individual batches were manufactured to be minimal where the % RSD values for weight variation for most batches was less than 5%.

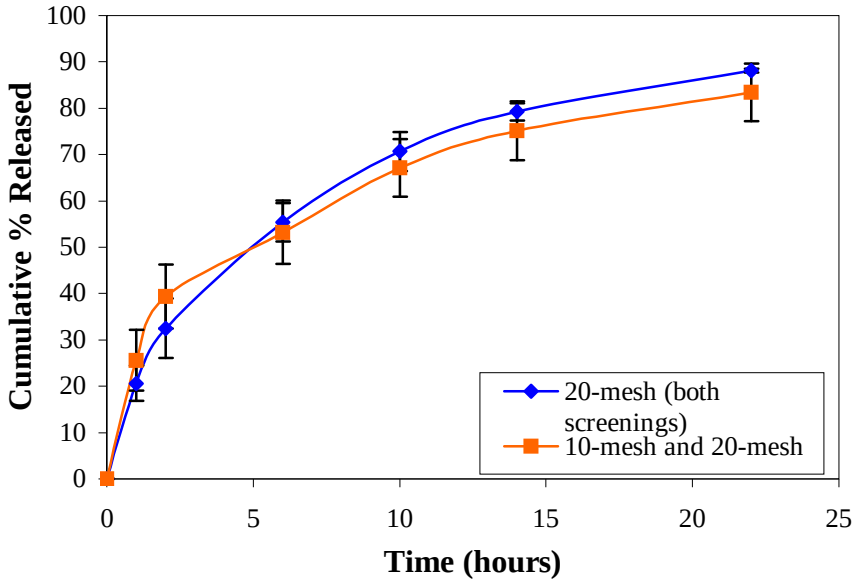


Figure 3.16: The effect of screen mesh size on the drug release profile (n=6)

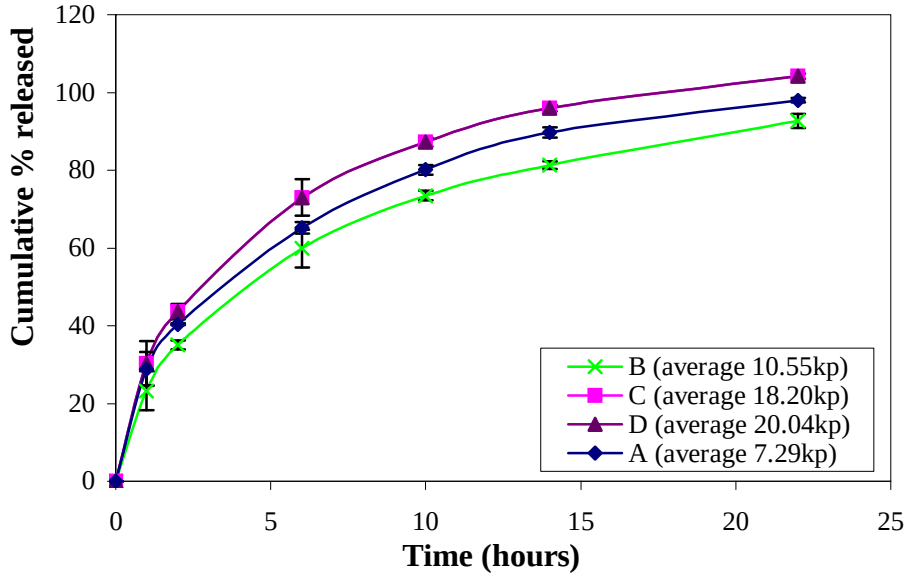


Figure 3.17: Comparison of the release profiles of tablets compressed to different target hardness values (n=6)

Tablet Hardness

Tablet hardness was more variable than tablet weight and %RSD values were found to exceed 10% for most batches. This was more than likely due to inefficient die filling, which may have been a consequence of the small batch sizes compressed. Furthermore, an instrumented press was unavailable therefore only the compression force could be altered to achieve and maintain the desired tablet hardness within a specific range. As a result of the large deviations in tablet hardness, the effect of tablet hardness on drug release was assessed. The hardness of tablets from one batch (M01270504) was varied from 7.29 to 20.04kp, and the release profiles developed are depicted in Figure 3.17.

Theoretically, differences in hardness are an indication of differences in tablet density and porosity, and such differences may affect the initial penetration of dissolution media into a dosage form. In the case of matrix tablets, this may affect the rate of gel layer formation and subsequently, the rate of drug release from the dosage form [179]. In this formulation, it appeared that drug release was slower from tablets with a lower hardness (7.29kp and 10.55kp) as compared to tablets with hardness of 17.94 and 20.04kp. The release profiles for the harder tablets were almost super-imposable, indicating those small differences in hardness had almost no impact on drug release. To assess whether the differences in hardness of tablets were significant, the f1 and f2 difference and similarity factors were used. It was found that none of the f1 and f2 values obtained fell out of the acceptance limits for similarity (Table 3.6), that is, none were > 15 or <50, indicating that the release profiles were not significantly different. This may indicate that the hardness of these tablets has a minimal impact on drug release from this formulation.

Table 3.6: f1 and f2 Values for the comparison of the same batch with varying hardness

	f1	**f2**
A vs B	9.1	60.2
A vs C	2.2	83.7
A vs D	8.0	61.5
B vs C	7.6	62.5
B vs D	9.1	60.2
C vs D	9.7	59.0

Content Uniformity

Content uniformity data for batches assessed are included in Table 3.7. As the tablet weight was not maintained for earlier batches, the calculated theoretical amounts and the actual percentages determined are included in the table for comparison.

Table 3.7: Content uniformity data from randomly selected batches

Batch	Theoretical	Actual	% RSD	% Determined
M011210	100.05	96.45 ± 4.16	4.46	96.40
M01270503	93.72	85.96 ± 5.40	6.28	91.72
M01270504	100.04	98.30 ± 5.79	5.89	98.26
M01270501 (200mg)	197.54	178.34 ± 6.56	3.67	90.28
M01270502 (50 mg)	59.22	55.43 ± 4.19	5.67	93.60

(Actual and Theoretical amounts obtained are expressed in mg)

As per the USP recommendations, a batch is passed for content uniformity if the amounts determined lie between 85-115% of the theoretical value, with a % RSD value <6% [73]. All of the batches assessed were analyzed and found to fall within the specifications, however the %RSD obtained for batch M01270503 was not below the 6% limit set in by the USP, indicating a failed batch. The content uniformity of batch M01270503 was found to be lower than for other batches containing 100mg of drug. This may have been a function of the altered percentages of DCP and MCC in the granules, and may indicate that powder flow might have been affected by altering the proportions of these excipients in the formulation. Flow rates of powders were not assessed in thses experiments.

3.2.4 Conclusions

Based on the evaluation of the batches described in § 3.2.3.3, the formulation selected for the tablet core is described in Table 3.8. Batches M011210, M021711 and M01270504 are replicates of this particular formulation. The formulation was found to be capable of sustaining the release of MPTA over a 22-hour period using USP Apparatus III. The results of physical testing reveal that the tablets were resilient and displayed good reproducibility on re-manufacturing. Drug release from

Batch M01270504 was significantly faster than the other batches of the same formulation, however this was more than likely due to a smaller quantity of Surelease® being used in the granules. This indicates that the amount of granulating fluid added is an important consideration, with deviations of greater than 15% from the optimized value resulting in significant changes in the drug release profile as discussed in § 3.2.3.3.2. Although the hardness of the formulation was kept within a target range, it was not found to have a significant impact on drug release from this formulation.

Table 3.8: Formulation composition for a sustained release MPTA matrix tablet

Excipient	**Percent (w/w)**
Water-soluble drug (MPTA)	20
HPMC	10
MCC	37.5
DCP	32.5
Surelease® (g suspension/g powder blend)	0.18-0.21
Granules	**62**
HPMC	20
MCC	7
DCP	10
Magnesium stearate	1

The matrix formulation listed in Table 3.8 was found to be capable of retarding the release of MPTA over a 22-hour period and may be used as a stand-alone once daily dosage form of MPTA. The formulation may also be considered for subsequent development of a combination dosage form containing both HCTZ and MPTA.

CHAPTER FOUR
THE IN VITRO RELEASE OF METOPROLOL TARTRATE FROM MATRIX TABLETS

4.1 INTRODUCTION

Dissolution testing is a valuable surrogate and tool for drug release rate characterization from a dosage form in vivo. Drug dissolution from a dosage form is one of the prerequisites for its absorption, bioavailability and ultimate efficacy in vivo [183]. It is used in the pharmaceutical industry during formulation development, stability testing and preclinical trials as a quality control tool. More specifically, it is used in the developmental stages to determine the best formulations for further development [184]. For a marketed product dissolution rate testing is widely used to assess batch to batch quality [183,185], provide control over the manufacturing process and quality assurance of a product [185], and such tests may also be used to assess whether further bioequivalence studies may be required after scale-up and post-approval changes (SUPAC) [185,186]. It has also become particularly valuable in the development of sustained-release products [183,186], where certain dissolution conditions may be chosen to simulate the passage of the dosage form in vivo, more closely, thereby allowing for better characterization of the behaviour of the dosage form in the GIT. The biopharmaceutical relevance of dissolution testing has increased in recent decades and its use in developing the biopharmaceutics classification (BCS) [187] and in establishing in vitro-in vivo correlations (IVIVC) is increasing for certain drug classes [187-189].

4.2 THE BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS) AND IN VITRO-IN VIVO CORRELATIONS (IVIVC)

The BCS is based on the concept that two drug products that exhibit the same concentration profile along the GIT will have similar concentration profiles following oral administration [190]. It allows for estimation of the influences of dissolution, solubility and intestinal permeability on oral drug absorption from immediate release products [191]. The BCS has enabled the classification of drugs into four classes [190], which are listed in Table 4.1. Table 4.1 also includes some examples of drug candidates. Of particular interest are the compounds of interest for this work, namely metoprolol and HCTZ, which are classified as Class I, and Class IV drugs, respectively.

Table 4.1: The Biopharmaceutics Classification System

(Class I)	**(Class II)**
High solubility, High permeability Eg. Metoprolol tartrate, Propranolol hydrochloride [190,192]	Poor solubility, High permeability Eg. Carbamazepine [190]
(Class III)	**(Class IV)**
High solubility, Poor permeability Eg. Atenolol [190]	Poor solubility, Poor permeability Eg. Hydrochlorothiazide [190]

A draft guidance on biowaivers based on the BCS suggests that the documentation of bioequivalence by using dissolution studies may be appropriate for oral immediate release products which contain highly soluble and permeable drugs within a rapidly dissolving dosage form [185,190,193]. Consequently, the need for expensive and difficult analytical procedures that are required to analyze drugs in biological fluids following bioequivalence studies is eliminated. At present biowaivers, may only be granted for Class I immediate release products [193], whereas modified release must be assessed for in vivo bioequivalence and in vitro release characterized by dissolution testing [194]. However, the applicability of the BCS with respect to modified release systems is debatable and additional considerations of the product, including transit time, the non linearity of transport systems, the metabolic transformation process and the addition of certain adjuvants which may alter gastric transit, membrane transport or transformation variables are required [195].

IVIVC aid in product development as it allows for a prediction of the plasma profile of a drug product without the need for a biostudy. In this case dissolution testing is used as a surrogate measure for predicting the in vivo behaviour of a particular product [196,197]. The establishment of an IVIVC is dependent on several factors and differs for the different drug classes described in Table 4.1 [187], where the design of an IVIVC increases in complexity for the less soluble or permeable (Class II, III and IV) drugs, and more especially for modified release products [196].

For modified release dosage forms, the in vitro test conditions must be selected to be representative of the in vivo conditions of the human gastro-intestinal tract, and are in general, more rigorous than for immediate release dosage forms. A weak dissolution test design may result

in a poor correlation with in vivo behaviour of the dosage form [197]. The implication is that a larger number of in vitro variables, including pH, agitation, physical stress and food effects must be evaluated to establish a predictive correlation with in vivo conditions [195].

The validation of an IVIVC is also important as it allows for certain post-approval changes as described in the Scale-up and Post Approval Changes for Modified Release (SUPAC-MR) to be made, and may therefore justify biowaivers for such changes to be made [186,198]. The validation of a dissolution method, as described by Eddington *et al* allows for the identification of optimal dissolution conditions to be identified for a particular drug [199].

4.3 COMPARISON OF DISSOLUTION PROFILES

Dissolution testing therefore forms an integral part both product development and manufacture and throughout the shelf life of a product. Consequently, an effective and accurate means for the comparison of dissolution profiles is necessary. A number of methods to compare dissolution profiles are available and may be categorized either as model independent or model dependent approaches for comparison. Model independent approaches include the ratio test, pair-wise test procedures and analysis of variance (ANOVA) [200]. Some authors have described ANOVA analysis as a separate category [201]. Model dependent approaches may be based on any number of models including zero-order, first-order Hixson-Crowell, Higuchi, Weibull and others [200,202].

Pair-wise test procedures include the difference factor (f1) [200,201], similarity factor (f2) [200,201] and two indices of Rescigno [200,201,203]. Pair-wise procedures are used for comparison of a pair of profiles and employ a 90% confidence interval approach [201].

The f1 and f2 indices or fit factors, as they are more commonly referred may be calculated using Equations 4.1 and 4.2 [203]. f1 approximates the percentage error between two curves, and increases proportionally with increasing dissimilarity between curves. f2 on the other hand is a logarithmic transformation of the sum of the squared error and approaches zero as dissimilarity increases [202,204].

$$f1 = \{ [\sum_{t=1}^{n} R_t - T_t] / [\sum R_t] \} \times 100 \qquad \text{Equation 4.1}$$

$$f2 = 50 \log \{[1 + 1/n \sum_{t=1}^{n} w_t (R_t - T_t)^2]^{-0.5} \times 100\} \qquad \text{Equation 4.2}$$

Where,

R_t and T_t = assay values of the reference and test at time t, respectively
n = the number of pull points
wt = an optional weight factor which can be used to give more weight to a value/s that are considered more important than others.

f1 and f2 data must be generated from a minimum of 12 replicates [183,205] and a limit of one sampling time after 85% drug dissolution is recommended [205,206]. f2 may be calculated using mean data as calculations using both individual and mean data were not found to be statistically different [207], and dissolution measurements of two products must be made under exactly the same test conditions [205].

The similarity factor is more commonly quoted [201,208-210], as it is the simplest to use [205]. Furthermore the FDA recommends the similarity factor, for the comparison of dissolution profiles [205]. f1 values < 15 and f2 values > 50 are considered to be the limits for similarity between to curves, where f2 = 100 indicates that two profiles are identical and f2 =50 indicates that there is a 10% difference at all measured time points [205]. Some have suggested that the acceptance criteria of 50-100 is too conservative [200], however this has not been altered to more liberal limits at present. Limitations of f2 include the disregard for the time and duration over which differences are found [211] and the failure to take into account differences in dissolutions between test and reference batches [206,212]. A new multivariate procedure for testing the similarity or equivalence between two dissolution profiles through Hotelling's T^2 statistic has recently been proposed, and has been found to eliminate some of the shortcomings of the f2 similarity factor [206].

Despite possible shortcomings of the f1 and f2 fit factors, these were chosen for comparison of dissolution profiles and were used either alone or in combination with certain model dependent

procedures, the Higuchi model in particular was considered. No extra weighting was given to any time for the analyses using f2 in this work

4.4 SELECTION OF THE DISSOLUTION APPARATUS

4.4.1 Factors affecting the choice of a dissolution apparatus

The type of apparatus used mainly in dissolution testing of sustained release products includes the basket (USP Apparatus I) and the paddle (USP Apparatus II), however the reciprocating cylinder (USP Apparatus III) or the flow through cell (USP Apparatus IV) may also be used [179], and all four pieces of equipment are listed in the FDA Guidance for Industry: Oral extended release dosage forms, In Vivo Bioequivalence and In Vitro dissolution testing. [188,194].

In terms of establishing an IVIVC for controlled release products, dissolution apparatus that allow for alteration of the dissolution medium with respect to pH, molarity, anions, cations, viscosity, buffers and surface-active agents, are becoming more valuable [188,213]. The Bio-Dis® (Apparatus III) is useful in the development of controlled release products, as it exposes the product to both mechanical and various physicochemical conditions that may influence drug release along the GIT [213]. The Bio-Dis® is also said to be advantageous because it is not subject to variability cause by air bubbles and minor modifications in the geometry of the dissolution vessel as the pump action of the reciprocating tube in which the dosage form is held, negates such effects [213]. Dissolution rates from Apparatus I and II, on the other hand have been reported to be altered by air bubbles and the geometry of the dissolution vessel [213]. Problems such as clogging of basket screens in Apparatus I, and ‘coning’ observed in Apparatus II are also eliminated in Apparatus III. ‘Coning’ is known to reduce dissolution rates significantly, resulting in variable data that is often dependent on the position of the dosage form in the dissolution vessel [213], as only the upper surface of the tablet is subject to erosion caused by the paddles whilst the bottom of the tablet is stuck to the vessel and remains unexposed to the hydrodynamic stress that is created in the dissolution vessel [213].

4.4.2 Selection of dissolution apparatus

The selection of the dissolution apparatus for the assessment of a MPTA product or a combination product of MPTA and HCTZ was limited to a choice between Apparatus II and III, Apparatus III

was chosen for the advantages in the assessment of controlled release dosage forms as described above. Apparatus IV was not selected, as it is mainly applicable to poorly water-soluble drugs whereas Apparatus I is designed for dissolution testing of floating devices. The use of the basket may possibly alter release from a matrix tablet from which drug release is primarily controlled by three dimensional swelling of the polymer used in the dosage form.

Comparative dissolution profiles of two batches tested using both Apparatus II and III (Figure 4.1) show that drug release from tablets assessed in Apparatus III was greater from both batches when compared to the release determined when Apparatus II was used.

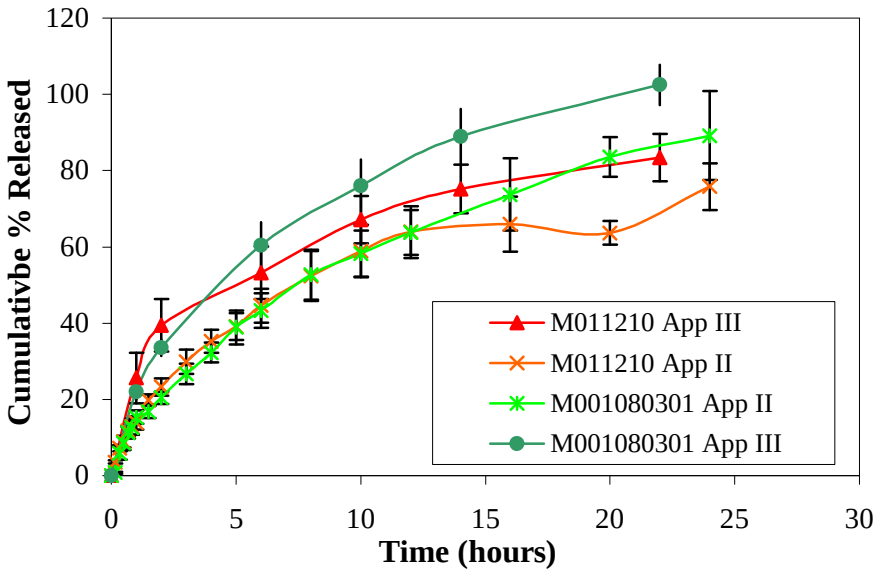


Figure 4.1: Comparison of release profiles of two batches in Apparatus II and III (n=6)

Furthermore, it was apparent that whilst both batches resulted in almost identical profiles over the first 12 hours of release in Apparatus II, as indicated by the overlapping error bars, whereas differences between the profiles were more apparent in Apparatus III. Calculated f1 and f2 values for the batches assessed in Apparatus II were 15.3 and 47.7, respectively and therefore indicated that the apparent differences in Apparatus III were significant. The hydrodynamics in these apparatus have been reported to be different. Madden *et al* found that of four hydrophilic matrix formulations assessed in Apparatus I-IV, only the release rate profiles in Apparatus III differed, indicating that the mechanism of drug release was possibly different. It was suggested that drug

release occurred by diffusion in the three apparatus that yielded similar profiles whereas erosion had a larger impact on drug release from the tablets assessed using Apparatus III [214]. Furthermore, it was found that the hydrodynamics of the Apparatus II also affected release, whilst this was not the case for the other dissolution apparatus [214]. This suggests that the Bio-Dis® may be better able to simulate the GIT conditions, especially the conditions in the stomach, which is a 'grinding' organ and may therefore contributes to greater initial erosion of a dosage form.

Apparatus III, the Bio-Dis® (G.B.Caleva Ltd, Ascot, England) was therefore selected as the apparatus of choice for dissolution testing as it was found to be more discriminating between batches and it allowed for a close simulation of the passage of the dosage form in vivo. Furthermore, the technology developed could be designed to be more rigid with testing in USP Apparatus III. Apparatus II was used in preliminary and follow-up evaluation of some batches for comparative purposes.

4.5 SELECTION OF THE DISSOLUTION TEST CONDITIONS

4.5.1 Introduction

The dissolution test may be affected by numerous variables such as the physico-chemical properties of the drug, the type of dosage form and other factors independent of the product itself. Drug properties that may influence the choice of dissolution conditions include particle size, solubility and ionization properties of the compound. Dosage form independent variables include the composition of the dissolution medium, agitation rates, pH, osmolarity and ionic strength of the dissolution medium and the surface area within the dissolution vessel [208,214,215]. The temperature of the dissolution medium is also an important consideration and is generally selected to simulate the in vivo conditions of 37°C [208]. The inclusion of additives such as surfactants to the dissolution medium may also affect the behaviour of the dosage form in these tests, thus the selection of dissolution conditions that allow for the establishment of a valid IVIVC are essential.

4.5.2 Dissolution test conditions for USP Apparatus III

The dissolution test conditions used in Apparatus III are described in Table 4.2. A 22-hour test period was selected, as this was the most suitable from the programme options available on the Bio-Dis®. The temperature was selected to simulate in vivo conditions and a volume of 185mL per

tube was selected based on the capacity of the tubes available in our laboratory. All calculations were performed on the assumption that 10mL of the sample was lost due to evaporation at the end of the 22-hour dissolution run.

Table 4.2: Dissolution conditions for Apparatus III

Parameter	Conditions	
	pH	**Time**
Medium (0.1M phosphate buffers)	1.6 3.4 4.7 6.8 7.2 7.2	1 1 4 4 4 8
Temperature	37 ± 0.5°C	
Volume of medium	185mL	
Agitation	20 dips/min	

Buffers were prepared by making up 0.1M phosphoric acid solution using 5.82mL of 85% ortho-phosphoric acid per litre of HPLC grade water. The pH was adjusted as required using sodium hydroxide pellets, as described in § 2.2.1.5.3 on mobile phase preparation.

As part of the selection process certain variables, including the effects of buffer molarity and agitation rate were assessed to ascertain whether those conditions as described in Table 4.2 were indeed capable of discriminating between dosage forms without erroneously altering the drug release profiles.

2.5.2.1 Effect of buffer molarity

The effect of buffer molarity on drug release from this formulation was conducted using 0.05M, 0.1M and 0.2M buffers. The similarity between the profiles was assessed using the f1 and f2 fit factors. Drug release was found to occur more rapidly in the 0.2M buffers than low molarity buffers, as depicted in Figure 4.2. The calculated f1 and f2 values as listed in Table 4.3 indicate that the profiles obtained for 0.05M and 0.1M buffers were similar whilst the release profile from drug dissolution testing in 0.2M buffers was significantly different from the 0.05M and 0.1M buffers.

Table 4.3: f1 and f2 comparison of buffer molarity

Comparison	f1	f2
0.05M vs 0.1M	3.1	81
0.05M vs 0.2M	27	38.3
0.1M vs 0.2M	30	36.3

It has been found that for two hydrophilic matrix tablet formulations containing a highly water soluble drug, a combination of a high ionic strength, resulting from increased buffer molarity, and a low pH resulted in significantly faster dissolution rates than at high pH [215]. This may explain the faster release rate from the tablets observed in 0.2M buffers, where it is likely that the combination of low pH in the first three rows of the Bio-Dis (pH < 5.0) and the increased molarity contributed to a greater drug release rate. No differences between 0.05M and 0.1M were found indicating that drug release may be unaffected by minor changes in buffer molarity. However increasing buffer molarity above a concentration of 0.1M may have implications for product dissolution testing.

2.5.2.2 Effect of agitation rate

Alteration of the paddle speed or agitation rate in Apparatus II and III respectively, will alter the hydrodynamics in the dissolution vessel, and may consequently impact on the dissolution behaviour of the drug. As Apparatus III was selected, the effect of altering the agitation rate using 10, 20 and 30 dips/minute was examined. These agitation rates were selected, as 10-30dips/min is known to be representative of the physiological conditions in the GIT [213,214]. As shown in Figure 4.3, alteration of the agitation rate appeared to impact significantly on the drug release profiles, and the calculated f1 and f2 values (Table 4.4) reveal that none of the release profiles are similar.

Table 4.4: f1 and f2 comparison of agitation rates

Comparison	f1	f2
10 vs 20 dips/min	17.9	43.9
10 vs 30 dips/min	34.4	28.7
20 vs 30 dips/min	67.3	20.2

It was expected that by increasing the agitation rate, drug dissolution rate would increase as a result of the more effective removal of the stagnant/unstirred layer of drug solution that surrounds drug

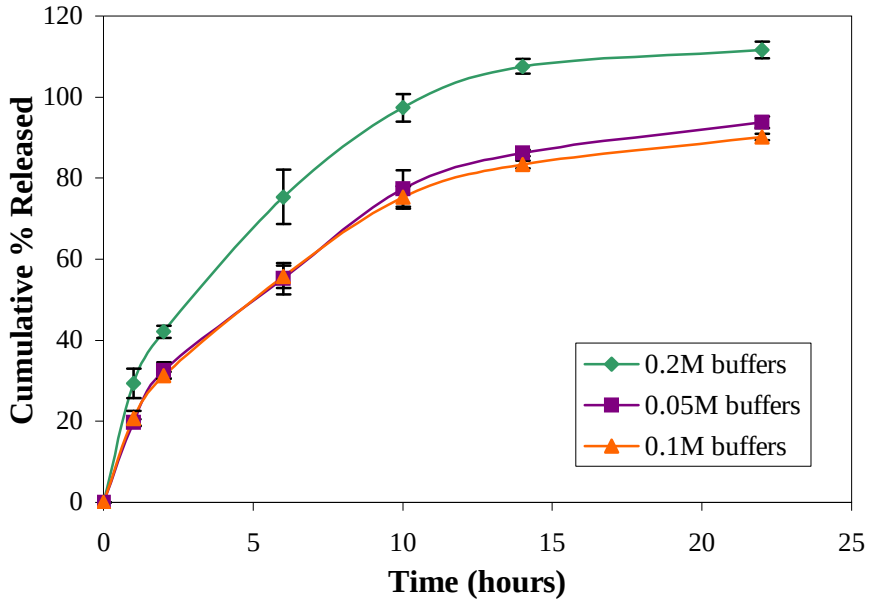


Figure 4.2: The effect of buffer molarity on the MPTA release profile (n=6)

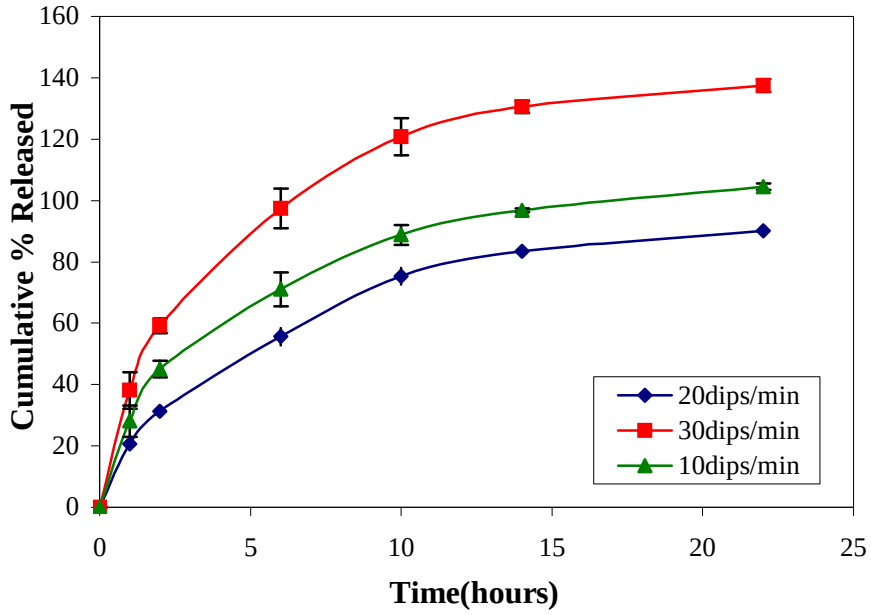


Figure 4.3: The effect of agitation rate on the MPTA release profile (n=6)

particles, hence improving sink conditions. The increased effects from erosion of the dosage form may also be expected to contribute to a more rapid drug release rate. Furthermore, it has been reported that less energetic agitation rates may favour more homogenous gelation initially and subsequent slower erosion, leading to slower release rates [216]. Madden *et al* also reported that increasing agitation rate resulted in a corresponding increased dissolution rate and suggested that erosion is more than likely the predominant mechanism of release in Apparatus III [214]. It was found that although tablets assessed at 30dips/min showed the fastest release rate, the trend was not obeyed by those assessed at 10 and 20 dips/min.

The choice of dissolution conditions for Apparatus III were therefore not altered as it was thought that tests using 0.1M buffers at an agitation rate of 20 dips/min were capable of adequately discriminating between batches without altering the hydrodynamics of the system and leading to false conclusions being drawn.

4.5.3 Selection of dissolution test conditions for USP Apparatus II

Apparatus II was used mainly for the assessment of immediate release products of both MPTA and HCTZ and for preliminary tests on the tablet core described in Chapter Three. Tests for immediate release products were conducted using the conditions depicted in Table 4.5.

Table 4.5: Dissolution conditions for Apparatus II

Medium	0.1M phosphate buffers pH 7.20
Rotation speed	100 rpm
Volume	900mL
Temperature	37°C

Dissolution testing of immediate release products of both drug compounds met the USP specifications when tested in 900mL of 0.1M buffer (pH 1.60), using a paddle speed of 100rpm.

It has been reported that drug properties including its ionization at different pH's does not alter the release characteristics of the drug from the product [217]. Sandberg *et al* [218] and Eddington *et al* [219] who both investigated metoprolol tartrate release from extended release formulations found that the rate of drug release was independent of the pH of the dissolution medium. Dissolution tests to determine whether this was also the case for MPTA release from the matrix formulation

developed, were therefore conducted. Tests were conducted in Apparatus II using 0.1M buffers of pH 1.60 and pH 7.20. Profiles for batches M001080301 and M011210 are depicted in Figure 4.4 and it is evident that pH appears to have no effect on drug release from the matrix tablet formulation developed on our laboratories. Drug release from this dosage form is pH independent, which is a desirable characteristic in a controlled release dosage form in which drug release must be sustained throughout the passage of the dosage form in the GIT, viz pH 1.2- pH 7.2.

4.6 MECHANISMS OF RELEASE FROM THE EXPERIMENTAL MATRIX FORMULATION

Drug release from HPMC hydrogel matrices is mainly controlled by diffusion resulting in Fickian release that is characterized by square root of time dependent release rates. The drug release profile is characterized by a fast initial rate of drug release that is followed by a decreasing release rate over time [220,221]. A typical release profile is depicted in Figure 4.5. Diffusion in such systems is best described by the Higuchi model, as was discussed § 3.1.3.1.1. Polymers such as HPMC, which are initially dry glassy polymers that swell upon absorption of water resulting in a rubbery outer layer, as a consequence of rearrangement of the polymer chains [220], may also exhibit Fickian release kinetics that is dependent on polymer relaxation alone [221,222].

When both diffusion and polymer relaxation processes are involved in drug release, the mechanism of release is altered and non-fickian or anomalous diffusion may occur. In these cases, drug is released by a combination of both diffusion and swelling effects and this type of release is often also referred to as first order release, and a typical profile is shown in Figure 4.5 [221,222].

Zero-order or Case II diffusion is also a type of non-Fickian diffusion, however the rate of drug release is constant over time, resulting in a linear profile, as shown in Figure 4.5. This type of release is controlled by a swelling mechanism only [220,221]. Super case II diffusion also exhibits constant release over time and in these instances release is dependent on both swelling and erosion of the polymer [223].

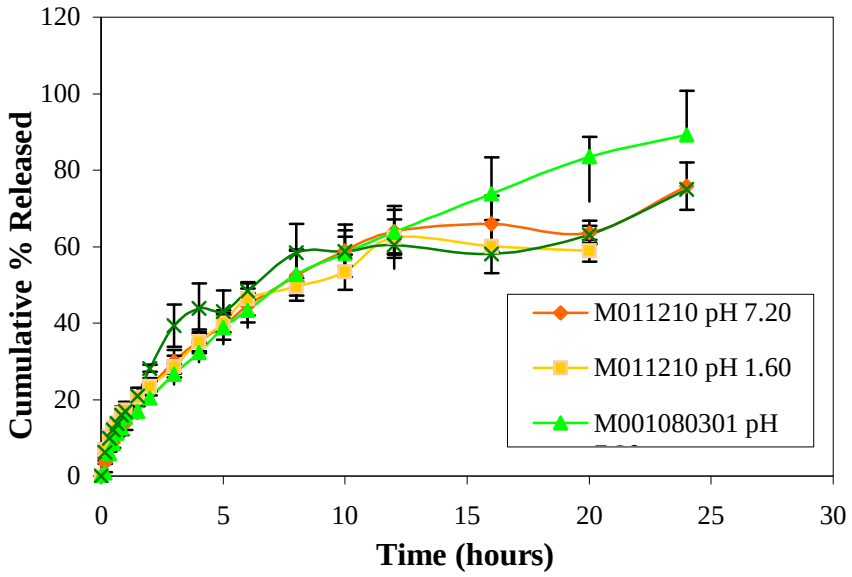


Figure 4.4: Effect of pH of the dissolution medium in USP Apparatus II on MPTA release profile

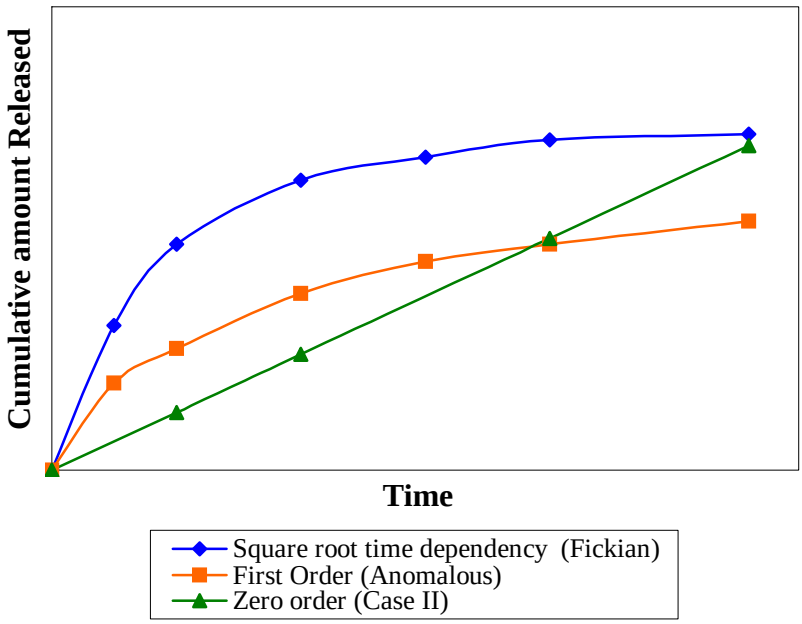


Figure 4.5: Typical release profiles representative of different drug dissolution kinetics

Drug release through a matrix tablet may therefore be characterized mathematically using the Power Law equation (Equation 4.3). This equation, which may is often referred to as the Korsemeyer-Peppas equation, has become commonly used to predict the mechanism of diffusional release from matrix systems [202,220,221,225].

$$\frac{M_t}{M_\infty} = kt^n \qquad \text{Equation 4.3}$$

Where,

M_t/M_∞ = the fraction of drug released at time t
k = kinetic constant which incorporates structural and geometric characteristics of the controlled release device
n = diffusional exponent which is indicative of the drug release mechanism

In the case of Fickian diffusion n = 0.5, whereas when n = 1.0 Case II or zero order transport is indicated. Values for n that fall between 0.5 and 1.0 are indicative of anomalous diffusion. The equation is also capable of predicting Super Case II diffusion when an n value greater than 1.0, is obtained [203,220,222-225]. However, this model is unable to explain release kinetics for n values less than 0.5 [225], and the equation can only be used to characterize 60% drug release from a dosage form as sample points where the drug concentration is > 60% are invalid and cannot be used in the calculation of the n value [223].

The use of the Higuchi equation in isolation, to characterize drug release from an HPMC matrix tablet may therefore not be capable of identifying the exact mechanism of drug release and therefore both the Higuchi model and the Power Law were used in an attempt to characterize drug release mechanisms from this formulation.

The n values obtained for each of the batches assessed are listed in Table 4.6. Only batches M001270502 and M001270504 produced n values of 0.5, indicating that drug was released by Fickian diffusion controlled mechanism. The majority of the batches assessed resulted in calculated n values of between 0.5 and 1.0, indicating that anomalous diffusion was primarily responsible for drug release from these formulations. The Power Law could not characterize the mechanisms of release of batches M010711, M011210 and M021711, which resulted in n values of less than 0.5 being calculated.

With respect to batches M01270502 and M01270504, the square root time plots (Figure 4.6) indicate that drug release is linear with square root time, therefore drug release from these batches may be best described as Fickian diffusion controlled. Drug release is therefore controlled entirely the by diffusion of drug through the fluid filled pores on the polymer matrix. This was unexpected for batch M01270504 that differed from batch M011210 with respect to the amount of granulating fluid added. Only 0.14gram/gram of Surelease® was used in M01270504, in comparison to 0.18gram/gram used in M011210. As was discussed in § 3.2.3.3.2, differences of greater than 15% from the target value resulted in an altered release profile and may imply that the larger amounts of Surelease® contribute to drug release control to a significant extent, thus altering the overall drug release mechanism from this dosage form. Due to the hydrophobic nature of the ethylcellulose polymer, penetration of fluid into the granules may have been hindered in batch M011210, hence delaying drug release. Drug release from batch M01270504 was controlled by the diffusion of drug through the fluid filled pores of the HPMC matrix only.

Table 4.6: Calculated n values and correlation coefficients (r^2) for square root time plots

Batch	n value	r^2 for $\sqrt{t}$ plots	Time Period
M001270502	0.50	0.9964	0-10 hrs
M0110508	0.57	0.9908	0-14 hrs
M012208	0.57	0.995	0-14 hrs
M013009	0.51	0.9965	0-10 hrs
M011711	0.57	0.9911	0-10 hrs
M023009	0.56	0.9962	0-10 hrs
M010711	0.29	0.9872	0-14 hrs
M011210	0.41	0.9849	0-14 hrs
M021711	0.43	0.9897	0-14 hrs
M001080301	0.57	0.9988	0-14 hrs
M001080302	0.53	0.9990	0-14 hrs
M001270501	0.57	0.9918	0-14 hrs
M001270503	0.67	0.9728	0-22 hrs
M001270504	0.50	0.9962	0-14 hrs
M00050601	0.62	0.8048	0-14 hrs

As was expected batch M01270502 displayed Fickian diffusion due to the larger percentage of HPMC used in comparison to the drug load, the primary result of greater percentages of HPMC was that a thicker gel layer through which the drug had to diffused was obtained.

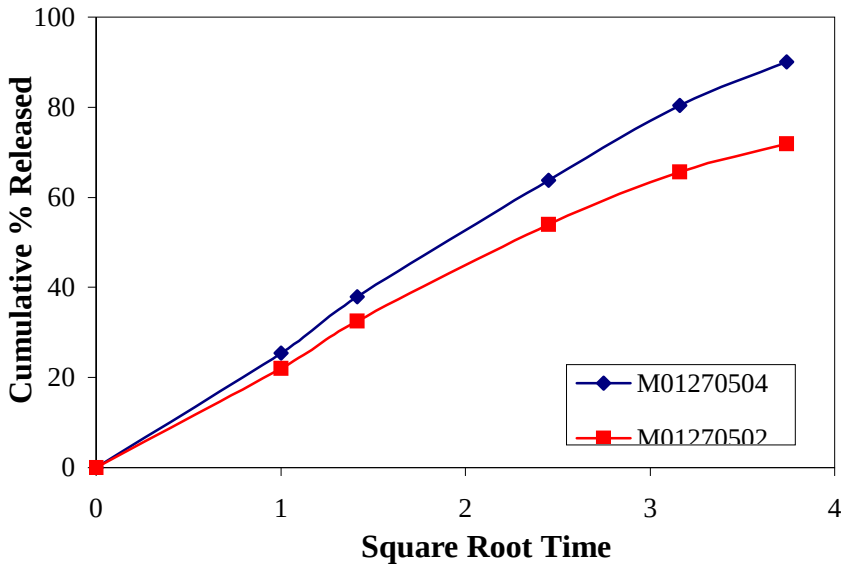


Figure 4.6: Higuchi plots for batches M01270502 and M01270504 (n=6)

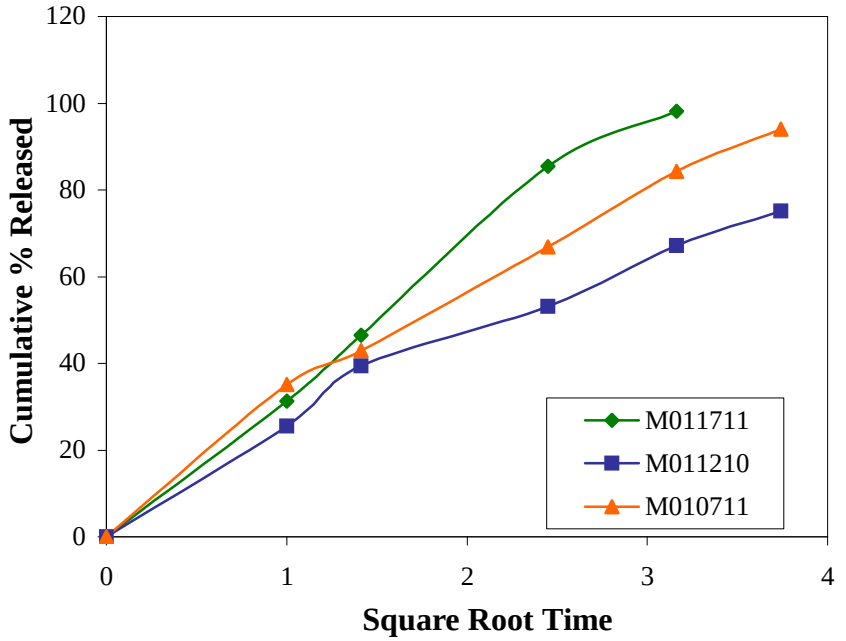


Figure 4.7: Higuchi plots for batches M101210, M011711 and M021711 (n=6)

The majority of the batches displayed anomalous diffusion behaviour indicating that drug release was controlled by both diffusion and by the three dimensional swelling and relaxation of the polymer. Drug release may therefore be better described by first order release kinetics, which is

characterized by a rapid initial release that declines over time. The initial release in this case is slower than for a Fickian diffusion controlled release rate.

Drug release from batches M011210, M010711 and M021711 could not be explained using the Power Law, as n values obtained were less than 0.5. Dissolution data was fitted to the Higuchi model (Figure 4.7) and although the correlation coefficients obtained were high, the data were not well fitted to the model. This may indicate that the drug release was controlled by mechanisms other than diffusion only, or it may be likely that these batches display a 'burst' effect [220], in which the drug is released from the tablet surface before the gel layer is formed. This may have as a consequence of more drug being present on the tablet surface due to breaking of the more rigid granules during the compression stage of manufacture. n values may be recalculated to exclude the initial times points over which rapid drug release occurs so as to obtain a new value which is more descriptive of the mechanism of drug release. The new values obtained were 0.50 for M011210 and 0.40 for the two remaining batches. Whilst the value obtained for the former batch may substantiate the idea of a burst effect, this could not be conclusively verified. These batches contained between 0.19-0.24 g/g of Surelease® and therefore it may also have been likely that the larger amounts of Surelease® retarded drug release initially, such that the drug would have to diffuse through the hydrophobic granules prior to diffusion through the fluid filled pores of the matrix. The ultimate result of this combination is an altered overall mechanism of release.

4.7 CONCLUSIONS

It was evident that drug release from the batches assessed was affected by certain dissolution conditions including buffer molarity and agitation speed of the reciprocating cylinders. However the drug release from the dosage form appeared to be independent of pH, which is advantageous for an extended release dosage form. The mechanism of drug release from the dosage form varied depending on the alterations made to the batches, and it was more than likely that anomalous diffusion was the most common mechanism of release in most batches. The mechanism of drug release from the batch selected for further modification was not conclusively identified, however it is likely that the mechanism of release is predominantly first order, and is controlled both by the high viscosity HPMC used as the matrix-former and the Surelease® used as the granulating fluid.

CHAPTER FIVE
STABILITY ASSESSMENT OF THE PROTOTYPE BATCH

5.1 INTRODUCTION

Stability testing of a drug substance or products is critical and as a result has become a mandatory procedure that is performed at various stages of development of a drug product [226-228]. It is of particular relevance in identifying any changes that may occur upon storage, and which may adversely affect the ultimate quality of the marketed product. Therefore stability testing is vital for obtaining information with respect to recommended storage conditions, shelf life and expiry date of a product [227-230]. With respect to controlled release dosage forms stability testing is necessary to identify a loss or alteration of sustained release characteristics that may lead to dose dumping and subsequent toxic or adverse effects following long term storage of these types of dosage forms.

The stability of a pharmaceutical product may be affected by any number of factors including stability of the active compound(s), possible interactions between the active and excipients, the packaging or storage conditions, the length of time between manufacture and use of the product and environmental conditions experienced during transport, storage and handling [227,229]. The specific chemistry of the functional groups of a drug compound will determine the susceptibility of the compound to various degradation reactions. Degradation processes that drugs are primarily susceptible to include hydrolysis, oxidation, thermolabile reactions and degradation by light (photolysis). These processes are accelerated by environmental conditions that include, the presence of water and oxygen, heat and light [227,229,231]. This indicates that both chemical and physical stability of the dosage form must be assessed, as both affect the chemical stability of the active drug.

According to the ICH Guidelines [226,228] on stability testing such tests should be conducted on a number of batches of the final product packaged in the containers and closures to be used in storage and distribution of the product. Recommended test storage conditions include isothermal and humidity conditions. Recommendations differ between countries, which have been divided into different climatic zones. South Africa falls in Zones I and II and therefore stability testing should be conducted under the conditions described in Table 5.1 [226-229, 232].

Table 5.1: Recommended conditions for stability testing in Zones I and II

	Recommended Conditions		Test length	References
	Temperature	RH		
Long Term	25 ± 2°C	60 ± 5%RH	1-5 years	226-229,232
Intermediate	30 ± 2°C	60 ± 5% RH	6 months reported, 12 months study	226,228,229
Accelerated	40 ± 2°C	75 ± 5% RH	6 months	226-229,232

Accelerated stability testing at 40°C for a period of six months is generally used as an assurance of the stability of a product upon storage at recommended conditions. Six months at accelerated conditions is considered to be equivalent to 30 months at 25°C [233]. Results from accelerated stability studies are therefore used to assess potential long-term chemical effects, however they may not always be predictive of physical changes that also need to be assessed [234].

5.2 EXPERIMENTAL

A stability study was performed on one of the experimental batches, to gain insight into the behavior of the dosage form under conditions of high humidity and temperature. This would provide information on the feasibility of the uncoated matrix as a once-daily sustained release dosage, and as a component in a combination dosage form of HCTZ and MPTA. Batch M001270504 was used for the study.

The study was conducted using humidity chambers prepared in desiccators with saturated salt solutions to achieve the required relative humidity conditions. The chambers were prepared using sodium chloride and sodium nitrite, which are reported to produce a relative humidity of 75% at 37°C to achieve humidity conditions required for accelerated stability. To achieve 60% RH required for long term and intermediate stability testing, sodium nitrite was selected as it is reported to produce a relative humidity of 66% at 20°C and 62% at 37°C [159]. The experiments were conducted using the recommended conditions for countries in Zones I and II, as indicated in Table 3.5, however, testing and was conducted for three months under all conditions.

The dessicator chambers were placed in ovens that were maintained at 30°C and 40°C. The assessment for long-term stability was conducted by storing the chamber in a dark cupboard in the laboratory that has a controlled temperature of 22°C. Tablets were placed in open glass jars and stored for the time periods described in Table 3.6. Although dosage forms should be tested in their final packaging, it was thought that these conditions would give a better indication of the ability of the dosage from to resist changes in humidity and temperature. Thermohygropens (Control Company, China) were used to monitor the temperature and humidity in each chamber, on a weekly basis. A plot depicting the temperature and humidity conditions for the period under investigation is shown in Figure 5.1.

The temperature or percent relative humidity (RH) in all chambers did not deviate by greater than 5% on any week that it was monitored. The temperature in the stability chamber designated to monitor ambient conditions was less than 25°C. The use of these humidity chambers was not optimum, however, samples were assessed for changes in stability under the storage conditions as described. The average of the actual conditions within each chamber are listed in Table 5.2.

Table 5.2: Actual Temperature and Humidity Conditions

	Actual Conditions		**Storage Period**
	Temperature (°C)	**Humidity (%RH)**	
Long term (Ambient)	19.14 ± 1.49	77.29 ±1.27	3 months
Intermediate	29.97 ± 0.46	74.36 ±1.22	3 months
Accelerated	40.28 ± 0.19	86.93 ±1.90	3 months

The stability batches were also assessed in terms of its changes in weight, hardness, moisture content, friability and visual appearance. Visual assessment of colour and overall appearance of a dosage form may be an early indication of drug decomposition, as has been reported from assessment of eighteen ticlopidine products [233]. Stored tablets were also assessed for potency, after each storage period. The release of MPTA from the tablets was assessed using the dissolution method described in § 4.5 to determine whether changes in the sustained release characteristics of the dosage form occurred on long-term storage. These results were compared to those obtained from evaluation of the dosage form prior to storage.

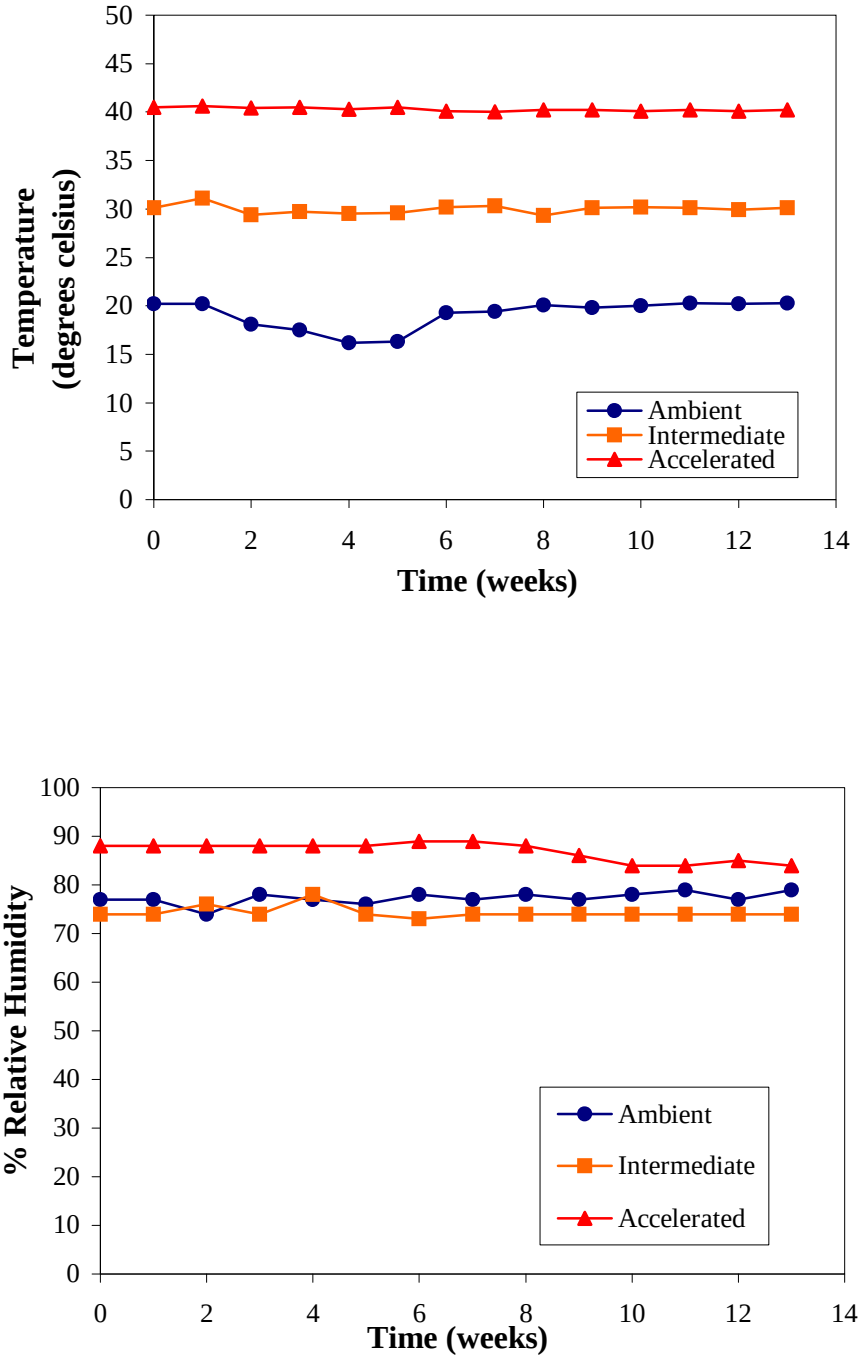


Figure 5.1: Graphical representation of temperature and humidity recordings over 3 months

5.3 RESULTS

5.3.1 PHYSICAL TESTS

5.3.1.1 Visual Appearance

In comparison to their appearance prior to storage, tablets stored at ambient and intermediate conditions displayed no obvious visual changes over the three-month period. However, in contrast those tablets stored at accelerated conditions showed differences after one month of storage. After one month, tablets appeared swollen and the surface appeared uneven and powdery and no changes in colour were observed. After two months, these changes were more pronounced and cracks were visible on all planes of the tablet, however tablets still retained their shape despite being difficult to handle. Tablets were also found to adhere to the jar walls and to each other. These effects were more pronounced after three months and at this stage a large amount of powder was found in the jar and the tablets were no longer completely intact.

5.3.1.2 Tablet Hardness

Tablet hardness was determined using an Erweka TBH 28 Hardness tester (Erweka-Apparatebau-GMBH, Germany). Results of hardness testing are listed in Tables 5.3.1-5.3.3. A comparison with the hardness of tablets determined prior to storage, all tablets assessed showed a decreased hardness at all time-points of the study, as was expected. It was found that the hardness of tablets stored at intermediate conditions showed a smaller decrease in hardness than those stored at ambient conditions for all stability testing points. This may indicate that tablets stored at ambient conditions may have absorbed larger amounts of water, resulting in softer tablets. Tablets stored at accelerated conditions showed significant decreases in hardness after one month of storage such that tablets could not be handled without damaging them. The changes caused by high humidity and temperatures were considered to be unacceptable as such tablets could not be used easily by patient and may impact on the release rate profile of the drug formulation.

Table 5.3.1: Hardness of tablets stored at ambient temperature (n=10)

	Average Hardness ± SD	% RSD
Before storage	19.30 ± 1.17 kp	6.08
1 month	13.81 ± 1.04 kp	7.51
2 months	12.71 ± 0.54 kp	4.22
3 months	11.99 ± 0.69 kp	5.77

Table 5.3.2: Hardness of tablets from intermediate stability batch (n=10)

	Average Hardness ± SD	% RSD
Before storage	19.30 ± 1.17	6.08
1 month	15.98 ± 1.35	8.42
2 months	15.66 ± 0.53	3.35
3 months	15.78 ± 0.32	2.04

Table 5.3.3: Hardness of tablets from accelerated stability batch (n=10)

	Average Hardness ± SD	% RSD
Before storage	19.30 ± 1.17	6.08
1 month	3.17 ± 0.67	21.17
2 months	1.70 ± 0.53	19.78
3 months	Tablets too soft to handle	

5.3.1.3 Tablet Weight

The weight of all stored tablets increased, as shown in Tables 5.4.1-5.4.3. Tablets stored under accelerated conditions showed the largest increases in weight whereas those stored at intermediate conditions showed comparatively smaller increases. These observations correspond with observations of hardness variations, and may also be an indication that the stored tablets absorbed more moisture that resulted in an increased tablet weight.

Table 5.4.1: Weight of tablets stored at ambient conditions (n=10)

	Average Weight ± SD	% RSD
Before storage	689.64 ± 10.88 mg	1.58
1 month	708.20 ± 9.40 mg	1.33
2 months	693.15 ± 22.51 mg	3.25
3 months	710.67 ± 14.15 mg	1.99

Table 5.4.2: Weight of tablets stored at intermediate conditions (n=10)

	Average Weight ± SD	% RSD
Before storage	689.64 ± 10.88 mg	1.58
1 month	699.46 ± 11.75 mg	1.68
2 months	690.27 ± 4.53 mg	0.66
3 months	699.79 ± 11.68 mg	1.67

Table 5.4.3: Weight of tablets stored at accelerated conditions (n=10)

	Average Weight ± SD	% RSD
Before storage	689.64 ± 10.88 mg	1.58
1 month	721.34 12.99 mg	1.80
2 months	Could not be accurately weighed	
3 months	Too soft to weigh	

5.3.1.4 Tablet Friability

The test was conducted using an Erweka Friabilator (Erweka-Apparatebau-GMBH, Germany) at 33 drops per minute for 3 minutes, and the results obtained are listed in Table 5.5. Tablets stored at intermediate conditions showed 0.15% loss after friability testing at all time points. At ambient temperature, the friability of tablets increased with increased exposure times, however, the percent weight lost during testing did not exceed 1%, as specified by the USP, and therefore the batch was still within specifications for this parameter. It was surprising that although tablets stored at accelerated conditions for a month showed a drastic decrease in hardness, only 0.57% of the tablet weight was lost after one month of storage. This was still within the acceptable limit and therefore is an indication that these tablets would possibly remain intact during transport and handling of the dosage form if exposed to these conditions.

Table 5.5: Friability Data for stability samples

	Friability* (n=10)		
	Ambient	Intermediate	Accelerated
1 month	0.15	0.15	0.57
2 months	0.29	0.15	-
3 months	0.28	0.15	-

* Friability prior to storage was 0.15%

5.3.1.5 Moisture Content

The uptake of moisture was determined using the Karl Fischer procedure. Testing was performed using a Mettler DL 18 Karl Fischer Titrator. The Karl Fischer titration technique is commonly used to determine small amounts of water in a sample [105]. Tablets tested before storage had average moisture content of 2.59%. No limits for the moisture content of MPTA tablets were found in the literature however, a literature search revealed that the upper limit of moisture content for a multi-

component nutritional supplement prepared by wet granulation was 3% [235]. Therefore this value was selected as a limit for moisture content of MPTA tablets that were tested prior to storage. as was expected, the water content in all tablets under all storage conditions assessed was increased. Tablets stored at intermediate conditions contained less moisture than those stored at a lower temperature. This correlates with the values obtained for hardness, weight and friability of these tablets. The average values for percent moisture content of all tablets are shown in Table 5.6.

Table 5.6: Moisture Content (n=3)

	Moisture Content (%) ± SD *		
	Ambient	**Intermediate**	**Accelerated**
1 month	5.49 ± 0.07	4.03 ± 0.14	6.48 ± 0.19
2 months	5.83 ± 0.12	5.49 ± 0.00	6.37 ± 0.28
3 months	5.91 ± 0.02	4.37 ± 0.10	6.79 ± 0.11

* Average moisture content before storage was 2.59 ± 0.18

5.3.1.6 Discussion and Conclusions

The results from each of the physical tests performed indicate that tablets stored under each of the conditions assessed show a definite increase in moisture content. This was confirmed by corresponding increases in tablet weight and friability and a decrease in tablet hardness. Tablets stored at intermediate conditions absorbed less moisture than those stored at ambient and at accelerated conditions. Temperature and humidity both have an effect on the physical properties of a dosage form, and optimal conditions for both must be specified. The results also indicate that packaging for the tablets would be a critical consideration.

The physical stability of the batch assessed is adversely affected by the absorption of moisture into the dosage form and it is likely that all other batches manufactured would behave in a similar manner. Mosquera *et al* [236] have found that increased moisture in HPMC tablets led to reductions in tablet hardness and an increase in tablet porosity and mean pore diameter, and this is probably likely for the batch assessed. Swelling can be attributed to the diffusion of water from the interparticulate spaces into the HPMC molecules [236]. Mosquera *et al* also found that both HPMC K4M and K100M exhibited similar properties [236], indicating that the HPMC in both the granules and matrix may have contributed to an increased moisture content in the dosage form. This increase may have also been facilitated by the inclusion MCC, another hygroscopic excipient in the

dosage form. MCC is capable of holding large amounts of water within its internal structure without changes in volume, and increases in water lead to increased particle volume with the subsequent creation of voids [237], which may have further contributed to the resultant appearance and characteristics of the tablets. Furthermore, MPTA itself is hygroscopic and may alsolead to increased intake into the dosage form.

As stability tests should be conducted on the dosage form in its final packaging, these tests represent extreme cases of each of the conditions assessed. These test allow for recommendations in terms of storage and packaging to be made. Due to the hygroscopic nature of the drug and certain excipients, the inclusion of a desiccant in the final packaging would be an essential requirement. Desiccants function to rapidly reduce humidity to a low level and to maintain this level during transport, storage and use of the dosage form [238]. An alternative may be the use of blister packaging that is designed to keep each dosage unit fresh until required for use [239]. This may be particularly beneficial as tablets stored under accelerated stability were observed to adhere to the jar and each other. The selection of such packaging would require further stability testing as certain packaging materials may interact with the dosage firm or may fail to protect the contents from environmental hazards [239]. It was found that humidity had a great impact on the integrity of the dosage form and therefore greater efforts should be made to minimize these effects, therefore suitable packaging and formulation compensation may be necessary.

5.3.2 ASSAY OF TABLETS

Content uniformity tests (n=6) were performed on the batch both prior to storage and after storage under each of the stipulated conditions, over one, two and three months. The amount of drug amount was compared to the reference samples using the Timm analysis [111] previously described in § 2.3.9.

A significant change in drug potency is defined by the Medicines Control Council of South Africa, as being a loss of potency of greater than 5% from the initial assay value of the batch and in this event, it is recommended that further stability tests be conducted at intermediate conditions [240]. However, 90% of label claim is generally regarded as the lowest acceptable value for potency [227,231]. As depicted in Figure 5.2 only of the samples assessed showed significant and possibly

relevant decrease in the amount of drug determined, whereas all other samples showed neither significant nor relevant changes over the storage periods. This indicates that there was no loss in potency of MPTA after three months of storage at ambient and intermediate conditions, as none of the samples assessed showed changes of greater than 10%. However, due to the significant decrease in response for the three month accelerated stability sample, further testing at intermediate conditions should be conducted, possibly for 6-12 months, in accordance with recommendations by the Medicines Control Council of South Africa [240].

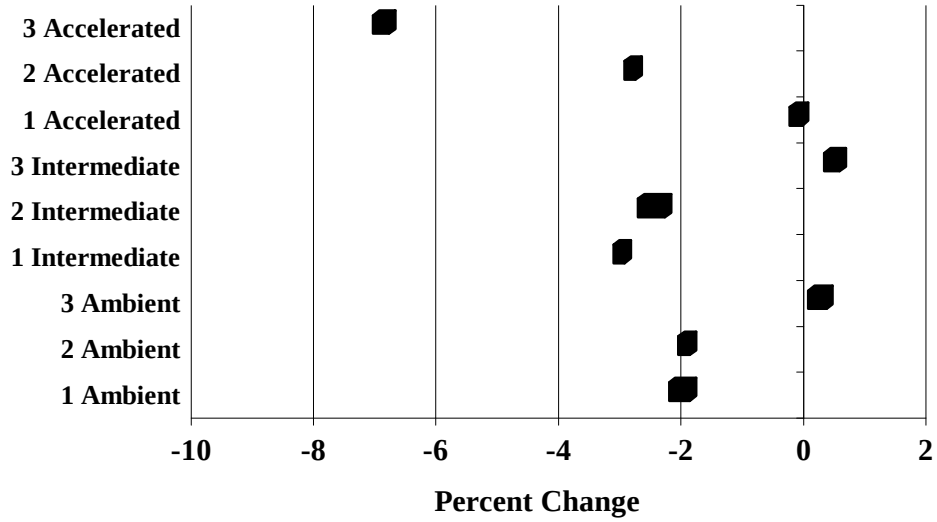


Figure 5.2: Timm analysis of stability samples

5.3.3 DISSOLUTION STUDIES

Dissolution studies were conducted as described in § 4.5. A dissolution study was conducted using six tablets of Batch M001270504 before storage and the release rate profile obtained was used as a reference for comparison for dissolution tests conducted on the stability samples. Profiles obtained for the stability samples are shown in Figures 5.3-5.5.

The release profiles obtained for all batches assessed showed no significant differences in terms of shape, from the initial release profile. At ambient conditions, none of the release profiles appeared to be significantly different from each other (Figure 5.3), however, calculated f1 and f2 values (Table 5.7) for comparisons of each of the curves stored for one, two and three months, with the reference, indicate that whilst the curves are similar to each other after three months of storage, the

dissolution profile after three months was significantly different from the reference dissolution profile, where a greater rate of drug release was observed after three months storage, indicating a potential loss of rate controlling effects of the formulation.

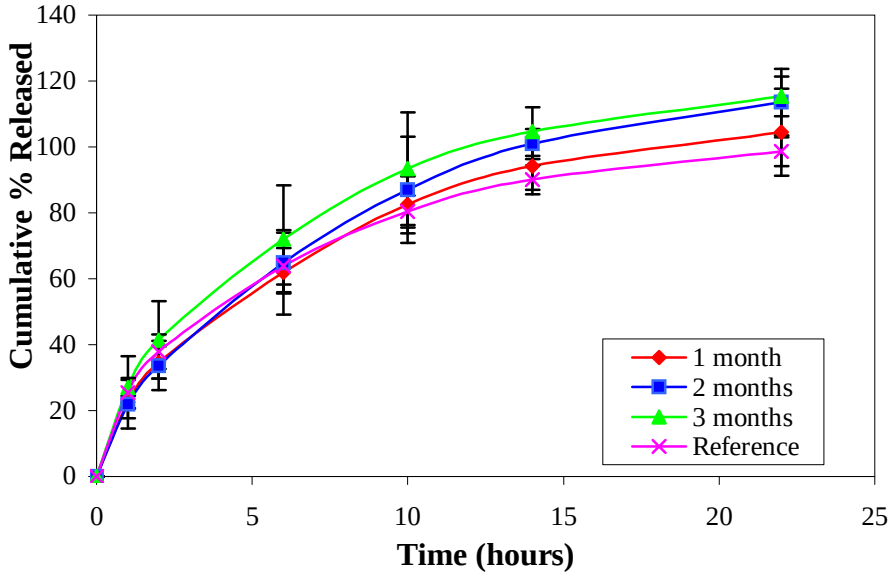


Figure 5.3: Dissolution profiles of stability samples stored at ambient conditions (n=6)

Table 5.7: f1 and f2 values for the comparison of dissolution profiles of stability batches

	f1	f1
1 AM vs 2 AM	6.2	63.9
1 AM vs 3 AM	13.5	51.4
2 AM vs 3 AM	7.4	62.4
Reference vs 1AM	5.3	70.6
Reference vs 2 AM	10.4	53.7
Reference vs 3 AM	14.6	47.5
1 IN vs 2 IN	9.8	54.9
1 IN vs 3 IN	4.3	71.6
2 IN vs 3 IN	13.6	50.0
Reference vs 1 IN	14.5	48.1
Reference vs 2 IN	5.2	67.5
Reference vs 3 IN	17.3	43.4
1 AC vs 2 AC	22.1	43.1
1 AC vs 3 AC	12.7	48.9
2 AC vs 3 AC	20.8	42.9
Reference vs 1 AC	9.3	60
Reference vs 2 AC	10.5	55.1
Reference vs 3 AC	15.2	47.5

(Where AM, IN and AC represent ambient, intermediate and accelerated conditions, respectively and 1,2,3, refers to the period of storage in months)

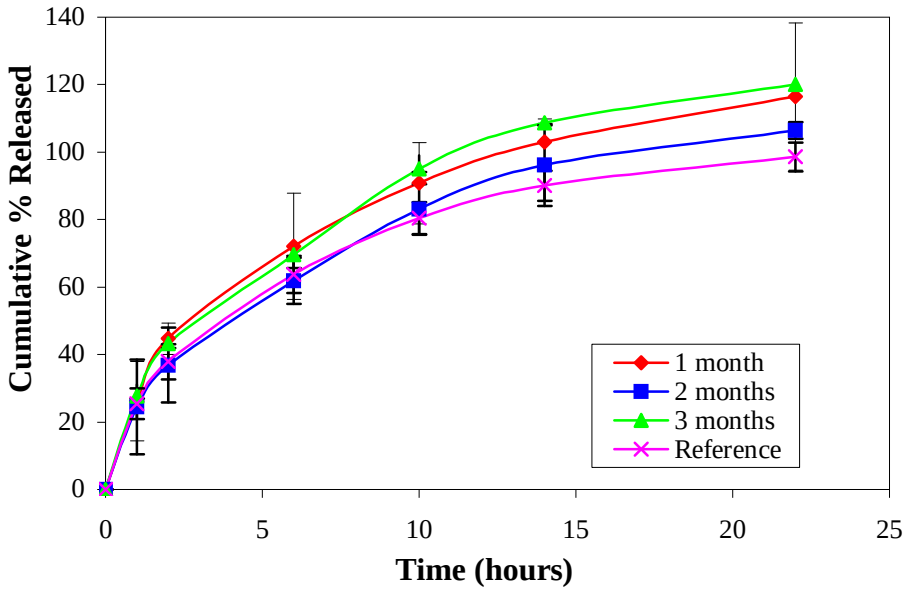


Figure 5.4: Dissolution profiles of stability samples stored at intermediate conditions (n=6)

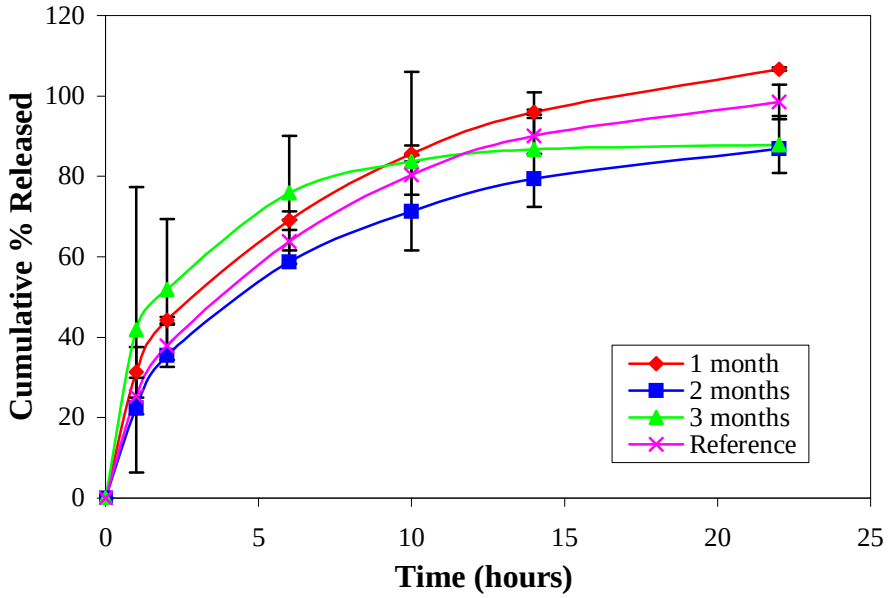


Figure 5.5: Dissolution profiles of stability samples stored at accelerated conditions (n=6)

With respect to tablets stored at intermediate conditions, it was also found that the dissolution profiles for the tablets stored under these conditions were similar, however there was also a significant difference between the profile obtained after one and three months from the reference, as f1 was <15 and f2 was > 50 (Table 5.7).

Following storage under accelerated conditions, all tablets showed a faster rate of drug release as is evident in Figure 5.5, however the calculated f2 values indicated that all profiles were significantly different from each other. Under these conditions it was noted that samples stored for two months released less drug than those stored for one and three months, and the calculated fit factor values indicate that whilst the release profiles for samples stored for one and three months were similar to each other, neither were found to be similar to the two month sample. Furthermore, samples stored for two months were similar to the reference samples, whereas those stored for three months exhibited significant differences (f1 and f2 in Table 5.7). These observations may have been a function of increased friability and decreased tablet hardness that may have resulted in loss of MPTA particles from the dosage form during handling of these samples. However, the variability observed for samples stored at intermediate conditions for one month and that observed for accelerated stability samples, may have also been a function of the inherent variability of sustained release dosage forms.

5.4 CONCLUSIONS

Evaluation of the physical properties of the tablets showed a trend of increasing friability and decreasing tablet hardness as the length of storage increased. Larger storage times also resulted in the absorption of larger amounts of moisture, which subsequently leads to softer tablets and it is likely that these effects may be exacerbated by the hygroscopic nature of the drug and other excipients as was discussed previously (§ 5.3.1.7). No loss in drug potency >10% was observed for any of the tablets tested after a three-month period under any of the storage conditions used in the stability assessment.

Dissolution studies revealed that there was an increased rate of drug release with increased storage time and after three months storage under all conditions, there was a significant difference between the test and reference profiles, indicating a possible alteration of sustained release characteristics of

the dosage form. Although, varying the hardness of these tablets was not found to significantly alter the drug release profile under ambient conditions (§ 3.2.3.3.6.2), it is likely that a combination of decreased tablet hardness and subsequent increased porosity as a result of the increased moisture content of the dosage form under conditions of extreme temperature and humidity may have contributed to an alteration in the drug release profile.

The observed softening of tablets due to the absorption of excess moisture may be problematic as handling of the dosage form would be difficult and the sustained release behaviour of the dosage form may be compromised. Therefore the final dosage form will require the inclusion of a dessicant in the packaged product. Subsequent manipulation of the formulation may also be required to prevent tablet softening and loss of sustained release characteristics over time, and therefore the addition of a coating may be necessary to minimize the potential environmental effects on the release rate controlling properties of the dosage form.

CHAPTER SIX
THE DEVELOPMENT, FORMULATION AND ASSESSMENT OF THE COMBINATION DOSAGE FORM OF MPTA AND HCTZ

6.1 INTRODUCTION

The use of controlled release systems for optimization of drug therapy has increased substantially in recent years. Consequently, methods to control release have become more diverse and efficient to meet the challenges of drug delivery. Whilst matrix systems are still the most commonly used, membrane and hybrid systems are also being increasingly used. This chapter focuses on such systems and the applicability of these technologies for the further development of the matrix MPTA tablet core previously discussed in Chapter Three.

This chapter also focuses on the formulation of a combination dosage form of MPTA and HCTZ. Fixed-ratio combination products are only deemed acceptable when the combination has a proven advantage over the single compounds administered separately in terms of therapeutic effect, compliance, safety, chemical relevance and overall cost [241]. The combination of a β-blocker and a diuretic into a single dosage form, has several advantages which include convenience to both the patient and physician, simplified drug dosing regimen and improved patient compliance, reduction of adverse effects and potentiation of the anti-hypertensive effects of the two drugs used in combination [242]. As discussed in § 1.3, the combination of MPTA and HCTZ into a single dosage form is beneficial as the two drugs exhibit a synergistic or additive blood pressure lowering effect as it was found that a combination of the two lowered blood more effectively than increased doses of either drug individually [62-69,243]. Furthermore, a combination of these drugs is beneficial as the diuretic acts to decrease the sodium and water retention caused by the β-blockers, whereas the β-blockers blunt the increase in plasma renin levels caused by the diuretic [243]. The increase in renin levels in especially advantageous in the South African context, as the majority of hypertensives are either black or elderly patients with low renin levels and are therefore poorly responsive to treatment with β-blockers, such as metoprolol, only.

A dose of 100mg daily was selected for the metoprolol component of the combination dosage form, as the dose has been found to be effective in controlling blood pressure when used in a once

daily controlled release dosage form [59,60]. HCTZ exhibits dose-dependent blood pressure reduction that decreases with higher doses. A dose of 25 mg daily has been found to be as effective as higher doses, however, it has also been reported that lower doses of 6.25mg and 12.5mg daily were capable of achieving adequate blood pressure reduction without the adverse metabolic effects of HCTZ that may counteract the beneficial cardiovascular effects of the drug, as observed with higher doses [243]. Consequently, a dose of 12.5mg of HCTZ was selected for the combination dosage form to be developed.

6.2 MEMBRANE SYSTEMS

Membrane systems (§ 3.1.3) consist of a rigid membrane surrounding a drug-containing core, and drug release from these systems may be controlled either by solution diffusion, osmotic pumping or dissolution of the membrane itself.

6.2.1 Solution diffusion

This mechanism of drug release is characteristic to most systems where the drug core is surrounded by a rate-controlling membrane [117,138]. It is characterized by drug dissolution at one interface of the membrane and then diffusion down a concentration gradient into the external medium. Fick's first law of diffusion, as described in Equation 6.1, describes diffusion through a membrane [117, 244] and the process of diffusion through the membrane is illustrated in Figure 6.1 [117,244].

$$J = -D \frac{\delta C}{\delta x} \qquad \text{Equation 6.1}$$

where,

J = flux/ amount of drug moving through a unit cross-sectional area of membrane per unit time
D = diffusion coefficient of the drug (incorporated partition coefficient between drug and membrane
C = drug concentration in the reservoir (Ci)
x = the perpendicular distance traveled /thickness of the membrane (ℓ)

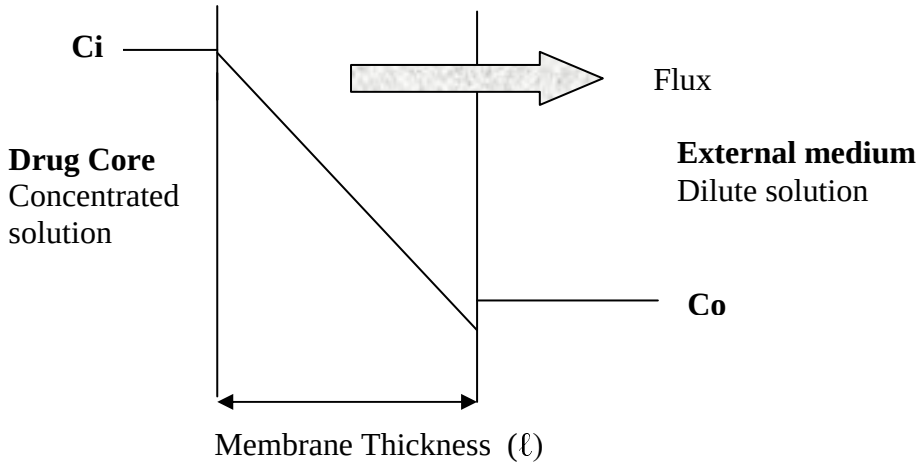


Figure 6.1: Schematic representation of drug diffusion through a membrane

At steady state, Equation 6.1 can be integrated to Equation 6.2, which is the more common form of Fick's first law when a water insoluble membrane is used to control release of a drug.

$$\frac{dM}{dt} = \frac{ADK\Delta C}{\ell} \qquad \text{Equation 6.2}$$

where,

A = area
D = diffusion coefficient
K = partition coefficient of drug into the membrane
ℓ = diffusion path length (membrane thickness)
ΔC = concentration gradient across the membrane (Ci-Co)

Zero order release kinetics are a characteristic of these systems, however a change in any of the variables shown in Equation 6.2 may result in non-zero order kinetics prevailing.

When a partially soluble membrane or a combination of water insoluble and soluble polymers are used, the water soluble components of the membrane dissolves, creating pores or channels through which drug can diffuse from the dosage form. Consequently, overall drug diffusion occurs through both the pores and the membrane itself [244]. The differences between drug diffusion through insoluble and partially soluble membranes is graphically depicted in Figure 6.2.

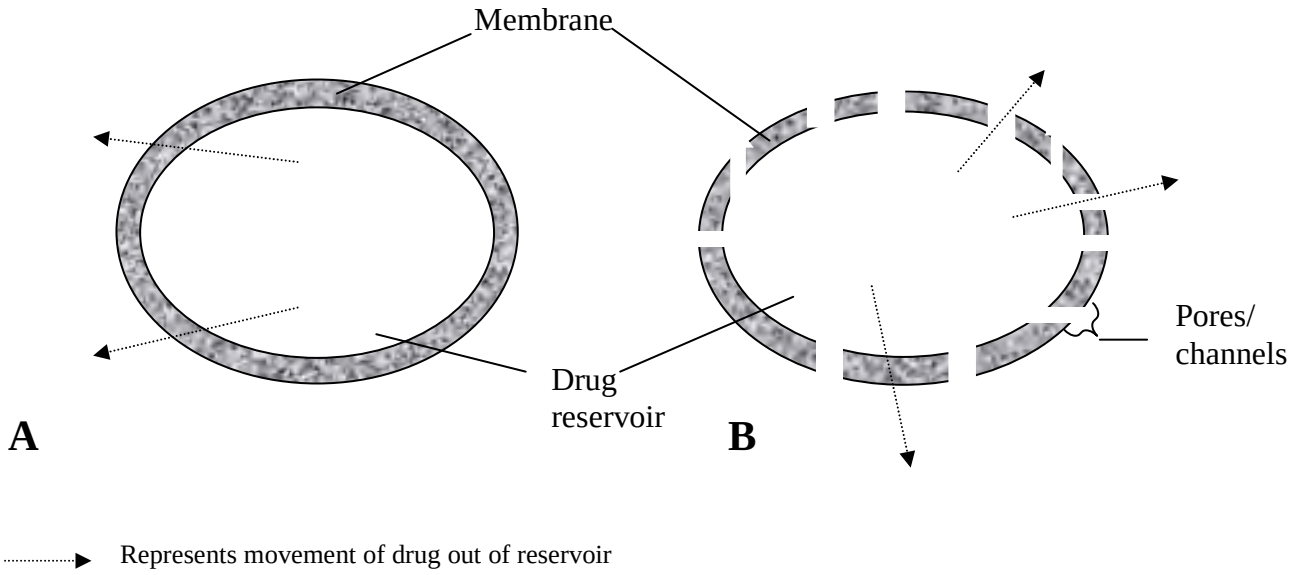


Figure 6.2: Drug diffusion through an insoluble (A) and partially soluble (B) membrane

6.2.2 Dissolution control

Drug release may be dissolution controlled in systems where the drug is surrounded by a membrane, which is itself dissolved or eroded over time [244]. In this instance, drug release is dependent on the rate of membrane erosion and dissolution and a constant rate of erosion will result in zero order release kinetics. The rate of release may therefore be altered by increasing membrane thickness or by layering drug and coating as is the case for repeat action dosage forms [244,245].

6.2.3 Osmotic Pumping

Osmotic systems may be one of two types. The first type is known as the elementary osmotic pump and is schematically represented in Figure 6.3.

This technology consists of a semi-permeable membrane that surrounds a particle, tablet or drug solution that provides a constant reservoir volume [117,244]. An osmotic pressure difference is created between the inside and outside of the tablet, and the drug solution is ‘pumped’ out through an orifice in the membrane after water is taken in through the semi-permeable membrane [244]. The orifice is usually a laser-drilled hole. The core contains excess drug and/or an osmotic agent, and exhibits a declining release rate as the concentration of drug in the reservoir falls below

saturation [117], usually after about 60-80% of the drug has been released. The osmotic pressure differential and the membrane regulate the osmotic water permeation, such that volume of drug solution delivered through the orifice is equivalent to water uptake of the membrane in a certain time interval. This is a simple system, which releases drug molecules by a zero-order mechanism irrespective of pH, however this elementary osmotic pump system is not well suited for water insoluble drugs [246,247].

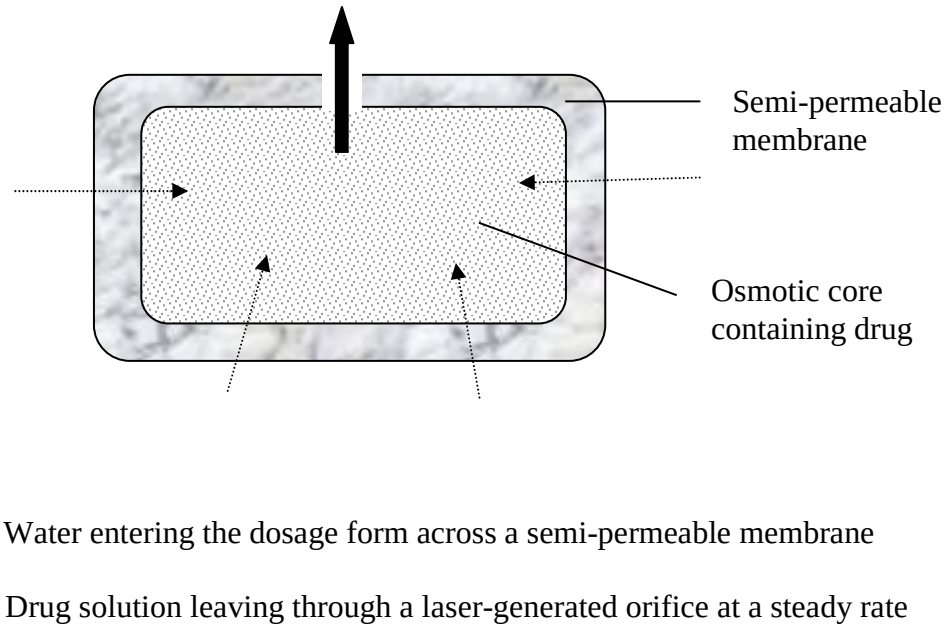


Figure 6.3: A schematic of an elementary osmotic pump system

As the delivery rate of the drug from the osmotic pump depends largely on the solubility of the drug, the inclusion of water-insoluble drugs into an osmotic pump system may require co-compression of the drug with excipients, such as swellable polymers, which can modulate the solubility of the drug within the core [248]. Multi-chamber osmotic pumps have also been developed for delivery of water-insoluble drugs over extended periods. The most commonly used of these is the push/pull design that consists of two compartments, one containing the drug and the other containing an osmotic agent in an expandable chamber [247,248]. In these systems, depicted in Figure 6.4, the upper compartment contains the drug and is connected to the outside environment via a delivery orifice. The polymeric osmotic agent is contained in the lower compartment has no direct contact with the outside environment. Upon contact with aqueous

media, both layers imbibe water, however the polymeric layer swells and pushes into the drug chamber and drug release through the orifice is effected [248,249].

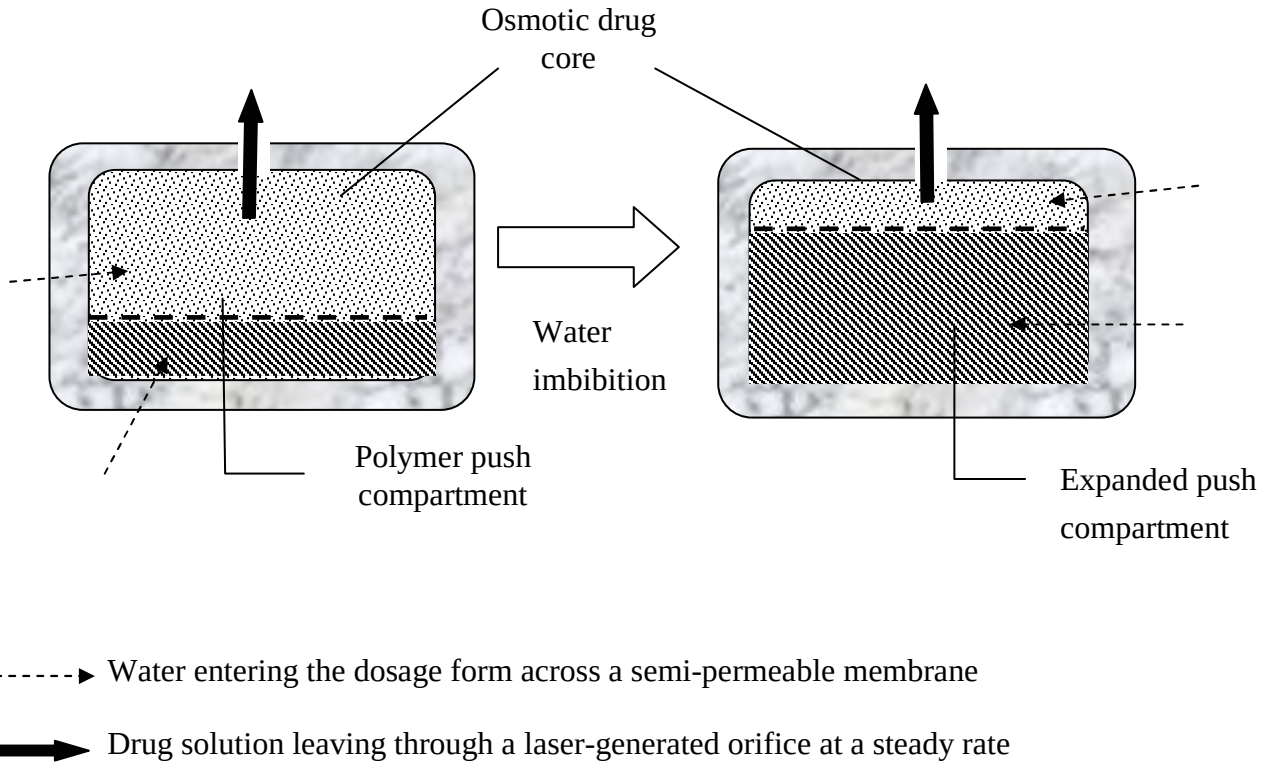


Figure 6.4: A schematic of a push/pull osmotic system before and after water imbibition

6.3 HYBRID SYSTEMS AND MULTIPLE-UNIT DOSAGE FORMS

Hybrid systems are usually combinations of membrane and matrix systems and may occur either as single or multiple-unit dosage forms [117]. Drug release from these systems may therefore occur via a combination of mechanisms as described for matrix, membrane and perhaps even osmotic systems.

Multiple-unit dosage forms

These dosage forms are particularly advantageous as there is a lower risk of spontaneous drug release than from single units and there is a more reproducible dispersion of units throughout the GIT is prevalent, possibly lowering intra- and inter-subject variability [250]. Multi-unit systems typically comprise are typically comprised of microspheres in hard gelatin capsules, however

newer systems include microspheres within matrix tablets [251], matrix pellets [252] and coated pellets [245], and coated pellets incorporated into divisible tablets [253].

6.4 TABLET COATING FOR CONTROLLED RELEASE DOSAGE FORM PRODUCTION

6.4.1 Introduction

The process of tablet and pellet coating has become increasingly utilized for a variety of purposes including protection of active ingredients from environmental factors, taste masking and as functional coats [254-257]. A coat may also impart resistance to abrasion and may be used to promote easy identification of a product [254]. Functional coats are of particular interest as these may be used to impart enteric or controlled release properties to a dosage form. Enteric coating is used to prevent drug release in the acidic environment of the stomach in order for release to occur once the dosage form has passed into the small intestine in which the pH is alkaline [254,255].

Three types of coating processes are commonly used for tablet dosage forms. The use of sugar coating, which is a panning technique, has decreased substantially and as a result, film coating has become increasingly used [254,256]. Compression coating may be utilized for highly water-sensitive drugs or to separate materials where chemical incompatibilities are likely to occur [254].

6.4.2 Film Coating

The coating technique of choice when considering the application of a coat to controlled release dosage forms is film coating. The obvious advantages include simplicity and automation of the process, as a single manipulation in the coating process is required as opposed to conventional sugar coating techniques. Furthermore, the possibility of moisture exclusion in the case of water-sensitive drugs and the ability to exhibit distinctive markings, are other distinct advantages of film coating. A number of polymeric materials have been developed and are available for application to dosage forms to achieve membrane control of drug release [255,256]. More recently, film coating materials have been used for application to granules, pellets and powders that are then incorporated into capsules, suspensions or tablets as a multiparticulate or hybrid system for controlling release of the active pharmaceutical ingredient [257]. Film coating materials have been used in the preparation and manufacture of osmotic systems, as described in § 6.2.1.3. Drug release from

membrane systems occurs via diffusion across the membrane, where the drug may diffuse through the membrane itself or through pores created in the membrane as depicted in Figure 6.2.

6.4.2.1 Film coating polymers

A number of synthetic polymeric materials, including cellulose derivatives, are now available for film coating [254,257]. These are selected on the basis of their low toxicity, solubility characteristics in-vivo, and their ability to form non-tacky mechanically strong coats that are resistant heat, light and moisture [254,257]. These types of polymers used for enteric coats include cellulose acetate phthalate and the polymethacrylates [254,257]. The Eudragits® [258,259], in particular methacrylic acid copolymers [260,261] and cellulose ester derivatives such as cellulose acetate phthalate [262] may also be used for their enteric functionality.

Polymeric materials that are used for controlled release dosage forms include the cellulosics such as HPMC [263] and ethylcellulose [263,264] and the acrylics such as the Eudragits® [263]. Water-soluble polymers however, are generally not well suited for controlling drug release [265]. The most predominantly used for controlled release coatings therefore include ethylcellulose or the polymethacrylates [265]. These polymers may be used individually or in combination with other polymers [258,266] with or without plasticisers [254]. As the use of organic solvents has decreased for environmental and safety reasons, consequently, latex or pseudolatex technologies have been developed and allow for such polymers to be dispersed in aqueous vehicles [267,268]. Psuedolatex products which are available include Aquacoat®, Surelease® and the Eudragits® [263,264].

6.4.2.2 Film coating plasticisers

The use of plasticisers to modify the physical properties of the film-forming polymers [254,257,269], such as reduction of the minimum film formation temperature to below the coating temperature [267], is well known. Film properties such as permeability, solubility, workability and flexibility may be optimized [254,257,269, 270]. The choice of plasticiser is dependent on the polymer used and on the desired drug release profile. Commonly used plasticisers include the polyols, PEG's, fixed oils such as oleic acid and organic esters such as triethyl citrate (TEC), acetyltriethyl citrate and dibutyl sebacate (DBS) [270]. Plasticisers are generally required for pseudolatex deformation to improve film coalesence, where an increase in plasticiser content

results in a decreased drug release in most cases [271].

6.4.2.3 The Coating Process and Apparatus

The coating process is characterized by formation of droplets of the coating solution, which then come into contact with the core to be coated. The droplets spread along the surface of the core and coalescence of the droplets then occurs. The drying conditions must then allow for solvent evaporation, leaving a thin film of polymer around the core [254,272]. The coating layer is not applied in a single step but requires the dosage form to pass through the coating zone several times for a complete film to form.

Film coating may be carried out in a coating pan or in a fluidized bed system [254,257], the fluidized system being the preferred technique for coating of small units. The principle of the fluidized bed system is that tablets are suspended in an upward stream of air that is moving at a rate that is great enough to set the tablets in motion so that they are not in contact with one another [257]. The coating solution is introduced onto the tablets by means of a spray atomizer located at the base of the coating chamber, and cores are then carried away from the spray area and dried by the fluidized air [257].

Various types of fluidized bed systems may be used. These may exhibit incipient or smooth fluidization, bubbling, slugging or spouting fluidization. The fundamental difference between these systems is the difference in airflow in each region of the bed. Incipient fluidization refers to that point at which the bed just becomes fluidized and the system cannot be utilized in this state of fluidization. Bubbling fluidization beds provide random mixing of particles and efficient rates of evaporation and may be used to achieve good quality films. The spouting bed in which the air forms a single opening, through which particles flow and then fall out of, is more commonly used and is the bed-type used in the Wurster coating process [272].

Coating or granulation solutions may be introduced by one of three inserts, which may be selected based on the cores that are to be coated. Each of these differs in terms of the ultimate spray patterns achieved. In top spray systems, the nozzle for spraying of coating/granulating solutions is located at the top of the chamber. This is the method of choice for wet or spray granulation [273], however it is not suited for sustained release coatings as the droplet distance traveled cannot be controlled

due to random fluidization pattern [272]. However, this method may also be used for hot-melt coating with PEG's or waxes [274].

Nozzles may also be fixed tangentially within the bed. This insert is comprised of two nozzles that may be used to perform different operations such that one nozzle may be used to spray the powder into the chamber and the other to deliver the coating/granulation liquid. A tangential insert may be used for coating, granulation, pellet formation and drug loading onto non-pareil beads [272]. However, the use of such an insert may be undesirable in the coating process due to the potential for strong mechanical forces, which may cause damage and abrasion to the coated cores [273].

The Wurster or bottom spray process is applicable to beads, pellet, particle and powder coating [272], and more recently for processes such as thermosensitive microcapsule preparation [275]. In addition small tablet cores can be coated. The components of the Wurster system are illustrated in a schematic diagram, Figure 6.5. The system is comprised of a coating chamber inside which is a partition (Wurster column), which has a diameter that is approximately half the diameter of the base of the chamber. The spray nozzle is located at the bottom of the chamber and is surrounded by a nozzle surround, which keeps the particles from entering this region, as it is here that droplet density is the highest and contact at this region may lead to uneven film formation or excessive particle deposition at certain points close to the spray nozzle. An air distribution plate and a fine screen are located at the bottom of the Wurster insert [272]. There is a region of high volume and air velocity below the column, which causes the particle to move upwards through the spray zone. As the particles enter a region of lower velocity outside the column, they fall back to the bottom of the column and are simultaneously dried. Particles may then re-enter the spray zone and the process is repeated [272,276].

The Wurster process is considered advantageous as both small and large particles can be uniformly coated [276], more rapid drying rates are achieved and accurate fluid pumping and efficient methods for heating large volumes of air are achieved [277]. A Strea-1 fluid bed drier with a wurster insert was used for coating tablets in our laboratory.

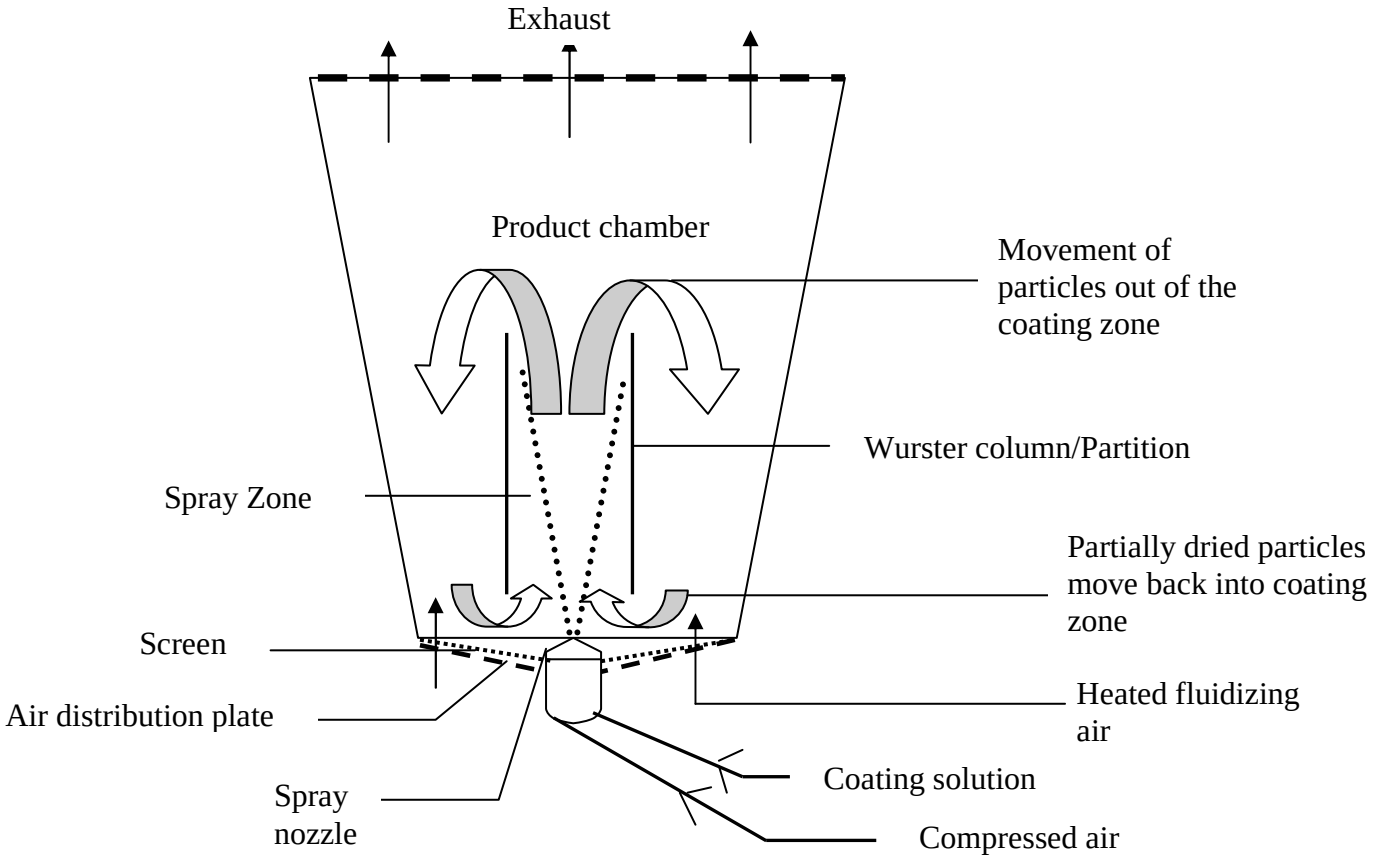


Figure 6.5: Schematic of a Wurster coating apparatus

6.4.2.4 Variables affecting the coating process

In terms of controlled release dosage forms, film coating must produce a uniform and coherent film that is stable on storage [278]. It is therefore of vital importance that the coating process is optimized to ensure the integrity of the films that are produced. The coating process may be affected by a number of variables, which may be categorized into process, and product variables. With respect to the product, substrate particle size, shape, porosity, density and friability are important considerations [272].

Certain process variables that must be considered are discussed in further detail below. The volume of the fluidization air affects the velocity and fluidization pattern of all particles within the coating chamber and must therefore be consistent between different batches that are coated [272]. Poor

fluidization of the tablet bed results in poor movement of the cores through the spray zone and therefore inconsistent and a non-uniform coat thickness may result [278]. Therefore, the configuration of the air distribution plate is one of the critical process considerations.

The polymer and the type of coating solution used can also influence the fluidization air temperature, and therefore selection of the appropriate fluidization air temperatures must be selected upon consideration of these factors. For aqueous-based coating systems, a low temperature may result in fluctuating drying capacity with seasonal changes [272]. The air temperature affects the drying capacity of the system and consequently impacts on the drying time [269]. Although the fluidization air temperature can be manipulated, temperature of the product bed and exhaust air must also be monitored. Differences in substrate and film expansion and contraction during the heating and cooling phases of the coating process may result in internal stresses within the film, which may lead to cracking, edge splitting and peeling [279]. The selection of a column of appropriate height in relation to the air distribution plate must be considered as must the distance between the plate and the column [280]. The selection of appropriate column height and air distribution plate affects particle fluidization [273].

Excessive spray rates may result in conditions such as film picking [263,278], which may also be a function of under-drying. Conversely rapid drying may result in an orange-peel effect in which the resultant coat has a rough appearance [263]. The spray rate must be selected based on consideration of the tackiness of the coat being applied, the speed at which particles travel through the coating zone and the capacity of air to remove the carrier solvent being used [272].

The viscosity of the coating solution must be a considered as solutions with a very high viscosity may result in a large droplet size resulting in low surface area for evaporation leading to poor film formation, whilst low viscosity solutions will have the opposite effect [281]. In contrast, coating solutions containing high levels of HPMC as the fixing agent can lead to agglomeration of droplets due to increased viscosity of the coating solution [282].

Humidity may affect drying of both aqueous and organic coating systems. In cases where low temperature and high humidity are used for aqueous-based systems, lower spray rates may be

required [272], whereas for organic systems increases in humidity may decrease air temperature resulting in condensation of the solvent on the substrate surface [272], which results in poor film formation.

6.5 DESIGN OF A COMBINATION DOSAGE FORM

6.5.1. PROPSED DESIGN

The matrix tablet core developed for MPTA (Table 3.8) was found to be capable of retarding drug release over an extended period, however the large initial release observed in the first two hours would probably lead to 100% drug release in less than 24 hours. Furthermore, HCTZ could not be incorporated directly into the formulation, as the polymeric matrix would retard its release. The option of dispersing the HCTZ in a water-soluble coating, which would rapidly dissolve to release drug, was excluded due to the rapid hydrolysis of HCTZ in aqueous solution. A multi-unit hybrid formulation that would involve the compression of the matrix formulation into mini-tablets was considered. To achieve zero-order release the mini-tablets could be coated to varying levels to, and subsequently incorporated into a capsule. HCTZ could then be included as a single mini-tablet or loose powder into the capsule, an approach that would minimize potential incompatibility problems between the two drug components, as identified in § 3.2.2.1.

6.5.2 COMPRESSION AND ASSESSMENT OF MINI-TABLETS

6.5.2.1 Materials and Methods

The mini-tablets were manufactured as per the method described in § 3.2.3.2, utilizing the formulation described in Table 3.8. Granules were screened through the smaller mesh size both prior to and after drying. The tablets were compressed on a Manesty F3 single punch press, using a 3mm die and biconcave punches. The tablets were compressed to a target hardness of 7-8kp and weight of 120mg such that each tablet would be equivalent to approximately 16.67mg MPTA. Therefore six tablets could be included into a capsule to achieve 100mg overall MPTA load and this would represent one dosage unit.

6.5.2.2 Evaluation of Mini-tablets

The mini-tablets resembled the larger tablets in appearance. Batch manufacturing records for these tablets are included in Appendix II. The resultant hardness ranged from 6.80 –8.50kp for all

batches manufactured. Friability was less than 1% for all batches, indicating the suitability of the tablets for a coating procedure. Highly friable tablets were not desirable due to the abrasive nature of the coating procedure in a fluidized bed system. Weight variation was also found to be minimal. Ten dosage units, which consisted of six mini-tablets per unit, from batches M00110601M and M00020901M were assessed for uniformity of content using the method described in § 3.2.3.2. Batch M00020901M contained 98.35mg of drug per dosage unit and therefore the batch was accepted for uniformity of content as the amount of drug determined represented 105% of the theoretical amount. Batch M00110601 contained 90.54% of the theoretical amount, however it was used for further work and the cumulative percentages of drug released were calculated according to average values obtained from content uniformity tests. Release profiles for three batches of mini-tablets that were manufactured using the same formulation, are shown in Figure 6.6. The profiles are characterized by rapid initial release such that almost the entire amount of drug was released within 6 hours and f1 and f2 values (Table 6.1) indicate that all batches were similar to each other with respect to their release characteristics.

Table 6.1: f1 and f2 comparison between batches of mini-tablets

Comparison	f1	f2
M00200910M vs M00110601M	9.8	55.4
M00110601M vs M00050601M	7.9	62.0
M00200910M vs M00050601M	13.1	50.6

When compared to the release profiles for larger tablets (Figure 6.7) which were manufactured using the same powder blend as for the mini-tablets, release from the mini-tablets was significantly more rapid, and the difference between the release profile was significant, yielding f1 and f2 values (Table 6.2) which were not within the limits for acceptance of similarity between batches. Initial release from the uncoated mini-tablets was much greater than from the larger tablet cores, and approximately 65% of the drug was released within the first two hours of dissolution from the small tablets, which were not contained within a gelatin capsule. The increase in release rate may be a function of the larger surface area available for drug release. Murakami *et al* also reported that an increase in the surface area available for drug dissolution resulted in increased release of theophylline from matrix tablets, where surface area had the largest effect on drug dissolution when compared to the effects of tablet weight and drug loading [283].

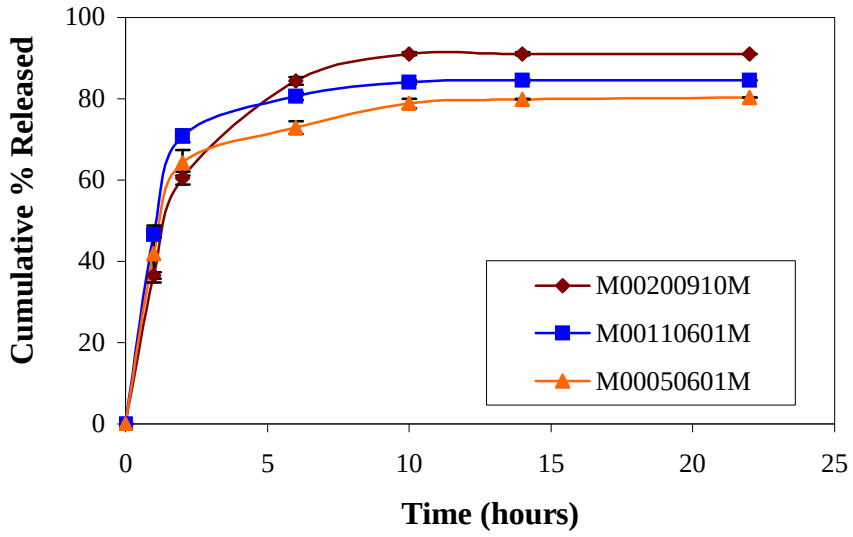


Figure 6.6: Release profiles of three batches of mini-tablets manufactured using the same formula (n=6)

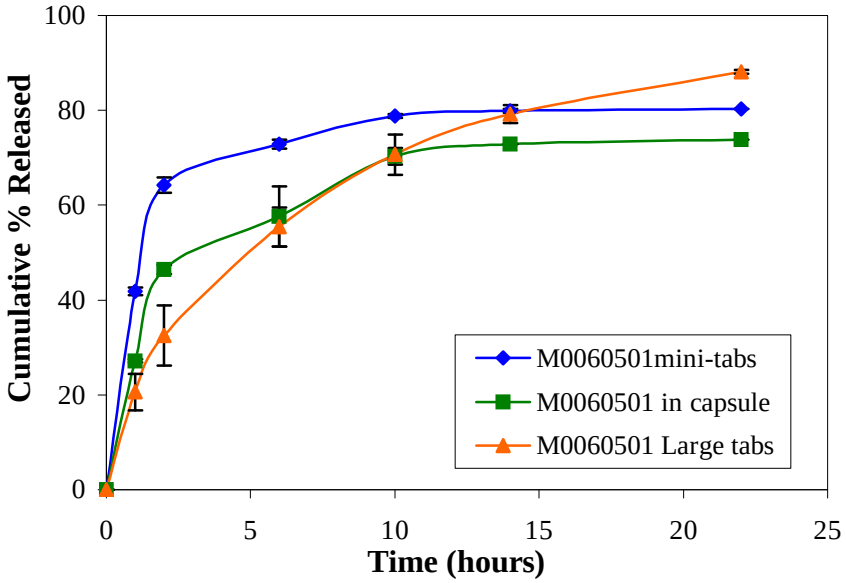


Figure 6.7: Release profiles of large matrix tablets and mini-tablets enclosed in a capsule and without a capsule (n=6)

Table 6.2: f1 and f2 comparisons of large tablets and mini-tablets with/without a capsule

Comparison	f1	f2
Large tablets vs mini-tabs (no capsule)	20.81	37.5
Mini-tabs (capsule) vs mini-tabs (no capsule)	16.7	45.2
Large tablets vs mini-tabs (capsule)	12.6	52.1

Tablets were also enclosed in a size 00 capsule and in comparison to the profiles obtained for tablets tested without a capsule, drug release rate was decreased (Figure 6.7). f1 and f2 values (Table 6.2) indicate that there was no difference between the mini-tablets which were filled into a gelatin capsule and the larger matrix tablets. The capsule therefore had a significant retarding effect on the release from the mini-tablets. The capsule shell might have acted to prevent the initial removal of drug from the tablet surface before the formation of the gel layer. It may also be likely that upon dissolving, the gelatin acted to bind the small tablets together, as depicted in Figure 6.8 C, resulting in a surface area that was smaller than for the tablets without the capsule, but that was comparable to the surface area of the larger tablet cores.

The mechanism of release from the mini-tablets occurred by anomalous or first order diffusion, as indicated by the calculated n values of 0.73 and 0.55 for batches M00200910M and M00050601M, respectively. The n value for batch M00110601M could not be calculated as greater than 70% drug was released within 2 hours. This corresponded with the release from most batches of the large tablets assessed. It was interesting to note that the n value obtained for drug release from tablets included in a capsule was 0.42, which was closer to the value obtained for the final formulation of the large tablets selected. This indicates that there is also an initial rapid release portion followed by a slower release component after a certain time point. This pattern was also observed for metoclopramide release from a matrix tablet in which case the release was characterized by a biexponential model in which both the rapid initial phase and the slow phase were separately accounted for [225].

It was thought that the difference in the spatial arrangement of tablets within the dissolution vessel may have had an impact on the drug release profile, thus the release profiles resulting from the dissolution of tablets with different configurations, as depicted if Figure 6.8, were compared. It was

thought that the configuration of the tablets would alter the surface area of the dosage form available for drug release to occur, however the release of MPTA from the tablets tested in USP Apparatus II using 0.1M buffer with a pH of 7.2 was found to be similar as can be seen from the overlapping error bars at all points. Therefore, it was concluded that the orientation of tablets in this dissolution vessels did not have a significant impact on drug release rates. The orientation of tablets in USP Apparatus III could not be isolated as a result of the manner in which dissolution testing is conducted. However, in most cases tablets adopted the shape depicted in Figure 6.8C. However the small standard deviations and the close similarity obtained from six tablets in Apparatus II (Figure 6.9) may indicate that the orientation of the tablets also has minimal impact on drug release in this dissolution apparatus.

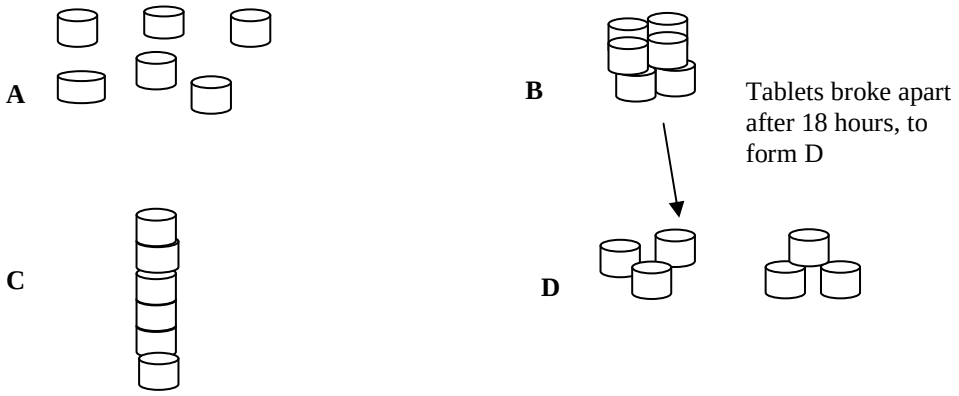


Figure 6.8: Schematic representation of the orientation of mini-tablets in dissolution testing apparatus

6.5.2.3 Conclusions

The inclusion of six tablets with smaller diameter led to increased drug release especially during the first two hours of dissolution rate testing. The rate of release was considered to be too rapid for the production of a once-daily dosage form of HCTZ and MPTA and consequently a coating was considered necessary to further decrease the initial release of MPTA and to ensure that the release of the drug is sustained over the desired period. It has been reported that low tablet friability is more a prerequisite for coating than high tablet hardness [263]. Therefore the batches of tablets manufactured on the single punch press could be used in a coating process, as the friability was low for all batches.

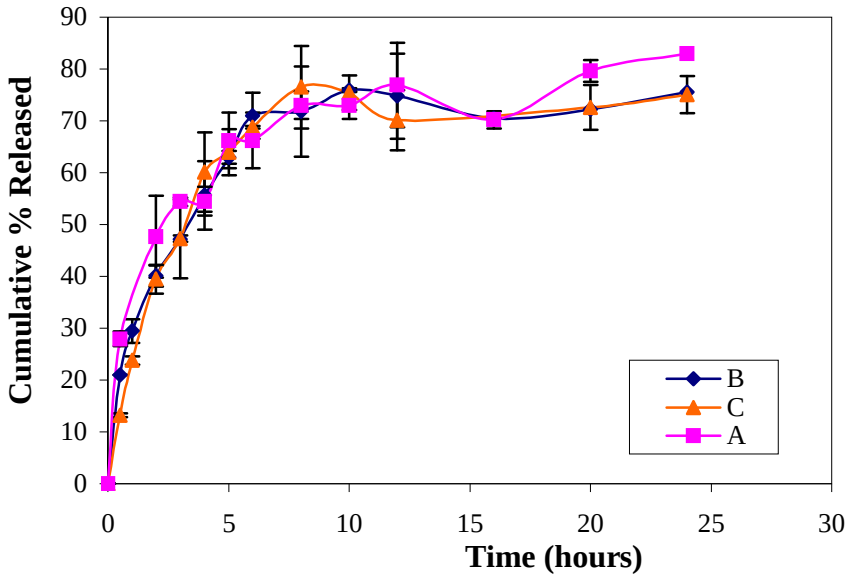


Figure 6.9: The Effect of tablet orientation on drug release in Apparatus II (n=6)

6.5.3 COATING OF MINI-TABLETS

6.5.3.1 Materials and Methods

To further retard the release of MPTA, a hydrophobic coating material was selected. Ethylcellulose and polymethacrylates are routinely used for sustained release film coating of tablets and ethylcellulose is particularly suited for use in oral dosage forms as it is odourless, tasteless and it possesses physical and chemical properties that make it ideal for film coating [263].

Ethylcellulose was selected for the coating experiments and was used in the form of Surelease® grade E-7-19010 (Colorcon, Kent, UK). Surelease® is an aqueous dispersion of ethylcellulose which consists of approximately 25%w/v ethylcellulose dispersed in ammoniated water, oleic acid, and dibutyl sebacate (a plasticiser). Large amounts of plasticiser are generally required for ethylcellulose coating suspensions to enhance film formation and flexibility of the films [263]. No pigments or opacifiers were included, as they are known to influence film permeability and behaviour [263]. Surelease® was diluted to approximately 15%w/v solids with distilled water prior to use to facilitate spraying and droplet coalescence. An additional amount of plasticiser was added to the coating solution as 10%w/w of the solids component. Triethyl citrate (TEC) (Morflex, North Carolina, USA) was selected, as it has been reported to result in good film formation and resulted

in further retardation of release of psuedoephedrine sulfate which is also highly water-soluble, in comparison to drug release from films that did not contain plasticiser [284].

The coating was performed using an Aeromatic Strea-1 fluidised bed drier (Aeromatic AG, Switzerland) fitted with a Wurster insert 150mm in length. A peristaltic pump (MaterflexL/S, Cole-Palmer instruments, Illinois, USA) was used to deliver the coating suspension, and the coating parameters are summarized in Table 6.3.

Initially the tablets were coated to varying levels and drug release from 6 dosage units was assessed at each of these levels. Tablets coated to different levels were then assessed in different combinations in order to achieve the desired release profile.

Table 6.3: Coating Parameters

Parameter	
Inlet air temperature	46-48°C
Outlet air temperature	40°C
Product bed temperature	43-45°C
Atomizing air pressure	20 psi
Spray rate	1.25-1.30g/min
Drying temperature	42°C
Drying time	15 minutes

6.5.3.2 Results and Discussions

Dissolution studies were performed in USP Apparatus III on 6 dosage units. Batches M00110601M and M0020901M were coated to varying levels using 100g of tablets for each coating experiment. In the initial stages the tablets were coated to actual weight gains of 4.8%, 6.8% and 10.4% and all three coating levels appeared to further retard drug release (Figure 6.10).

The 4.83% coat displayed sustained drug release characteristics for approximately 10 hours after which point no further drug release occurred possibly as result 100% drug release from the dosage form. The coating was observed to split along the tablet edges, indicating that tablet swelling due to the HPMC in the matrix occurred predominantly in the axial direction. This may have also been a function of the coating process, which resulted in coats that may have been thinner at the tablet

edges than on the surface. Drug release occurred by Case II or zero order transport (n=1.0), which indicates that drug release was dependent on polymer relaxation and swelling characteristics. This may have been a function of the hydrophobic polymer used in the coating such that drug release was permitted only after relaxation and swelling of this polymer subsequent to the wetting of the coat.

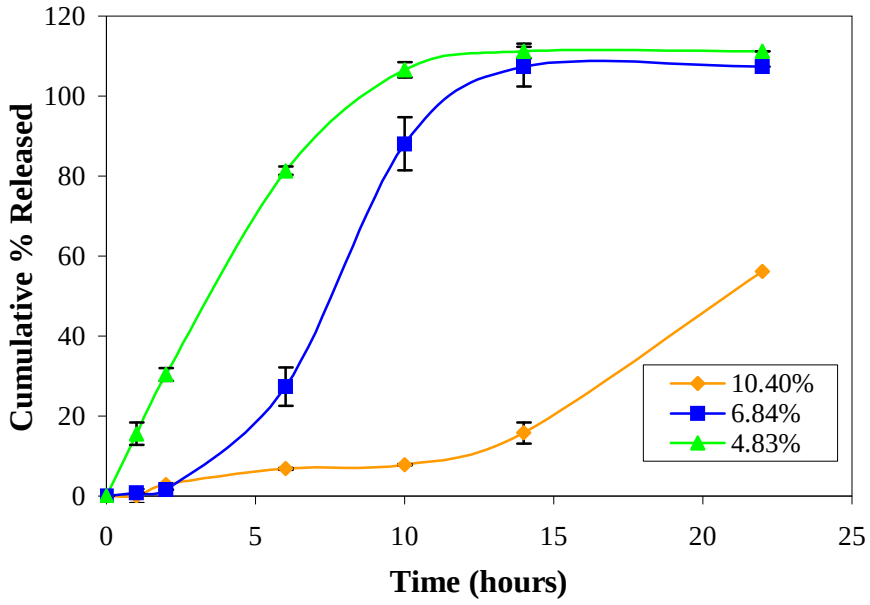


Figure 6.10: MPTA release profiles from tablets coated to 4.83, 6.84 and 10.40% weight gain, respectively (n=6).

Tablets coated to 6.84% resulted in an S-shaped dissolution profile, which was characterized by a lag of approximately two hours followed by rapid release up to 14-16 hours and a subsequent plateau. This coating level demonstrated a greater ability to control drug release over an extended period than the tablets coated to 4.83%. Drug was released by a Case II diffusion mechanism (n=1.0).

Tablets coated to 10.4% released approximately 55% of the drug within the 22-hour dissolution period. Although a small percentage of drug was released before 14 hours, this was taken to be representative of a lag phase, and the initial release may have been due to MPTA particles lost

from the tablet surface during coating and which may have subsequently been incorporated into the coat. This initial release may also have been a function of imperfect coalescence of the coat or the presence of cracks or holes, which may have resulted in increased drug release. A scanning electron micrograph (SEM) showing a coat that split along the tablet surface reveals that apart from imperfections in the coat, coat splitting may have also contributed to greater initial release is shown in Figure 6.11.

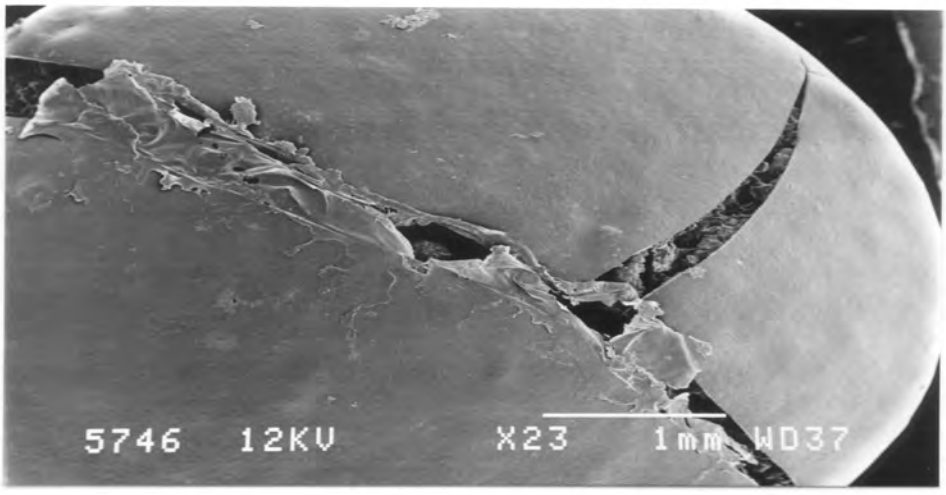


Figure 6.11: SEM of a coat split along the surface of a tablet after dissolution

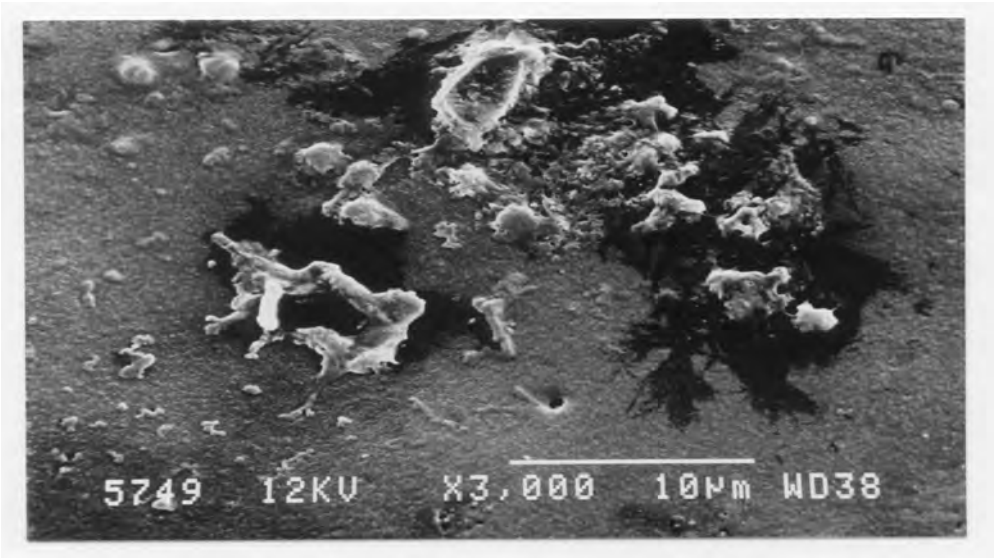


Figure 6.12: SEM of a membrane after dissolution testing, showing pores created in the membrane

Drug release occurred by a zero-order mechanism where n=1.31 indicating Super Case II transport. The resultant correlation coefficient (r^2) 1.0 from a plot of drug release against time verified a zero-order release mechanism. Drug release from all coated tablets, however, was characterized by the formation of pores within the coat throughout which the drug could diffuse into the dissolution medium. These pores are clearly visible in the membrane on the tablet surface, photographed following the dissolution test (Figure 6.12)

In order to optimize drug release additional coating levels of 3.10%, 6.97%, 6.93% and 9.13% weight gain, respectively (Figure 6.13) were assessed in Apparatus III. As expected tablets coated to 3.10% showed faster release than tablets coated to all other levels, which was a function of the lower coating, hence allowing for the passage of more drug through the membrane. Furthermore, the more rapid swelling of the matrix, in tablets with a lower coating may have contributed to faster splitting of the coat, as was observed for all tablets coated to a low level, causing drug to be released both by diffusion across the membrane and through the matrix itself.

The n values that were calculated using the Korsemeyer-Peppas equation (Table 6.4) indicate that drug was released from the coated tablets mainly by super case II transport and is therefore characterized by a zero-order mechanism, however the 9.13% coat resulted in drug release characterized primarily by Fickian diffusion.

Table 6.4: Calculated n values for tablets coated to different levels

Coating Level (% weight gain)	n
3.12	1.13
6.93	1.94
6.97	1.63
9.13	0.50

It was interesting to note that small increases in the applied coat had a significant retarding effect on drug release, as was observed with tablets coated to 6.84%, 6.93% and 6.97%, respectively. Tablets coated to 6.84% released almost 30% drug within 6 hours, whereas those coated to the higher levels showed little or no drug release. Tablets coated to 6.93% displayed a similar release profile to that of the 6.84% coat although a smaller amount of drug was released at all sample points in the dissolution test, whereas the 6.97% coat retarded drug release such that only about

60% drug was released within 22 hours. This indicates that the coating process is required to be highly efficient and accurate to achieve reproducible results, as minimal changes in the coating levels may have a significant impact on the resultant drug release profile.

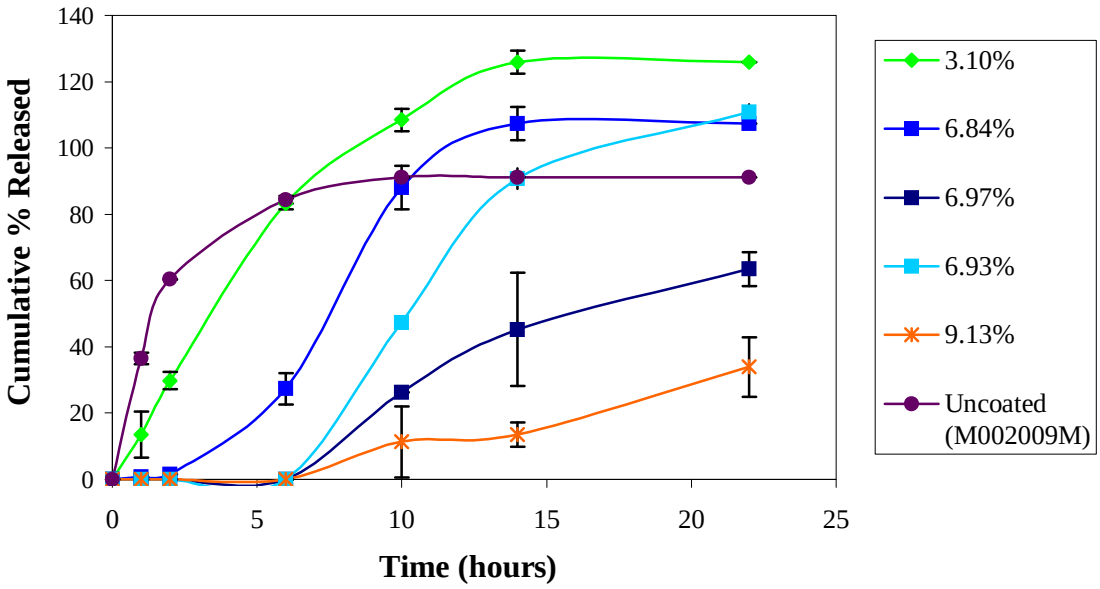


Figure 6.13: Comparison of release profiles of mini-tablets coated to 3.10, 6.84, 6.93, 6.97 and 9.13% weight gains, respectively and uncoated mini-tablets (n=6)

The coating process in this system was found to be highly inefficient, as can be seen from the SEMs in Figures 6.14 and 6.15. Figure 6.14 shows areas where the coating solution agglomerated to form regions where the coat thickness will be greater, as well as tears or holes within the coat. Figure 6.15 clearly shows that the deposition of the coating solution was non-uniform resulting in areas where no coat was applied whilst the coating solution was concentrated in certain areas.

A similar conclusion was made upon assessment of tablets coated to 9.13% and10.40% actual weight gain, where assessment of randomly chosen tablets yielded a lower rate of release from tablets with lower coating levels, such that tablets coated to 9.13% released 34% in 22 hours whereas tablets coated to 10.40% released an average of 56% in the same test period. Dissolution test results for tablets coated to both 9.13% and 10.42% weight gain had a characteristic non-or-slow release profile in the early stages of the experiment, which may be representative of a lag phase. The drug release was further characterized by zero-order release after 14 hours had elapsed.

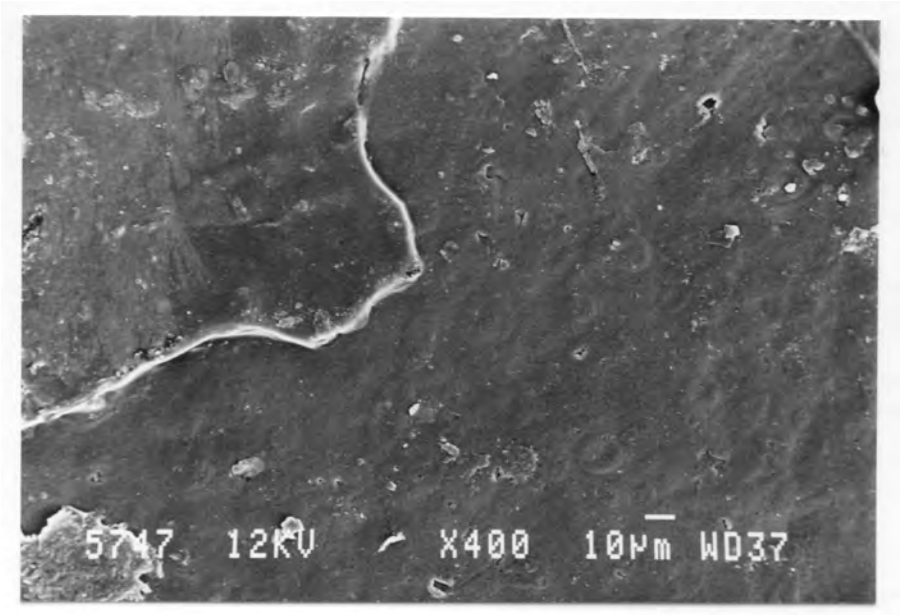


Figure 6.14: SEM of a coat showing agglomeration of the coating solution droplets

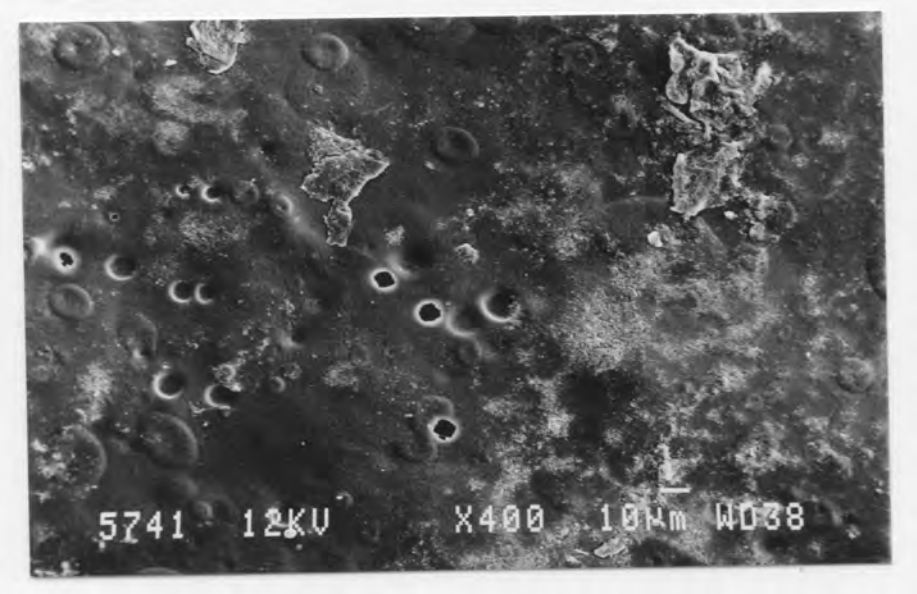


Figure 6.15: SEM showing a non-uniform coat with holes and areas where over-spaying occurred

None of the coating levels assessed retarded drug release over the 24 hour period desired, therefore drug release rates from combinations of tablets coated to varying levels were also evaluated in order to achieve a zero-order release profile for MPTA over a 22 hour period.

6.5.4 COMBINATIONS OF COATED MINI-TABLETS OF MPTA

Six tablets coated to different levels were incorporated into a capsule to make up the sustained release MPTA portion of the dosage form. The capsules were manually filled with the desired combination of tablets. The various combinations that were tested are listed in Table 6.5.

Table 6.5: List of combinations tested

Combination	Number of tablets used per coating level (%weight gain)							
	Uncoated	**3.10**	**4.83**	**6.84**	**6.93**	**6.97**	**9.13**	**10.42**
C1			2	2				2
C2	2			2				2
C3	1		1	2				2
C4	1	1		2				2
C5	1		1	3				1
C6				3				3
C7			2				2	2
C8			2	2			2	
C9	2		1	1			2	
C10	1		1	2			2	
C11			2	1	3			
C12			2	2	2			
C13	1			2		3		
C14			2			4		

Combinations of the 4.83%, 6.84% and 10.40% coated tablets were initially assessed, and selection of the combinations were based on the profiles obtained for the coated tablets run individually. For example, it was thought that the 10-14 hour lag for the 10.40% tablets would be compensated for by tablets with a lower coating level and from which 100% of the drug would be released within 14 hours.

In contrast to uncoated mini-tablets, the release profiles for the combinations displayed definite sustained release characteristics. Profiles for C1-C5 are shown in Figure 6.16.

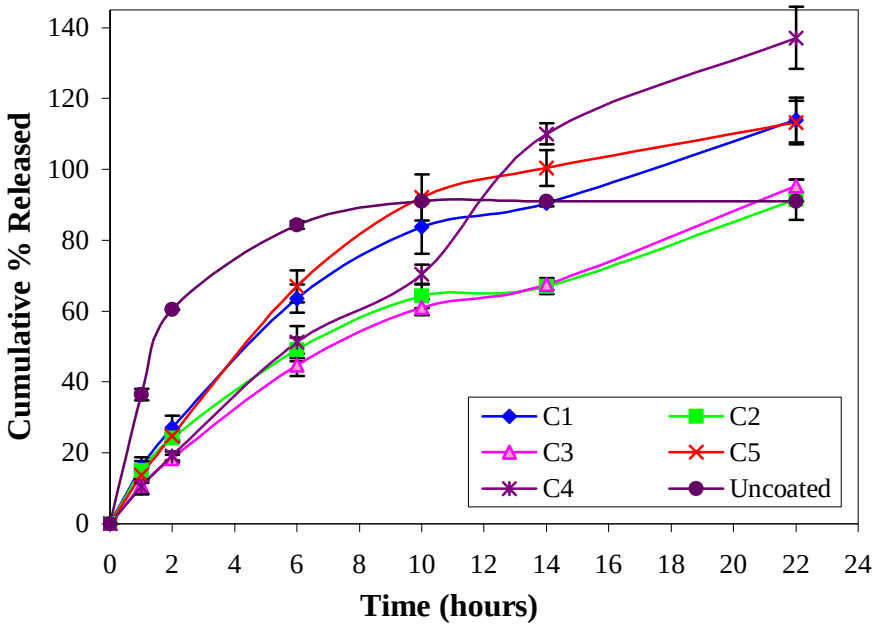


Figure 6.16: Release profiles of combinations C1-C5 in Apparatus III (n=6)

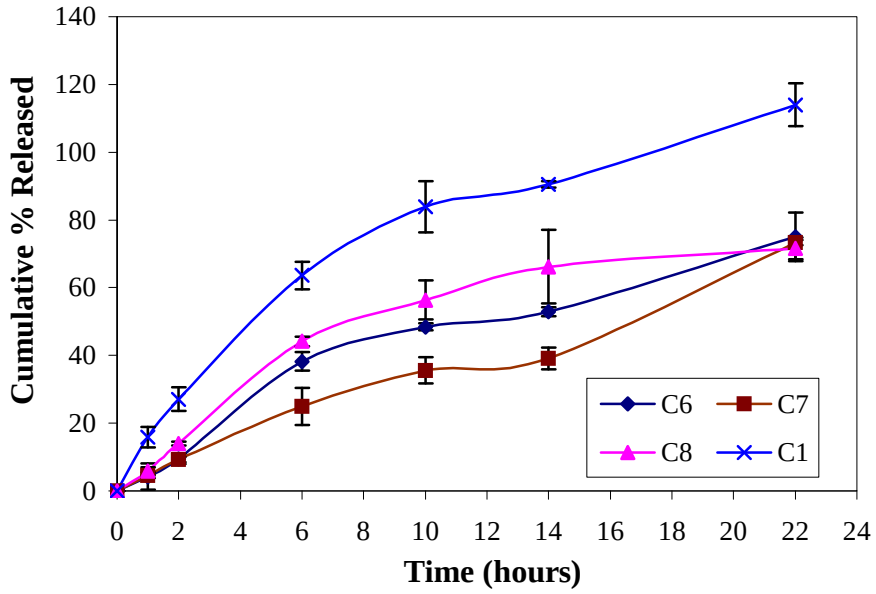


Figure 6.17: Release profiles of combinations C6-C8 compared to the profile of C1 (n=6)

The release from C1 was considered to be too rapid as 50% of the drug was released in approximately 4 hours. Combinations C2-C4 showed a more sustained release initially, however no drug was released between 10-14 hours after the start of the dissolution test.

The release profile from combination C5 was more acceptable however, drug release did not occur via a zero order release mechanism. Furthermore, the profile was not comparable to the prototype batch for the large tablets cores (M011210) as indicated by the fit factor values listed in Table 6.6. Therefore this combination was not selected for further development, as it was hoped that a combination could be produced to release 50% and 100% drug within 8-12 and 24 hours, respectively.

The release from combinations C6-C8 are depicted in Figure 6.17 and showed greater sustained release behaviour when compared to the previous combinations. However approximately 70% of the drug was released within 22 hours for each of these combinations. This indicated that although these combinations did not exhibit the desired release characteristics, the potential for further modification such that 100% drug was released within 22 hours, was feasible.
The release from combinations C9 and C10 (Figure 6.18) were found to be similar to batch M011210 (Table 6.6), and were considered for incorporation into the final combination product.

Table 6.6 Fit factors for the comparison of combinations C5, C9 and C10 with M011210

Comparison	**f1**	**f2**
M011210 vs C5	35.0	33.7
M011210 vs C9	7.9	64.3
M101210 vs C10	11.6	54.0

Resultant release profiles from combinations C11-C13 are depicted in Figure 6.19. In this combination tablets coated to 10.40% weight gain were replaced with tablets coated to either 6.93% or 6.97%. This resulted in release profiles in which 50% MPTA was released between 6-7 hours and almost all drug was released in 22 hours for combinations C11 and C12. Zero order release was observed for all these combinations and the correlation coefficients are included in Table 6.7. Zero order kinetics were not followed after 14 hours which may have a function of either too rapid initial release or slow, incomplete release from tablets with higher coating levels.

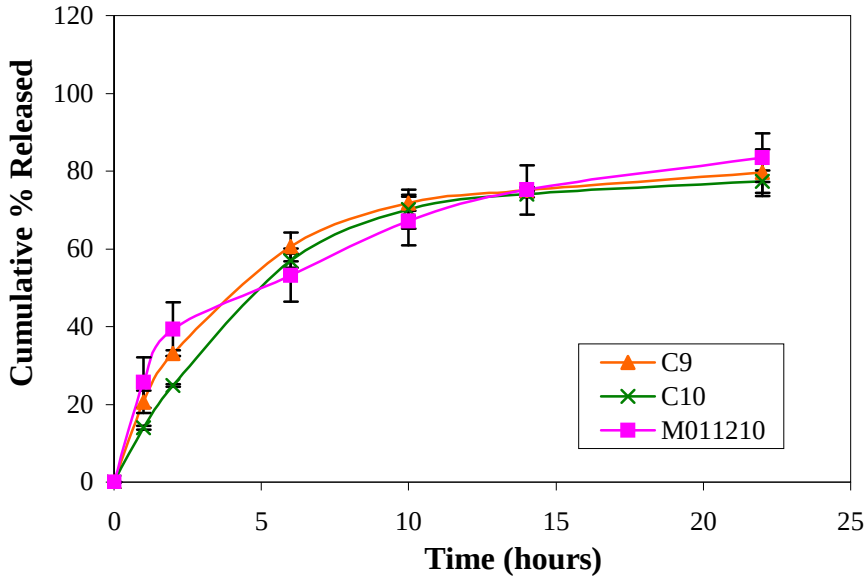


Figure 6.18: Comparison of drug release profiles of combinations C9 and C10 with combination C1 and the large matrix tablets (Batch M011210) (n=6)

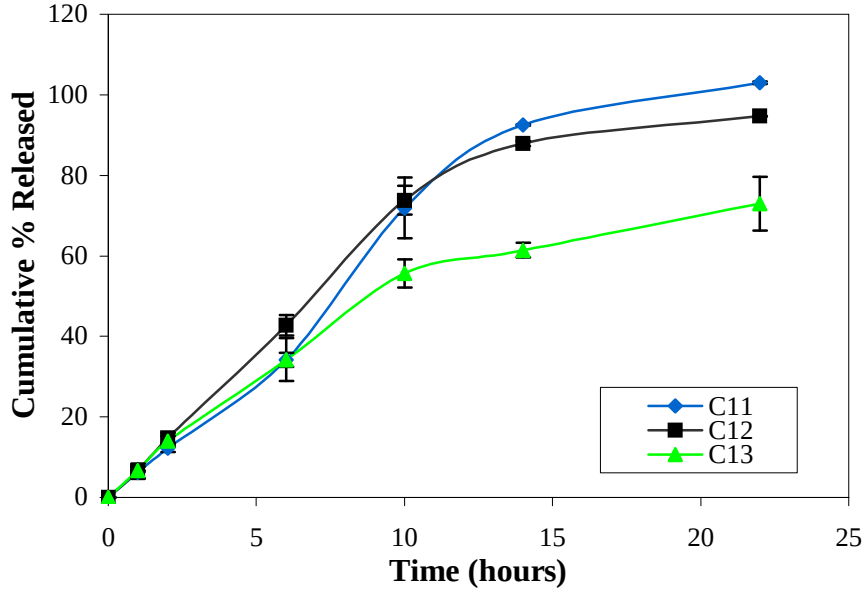


Figure 6.19: Drug release profiles of combinations C11-C13 (n=6)

Table 6.7: Correlation coefficients (r^2) for drug release from combinations C11-C14

Combination	r^2 value	Linearity	Time period
C11	0.9919	Time	1-14 hrs
C12	0.9846	Time	0-14 hrs
C13	0.9978	Time	0-10 hrs
C14	0.9946	Time	0-22 hrs

The release profiles for combination C14 is depicted in Figure 6.20 and showed zero order kinetics throughout the 22-hour test period, as was indicated by the correlation coefficient following linear regression analysis of the release profile. This combination was therefore chosen for the final product. In comparison to the uncoated mini-tablets and the larger matrix tablet, the rapid initial release observed with the uncoated tablets was reduced by the addition of a film coating. Furthermore, 85% drug was released in 22 hours, which would possibly allow for the remaining drug to be released within a 24-hour period.

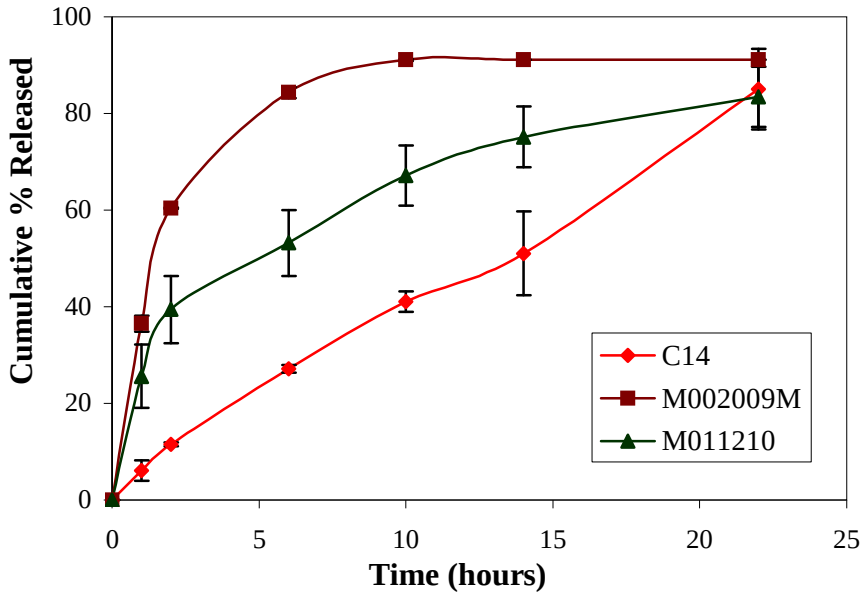


Figure 6.20: Comparison of release profiles of combination C14, large matrix tablets (Batch M011210) and uncoated mini-tablets (Batch M00200901M) (n=6 for all batches)

6.5.5 INCLUSION OF THE IMMEDIATE RELEASE HCTZ COMPONENT

Initially, the inclusion of HCTZ as a loose powder was considered. This method would be easier and would save time and resources, as the number of manipulations during the manufacturing process would be reduced, however the complexity of filling both tablets and a powder may be problematic for large-scale production.

6.5.5.1. Materials and Method

MCC was selected as the diluent for the powder blend as it would serve to enhance the flow properties of the mixture. The mixture was blended in a cube blender for 20 minutes at 20 rpm. Magnesium stearate was then incorporated as 0.5% of the mixture and the mixture was blended for a further 3 minutes. HCTZ and MCC were added in a ratio of 1:7 so that 100mg of this blend would include 12.5mg HCTZ, which is the desired dose. The release of HCTZ was assessed over a two-hour period in both USP Apparatus II and III. The powder blend was accurately weighed into a gelatin capsule containing the desired combination of 6 mini-tablets (C14).

6.5.5.2 Results and Discussion

HCTZ release in both Apparatus II and III was found to be within USP specified limits for an immediate release product and the profiles obtained are depicted in Figure 6.21. The USP states that not less than 60% of the dose label claim must be released within 60 minutes [73], however 85-100% HCTZ was released within the first hour from all samples tested. This release was comparable with that observed for a commercial immediate release product tested in Apparatus II, as indicated by the overlapping error bars.

Consequently, HCTZ inclusion into the dosage form was performed using this method. Herman *et al* found that a mixture of HCTZ and MCC for use in a sustained release pellet formulation resulted in slower release of HCTZ [285], however no sustained release effect was observed with the powder blend used. This may be a result of incorporation of the mixture as a loose powder in the dosage form as compared to the pellet formulation which undergoes a number of processes including granulation, extrusion and spheronisation, and more than likely resulted in a greater interaction and possible binding of the HCTZ, thus prolonging release.

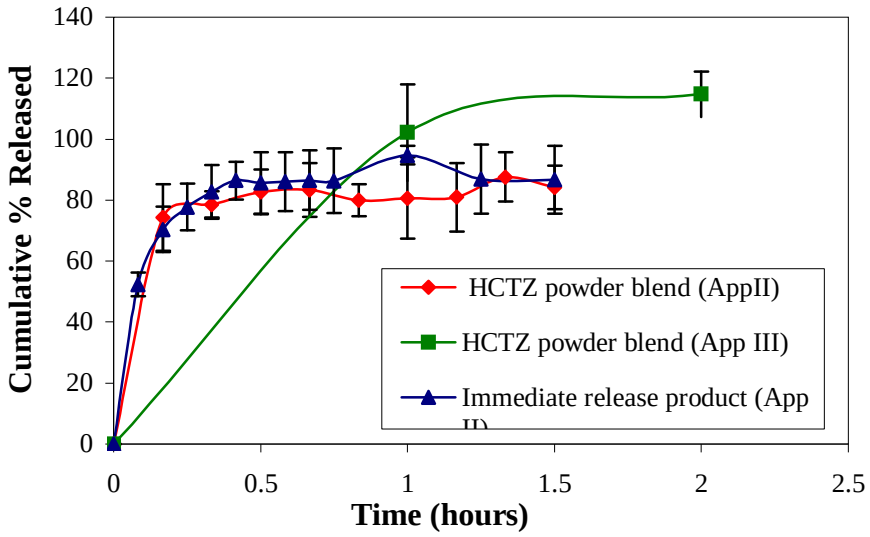


Figure 6.21: Release profiles of HCTZ in from capsule in Apparatus II and II compared to an immediate release HCTZ product (n=6)

6.6. CONCLUSIONS

The combination dosage form developed consisted of six mini-tablets that were coated to two different coating levels (*viz* two tablets were coated to 4.83% and four tablets to 6.97%), and an immediate release HCTZ component that was included into the capsule as a powder blend. MPTA release from the dosage form was retarded a 22-hour period, whereas almost 100% of the HCTZ was released within the first hour (Figure 6.22).

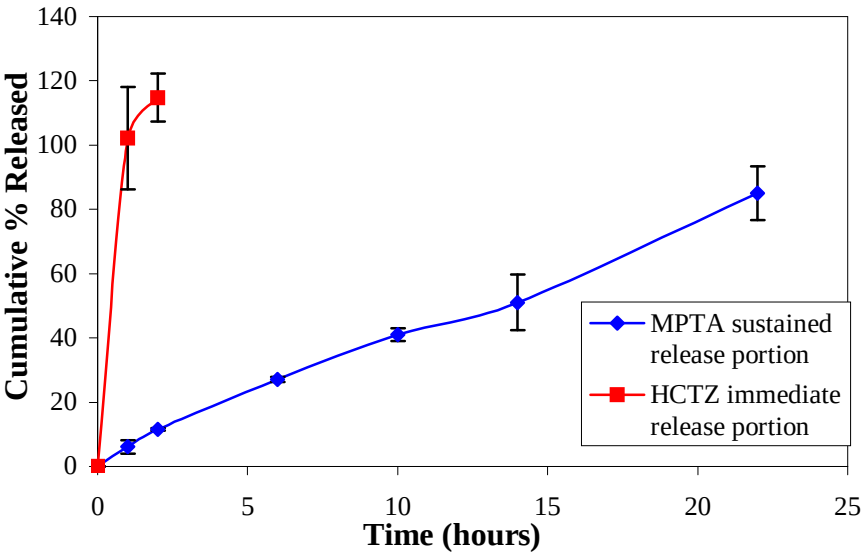


Figure 6.22: Release profile of HCTZ and MPTA from the combination dosage form (n=6)

The release from the coated tablets occurred mainly via a zero order mechanism, as was expected for these coated tablets, suggesting that the application of a film coat resulted in an alteration of the mechanism of drug release from first order that was observed with the majority of the large matrix tablets and the uncoated mini-tablets, to a zero-order mechanism. This combination product would be useful for once daily dosing of MPTA and HCTZ.

CHAPTER SEVEN
CONCLUSIONS

In this study two sustained release dosage forms were developed for the treatment of chronic hypertension. A hydrophilic matrix system was used to produce a modified release MPTA tablet that could be used as a once daily dosage form, particularly for patients on long-term β-blocker therapy. The second dosage formed consisted of an immediate release HCTZ component, delivered in the form of a powder blend of the drug and MCC, and a sustained release MPTA component which was formulated as six mini matrix tablets coated to different levels, which were subsequently incorporated into a size 00 gelatin capsule. The combination dosage form would be particularly beneficial for hypertensive patients who are unresponsive to monotherapy.

Dissolution testing of both the separate MPTA and combination dosage forms was performed in USP Apparatus III, which was found to be more discriminating than USP Apparatus II, and which was used as it is able to simulate the passage of the dosage form in-vivo, more closely. Samples were analyzed using a validated HPLC method that is described in Chapter Two.

Modification of a sustained release matrix tablet formulation that was developed to sustain the release of psuedoephedrine sulfate [146], resulted in the effective retardation of MPTA release over the over a 22-hour dissolution test period. This matrix formulation was also successfully manipulated to produce mini-tablets, which were subsequently coated to appropriate levels to achieve zero order release of MPTA over an extended period. HCTZ was successfully incorporated into the capsule as a powder blend such that approximately 100% of this drug was released within the first hour of dissolution testing.

The rate and mechanisms of drug release were found to be altered by formulation variables that include the amount of Surelease® added in the granulation process, drug load in the dosage form and the amount of HPMC included in the matrix. This indicated that drug release was not controlled entirely by the matrix composition itself, but via a combination of effects exerted by the hydrophobic ethylcellulose polymer in the granulation fluid and the hydrophilic HPMC polymer contained in the matrix.

Preformulation studies using DSC as a tool for identifying potential incompatibilities between the drug (MPTA) and tableting excipients, revealed that the potential for interaction of MPTA with magnesium stearate and dibasic calcium phosphate, existed and may subsequently result in issues with the stability of the dosage form on long term storage. Stability testing conducted over a three-month period at ambient, intermediate and accelerated conditions indicated that drug release from the dosage form was altered after three months storage at conditions of extreme temperature and humidity (40°C/ 87% RH), however the drug appeared to be chemically stable as there was no loss in potency of the drug when stored under the extreme conditions of temperature and humidity for three months. Further manipulation of this formulation, by the addition of a coating, was therefore recommended to improve the shelf life of the product. It is possible that a 1-2% coat may be beneficial in minimizing the effects of environmental conditions on long term storage, that alter drug release from this formulation, and to further prevent the 'burst' release observed over the first two hours of the dissolution test period.

The matrix dosage forms exhibit the potential for further optimization and development.
It was identified that Surelease® contributed towards controlling the release of MPTA from the formulation and therefore further investigation of this variable is suggested. It was found that increasing the amount of granulating fluid resulted in slower drug release rates. Consequently the scope for the use of ethylcellulose as the rate-controlling polymer in this formulation exists. Increasing the amount of granulating fluid, was precluded for this formulation as the use of HPMC within the granules resulted in an unworkable mass following granulation with a corresponding low yield of granules from the granulation process. The exclusion of HPMC and the possible inclusion of ethylcellulose into the granules may be more effective in achieving sustained release characteristics from highly water-soluble drugs such as MPTA, and these suggestions are included as topics for further investigation.

It has been widely reported that the drug to polymer ratio significantly impacts on drug release behaviour from a matrix system, consequently it was found that even small increases in the amount of matrix forming polymer (HPMC K100M) resulted in a significant decrease of drug release rates. However, further characterization of this variable may allow for the determination of an optimal

drug: polymer ratio for sustaining the release of MPTA from this formulation. This approach may enable the development of a system from which drug release is controlled entirely by the hydrophilic polymer, HPMC.

Process variables, including the impact of granule drying time and compression force on drug release were evaluated. Shorter drying times (6 hours) resulted in faster drug release possibly due to the creation of more pores within the matrix as a result if residual moisture. In this case faster diffusion through the tablet was suspected. A granule drying time of 17 hours did not exhibit significant differences in release rates from drying times of 12 hours when incorporated into the tablet. It is likely that drying times greater than those assessed may also impact on drug release profiles, and should be investigated further. Compression force was varied to achieve tablets of different hardness, however similarity and difference factor values obtained from comparison of these batches were within the limits for acceptance, indicating that there was no significant differences between the drug release profiles of these batches. The evaluation of process variables is particularly important in the scale-up stages of product development and would require further investigation.

The multiparticulate combination dosage form of HCTZ and MPTA resembled a hybrid system, in which a combination of matrix and membrane technologies was used to achieve zero order release of MPTA. The immediate release HCTZ component was incorporated into the gelatin capsule as a loose powder blend. Compression of the matrix formulation into smaller tablets (3.5mm diameter) such that six tablets contained an equivalent drug loading to that of the larger 100mg tablets (11.5mm diameter), resulted in drug release rates that were significantly faster from the smaller tablets. The use of a hydrophobic coat was found to retard drug release from the mini-tablets and an increase in the coating level resulted in a corresponding decrease in the rate of drug release from the tablets. Six mini-tablets coated to the same percentage weight gain did not provide the required sustained release characteristics over the 22-hour period of testing. The use of combinations of coated tablets resulted drug delivery profiles that were more closely represented of zero order release kinetics. Combination C14, containing tablets coated to 4.83% weight gain (2 tablets) and 6.97% weight gain (4 tablets) resulted in linear drug release over 22 hours and linear regression analysis resulted in a $r2 = 0.9946$. This is an indication that drug release from the dosage form

followed zero order kinetics.

No difference in release between HCTZ included as a powder in the capsule and a commercially available immediate release tablet were observed. Thus, the combination, which consisted of six coated matrix tablets consisting of MPTA and a HCTZ powder blend enclosed within a gelatin capsule, was considered feasible for use as a once-daily combination dosage form for the treatment of hypertension and subsequently requires in vivo evaluation.

The feasibility of the combination dosage for large scale manufacture may be compromised by the inclusion of both powder and tablet components into the capsule. Consequently, compression of the powder into a mini-tablet may also be necessary to facilitate capsule filling on a large scale.

Characterization of the film coats may also be beneficial in determining the optimal coating levels to achieve zero order release over the 22-hour period. For this formulation, the coating solution contained 10% w/w triethyl citrate as a plasticiser. Plasticisers are known to alter drug release, and assessment of altering the type and content of the plasticiser, may be useful and may aid in identifying a single coating level that would retard MPTA release over the test period. Similarly, the incorporation of a channeling agent such as HPC or HPMC into the coating solutions may also be beneficial in this respect. It was also thought that a more facile dosage form could be produced by the addition of a second enteric coat onto the mini-tablets coated to 4.83% weight gain, where 3 tablets would be coated with the enteric polymer such that bimodal drug release may be achieved. This may also eliminate some of the difficulties experienced with the coating process and is under further investigation.

Stability testing of the combination dosage form is also necessary to evaluate the effectiveness of the coat in minimizing the impact of adverse environmental factors on drug release and to identify possible instabilities of the powder blend on long term storage and to determine whether the possible interaction between MPTA and HCTZ identified by DSC, is valid.

It is evident that the formulation composition of both dosage forms was capable of sustaining the release of MPTA over a 22-hour period and may be used as once daily dosage forms for the

sustained release of MPTA alone or in combination with other drugs. The inclusion of HCTZ as a powder blend in the combination dosage form resulted in rapid release of the drug and may be beneficial in achieving a rapid reduction of blood pressure to within normal limits. Several recommendations for optimization of the formulation may provide alternative means of sustaining drug release via simple and economic processes and are currently being considered.

APPENDIX I: BATCH DATA FOR MATRIX TABLETS

BATCH: M0022005

Date of Manufacture: 20/05/00
Press: Manesty B3B (Tableted 21/05/00)
Batch Size: 250g

Composition (%)

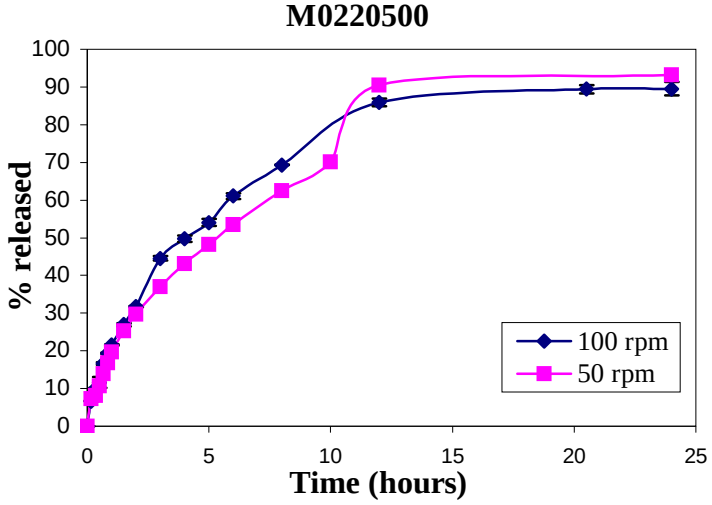
Metoprolol tartrate	15
Methocel® K_4M	10
Emcompress®	40
Emcocel® 90 M	35
Surelease®	0.28 gram/gram of granules
Methocel® K100M	15
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	487± 7.18	1.47

Friability	**passed**
Weight before (20 tablets)	16.24g
Weight after 100 drops	16.22g
Percent lost	0.12

Dissolution Profile (USP Apparatus II)



BATCH: M011508

Date of Manufacture: 15/08/00
Press: Manesty B3B (Tableted 16/08/01)
Batch Size: 400g

Composition (%)

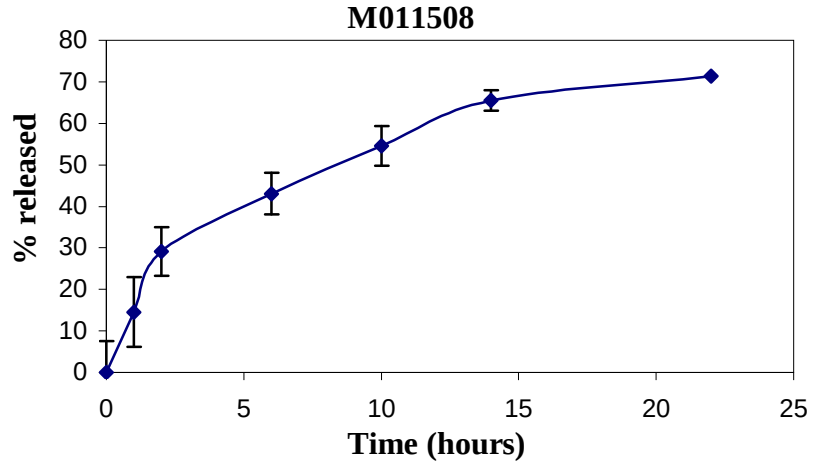
Metoprolol tartrate	15
Methocel® K_4M	10
Emcompress®	40
Emcocel® 90 M	35
Surelease®	0.36 gram/gram of granules
Methocel® K100M	15
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	516 ± 16.57	3.79
Hardness (kp)	5.89 ± 0.79	13.66

Friability	**passed**
Weight before (20 tablets)	10.30g
Weight after 100 drops	10.27g
Percent lost	0.29

Dissolution Profile (USP Apparatus III)



BATCH: M012208

Date of Manufacture: 22/08/00
Press: Manesty B3B (Tableted 23/08/00)
Batch Size: 287.5g

Composition (%)

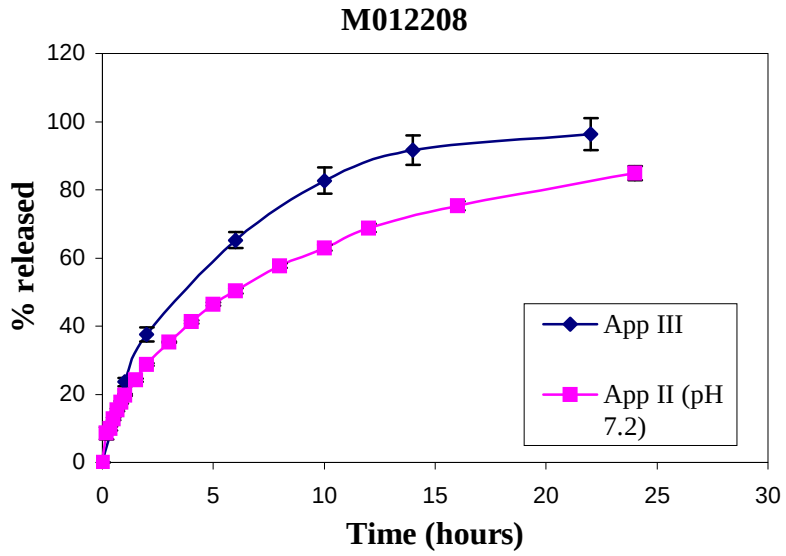
Metoprolol tartrate	26.00
Methocel® K_4M	8.69
Emcompress®	34.78
Emcocel® 90 M	30.43
Surelease®	0.18 gram/gram of granules
Methocel® K100M	15
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	507.44 ± 12.48	2.46
Hardness (kp)	10.04 ± 0.52	5.18

Friability	**passed**
Weight before (20 tablets)	10.21g
Weight after 100 drops	10.21g
Percent lost	0.00%

Dissolution Profile (Apparatus II and III)



BATCH: M013009

Date of Manufacture: 30/09/00
Press: Manesty B3B (Tableted 02/10/00)
Batch Size: 250g
Comments: Granules dried for 17 hours

Composition (%)

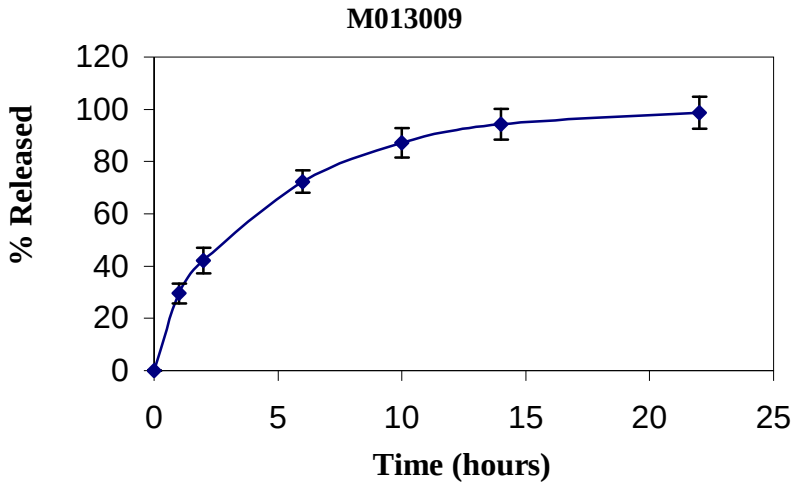
Metoprolol tartrate	26
Methocel® K_4M	8.7
Emcompress®	35
Emcocel® 90 M	30.5
Surelease®	0.19 gram/gram of granules
Methocel® K100M	15
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	543 ± 7.33	1.35
Hardness (kp)	10.81 ± 0.70	6.51

Friability	**passed**
Weight before (20 tablets)	10.86
Weight after 100 drops	10.84
Percent lost	0.18

Dissolution Profile



BATCH: M023009

Date of manufacture: 30/09/00
Press: Manesty B3B (Tableted 03/10/00)
Batch Size: 250g

Composition (%)

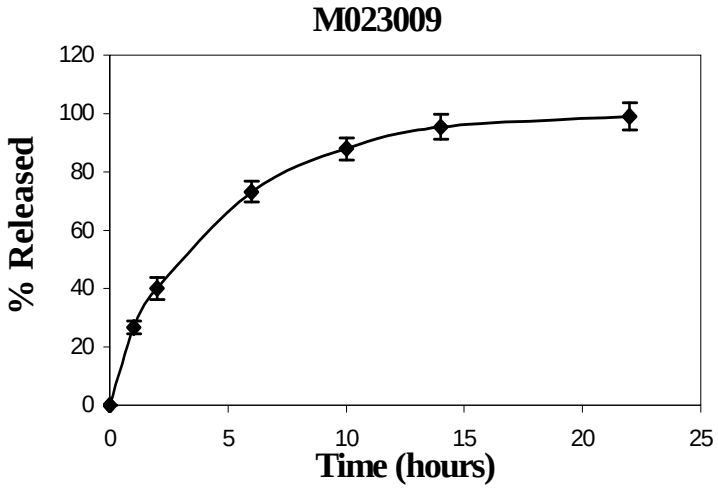
Metoprolol tartrate	30
Methocel® K_4M	8.2
Emcompress®	32.8
Emcocel® 90 M	29
Surelease®	0.20 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	9.33
Emcompress®	13.33
Magnesium stearate	1.00

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	490.5 ± 7.59	1.55
Hardness (kp)	7.95 ± 0.43	5.44

Friability	passed
Weight before (20 tablets)	9.89
Weight after 100 drops	9.88
Percent lost	0.10

Dissolution Profile



BATCH: M011210

Date of Manufacture: 12/10/00
Press: Manesty B3B (Tableted 13/10/00)
Batch Size: 250g

Composition (%)

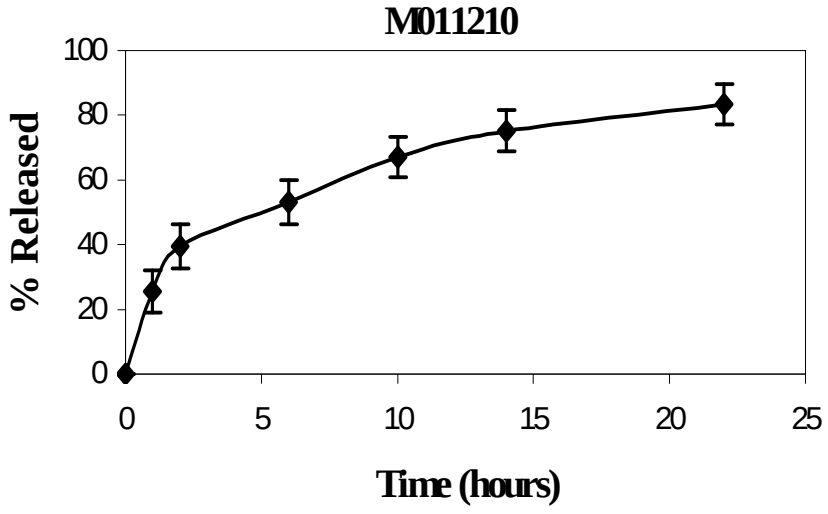
Metoprolol tartrate	20
Methocel® K_4M	9.4
Emcompress®	37.65
Emcocel® 90 M	33
Surelease®	0.19 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	689.5 ± 12.34	1.79
Hardness (kp)	15.43 ± 1.94	12.55

Friability passed
Weight before (20 tablets) 13.79
Weight after 100 drops 13.78
Percent lost 0.07
Content Uniformity (n=10) = 96.45mg

Dissolution Profile



BATCH: M010711

Date of Manufacture: 07/11/00
Press: Manesty B3B
Batch Size: 200g
Comments: Capping occurred, Granules dried for 6 hours

Composition (%)

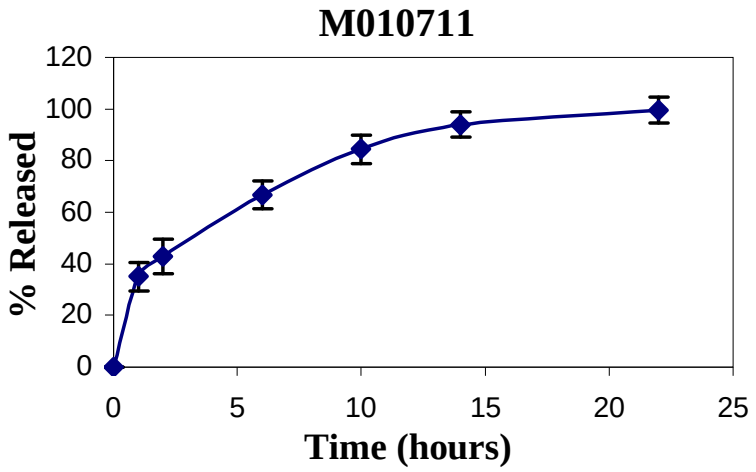
Metoprolol tartrate	23
Methocel® K_4M	9.1
Emcompress®	36.24
Emcocel® 90 M	31.70
Surelease®	0.24 gram/gram of granules
Methocel® K100M	18
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	578.00 ± 7.68	1.33
Hardness (kp)	12.47 ± 3.37	27.02

Friability	**passed**
Weight before (20 tablets)	12.06g
Weight after 100 drops	12.05g
Percent lost	0.08%

Dissolution Profile



BATCH: M011711

Date of Manufacture: 17/11/00
Press: Manesty B3B (Tableted 10/11/00)
Batch Size: 275g

Composition (%)

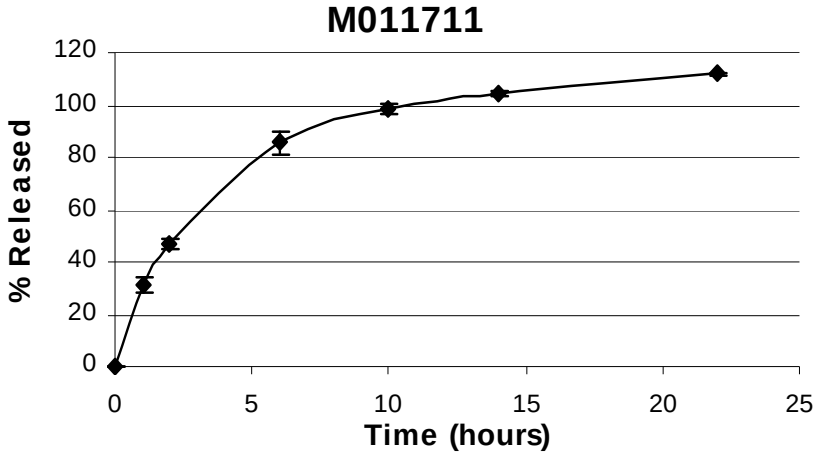
Metoprolol tartrate	26
Methocel® K_4M	8.7
Emcompress®	34.8
Emcocel® 90 M	30.5
Surelease®	0.18 gram/gram of granules
Methocel® K100M	15
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	540.5 7.39	1.37
Hardness (kp)	10.56 ± 0.49	4.65

Friability	**passed**
Weight before (20 tablets)	10.76g
Weight after 100 drops	10.75g
Percent lost	0.09%

Dissolution Profile



BATCH: M021711

Date of Manufacture: 17/11/00
Press: Manesty B3B (Tableted 19/11/00)
Batch Size: 275g

Composition (%)

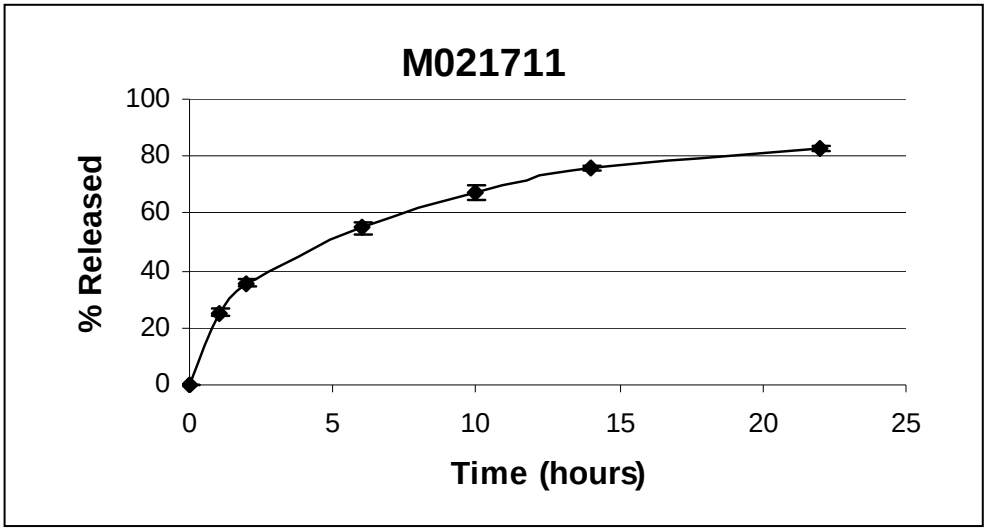
Metoprolol tartrate	20
Methocel® K_4M	9.41
Emcompress®	37.65
Emcocel® 90 M	32.94
Surelease®	0.21 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	667.17 ± 14.53	2.18
Hardness (kp)	15.73 ± 1.55	9.87

Friability	**passed**
Weight before (20 tablets)	13.25g
Weight after 100 drops	13.25g
Percent lost	0.00%

Dissolution Profile



BATCH: M001080301

Date of Manufacture: 08/03/01
Press: Manesty B3B (Tableted 13/03/01)
Batch Size: 200g

Composition (%)

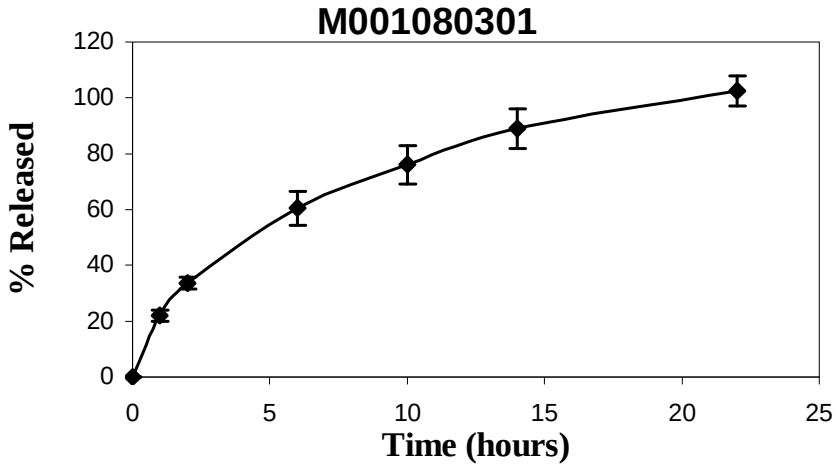
Metoprolol tartrate	20
Methocel® K_4M	9.41
Emcompress®	37.65
Emcocel® 90 M	33
Surelease®	0.16 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	670.27 ± 41.92	6.25
Hardness (kp)	15.11 ± 1.40	9.26

Friability	passed
Weight before (20 tablets)	13.41g
Weight after 100 drops	13.41g
Percent lost	0.00%

Dissolution Profile



BATCH: M001080302

Date of Manufacture: 08/03/03
Press: Manesty B3B (Tableted 13/10/01)
Batch Size: 300g

Composition (%)

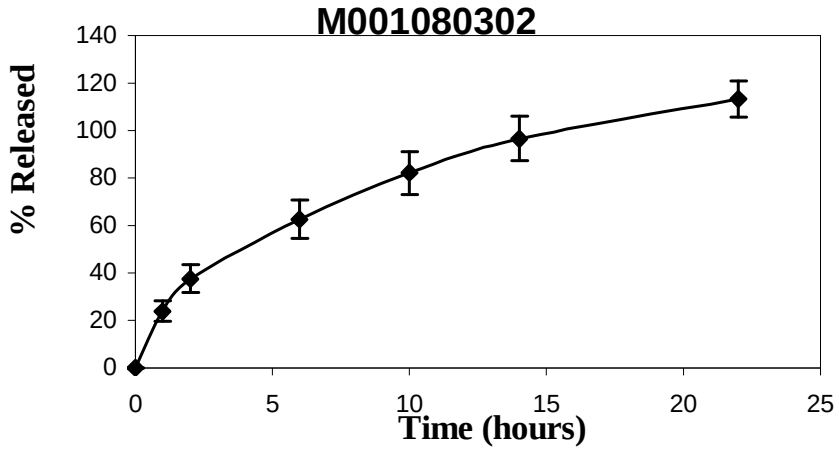
Metoprolol tartrate	40
Methocel® K_4M	7.1
Emcompress®	28.34
Emcocel® 90 M	24.70
Surelease®	0.25 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	695.99 ± 15.13	2.17
Hardness (kp)	12.90 ± 1.71	13.28

Friability	passed
Weight before (20 tablets)	13.82g
Weight after 100 drops	13.81g
Percent lost	0.07%

Dissolution Profile



M001270501

Date of Manufacture: 27/05/01
Press: Manesty B3B (Tableted 28/05/01)
Batch Size: 250g

Composition (%)

Metoprolol tartrate	40
Methocel® K_4M	10
Emcompress®	30
Emcocel® 90 M	20
Surelease®	0.21 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

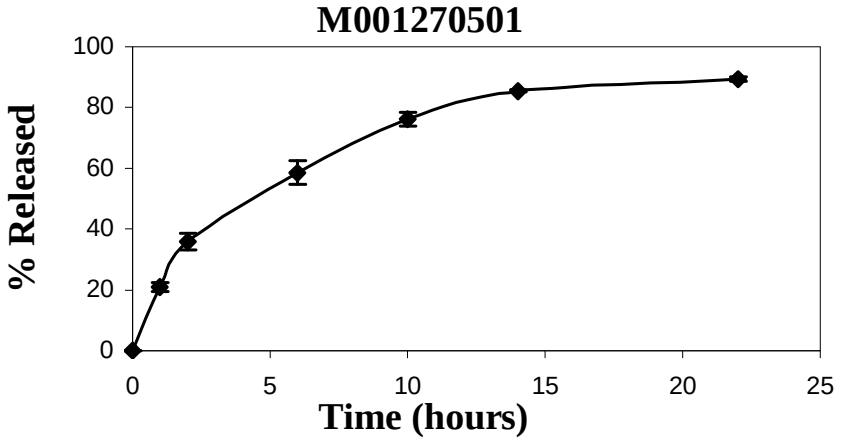
Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	681.16 ± 35.17	5.16
Hardness (kp)	13.67 ± 1.48	10.79

Friability	**passed**
Weight before (20 tablets)	13.57g
Weight after 100 drops	13.54g
Percent lost	0.22%

Content Uniformity: 178.34 ± 6.56 mg (200mg tablets)

Dissolution Profile



BATCH: M001270502

Date of Manufacture: 27/05/01
Press: Manesty B3B (Tableted 29/05/01)
Batch Size: 500g

Composition (%)

Metoprolol tartrate	15
Methocel® K_4M	10
Emcompress®	40
Emcocel® 90 M	35
Surelease®	0.18 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

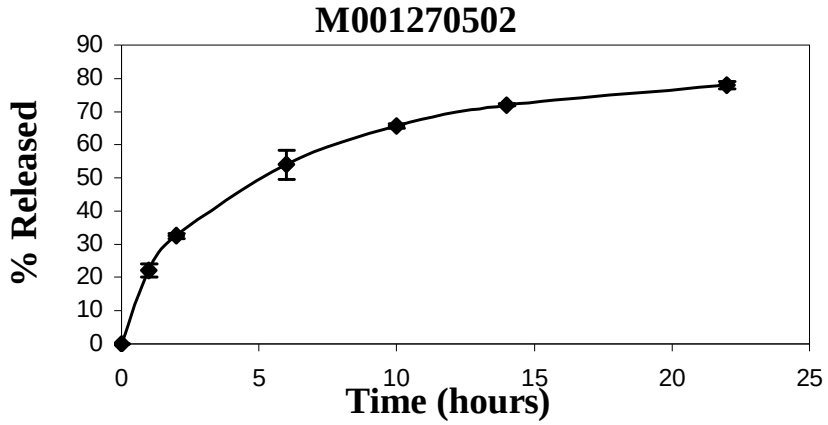
Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	544.78 ± 10.67	1.96
Hardness (kp)	7.22 1.02	13.18

Friability	**passed**
Weight before (20 tablets)	10.79g
Weight after 100 drops	10.76g
Percent lost	0.28%

Content Uniformity: 55.43 ± 4.19 mg (50mg tablets)

Dissolution Profile



BATCH: M001270503

Date of Manufacture: 27/05/01
Press: Manesty B3B (Tableted 29/05/01)
Batch Size: 250g

Composition (%)

Metoprolol tartrate	20
Methocel® K_4M	10
Emcompress®	30
Emcocel® 90 M	40
Surelease®	0.26 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

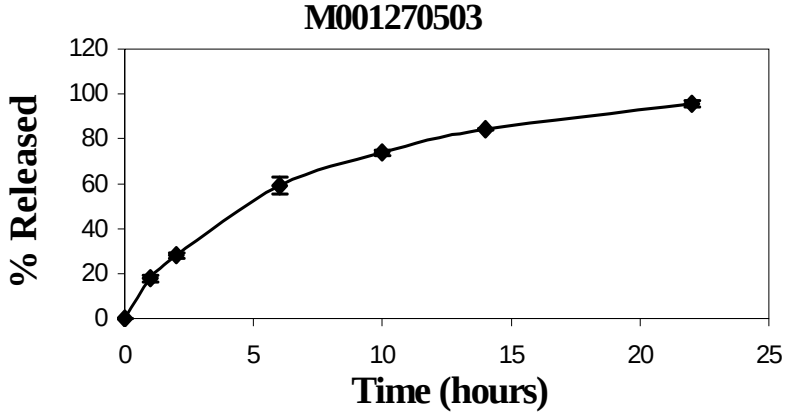
Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	646.31 ± 22.09	3.42
Hardness (kp)	15.02 ± 2.03	13.49

Friability	**passed**
Weight before (20 tablets)	11.82g
Weight after 100 drops	11.81g
Percent lost	0.085%

Content Uniformity: 85.96 ± 5.40mg

Dissolution Profile



BATCH: M001270504

Date of Manufacture: 27/05/01
Press: Manesty B3B (Tableted 28/05/01)
Batch Size: 1000g
Comments: Compressed to varying hardness

Composition (%)

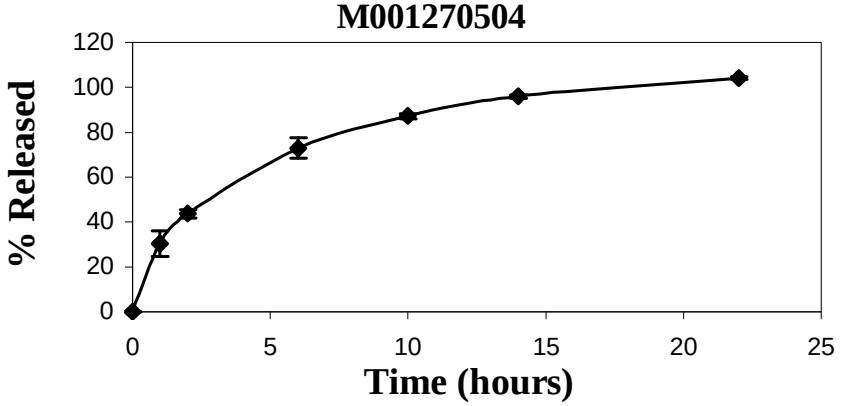
Metoprolol tartrate	20
Methocel® K_4M	10
Emcompress®	37.5
Emcocel® 90 M	32.5
Surelease®	0.39gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	675.60 ± 10.15	1.50
Hardness (kp)	7.29 ± 0.87	11.93
	10.55 ± 0.83	7.95
	17.94 ± 2.93	16.13
	20.04 ± 0.76	3.77

Friability	**passed**
Weight before (20 tablets)	13.57g
Weight after 100 drops	13.55g
Percent lost	0.15%

Dissolution Profile (for tablets with hardness of 18.02 ± 2.93kp)



BATCH: M00050601

Date of Manufacture: 05/06/01
Press: Manesty B3B (Tableted on 05/06/01)
Batch Size: 250g
Comments: Both wet and dry granules screened through 20-mesh screen on oscillating granulator

Composition (%)

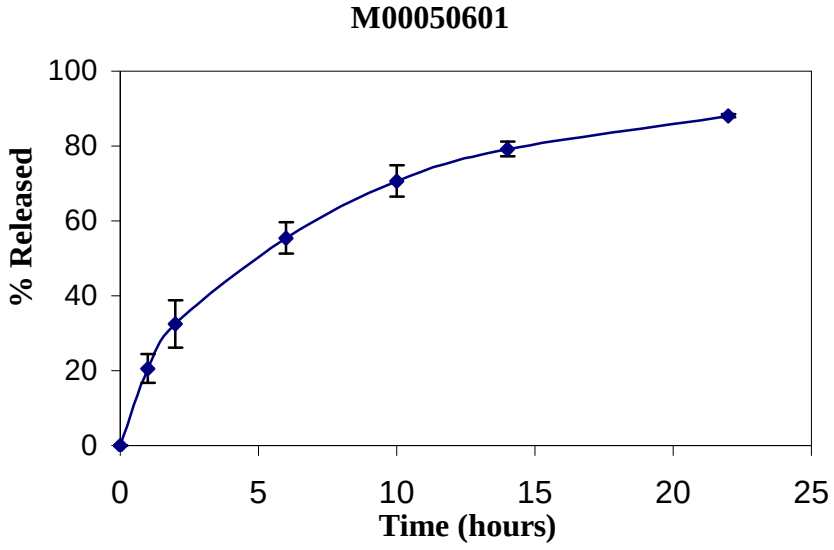
Metoprolol tartrate	20
Methocel® K_4M	10
Emcompress®	37.5
Emcocel® 90 M	32.5
Surelease®	0.19 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	649.20 ± 6.37	0.98
Hardness (kp)	12.51 ± 1.10	8.80

Friability	**passed**
Weight before 20 tablets)	12.89g
Weight after 100 drops	12.88g
Percent lost	0.08%

Dissolution Profile



APPENDIX II: BATCH DATA FOR MINI-TABLETS

BATCH: M00050601M

Date of Manufacture: 05/06/01
Press: Manesty F3 (Tableted on 06/06/01)
Granules manufactured: 300g

Composition (%)

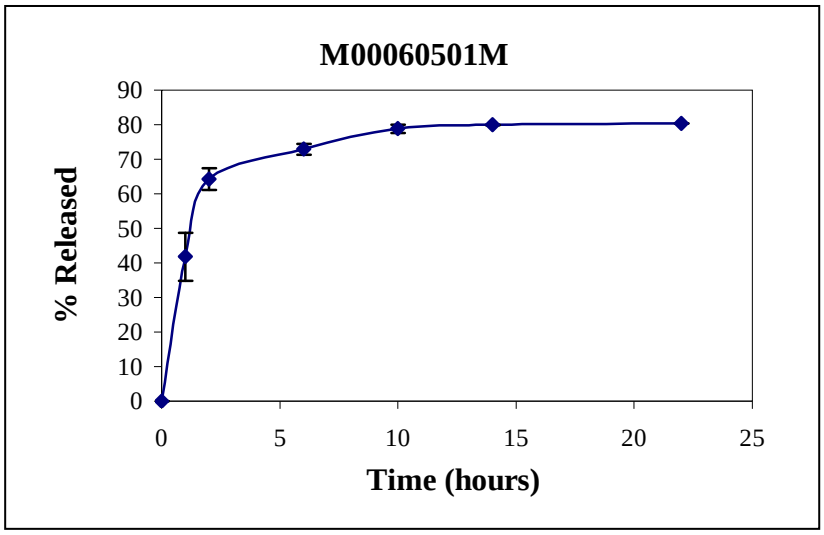
Metoprolol tartrate	20
Methocel® K_4M	10
Emcompress®	37.5
Emcocel® 90 M	32.5
Surelease®	0.19 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	106.27 ± 6.56	6.17
Hardness (kp)	7.12 ± 0.81	12.14

Friability	**passed**
Weight before (40 tablets)	4.22g
Weight after 100 drops	4.21g
Percent lost	0.078%

Dissolution Profile



BATCH: M00110601M

Date of Manufacture: 11/06/01
Press: Manesty F3 (Tableted on 12/06/01)
Granules manufactured: 650g

Composition (%)

Metoprolol tartrate	20
Methocel® K_4M	10
Emcompress®	37.5
Emcocel® 90 M	32.5
Surelease®	0.20 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

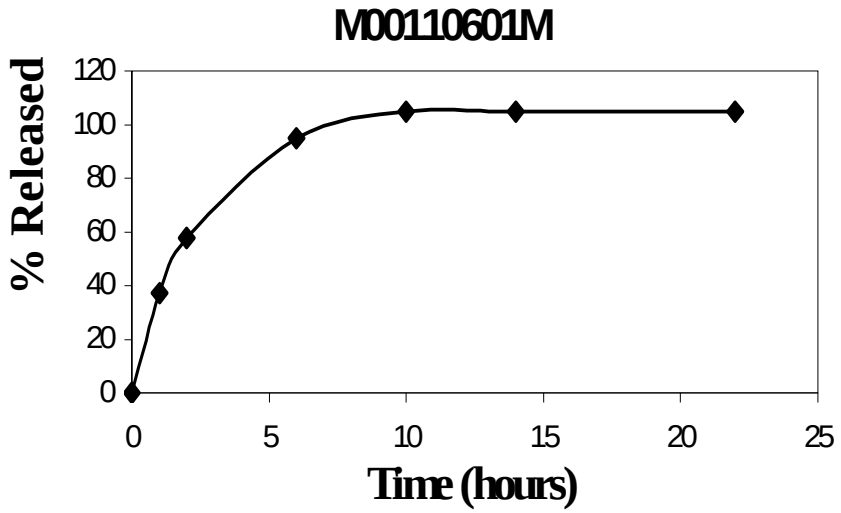
Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	108.42 ± 3.04	2.80
Hardness (kp)	6.82 ± 0.75	11.05

Friability	**passed**
Weight before (20 tablets)	2.09g
Weight after 100 drops	2.08g
Percent lost	0.48%

Content Uniformity: 90.54mg

Dissolution Profile



BATCH: M00200910M

Date of Manufacture: 02/09/01
Press: Manesty F3 (Tableted on 04/09/01)
Granules manufactured: 1250g

Composition (%)

Metoprolol tartrate	20
Methocel® K_4M	10
Emcompress®	37.5
Emcocel® 90 M	32.5
Surelease®	0.20 gram/gram of granules
Methocel® K100M	20
Emcocel® 90M	7
Emcompress®	10
Magnesium stearate	1

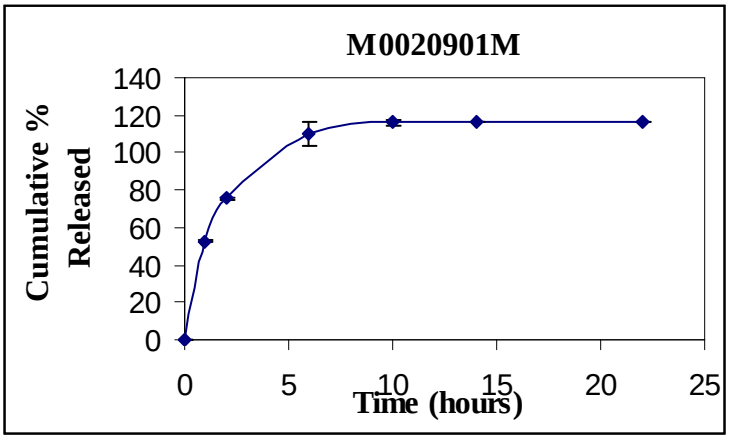
Physical Tests :

	Mean ± SD	% RSD
Weight (mg)	121.58 ± 1.29	1.06
Hardness (kp)	6.80 ± 0.71	10.39

Friability	**passed**
Weight before (20 tablets)	2.42g
Weight after 100 drops	2.41g
Percent lost	0.41%

Content Uniformity: 98.35 mg (100mg)

Dissolution Profile



APPENDIX III

BATCH PRODUCTION RECORDS

Only one coating record is included for this batch. The records for the other batches and other coating levels are available on request.

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate granules
Batch #: M00 200910 M

Page 1 of 8
Batch size: 1250g

MANUFACTURING APPROVALS

Batch record issued by: _____ Date: 02/09/01
Master record issued by: _____ Date: /

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate granules
Batch #: M002009l0M

Page 2 of 8
Batch size: 1250g

MASTER FORMULA AND BATCH FORMULA

Quantity (w/w)	Component Name	RM #	Lot #	Amount/ Batch	Amount Dispensed	Dispensed By	Checked by
20	Metoprolol tartrate (MPTA)	RM000052	505242	250.00g	250.12g		TJW
10	Methocel K4M		M0539	125.00g	125.01g		TJW
37.5	Emcompress			486.75g	468.85g		TJW
32.5	Emcocel 90M			406.25g	406.32g		TJW
q.s	Surelease	RM000010	IN500647		290.46g		TJW

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate granules
Batch #: M002009l0M

Page 3 of 8
Batch size: 1250g

EQUIPMENT VERIFICATION

Description	Type	Verified By	Confirmed by
Sieves	#20 mesh		TJW
Scale	Precisa 4000		TJW
Blender	Kenwood Major		TJW
Pump	Masterflex		TJW
Tubing	Masterflex LS14		TJW
Granulator	Erweka Oscillating		TJW
Oven			

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate granules
Batch #: M00200910M

Page 4 of 9
Batch size: 1250g

MANUFACTURING DIRECTIONS

Step	Procedure	Weight	Time	Date	Done by	Checked by
1	Separately screen the following materials through a 20 mesh screen.			02-09-01		TJW
	Metoprolol tartrate	125.49g	06:59			
	Methocel K4M	125.01g	06:51	02-09-01		TJW
	Emcompress	486.84g	06:56	02-09-01		TJW
	Emcocel 90M	406.31g	06:54	02-09-01		TJW
2	Place the following materials in the Kenwood bowl.					
	Metoprolol tartrate	125.49g	07:00	02-09-01		TJW
	Methocel K4M	125.01g	06:52	02-09-01		TJW
	Emcompress	486.84g	06:58	02-09-01		TJW
	Emcocel 90M	406.31g	06:56	02-09-01		TJW
3	Blend the materials in step 2 for 2 minutes at low speed.					
	Time started: 07:12					
	Time completed: 07:14					
	Total blending time: 2 minutes					
	Speed setting: 1			02-09-01		TJW

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate granules
Batch #: ①~~M00200910~~ M00200910M

Page 5 of 8
Batch size: 1250g

MANUFACTURING DIRECTIONS

Step	Procedure	Weight	Time	Date	Done By	Checked By
4	Place the Surelease in a tared beaker and insert pump tubing.	(beaker + Surelease) 540.06g	②07:13	02-09-01		TJW
5	With the blender on low speed, add the Surelease at a pump rate of 8 – 9 for a total time of 10 minutes.					
	Time started: 07:13					
	Time completed: 07:27					
	Total time taken: 14 mins					
	Blender speed: 1					
	Pump setting: 6					
	Amount of Surelease added: 290.84g = 0.239g/g (72.62g ethylcellulose added).					TJW
6	Transfer the granules to the granulator and screen as follows, using 20 mesh screen and 100 rpm motor speed.					
	Speed: 49 – 52 rpm		07:40	02-09-01		TJW

① Typographical error - wrong batch number entered JJ
② Typographical error JJ

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate granules
Batch #: M00200910M

Page 6 of 8
Batch size: 1250g

MANUFACTURING DIRECTIONS

Step	Procedure	Weight	Time	Date	Done by	Checked by
7	Place the granules on weighing paper and dry in the oven at 60 degrees for 12 hours Time started: 08:30 Time completed: 21:00 Total drying time: 12.5hours Oven temperature: 60 °C		08:30 21:00	02-09-01 02-09-01		TW TW
8	Remove the dried granules from the oven, and rescreen using the oscillating granulator (20 mesh, speed ~~100~~). 50 rpm ② Speed: 50rpm		21:22	02-09-01		TW

② 50rpm used

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate granules
Batch #: M00200910M

Page 7of 8
Batch size: ~~750g~~ 1250g ③

MANUFACTURING DIRECTIONS

Step	Procedure	Time	Date	Done By	Checked By
9	Record the weight of acceptable granules obtained. Gross weight: 1383.84g Tare weight: 175.01g Net weight: 1208.84g	21:30	02-09-01		TW
10	Work out the percent yield as follows. Weight acceptable granules (AG): 1208.84g Other weight accounted for (describe): 64.82g Total weight accounted for (TW): 1273.66g Percent accountability = (TW/ 1250) x 100% = 101.89 % Percent yield = (AG/1322.62 x 100% = 91.40 %				
11	Transfer granules to airtight container until tableting.	21.40	02-09-01		TW

③ Batch size incorrectly typed Actual = 1250g

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate granules
Batch #: M00200910M

Batch size: 1250g

SIGNATURE AND INITIAL REFERENCE

Full Name (print)	Signature	Initials	Date
J. ARJUN			02-09-01
T. WILD			02-09-01

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M002009 10 M

Page 1 of 6
Batch size: 1313g

MASTER FORMULA AND BATCH FORMULA

Quantity (w/w)	Component Name	RM #	Lot #	Amount/ Batch	Amount Dispensed	Dispensed By	Checked by
62	Metoprolol tartrate granules		M0020901G	① ~~1208.849~~ ~~951.619~~	951.619		TDW
20	Methocel K100M		M0539	190.329	190.399		TDW
10	Emcompress		24K	95.169	95.179		TDW
7	Emcocel 90M			66.619	66.639		TDW
1	Magnesium stearate			9.529	9.529		TDW

① Incorrectly entered

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M002009 10 M

Page 2 of 6
Batch size: 1313g

EQUIPMENT VERIFICATION

Description	Type	Verified By	Confirmed by
Sieves	#20 mesh, #44 mesh		TDW
Scale	Precisa 4000		TDW
Blender	Cube blender (Erweka)		TDW
Tablet press	Manesty F3		TDW

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: MCO2009 10 M

Page 3 of 6
Batch size: 13139

MANUFACTURING DIRECTIONS

Step	Procedure	Weight	Time	Date	Done by	Checked by
1	Screen the following materials through a #20 mesh.			~~03-09-01~~ 04-09-01 ②		TJW
	Methocel K100M	190.39g	11:20			
	Emcompress	95.17g	11:22	04-09-01		TJW
	Emcocel 90M	66.62g	11:24	04-09-01		TJW
2	Place the following in the cube blender.					
	Metoprolol tartrate granules	951.61g	11:21	04-09-01		TJW
	Methocel K100 M	190.39	11:23	04-09-01		TJW
	Emcompress	95.17g	11:25	04-09-01		TJW
	Emcocel 90M	66.62g	11:25	04-09-01		TJW
3	Blend the materials in step 2 for 20 minutes at speed 50.					
	Time started: 11:31					
	Time completed: 11:51					
	Total blending time: 20 min					
	Speed: 49-51 rpm			04-09-01		TJW

② Typographical error · Actual date = 04-09-01

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: MCO2009 10 M

Page 4 of 6
Batch size: 13139

MANUFACTURING DIRECTIONS

Step	Procedure	Weight	Time	Date	Done by	Checked by
4	Screen the magnesium stearate using a #44 mesh.	9.52g	11:49	04-09-01		TJW
5	Add the magnesium stearate to the blender and blend for 3 minutes at 50 rpm.					
	Time started: 11:53					
	Time completed: 11:56					
	Total blending time: 3 min					
	Speed: 49-52 rpm			04-09-01		TJW
6	Calculate the percent accountability and yield.					
	Gross weight (blend): 1497.25g					
	Tare weight: 186.25g					
	Net weight: 1311g					
	Other weight (Describe): N/A					
	Total weight accounted for (TW) = net weight + other weight = N/A					
	Percent accountability = (TW/ 13139) x 100% = NA %					
	Percent yield = (blend/ 1311g) x 100% = 99.85 %					
7	Store in airtight container until compression.			04-09-01		TJW

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M002009 10M

Page 5 of 6
Batch size: 1313g

MANUFACTURING DIRECTIONS

Step	Procedure	Time	Date	Done by	Checked by
8	Tablet the blend on the tablet press according to the standard operating procedures. Desired Hardness: 6-8kp Desired Weight: ①120mg		04-09-01 04-09-01		TJW
9	Sample 4 tablets every 5 minutes and check hardness and weight. Enter results on the in-process results sheet.		04-09-01		TJW
10	Perform physical tests of hardness, friability and weight uniformity on the final batch. Enter the results on the bulk product test reports.		04-09-01		TJW
11	Store product in an airtight container.		04-09-01		TJW

① 15 mins used because single punch press was utilised ...run longer

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M002009 10M

Page 6 of 6
Batch size: 1313g

MANUFACTURING DIRECTIONS

Step	Procedure	Time	Date	Done By	Checked By
12	Record the weight of acceptable tablets obtained. Gross weight: 1247.14g Tare weight: 300.12g ① Net weight: ~~954~~ 947.02g		04-09-01		TJW
13	Work out the percent yield as follows. Weight acceptable tablets (AT): 947.02g Other weight accounted for (describe): 31.08g (Discarded) Total weight accounted for (TW): 978.1g Percent accountability = (TW/ 1313g) x 100% = 74.49 % Percent yield = (AG/ 1313) x 100% = 72.13 %		04-09-01		TJW

① Weight incorrectly entered

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M00 2009 10M Batch size: 131 3g

IN-PROCESS TABLETING REPORT

Date	Time	Hardness (kp)	Weight (mg)
04-09-01	14:01	6·01	122·6
04-09-01		5·81	122·5
		6·72	120·3
		6·03	121·1
	14:20	6·52	119·9
		7·24	119·6
		7·14	120·7
		7·18	120·4
	14:37	6·81	121·3
		7·12	120·4
		7·24	119·6
		8·10	121·0
	15:00	5·81	122·5
		6·01	121·8
		7·28	120·9
		5·91	122·5
	15:21	7·24	120·3
		7·14	121·1
		5·98	124·1
		6·02	122·5
	15:44	4·89	121·0
		5·10	122·5
		5·61	119·8
		6·01	124·1

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M00200910M

Page 1 of 4
Batch size: 100 g

MASTER FORMULA AND BATCH FORMULA

Quantity	Component Name	RM #	Lot #	Amount/ Batch	Amount Dispensed	Dispensed By	Checked by
100g	Metoprolol tartrate tablets		M0020901M	100g	100.06g		TZW
15% soln	Surelease			-			TZW
10% w/w	TEC			-			TZW

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M00200910M

Page 2 of 4
Batch size: 100g

EQUIPMENT VERIFICATION

Description	Type	Verified By	Confirmed by
Fluid bed drier	Aeromatic Strea-1		TZW
Scale	Mettler		TZW
Mixer	Collenkamp		TZW
Peristaltic pump	Masterflex, LS 13 tubing		TZW
Digital Thermometer	Lutron		TZW

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M00 a0019M

Page 3 of 4
Batch size: 100g

MANUFACTURING DIRECTIONS

Step	Procedure	Weight	Time	Date	Done by	Checked by
1	Place the tablets in the product container	100.06g	11:02	14/09/01		TJW
2	Start the fluidising air at a rated temperature of 60 degrees and allow the tablets to circulate for 5 minutes until a product bed temperature of 40 degrees is reached. Time started: 11:05 Time completed: 11:09 Total time: 4min					TJW
3	Begin spraying by turning on the pump at a speed setting of 1.0 and turning on the atomising air to a pressure of 20 psi. Pump speed: 1.0 Atomising air pressure: 20psi Actual spray rate: 1.84g/min					TJW
4	Spray the product until a theoretical weight gain of 5%. If the product becomes tacky and no longer fluidises effectively, turn off the pump and allow the tablets to circulate freely for 2-3 minutes before recommencing spraying. Record product bed temperature, weight of Surelease® and time of any pauses.		11:10 – 11:22 11:23 – 11:30	(550.20 – 526.80g) (525.62 – 440.00g) 14-09-01		TJW

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M00 20019 10M

Page 4 of 4
Batch size: 100g

MANUFACTURING DIRECTIONS

Step	Procedure	Time	Date	Done By	Checked By
5	Record the following parameters at 5 minute intervals on the in process record sheet. Inlet air temperature Outlet air temperature Product temperature Atomising air pressure		14-09-01		TJW
6	Allow the tablets to fluidise for a drying time of 20 minutes once spraying has finished.		14-09-01		TJW
7	Record the weight of coated tablets obtained. Gross weight: 328.91g Tare weight: 224.02g Net weight: 104.89g		14-09-01		TJW
8	Work out the percent weight gain as follows. Weight coated tablets (CT): 104.89g Other weight accounted for (describe): — Total weight accounted for (TW): 104.89g Percent weight gain = (TW - 100.06 / 100.06) x 100% = 4.83% %				
9	Store product in an airtight container.		14-09-01		TJW

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Metoprolol tartrate tablets
Batch #: M00200910M Batch size: 13139

IN-PROCESS COATING REPORT

Time	Inlet Air Temperature	Outlet Air Temperature	Product Temperature	Atomising Air
11:10	52°C	42°C	46°C	20psi
11:15	52°C	43°C	47°C	19psi
11:20	52°C	42°C	47°C	20psi
11:25	52°C	44°C	48°C	19psi
11:30	52°C	42°C	48°C	20psi

PHARMACEUTICS DEPARTMENT
Faculty of Pharmacy, Rhodes University
Grahamstown, 6140

BATCH PRODUCTION RECORD

Product name: Hydrochlorothiazide powder blend
Batch #: H02 Batch size: 200g

MASTER FORMULA AND BATCH FORMULA

Quantity (w/w)	Component Name	RM #	Lot #	Amount/ Batch	Amount Dispensed	Dispensed By	Checked by
12.5	Hydrochlorothiazide	RM0000 70	X00137.5	25g	25.03g		TJW
87.5	Emcocel 90M			175g	175.31g		TJW
0.5	Magnesium stearate			1g	1.01g		TJW

EQUIPMENT VERIFICATION

Description	Type	Verified By	Confirmed by
Sieves	#20 mesh, #44 mesh		TJW
Blender	Cube blender		TJW
Scale	Precisa 4000		TJW

MANUFACTURING DIRECTIONS

Step	Procedure	Weight	Time	Date	Done by	Checked by
1	Separately screen the following materials through a 20 mesh screen. Hydrochlorothiazide Emcocel 90M	 25.03g 137.31g	 11:51 11:53	 06-09-01 06-09-01		TJW
2	Blend in cube blender at 50 rpm for 20 minutes. (51rpm) Time started: 11:57 Time completed: 12:14 Total blending time: 20minutes					TJW
3	Separately screen magnesium stearate through a 44 mesh screen	1.01g	12:12	06-09-01		TJW
4	Blend in a cube blender at 50 rpm for 3 minutes. Time started: 12:15 Time completed: 12:18 Total blending time: 3mins			06-09-01		TJW
5	Store product in a airtight container			06-09-01		TJW

REFERENCES

1. J.R.Luch. Metoprolol Tartrate. In K.Florey (ed.) *Analytical Profiles of Drug Substances,* **Volume 12**, Academic Press, New York, USA pp 326-354 (1983)

2. http://www.becker.wustl.edu

3. wysiwyg://38/http://www.parkinsons-inf…nge-network-online.com.drugb/085.html

4. http://www/rxlist.com/cgi/generic/meto.htm

5. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, J.G.Hardman, L.E.Limbird (editors-in-chief), P.B.Molinoff, R.W.Ruddon (eds.), A.Goodman Gilman (consultant ed.), McGraw-Hill, USA, 9th Edition, 1996, pp 235-239

6. P.Modamio, C.F.Lastra, E.L.Mariño. A comparitive in vitro study of percutaneous penetration of β-blockers in human skin. *Int. J. Pharm*. **194**: 249-259 (2000)

7. S.Ablofazl Mostafavi, Richard.Z.Lewanczuk, Robert.T.Foster. Influence of Acebutolol and Metoprolol on Cardiac Output and Regionall Blood Flow in Rats. Biopharm. *Drug. Dispos* **21**: 121-128 (2000)

8. L.V.Allen. Stability of extemporaneous Prepared Pediatric Formulations Using Ora-Plus with Ora-Sweet SF – Part II. *Secundum Artem* **6**(1) Retrieved from http://www.paddocklabs.com/publications/secundum/secart61.html on 03/04/2001

9. A.Albin, A.Markus. Z.Ben-Zvi, Z.Pelah. A new slow release formulation of metoprolol: in-vitro and in-vivo evaluation in dogs. *J. Contr. Rel*. **23**: 1-12 (1993)

10. *The Merck Index*, S.Budavari, M.J.O'Neil, A.Smith, P.E.Heckelman and J.F.Kinneary (eds.), Merck and Co, 1996, pp 1050

11. http://www.nursespdr.com.members/database/ndrhtml/metoprololsuccinate.html

12. P.Benfield, S.P.Clissold, R.N.Brogden. Metoprolol: An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in Hypertension, Ischaemic heart disease and related cardiovascular disorders. *Drugs* **31**: 376-429 (1986)

13. S.Agewall, M.Kendall. Treatment with β-blockers-the value of an even plasma concentration over 24h. *Journal of Clinical Pharmacy and Therapeutics*. **22**: 171-179 (1997)

14. *The Merck Manual*, Donald F.de Korte (ed.), Merck and Co, 1992, pp 413-429

15. R.M.Myerson. Part II The Treatment of Hypertension: An Independent CME Study Course For Healthcare Professionals. Retreived from http://www.arcmesa.com/pdf/hyptnsn2_book.htm on 20/11/01

16. B.N.C.Prichard, B.Graham, J.M.Cruickshank. Beta-Blockers in the Third Millenium – When are They Really Indicated? *J. Clin. Basic.Cardiol* **4**: 3-9 (2001)

17. C.Spencer. Hypertension (3) management of the hypertensive patient. *Pharmaceutical Journal*. **263** (7062): 383-386 (1999)

18. I.Lager, U.Smith, G.Blohme. Effect of Cardioselective and Non-Selective ß-Blockade on the hypoglycaemic response in Insulin-Dependent Diabetics. *The Lancet*. 3 March: 458-462 (1979)

19. J.Wikstrand. Initial therapy for mild hypertension. *Pharmacotherapy* **6** (2): 64-71 (1986)

20. J.Wikstrand. New concepts in the treatment of elderly hypertensive patients. *American Heart Journal* **116**(No.1, part 2): 296-300 (1988)

21. A.J.Smith, J.S.Wehner, H.J.Manley, A.D.Richardson, J.Beal, P.J.Byrant. Current role of β-adrenergic blockers in the treatment of chronic congestive heart failure. *Am. J. Health-Syst. Pharm*. **58**: 140-145 (2001)

22. L.LaPalio, A.Schark, S.Glasser, C.Tifft. Safety and efficacy of metoprolol in the treatment of hypertension in the Elderly. *JAGS* **40**(4): 354-358 (1992)

23. P.D.McSorley, D.J.Warren. Effects of propranolol and metoprolol on the peripheral circulation. *B.M.J.* 9 December: 1598-1600 (1978)

24. http://www/vitamins.com/encyclopedia 10mg/metoprolol.htm

25. *Basic and Clinical Pharmacology*, Bertram G. Katzung (ed.), Appleton and Lange, 7th Edition , 1998, pp144, 165

26. Michael.W.Rich, Lawrence LaPalio, Anthony Schork. The Safety After Fifty Evaluation trial: Evaluation of he safety and efficacy of antihypertensive therapy with metoprolol in patients 50 to 75 years of age: Study design. *American Heart Journal* Volume 116 1(2): 301-304 (1988)

27. W.A.Ritschel. Compilation of pharmacokinetic parameters of beta-adrenergic blocking agents. *Drug Intelligence and Clinical Pharmacy*. **14**: 747-755 (1980)

28. K.H.Yuen, K.L.Chan, W.T.Toh, K.K.Peh, H.H.Ang, B.L.Ong. Pharmacokinetics and Comparative Bioavailability of Two Metoprolol Tablet Preparations. *Drug. Dev. Ind. Pharm*. **22**(4): 329-333 (1996)

29. Z.Linze, C.Mel, L.Hua, Z.Xianglan and F.Xia. Preparation and Pharmacokinetic Characterization of a Controlled Release Dosage Form of Metoprolol. *HKPJ*. **6**(2): 54-58 (1997)

30. J.F.Dasta. Drug Evaluation data. *Drug Intelligence and Clinical Pharmacy*. **13**:320-322 (1979)

31. R.Loebenberg, J.S.Kim, J.Crison, G.L.Amidon. *Controlled release of metoprolol: Influence of motility and food*. Poster presented at 26th Annual Symposium on the Controlled release of Bioactive Materials, Paris, France, 9-13 July (2000)

32. S.Abolfazl Mostafavi, Robert.T.Foster. Pharmacokinetics of metoprolol enantiomers following single and multiple administration of racemate in rat. *Int. J. Pharm*. **202**: 97-102 (2000)

33. B.Oosterhuis, J.H.G. Jonkman, F.A.Kerkhof. Pharmacokinetic and Pharmacodynamic comparison of a new controlled release formulation of metoprolol with a traditional slow-release formulation. *Eur. J. Pharmacol*. **38**(suppl): S15-S18 (1988)

34. B.Mistry, J.Leslie, N.E.Eddington. A sensitive assay of metoprolol and its major metabolite α-hydroxy metoprolol in human plasma and determination of dextrmethorphan and its metabolite dextrorphan in urine with high performance liquid chromatography and fluorometric detection. *J. Pharm. Biomed. Anal*. **16**:1041-1049 (1998)

35. S.Madani, M.F.Paine, L.Lewis, K.E.Thummel, D.D.Shen. Comparison of CYP2D6 Content and Metoprolol Oxidation Between Microsomes Isolated from Human Livers and Small Intestines. *Pharm. Res*. **16**(8): 1199-1205 (1999)

36. *South African Medicines Formulary*, C.J.Gibbon (eds.), South African Medical Association, health and Medical Publishing Group, 2000, pp 138

37. Hans Peter Deppeler. Hydrochlorothiazide. In K.Florey (ed.) *Analytical Profiles of Drug Substances*,**Volume 10**, Academic Press, New York, USA pp 405- (1983)

38. W.J.A.Vanderheuvel, V.F.Gruber, R.W.Walker, F.J.Wolf. GLC analysis of HCTZ in blood and plasma. *J. Pharm. Sci*. **64**(8): 1309-1312 (1975)

39. *Standard Treatment Guidelines and Essential Drugs List for South Africa, Adult Hospital Level*, The National Department of Health, SA, 1998, pp 67-68

40. *The British Pharmacopoeia*, The Stationary Office, London, 1998, pp 684

41. U.G.Hennig, R.E.Moskalyk, L.G.Chatten, S.F.Chan. Semiaqueous Potentiometric Determinations of Apparent pKa1 Values for Benzothiadiazines and Detection of Decomposition during Solubility Variation of pH Studies. *J. Pharm. Sci*. **70**(3): 317-319 (1981)

42. J.K.Pandit, B.K.Khakurel. In-vitro and in-vivo evaluation of some fast release dosage forms of hydrochlorothiazide. *Drug. Dev. Ind. Pharm*. **10**(10): 1709-1724 (1984)

43. E.Ridalieu, K.K.H.Chan, V.Tipnis, S.B.Zak, T.G.Gilleran et al. Kinetics of hydrochlorothiazide absorption in humans. *J. Pharm. Sci*. **74**(7): 765-767 (1985)

44. *The Pharmaceutical Codex, Principles and Practice of Pharmaceutics*, Walter Lund (ed.), The Pharmaceutical Society, London, 12th Edition, 1994, pp 899-901

45. V.Ulvi, S.Tammilehto. Photodecomposition studies on chlorothiazide and hydrochlorothiazide. *Acta. Pharm*. *Nordica*. *1*(4): 195-200 (1989)

46. J.A.Mollica, C.R.Rohm, J.B.Smith, H.R.Govan. Hydrolysis of benzothiadiazines. *J. Pharm. Sci* **6**(9): 1381-1384 (1971)

47. *Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists.* K.A.Connors, G.L.Amidon, V.J.Stella (eds.), John Wiley and Sons, USA, 1986, pp 478-482

48. S.R.Tamat, D.E.Moore. Photolytic decomposition of hydrochlorothiazide. *J. Pharm. Sci*. **72**(2): 180-183 (1983)

49. Douglas E.Moore. Photosensitization by Drugs. *J. Pharm. Sci*. **66**(9): 1282-1284 (1977)

50. Scott J.Weir, Dan C.Dimmitt, Robert C.Lamman, M.Bruce.Morrill and Dennis H.Geising. Steady-State Pharmacokinetics of Diltiazem and Hydrochlorothiazide Administered Alone and in Combination. *Biopharm. Drug Dispos*. **19**: 365-371 (1998)

51. http;//www.rxlist.com/cgi/generic/hydrochlorothiazide/htm.

52. Hunter Hypertension Research Group. Metoprolol or Hydrochlorothiazide in patients with hypertension aged 60-75 years, With special reference to assessment of compliance. *The Medical Journal of Australia*. **Volume 145** Nov17, 1986

53. Britta Hylander, Mats Danielson and Keith Eliasson. Comparison of Hydrochlorothiazide and Salow Release Furosemide as Adjuvant Therapy to ß-blockers in the Treatment of Moderate Hypertension. *Acta. Med. Scand*. **222**: 137-142 (1987)

54. C.Bernal, R.Patarca. Hydrochlorthiazide-Induced Pulmonary Edema and associated Immunologic Changes. *Annals of Pharmacotherapy*. **33**: 172-173 (1999)

55. W.Möhrke, H.Knauf, E.Mutschler. Pharmacokinetics and pharmacodynamics of triamterene and hydrochlorothiazide and their combination in healthy volunteers. *International Journal of Clinical Pharmacology and Therapeutics,* **35**(10): 447-452 (1997)

56. Sam Corveleyn, Jean Paul Remon. Bioavailability of hydrochlorothiazide: conventional versus freeze-dried tablets. *Int. J. Pharm*. **173**: 149-155 (1998)

57. http://www. micromedex.com/cust_center/drdex/irbes.htm Retrieved 25/10/01

58. A. Sandberg, G.Ragnarsson, U.E.Jonsson, J.Sjögren. Design of a new multi-unit controlled-release formulation of metoprolol – Metoprolol CR. *Eur. J Clin Pharmacol*. **33**(suppl):S3-S7 (1988)

59. M.J.Kendall. Metoprolol – Controlled release, Zero order kinetics. *Journal of Clincal Pharmacy and Therapeutics*. **14**: 159-179 (1989)

60. G. Carruthers, R.Shearer, W.Taylor. A Comparison of a controlled release (CR/ZOK) Formulation of metoprolol, once daily, with conventional metoprolol tablets, twice daily, in mild to moderate hypertension. *J Clin Pharmacol* **30**: S61-S65 (1990)

61. O.Lederballe Pedersen. Comparison of metoprolol and hydrochlorothiazide as antihypertensive agents. *Europ. J. Clin. Pharmacol*. **10**: 381-385 (1976)

62. S. Rasmussen and K. Rasmussen. Influence of metoprolol alone and in combination with a thiazide diuretic, on blood pressure, plasma volume, extracellular volume and glomerular filtration rate in essential hypertension. *Europ. J. Clin. Pharm*. **15**: 305-310 (1979)

63. TH.J.J.M.Bloem, R.P.Disch, P.C.J.M Lindner and V.D.Kerkhof. Antihypertensive effects of metoprolol and a fixed-ratio combination of metoprolol and hydrochlorothiazide given once daily. *Curr.Ther. Res.* **24**(1): 26-30 (1978)

64. J.G.Smilde. Comparison of the Antihypertensive effect of a double dose of metoprolol versus the addition of hydrochlorothiazide to metoprolol *Eur. J. Pharmacol*. **25**: 581-583 (1983)

65. Paula Mitenko, John K. McKenzie. The treatment of mild to moderate hypertension with the combination of metoprolol and hydrochlorothiazide. *Curr. Ther. Res.* **14**(6): 1029-1036 (1983)

66. P.Lundborg, B.Abrahamsson, I.Wieselgren, M.Walter. The Pharmacokinetics and pharmacodynamics of metoprolol after conventional and controlled-release administration in combination with hydrochlorothiazide in healthy volunteers. *Eur J. Clin Pharmacol*. **45**: 161-163 (1993)

67. L.Jordo, G.Johnsson, P.Lundborg, B.A.Persson, C.G.Regardh and O.Ronn. Bioavailability and Disposition of Metoprolol and hydrochlorothiazide combined in one tablet of separate doses of hydrochlorothiazide. *Br. J.Clin. Pharmacol*. **7**: 563-567 (1979)

68. R.T.Owen. Beta-Blocker/Diuretic combinations. *Pharmacy International*. January, pp 17-21 (January 1981)

69. S.Hutchinson, L.M.Campbell. Beta-Blockers and the elderly. *J. Clin. Hosp. Pharm*. **8**: 191-199 (1983)

70. B.Abrahamsson, M.Alpsten, U.E.Jonsson, P.J.Lundberg, A.Sandberg, M.Sundgren, A.Svenheden, J.Tolli. Gastro-intestinal transit of a multi-unit formulation (metoprolol CR/ZOK) and a non-disintegrating tablet with the emphasis on colon. *Int. J. Pharm* **140**: 229-235 (1996)

71. M.T.Rosseel, F.M.Belpaire, I.Bekaert and M.G.Boagaert. High-Performance liquid chromatographic determination of metoprolol in plasma. *J. Pharm. Sci*. **71**(1): 114-115 (1982)

72. J Kirschbaum, S Perlman. Analysis of captopril and hydrochlorothiazide combination tablet formulations by liquid chromatography. *J. Pharm. Sci*. **73**(5): 686-687 (1984)

73. *United States Pharmacopoeia incorporating 'The National Formulary'*, United States Pharmacopoeial Convention, Maryland, 24th Edition, 1999, pp 820-821, 1101-1104, 2000-2002

74. R.Soltero, J.Robinson, D.Adair. Dissolution Profile Determination of a multicomponent product using a rapid liquid chromatographic analysis. *J. Pharm. Sci*. **73**(6): 799-803 (1984)

75. V.DasGupta, J.Maswoswe. Quantitation of Metoprolol tartrate and Propranolol hydrochloride in pharmaceutical dosage forms: Stability of metoprolol in aqueous mixtures. *Intl J. Pharm Comp.* **1**(2): (March/April 1997)

76. R.Ghanem, MA.Bello, M.Callejon, A.Guiraum. Determination of beta-blocker drugs in pharmaceutical preparations by non-suppressed ion chromatography. *J. Pharm. Biomed. Anal*. **15**: 383-388 (1996)

77. R.Groning. Computer-controlled release of metoprolol from capsules. *Int. J. Pharm*. **87**: 89-93 (1992)

78. AFM El Walily, SF Belal, EA Heaba, AE Kersh. Simultaneous determination of enalapril maleate and hydrochlorothiazide by first-derivative ultraviolet spectrophotometry and high-performance liquid chromatography. *J. Pharm. Biomed. Anal*. **13**: 851-856 (1995)

79. V.Ulvi, H.Keski-Hynnila. First-derivative UV spectrphotometric and high performance liquid hromatographic analysis of some thiazide diuretics in the presence of their photodecomposition products. *J. Pharm. Biomed. Anal*. **12**(7): 917-922 (1994)

80. S.I.Daniels, A.J.Vanderwielen. Stability-Indicating assay for Hydrochlorothiazide. *J. Pharm. Sci*. **70**(2): 211-215 (1981)

81. A.A Fatmi, G.V Williams. Determination of hydralazine hydrochloride and hydrochlorothiazide in dosage forms by high performance liquid chromatography. *Drug. Dev. Ind. Pharm*. **16**(5): 779-789 (1990)

82. L.Jordo, G.Johnsson, P.Lundborg, B.A.Persson, C-G.Regardh, O.Ronn. Bioavailability and disposition of metoprolol and hydrochlorothiazide combined in one tablet and of separate doses of hydrochlorothiazide. *Br. J. Clin. Pharmac.* **7**: 563-567 (1979)

83. S.Lindsay. *High performance liquid chromatography*. John Wiley & sons, Chicester, UK, 1987, pp 1-207

84. http://www.wpi.edu/Academics/Depts/Chemistry/Courses Retreived on 21/04/2001

85. P.McKay. *An Introduction to Chromatography* Retrieved from http://www.accessexcellence.com/TSN/SS/chromatography_background.html on 20/04/2001

86. http://www.kerouac.uky.edu/asrg/hplc/history.html Retrieved on 20/04/2001

87. http://www.tamuk.edu/fplc/ion.html Retrieved on 20/04/2001

88. P.Modamio, C.F.Lastra, O.Montejo, E.L.Mariño. Development and validation of liquid chromatography methods for the quantitation of propranolol, metoprolol, atenolol and bisoprolol: application in solution stability studies. *Int. J. Pharm*. **130**: 137-140 (19966)

89. M.Zecevic, L.J.Zivanovic, S.Agatonovic-Kustrin, D.Ivanovic, M.Maksimovic. Statistical optimization of a reversed-phase liquid chromatographic method for the analysis of amiloride and hydrochlorothiazide in tablets. *J. Pharm. Biomed. Anal.* **22**:1-6 (2000)

90. http://www.micromass.co.uk/basics/Gclcth.2.html Retrieved on 20/04/2001

91. N.Hadden, F.Baumann, F.MacDonald, M.Munk, R.Stevenson, D.Gere, F.Zamaroni, R.Majors. *Basic Liquid Chromatography*. Varian Aerograph, U.S.A 1971 pp 1.1-12.4

92. David. V.McCalley. Reversed-Phase HPLC of Basic Samples – An Update. *LC-GC* **17**(5): 440-456 (1999)

93. Jacques Crommen. Ion-pairing detection technique in reversed-phase high-performance liquid chromatography of drugs and related compounds. *J. Pharm. Biomed. Anal*. **1**(4): 549-555 (1983)

94. J.A.Adamovics. *Chromatographic analysis of pharmaceuticals* **Volume 49** ,Marcell Dekker, (1990) pp 6

95. J.M.Green. *A Practical Guide to Analytical Method Validation*. Retrieved from http://pubs.acs.org/hotaartcl/ac/96/may/may.html on 28/04/2001

96. P.A.D.Edwardson, G.Bhaskar, J.E.Fairbrother. Method validation in pharmaceutical analysis. *J. Pharm. Biomed. Anal*. **8**(8-12): 929-933 (1990)

97. G.C. Hokanson, A life Cycle Approach to the Validation of Analytical Methods during pharmaceutical product development, Part I: The initial method validation process. *Pharm. Tech.* **18(9)**: 118-130 (1994)

98. J.R.Lang, S.Bolton. A comprehensive method validation strategy for bioanalytical applications in the pharmaceutical industry – 1. Experimental considerations. *J. Pharm. Biomed. Anal*. **9**(5): 357-361 (1991)

99. *Analytical Method Development and Validation*, M.E Swatz and I.S.Krull, Marcell Dekker Inc, 1997, pp 17-83

100. W.Lindner, I.W.Wainer, Requirements for initial assay validation and publication in Journal of Liquid chromatography B. *Journal of Liquid Chromatography B* No.1 March 1998

101. A Zarzuelo, M.J.Sayalero, F.G.López, J.M.Lanao, Determination of Ambroxol hydrochloride by HPLC. *J. Liq. Chrom. & Rel. Tech*. **24** (7): 1007-1014 (2001)

102. S. Wielinski, A. Olszanowski. Development and validation of HPLC method for simultaneous determination of fat-soluble vitamins in capsules. *J. Liq. Chrom. & Rel. Tech*. **24** (2): 201-213 (2001)

103. Center for Drug Evaluation and Research Reviewer Guidance: Validation of chromatographic methods, FDA, November 1994

104. Richard. D. Bunnell. Using Computer Simulated Results of a Bulk Drug Substance Assay to Determine Acceptance Criteria for Method Validation. *Pharm. Res*. 14(2): 156-163 (1997)

105. Vogel's Textbook of Quanitative and Chemical Analysis. G.H.Jeffry, J.Bassett, J.Mendham, R.C.Denney (eds.), Longman Scientific and Technical, London, 5th Edition (1989) pp. 637-638, 647-652

106. D.H.Weed. A statistically integrated approach to analytical method validation. *Pharm. Technol*. **23**(10: 116-129 (1999)

107. A.Segall, M.Vitale, V.Perez, F.Hormaechea, M.Palacios, M.T. Pizzorno. A Stabilty-Indicating HPLC Method to determine Cyproterone Acetate in Tablet Formulations. *Drug, Dev. Ind. Pharm*. **26**(8): 867-872 (2000)

108. G.S.Clarke. The validation of analytical methods for drug substances and drug products in UK pharmaceutical laboratories. *J. Pharm. Biomed. Anal*. **12**(5): 643-652 (1994)

109. A.P.Argekar, J.G.Sawant. Simultaneous Dtermination of Pyridixine Hydrochloride and Dixylamine Succinate from Tablets by Ion Pair Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). *Drug. Dev. Ind*. **Pharm**. **25**(8): 945-950 (1999)

110. J.R.Lang, S.Bolton. A comprehensive method validation strategy for bioanalytical applications in the pharmaceutical industry – 2. Staistical analysis. *J. Pharm. Biomed. Anal.* 9(6): 435-442 (1991)

111. U.Timm, M.Wall, D.Dell. A New Approach for dealing with the stability of drugs in Biological Fluids. *J. Pharm. Sci*. **74**(9): 972-977 (1985)

112. G.C. Hokanson, A life Cycle Approach to the Validation of Analytical Methods during pharmaceutical product development, Part II: Changes and the need for additional validation. *Pharm. Tech.* 18(10): 92-100 (1994)

113. T.Thrash. Controlled-Release Drug Delivery Systems: Mechanisms of Intestinal-Specific Polymeric Degradation. http://www.denison.edu/chem./DCS/journal/thrashvln1.shtml Retrieved on 23/04/01

114. F.W.H.M Merkus. *Controlled and Rate-Controlled Drug delivery: Principal Characteristics, Possibilities, and Limitations*. In H.A.J.Struyker-Boudier (ed.), *Rate-Controlled Drug Administration and Action.* CRC Press, 1986, pp 15-47.

115. V.H.K.Li, J.R.Robinson, V.H.L.Lee. Influence of Drug Properties and Routes of Drug Administration on the design of Sustained and Controlled Release Systems. In J.R.Robinson, V.H.L.Lee (eds.) *Controlled Drug Delivery: Fundamentals and Applications* **Volume 29** Marcel Dekker, New York and Basel, 1987, pp 3-94

116 Paul.A.Steward. Review of Pharmaceutical Controlled Release Methods and Devices. http://www.initium.demon.co.uk/rel-nf.htm Retrieved on 23/04/01

117. Ping I.Lee. Oral ER Technology: Mechanism of Release. In G.L.Amidon, J.R.Robinson, R.L.Williams (eds.) *Scientific Foundations for Regulating Drug Product Quality*, AAPS, USA, 1997, pp221-230

118. R.Eyjolfsson. HPMC mixtures: Effects and kinetics of release of an insoluble drug. *Drug. Dev Ind .Pharm*. **25**(5): 667-669 (1999)

119. P.Mura, G.Bramanti, L.Fabbri, M.Valleri. Controlled release matrix tablets of ketoprofen. In J.I.Wells, M.H.Rubinstein (eds.) *Pharmaceutical. Technology: Controlled Drug Release*, **Volume 2**, Ellis Horwood Ltd, Chicester, 1991, pp 73-80

120. K.K.Peh, C.F.Wong, K.H.Yuen. Possible mechanism for drug retardation from glyceryl monostearate matrix system. *Drug. Dev .Ind. Pharm*. **26**(4): 447-450 (2000)

121. L.G.Martini, K.Coles, K,Gravell, S.Stephenson, C.M.Thomson. The use of a hydrophobic matrix for the sustained release of a highly water-soluble drug. *Drug .Dev. Ind. Pharm*. **26**(1): 79-83 (2000)

122. G.Shlieout, G.Zessin. Investigation of ethylcellulose as a matrix former and a new method to regard and evaluate the compaction data. *Drug. Dev. Ind..Pharm*. **22**(4): 313-319 (1996)

123. S.Puttipipatkhachorn, J.Nunthanid, K.Yamamoto, G.E.Peck. Drug physical state and drug-polymer interaction on drug release from chitosan matrix films. *J. Contr. Rel*. **75**: 143-153 (2001)

124 N.K.Ebube, A.H.Hikal, C.M.Wyandt, D.C.Beer, L.G.Miller and A.B.Jones. Effect of drug, formulation and process variables on granulation and compaction characteristics of heterogenous matrices: Part II: HPMC and PVP systems. *Drug Dev. Ind. Pharm* **22**(7): 561-567 (1996)

125. Robert.T.C.Ju, Phillip.R.Nixon, Mahesh.V.Patel. Drug release from hydrophilic matrices. 1. New scaling laws for predicting polymer and drug release based on the polymer disentanglement concentration and the diffusion layer. *J. Pharm. Sci*. **84**(12): 1455-1463 (1995)

126. G.Yan, H.Li, R.Zhang, D.Ding. Preparation and evaluation of a sustained release formulation of nifedipine HPMC tablets. *Drug. Dev. Ind. Pharm*. **26**(6): 681-686 (2000)

127. K.A.Mehta, M.S.Kislalioglu, W.Phuapradit, A.Waseem Malick, N.H.Shah. Effect of formulation and process variables on porosity parameters and release rates from a multi unit erosion matrix of a poorly water soluble drug. *J. Contr. Rel*. **63**: 201-211 (2000)

128. M.L.González, *et al*. Channeling agent and drug release from a central core matrix tablet. *Drug. Dev. Ind. Pharm*. **27**(5): 439-446 (2001)

129. A.Struebel, J.Siepmann, N.A.Peppas, R.Bodmeier. Bimodal release achieved with multi-layered matrix tablets: transport mechanisms and device design. *J. Contr. Rel*. **69**: 455-468 (2000)

130. A.Struebel, J.Siepmann, A.Dashevsky, R.Bodmeier. pH-independent release of a weakly basic drug from water-insoluble and soluble matrix tablets. *J. Contr Rel*. **67**: 101-110 (2000)

131. A.Charlier, B.Leclerc, G.Courraze. Release of mifeprostine from biodegradable matrices: experimental and theoretical evaluations. *Int .J. Pharm*. **200**: 115-120 (2000)

132. V.Pillay, R.Fassihi. A novel approach for constant rate delivery of highly soluble bioactives from a simple monolithic system*. J. Contr. Rel*. **67**: 67-87 (2000)

133. G.S.Banker. Controlled Release of Effectors from Polymers and Microcapsules. In R.J.Kostelnik (ed.) *Polymeric delivery systems*, Gordon and Breach Science Publishers, New York, 1978, pp 25-58

134. W.R.Good. Diffusion of water soluble drugs from initially dry hydrogels. In R.J.Kostelnik (ed.) *Polymeric delivery systems*, Gordon and Breach Science Publishers, New York, 1978, pp 139-156

135. B.Gander, R.Gurny, E.Doelker. Crosslinked poloxamers as a versatile monolithic drug delivery system. In M.H.Rubinstein (ed.) *Pharmaceutical. Technologyl: Controlled drug release*, **Volume 1**, Ellis Horwood Ltd, Chicester, 1987, pp34-40

136. A.R.Fassihi, M.S.Parker. Controlled drug release from a compressed heterogenous polymeric matrix: kinetics of release. In M.H.Rubinstein (ed.) *Pharmaceutical Technology*: *Controlled drug release*. **Volume 1**, Ellis Horwood Ltd, Chicester, 1987, pp 64-71

137. M.E.Sangalli, P.Giunchedi, L.Maggi, U.Conte, A.Gazzaniga. Inert monolithic device with a central hole for constant drug release. *Eur. J. Pharm. Biopharm*. **40**(6): 370-373 (1994)

138. Ho-Wah Hui, Vincent H. L. Lee, Joseph R. Robinson. Design and fabrication of Oral Controlled Release Drug Delivery Systems. In In J.R.Robinson, V.H.L.Lee (eds.) *Controlled Drug Delivery: Fundamentals and Applications* **Volume 29** Marcel Dekker, New York and Basel, 1987, pp 373-432

139. M.A.Ibrahim, V.H.Dawes, A.B.Bangudu. The contributions of erosion, swelling, and porosity to ttheophylline release kinetics from *Cissus populnea* polymer matrices. *Drug.Dev.Ind.Pharm*. 26(5): 571-575 (2000)

140. A.Ainaoui, J.Siepmann, R.Bodmeier, J.M.Vergnaud. Calculation of the dimensions of dosage forms with release controlled by diffusion for in vivo use. *Eur. J. Pharm. Biopharm*. **51**: 17-24 (2001)

141. T.K.Mandal. The influence of binding solvents on drug release from HPMC tablets. *Drug. Dev. Ind. Pharm*. **21**(12): 1389-1397 (1995)

142. P.J.Sheskey, T.D.Cabelka, R.T.Robb, B.M.Boyce. The use of roller compaction in the preparation of controlled release hydrophilic matrix tablets containing methylcellulose and HPMC polymers. *Pharm. Tech*. 18(9): 132-150 (1994)

143. I.Katzhendler, K.Mader, M.Friedman. Structure and hydration properties of HPMC matrices containing naproxen and naproxen sodium. *Int. J. Pharm.* **200**: 161-179 (2000)

144. T.Ishikawa, Y.Watanabe, et al. Effect of Hydroxypropyl methylcellulose on the release profiles and bioavailability of a poorly water-soluble drug from tablets prepared using macrogols and HPMC. *Int. J. Pharm.* **202**: 173-178 (2000)

145. S.Baugartner *et al*. Optimisation of floating matrix tablets and evaluation of their gastric residence times. *Int. J. Pharm*. 195: 125-135 (2000)

146. J.J.Verner. *Formulation and Dissolution Assessment of a Novel Repeat Action Tablet containing a decongestant and an antihistamine.* MSc. Thesis, Rhodes University, November 2000

147. S.A.Botha and A.P.Lotter. Compatibility study between Naproxen and tablet excipients using differential scanning calorimetry. *Drug. Dev. Ind. Pharm* **16**(4): 673-683 (1990)

148. S.A.Botha and A.P.Lotter. Compatibility study between Ketoprofen and tablet excipients using differential scanning calorimetry. *Drug. Dev. Ind. Pharm* **15**(3): 415-426 (1989)

149. S.Venkataram, M.Khohlokwane, S.H.Wallis. Differential scanning calorimetry as a quick scanning technique for solid state stability studies. *Drug.Dev.Ind.Pharm* **21**(7): 847-855 (1995)

150. T.Durig A.R.Fassihi. Identification of stabilizing and destabilizing effects of excipient-drug interactions solid dosage form design. *Int. J. Pharm* 97: 161-170 (1993)

151. James.L.Ford, Peter.Timmins. *Pharmaceutical Thermal Analysis: Techniques and Applications* Ellis Horwood Limited, Chicester, 1989, pp 25-67, 213-216

152. E.K.Iyer, H.P.Tipnis. Preformulation compatibility study between metoprolol tartrate and tablet excipients using differential scanning calorimetry (DSC). *Indian J. Pharm. Sci.* 58(1): 22-24 (1996)

153. M.E.Brown, E.M.Antunes, B.D.Glass, M.Lebete, R.B.Walker. DSC screening of potential prochlorperazine-excipient interactions in preformulation studies. *J. Therm. Anal. Cal.* **56**: 1317-1322 (1999)

154. S.Wissing. D.Q.M.craig, S.A.Barker, W.D.Moore. An investigation into the use of stepwise isothermal high sensitivity DSC as a means of detecting drug-excipient incompatibility. *Int. J. Pharm.* **199**: 141-150 (2000)

155. J.Gabrielsson, A.Nyström, T.Lundstedt. Multivariate methods in developing an evolutionary strategy for tablet formulation. *Drug. Dev. Ind. Pharm* **26**(3): 275-296 (2000)

156. A.A van Dooren and B.W Muller. Purity determinations of drugs with DSC – A critical review. *Int J Pharm* **20**: 217-233 (1984)

157. P.Mura, M.T.Faucci, P.L.Parrini. Effects of grinding with mocrocrystalline cellulose and cyclodextrins on the ketoprofen physicochemical properties. *Drug. Dev. Ind. Pharm* 27(2): 119-128 (2001)

158. N.Passerini, D.Q.M.Craig. An investigation into the effects of residual water on the glass transition temperature of polyactide microsphers using modulated temperature DSC. *J. Contr. Rel*. **73**: 111-115 (2001)

159. *Handbook of Pharmaceutical Excipients.* A.Wade, P.J.Weller (eds.), The Pharmaceutical Press, Washington DC, USA, 1994, pp 56-60, 84-87, 229-231, 280-282, 628-629

160. T.Pesonen, H.Kanerva, J.Hirvonen, T.Nuuja, J.Pohjola. The incompatibilities between chlorhexidine diacetate and some tablet excipients. *Drug. Dev. Ind. Pharm*. 21(6): 747-752 (1995)

161. *Solid Pharmaceutics: Mechanical properties and rate phenomena*, J.T.Carstenson , Academic Press, New York, 1980, pp179-182

162. http://www.nbent.com/details.htm

163. X. Yu, R.H.Atalla. A staining technique for evaluating the pore structure variations of microcrystalline cellulose powders. *Powder Technology* **98**: 135-138 (1998)

164. P.W.S.Heng, J.N.Staniforth. The effect of moisture on the cohesive properties of microcrystalline celluloses. *J. Pharm. Pharmacol*. **40**: 360-362 (1988)

165. http://ethesis.helsinki.fi/julkaisut/mat/farma/vk.wetermarck/ch2.html

166. S.Yamamura, K.Terada, Y.Momose. Change of the microstructure of microcrystalline cellulose with grinding and compression. *J. Pharm. Pharmacol*. **49**: 1178-1181 (1997)

167. P.W.Heng, O.M.Y.Koo. A study of the effects of the physical characteristics of microcrystalline cellulose on performance in extrusion spheronisation. *Pharm, Res* **18** (4): 480-487 (2001)

168. R.E. O'Connor, J.B.Schwartz. Drug release mechanism from a microcrystalline cellulose pellet system. *Pharm. Res*. **10**(3): 356-361 (1993)

169. Peter Kleinebudde. Shrinking and swelling properties of pellets containing microcrystalline cellulose and low substituted hydroxypropylcellulose: I. Shrinking properties. *Int. J. Pharm* **109**: 209-219 (1994)

170. E.S.Ghali, G.H.Klinger and J.B.Shwartz. Modified drug release from beads prepared with combinations of two grades of microcrystalline cellulose. *Drug. Dev. Ind. Pharm*. **15**(9): 1455-1473 (1989)

171. A.M.Railkar, J.B.Schwartz. Evaluation and comparison of a moist granulation technique to conventional methods. *Drug. Dev. Ind. Pharm*. **26**(8): 885-889 (2000)

172. E.N.Hiestand. The Basis for Practical Applications of the Tableting Indices. *Pharm. Technol*. **Sept 1989**: 54-66

173. ShinEtsu. Metolose®, Water soluble cellulose ethers. *Product Information.* 12/1999

174. ShinEtsu. Metolose®, Sustained Release Agent for Matrix System. *Product Information*. 12/1999

175. Colorcon. Methocel®, *Product Information*. ME/C/00-02

176. Colorcon. Surelease® *Product Information* S/C/00-08 10/90

177. G.H.Klinger, E.S.Ghali, S.C.Porter, J.B.Schwartz. Formulation of controlled release matrices by granulation with a polymer dispersion. *Drug. Dev. Ind .Pharm*. **16**(9): 1473-1490 (1990)

178. P.W.S.Heng, L.W.Chan, M.G.Easterbrook, X.Li. Investigation of the influence of mean HPMC particle size and number of polymer particles on the release of aspirin from swellable hydrophilic matrix tablets. *J. Contr. Rel*. **76**: 39-49 (2001)

179. S.Şenel, Y.Çapan, A.A.Hncal. Factors affecting the formulation of sustained release potassium chloride tablets. In M.H.Rubinstein (ed.) *Pharmaceutical Technology*: *Controlled drug release*. **Volume 2**, Ellis Horwood Ltd, Chicester, 1991, pp 34-43

180. E.Horisawa, K.Danjo, H.Sunada. Influence of granulating methods on physical and mechanical properties, compression behaviour, and compactibility of lactose and MCC granules. *Drug. Dev. Ind. Pharm*. **26**(6): 583-593 (2000)

181. Ruey-ching Hwang, Gretchen.R.Peck. A systematic evaluation of the compression and tablet characteristics of various types of microcrystalline cellulose. *Pharm Tech*. **25**(3): 112-130 (2001)

182. D.Sixsmith. The properties of tablets containing microcrystalline cellulose. *J. Pharm. Pharmac*. **29**: 82-85 (1977)

183. M.C.Gohel, M.K.Panchal. Comparison of in vitro dissolution profiles using a novel, model-independent approach. *Pharm. Tech*. **24**(3): 92-102

184. B.Sievert, M.Siewert. Dissolution tests for ER products. *Dissolution Technology.* 5(4) (1999)

185. V.P.Shah, R.L.Williams. Roles of dissolution Testing: Regulatory, Industry and Academic Perspectives. Role of Dissolution Testing in Regulating Pharamaceuticals. *Dissolution Technologies*. 6(3) (1999)

186. Guidance for Industry, SUPAC-MR: Modified Release Solid Oral Dosage Forms, CDER, Div. Of Bioequivalence, FDA, OGD, Rockville, USA, (1997)

187. R.Löbenberg, G.L.Amidon. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. *Eur. J. Pharm. Biopharm*. **50**: 3-12 (2000)

188. J.R.Crison. Developing Dissolution tests for modified release dosage forms: General considerations. *Dissolution Technologies.* 6(1) (1999)

189. J.Dressman. Roles of dissolution testing: Regulatory, Industry and Academic perspectives, Future directions for academic research on dissolution testing. *Dissolution Technologies.* 6(2) (1999)

190. J.Dressman, J.Butler, J.Hempenstall, C.Reppas. The BCS: Where do we go from here? *Pharm Tech*. 25 **(6)**: 68-76 2001

191. A.S.Hussain, L.J.Lesko, K.Y.Lo, V.P.Shah, D.Volpe, R.L.Williams. The biopharmaceutics classification system: Highlights of the FDA's draft guidance. *Dissolution Technologies.* 6(2) (1999)

192. N.E.Eddington, G.Singh Rekhi, L.J.Lesko, L.L.Augsburger. Scale-Up Effects on dissolution and Bioavailability of propranolol hydrchloride and metoprolol tartrate tablet formulations. *AAPS PharmSci Technol* **1**(2) Article 14 (2000)

193. FDA Guidance for industry: Waiver of in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms containing certain active moieities/active ingredients based on biopharmaceutics classification system. July 1999

194. FDA Guidance for industry: Oral extended (controlled) release dosage forms *In vivo* bioequivalence and *in vitro* dissolution testing.

195. W.H.Barr. Modified release dosage forms: Proposals for dosage form classification systems. In G.L.Amidon, J.R.Robinson and R.L.Williams (eds.), *Scientific Foundations for Regulating Drug Product Quality*, AAPS, USA, 1997, pp 119-125

196. N.Sirisuth, N.D.Eddington. Influence of stereoselective pharmacokinetics in the development and predictability of an IVIVC for the enantiomers of metoprolol tartrate. *Pharm. Res*. **17**(8): 1019-1025 (2000)

197. L.H.Emara, B.S.El-Menshawi, M.Y.Estefan. In Vitro-In Vivo correlation and comparative bioavailability of vincamine in prolonged-release preparations. *Drug. Dev. Ind. Pharm.* **26**(3): 243-251 (2000)

198. L.P.Balant, M.Gex-Fabry. Modelling during drug development. *Eur. J. Pharm. Biopharm*. **50**:13-26 (2000)

199. N.D.Eddington, P.Marroum, R.Uppoor, A.Hussain, L.Augsburger. Development and internal validation of an *in vitro-in vivo* correlation for a hydrophilic metoprolol tatrate tablet formulation. *Pharm. Research*. **15**(3): 466-472 (1998)

200. J.E.Polli, G.Singh Rekhi. Methods to compare dissolution profiles. *Drug. Info. J.* **30**: 1113-1120 (1996)

201. J.E.Polli, G.Singh Rekhi, H.L.Augsburger, V.P.Shah. Methods to compare dissolution profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets. *J. Pharm. Sci* **86**(6): 690-700 (1997)

202. P.Costa, J.M.S.Lobo. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* **13**:123-133 (2001)

203. Aldo rescigno. Bioequivalence. *Pharm Res.* **9**(7): 925-928 (1992)

204. J.W.Moore, H.H.Flanner. Mathematical comparison of dissolution profiles. *Pharm. Tech*. **20**(6): 64-74 (1996)

205. V.P.Shah, Y.Tsong, P.Sathe, R.L.Williams. Dissolution Profile Comparison Using Similarity Factor, f2. **6**(3) (1999)

206. H.Sarandasa. Defining similarity of dissolution profiles through Hotelling's T^2 statistic. *Pharm Tech*. **25**(2): 46-54 (2001)

207. V.Pillay, R.J.Fassihi. Evaluation and comparison of dissolution data derived from different modified-release dosage forms: An alternative method. *J. Control. Rel*. **55**(1): 45-55 (1998)

208. T.X.Viegas, R.U.Curatella, L.L.Van Winkle, G.Brinker. Measurement of intrinsic drug dissolution rates using two types of apparatus. *Pharm. Tech.* **25**(2): 44-53 (2001)

209. P.Shesky, G.Sackett, L.Maher, K.Lentz, S.Tolle, J.Polli. Roll compaction granulation of a controlled-release matrix tablet formulation containing HPMC. In *Tabletting and Granulation Yearbook* **1999**

210. M-T Wang, F-H Tsai, D-P Wang, Formulation optiomization of controlled release pellets of metoclopramide hydrochloride using dissolution fit factor approach. *Drug. Dev. Ind. Pharm*. **26**(5): 577-581 (2000)

211. Frieder Langenbucher. IVIVC: Indices for comparing release and response profiles. *Drug. Dev. Ind. Pharm*. **25**(11): 1223-1225 (1999)

212. V.P.Shah, Y.Tsong, P.Sathe, J-P Liu. *In Vitro* dissolution profile comparison-Statistics and analysis of the similarity factor, f2. *Pharm Research*. **15**(6): 889-896 (1998)

213. I.Borst, S.Ugwu, A.H.Beckett. New and extended applications for USP Drug Release Apparatus 3. *Dissolution Technologies* February 1997

214. H.Madden, J.Butler, J.Devane. Impact of apparatus type and hydronamics on the release of a highly soluble drug from a hydrophilic matrix tablet. In *PharmSci*, AAPS, Virginia, **Volume 1**, November 1988, pp 643

215. H.Madden, J,Butler, J.Polli, S.Madden, O.Corrigan and J.Devane. Impact of dissolution medium on the release of a highly water soluble drug from a hydrophilic matrix tablet. In *PharmSci*, AAPS, Virginia, **Volume 1**, November 1998, pp643

216. M.T.Marin Bosća, J.Ollero Hinojosa, M.D.Contreas Claramonte, I.Ismail Salem. Statistical comparison of two methods of dissolution of sustained-release theophylline tablets. *Drug. Dev. Ind. Pharm*. **22**(7): 595-601 (1996)

217. K.K.Peh, C.F.Wong. Application of similarity factor in development of controlled-release diltiazem tablet. *Drug. Dev. Ind. Pharm*. **26**(7): 723-730 (2000)

218. A.Sandberg, B.Abrahmsson, J.Sjögren. Influence of dissolution rate on the extent and rate of bioavailability of metoprolol. *Int. J. Pharm*. **68**: 167-177 (1991)

219. N.E.Eddington, G.Singh Rekhi, L.J.Lesko, L.L.Augsburger. Scale-Up Effects on dissolution and Bioavailability of propranolol hydrchloride and metoprolol tartrate tablet formulations. *AAPS PharmSci Tech* **1**(2) Article 14 (2000)

220. S.Baumgartner, J.Kristl, F.Vrečer, P.Vodopivec, B.Zorko. Optimisation of floating matrix tablets and evaluation of their gastric residence time. *Int. J. Pharm*. **195**: 125-135 (2000)

221. BF Goodrich Bulletin 17: Controlled Release tablets and capsules. Retrieved from http://www.pharma.noveoninc.com/literature/bulletin/epb17.pdf

222. Tarun.K.Mandal. The influence of binding solvents on drug release from Hydroxypropyl methylcellulose tablets. *Drug. Dev. Ind. Pharm.* **21**(2): 1389-1397 (1995)

223. S.Li, S.Lin, Y.W.Chien, B.P.Daggy, H.L.Mirchandani. Statistical Optimization of gastric floating system for oral controlled delivery of calcium. *AAPS Pharm.Sci.Tech* **2**(1): Article 1 (2001)

224. C.S.Brazel, N.A.Peppas. Modeling of drug release from swellable polymers. *Eur. J. Pharm. Biopharm*. **49**: 48-57 (2000)

225. P.Frutos, C.Pabón, J.L.Lastres, G.Frutos. In Vitro Release of metoclopramide from hydrophobic matrix tablets. Influence of hydrodynamic conditions on kinetic release parameters. *Chem. Pharm. Bull* **49**(10): 1267-1271 (2001)

226 ICH Harmonised Tripartite Guideline on Stability Testing for New Drug and Products. November 1996.

227. B.Kommanaboyina, C.T.Rhodes. Trends in stability testing, with emphasis on stability during distrbution and storage. *Drug. Dev. Ind. Pharm*. **25**(7): 857-868 (1999)

228. S. Singh. Drug stability tesing and shelf-life determination according to international guidelines. *Pharm.Tech*. 18(6): 69-88 (1989)

229. B.R.Mattews. Regulatory aspects of stability testing in Europe. *Drug. Dev. Ind. Pharm.* **25**(7): 831-856

230. I.T.Some, P.Bogaerts, R.Hanus, M.Hanocq, J.Dubois. Improved kinetic parameter estimation in pH-profile data treatment. *Int. J. Pharm*. **198**: 39-49 (2000)

231. http://www.australianprescriber.com/magazines/vol17no2/expiry.htm Retrieved 14/06/01

232. S.Singh. Stability test storage conditions for Zones III and IV- some unresolved issues. *Pharm. Tech.* **23**(10): 130-142 (1999)

233. D.Peranac, F.Van Houtte, E.Roets, J.Hoogmartens. A Stability Study of Ticlopidine Products from 18 Countries. *Drug. Dev. Ind. Pharm.* **26**(4): 391-401 (2000)

234. http://www. cmcissues.com/stability/stability_related-definitions.htm Retrieved 14/06/01

235. S.R. Vaithiyalingam, V. Agarwal, I.K. Reddy, M.Ashraf, M.A.Khan. Formulation development and Stabilty Evaluation of a Multicomponent nutrional supplement. *Pharm.Tech*. **25**(4): 38-48 (2001)

236. M.J.Mosquera, S.Cal, C.Souto, A.Concheiro, R.Martinez-Pacheco, J.L.Gómez-Amoza. Effects of storage humidity on the mechanical, microstructural, and drug release properties of hydroxypropylmethylcellulose-based hydrophilic matrix tablets. *Drug. Dev. Ind. Pharm*. **23**(4); 403-406 (1997)

237. P. Luukkonen, T. Schaefer, F. Podczek, M. Newton, L. Hellén, J. Yliruusi. Characterization of microcrystalline cellulose and silicified microcrystalline cellulose wet masses using a powder rheometer. *Eur. J. Pharm. Sci*. 13: 143-149 (2001)

238. C.J.Taborsky, U.Mehta, M.Kusz, G.Caspersen. Multiple-Unit packaging for solid oral dosage forms. *Pharm.Tech*. 24(3): 44-51 (2000)

239. http://www.devicelink.com/pmpn/archive/99/04/002.html Retrieved 14/06/01

240. http://www.pharmnet.co.za/mcc/ph_analysis/A10-1997.htm Retrieved 28/10/01

241. Th. P.G.M. de Vries. R.H.Henning, H.V.Hogerzil, D.A.Fresle: WHO Guide to Good Prescribing – A Practical Manual. World Health Organization, 1995

242. S.Oparil, D.A.Calhoun. Managing the patient with Hard-to-Control hypertension. *American Family Physician*, 1 March, (1998)

243. N.S.Skolnik, J.D.Beck, M.Clark. *American Family Physician*. 15 May, 2000

244. J.M.Conrad, J.R.Robinson. Sustained Drug Release from Tablets and Particles Through Coating. In H.A.Lieberman, L.Lachman (eds.) *Pharmaceutical Dosage Forms: Tablets*, Volume 3, Marcel Dekker New York, 1982, pp 149-222

245. K.H.Yuen, A.A.Deshmukh, J.M.Newton. Development and in-vivo evaluation of a multiparticulate sustained release theophylline formulation. *Drug. Dev. Ind. Pharm*. **19**(8): 855-874 (1993)

246. F.Theeuwes, W.Boyce. Controlled Release Dosage Form Design. In J.Urquhart (ed.), *Controlled-Release Pharmaceuticals*, American Pharmaceutical Association, Washington, USA, 1981, pp 61-93

247. L.Liu, G.Khang, J.M.Rhee, H.B.Lee. Monolithic osmotic tablet system for nifedipine delivery. *J. Contr. Rel*, **67**: 309-322 (2000)

248. R.K.Verma, B.Mishra, S.Garg. Osmotically Controlled Oral Drug Delivery. *Drug. Dev. Ind. Pharm*. **26**(7): 695-708 (2000)

249. R.K.Verma, S.Garg. Current Status of Drug delivery Technologies and Future Directions. *Pharmaceutical Technology On-Line* **25**(2): 1-14 (2001)

250. J.T.Fell, L.Whitehead, J.H.Collett. Prolonged Gastric Retention Using Floating Dosage Forms. *Pharm Tech*. **24**(3): 82-90 (2000)

251. G.F.Plamieri, S.Michelini, P.Di Martino, S.Martell. Polymers with pH-Dependent Solubility: Possibility of Use in the Formulation of Gastroresistant and Controlled-Release Matrix Tablets. *Drug. Dev. Ind. Pharm*. **26**(8): 837-845 (2000)

252. K.K.Peh, K-H Yuen. Development and in-vitro evaluation of a novel multiparticulate matrix controlled release formulation of theophylline. *Drug. Dev. Ind. Pharm.* **21**(30: 1545-1555 (1995)

253. K.G.Wagner, M.Krumme, T.E.Beckert, P.C.Schmidt. Development of disintergrating multi-unit tablets on a high-speed rotary press. *Eur. J. Pharm*. **50**: 285-291 (2000)

254. *Pharmaceutics: The science of dosage form design*, M.E.Aulton (ed.), Churchill Livingstone, New York 1988, pp 669-677

255. *Tablets and Tabletting*, H.Burlinson (ed.), William Heinnemann Medical books Ltd, London, 1968, pp 48-54

256. K.Marshall, E.M.Ridric, Tablet Dosage Forms In *Modern Pharmaceutics*, Volume 40, G.S.Banker, G.T.Rhodes (eds.). Marcel Dekker Inc, 1990, pp 388-400

257. M.J.Robinson, Coating of pharmaceutical dosage forms. In *Remington's Pharmaceutical Sciences*, 16th Edition Arthur Osol, G.D.Chase, A.R.Gennaro, M.R.Gibson, G.L.Zink, S.C Harvey, R.E.King, A.N.Martin, E.A.Swinyard (eds.), Merck Publishing, Easton, Pennsylvannia, 1980

258. M.Z.I.Khan, H.P.Štedul, N.Kurjaković. A pH-Dependent colon-targeted oral drug delivery system using methacrylic acid copolymers. II.Manipulation of drug release using Eudragit® L100 and Eudragit S100 combinations. *Drug. Dev. Ind. Pharm*. **26**(5): 549-554 (2000)

259. Kachrimanis, I.Nikolakakis, S.Malmataris. Spherical crystal agglomeration of ibuprofen by the solvent-change technique in presence of methacrylic polymers. *J. Pharm. Sci*. **89**(2): 250-259 (2000)

260. C.Dangel, K.Kolter, H-B.Reich, G.Schepky. Aqueous enteric coatings with methacrylic acid copolymer Type C on acidic and basic drugs containing indomethacin and diclofenac sodium. *Pharm Tech.* **24**(4): 36-42 (2000)

261. C.Dangel, K.Kolter, H-B.Reich, G.Schepky. Aqueous enteric coatings with methacrylic acid copolymer Type C on acidic and basic drugs in tablets and pellets. Part I: Acetylsalicylic acid tablets and crystals. *Pharm. Tech*. **24** (4): 64-68 (2000)

262. R.O.Williams III, J.Liu. Influence of processing and curing conditions on beads coated with an aqueous dispersion of cellulose acetate phthalate. *Eur. J. Pharm. Biopharm*. **40**: 243-252 (2000)

263. C.Porter, J.E.Hogan. Tablet film-coating. *Pharmacy Int*. May: 122-127 (1984)

264. S.R.Béchard, J.C.leroux. Coated palletized dosage form: Effect of compaction on drug release. *Drug. Dev. Ind. Pharm*. **18**(18): 1927-1944 (1992)

265. F.Sadeghi, J.L.Ford, M.H.Rubinstein, A.R.Rajabi-Siahboomi. Comparative study of drug release from pellets coated with HPMC or Surelease. *Drug. Dev. Ind. Pharm*. **26**(6): 651-660 (2000)

266. F.Sadeghi, J.L.Ford, M.H.Rubinstein, A.R.Rajabi-Siahboomi. Study of drug release from pellets coated with Surelease containing hydroxypropylmethylcellulose. *Drug. Dev. Ind. Pharm*. **27**(5): 419-430 (2001)

267. M.Wesseling, R.Bodmeier. Drug release from beads coated with am aqueous colloidal ethylcellulose dispersion, Aquacoat®, or an organic ethylcellulose solution. *Eur. J. Pharm. Biopharm*. **47**: 33-38 (1999)

268. T.Wu, W.Pan, J.Chen, R.Zhang. Studies of the drug permeability and mechanical properties of free films prepared by cellulose acetate psuedolatex coating system. *Drug. Dev. Ind. Pharm*. **26**(1): 95-102 (2000)

269. D.M.Parikh. Fluid bed drying, agglomeration and coating technology. Workshop presented at the 1990 Powder and Bulk Solids Conference, Chicago, Illinois 1990

270. T.U.Okarter, K.Singla. The effects of plasticisers on the release of metoprolol tartrate from granules coated with a polymethacrylate film. *Drug. Dev. Ind. Pharm*. **26**(3): 323-329 (2000)

271. F.W.Goodhart, M.R.Harris, K.S.Murthy, R.U.Nesbitt. An evaluation of aqueous film-forming dispersions for controlled release. *Pharm. Technol* **April 1984**: 65-71

272. D.Jones. Air suspension coating for multiparticulates. *Drug. Dev. Ind. Pharm*. **20**(20): 3175-3206 (1994)

273. Orapin.P.Ribino. Fluid-Bed Technology overview and Criteria for Process selection. *Pharm. Tech*. **23**(6): 105-113 (1999)

274. A.Faham, P.Prinderre, N.Farah, K.D.Eichler, G.Kalantzis, J.Joachim. Hot-melt technology. I. Influence of Compritol 888 Ato and granule size on theophylline release. *Drug. Dev. Ind. Pharm*. **26**(2): 167-176 (2000)

275. H.Ichikawa, Y.Fukumori. A novel positively thermosensitive controlled-release microcapsule with membrane of nano-sized pol(N-isopropylacrylamide) gel dispersed in ethylcellulose matrix. *J. Contr. Rel*. **63**: 107-119 (2000)

276. D.E.Wurster. Particle-Coating Methods. In Pharmaceutical Dosage Forms: Tablets, Volume 3, H.A.Lieberman, L.Lachman (eds.), Marcel Dekker, New York, 1982, pp 120-148

277. N.R.Anderson, G.S.Banker, G.E.Peck. Principles of Improved Tablet Production System Design. In Pharmaceutical Dosage Forms: Tablets, Volume 3, H.A.Lieberman, L.Lachman (eds.), Marcel Dekker, New York, 1982, pp 41-46

278. R.C.Rowe. Film-coating-the ideal process for the production of modified-release oral dosage forms. *Pharmacy. Int*. Jan 1985: 14-17

279. R.C.Rowe. The expansion and contraction of tablets during film-coating – a possible contributory factor in the creation of stresses within the film? *J. Pharm. Pharmacol*. **32**: 851 (1980)

280. Lucy S.C.Wan, W.F.Lai. Factors affecting drug release from drug-coated granules prepared by fluidized-bed coating. *Int. J. Pharm*. **72**: 163-174 (1991)

281. R.C.Rowe. The effect of some formulation and process variables on the surface roughness of film-coated tablets. *J. Pharm. Pharmac*. **30**: 669-672 (1978)

282. G.S.Rekhi, R.W.Mendes, S.C.Porter, S.S.Jambekhar. Aqueous polymeric dispersions for controlled drug delivery- Wurster process. *Pharm. Tech*. **13**(3): 112-125 (1989)

283. H.Murakami, M.Kobayashi, H.Takeuchi, Y.Kawashima. Utilization of poly (DL-lactide-co-glycolide) nanoparticles for preparation of mini-depot tablets by direct compression. J. Contr. Rel. **67**: 29-36 (2000)

284. J.J Verner, R.B.Walker. Effect of an Ethylcellulose Coat on the Release of Pseudoephedrine Sulfate from Hydrophilic Matrix Tablets. Poster presented at The Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists, Indianapolis, Indiana, USA 27 October- 2 November 2000

285. J.Herman, J.P.Remon, R.Lefebvre, M.Bogaert, G.H.Klinger, J.B.Schwartz. The Dissolution Rate and Bioavailability of Hydrochlorothiazide in Pellet Formulations. *J. Pharm. Pharmacol*. **40**: 157-160 (1988)

.