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Optimisation of Once-a-Day Oral Sustained-Release Formulations for The Novel Antihypertensive Drug MT-1207

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Declaration

Unless otherwise acknowledged, the work presented in "**Optimisation of Once-a-Day Oral Sustained-Release Formulations for The Novel Antihypertensive Drug MT-1207**" is my own work. No part has been submitted for any other degree at The University of Nottingham or any other institution.

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Abstract

Background: Hypertension is a common chronic disease that increases the risk of cardiovascular disease. Sustained-release formulations are designed to maintain therapeutic levels of drugs throughout the course of hypertension treatment, thereby increasing patient compliance and enhancing patient outcomes. MT-1207 is a novel multi-target antihypertensive agent that has been indicated to be effective in treating hypertension.

Objective: This project is focused on the optimisation of a once-a-day oral sustained-release tablet of MT-1207 that may provide better properties, *in vitro* and *in vivo* sustained-release behaviour than the one developed previously by Vrettos et al.

Design and methods: Tablets containing different polymers, including HPMC K15M, HPMC K100M, Kollidon® SR and, Eudragit® RSPO, as well as combinations of them were prepared and characterised in terms of pre- and post-compression parameters, physicochemical properties and *in vitro* drug release. Apart from these tests, kinetic analysis was also conducted on the *in vitro* release data of the optimised formulation, along with *in vitro* release testing at 200 rpm.

Results: Each of the formulations tested in the project showed good flowability and compressibility. The most promising formulation containing 31% w/w HPMC K15M and 20%w/w Kollidon® SR provided a complete 24-hour sustained release of MT-1207 with more than 80% of the drug released at the 24-hour timepoint. The physicochemical characterisation showed that the drug was compatible with all the excipients. The drug release followed first-order model kinetics and the mechanism of release was found to be non-Fickian diffusion. Finally, the *in vitro* release data at 200 rpm had no statistically

significant difference to those collected at 75 rpm which indicated that the tablets could probably provide a robust sustained release for *in vivo* study.

Conclusion: In the present study, a new 24-hour sustained-release tablet for MT-1207 was successfully optimised and could be considered for further *in vivo* tests.

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List of Abbreviation

%	Percentage
°C	Degree Celsius
µg	Microgram
µL	Microlitre
µm	Micrometres
5-HT	Serotonin
ABPM	Ambulatory Blood Pressure Monitoring
ACE	Angiotensin Converting Enzyme
ACEI	Angiotensin Converting Enzyme Inhibitors
API	Active Pharmaceutical Ingredients
ARBs	Angiotensin Receptor Blockers
ATR-FTIR	Attenuated Total Reflectance Fourier-Transform Infrared
AV	Atrioventricular
Carr index	Compressibility Index
CCB	Calcium Channel Blockers
cm	Centimetre
d	Day
DBP	Diastolic Blood Pressure
DSC	Differential Scanning Calorimetry
DVB	Divinylbenzene
EOP	Elementary Osmotic Pump
f1	Difference Factor
f2	Similarity Factor
FDA	Food And Drug Administration
GIT	Gastrointestinal Tract
HBP	High Blood Pressure
HBPM	Home Blood Pressure Monitoring
HPLC-UV	High-Performance Liquid Chromatography-Ultraviolet
HPMC	Hydroxypropylmethylcellulose
HPMC K100M	Hydroxypropyl Methylcellulose Average Molecular Weight 120000
HPMC K15M	Hydroxypropyl Methylcellulose Average Molecular Weight 90000
IPA	Isopropyl Alcohol
kg	Kilogram
M	Molar
mA	Milliampere
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre

MR	Mineralocorticoid Receptors
mV	Millivolt
ng	Nanogram
NICE	National Institute For Health And Care Excellence
nm	Nanometre
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PEO	Polyethylene Oxide
PPOP	Push-Pull Osmotic Pump
PVA	Polyvinyl Acetate
PVP	Polyvinylpyrrolidone
PVP K30	Polyvinylpyrrolidone K30
RAAS	Renin-Angiotensin-Aldosterone System
rpm	Revolution Per Minute
SA	Sinoatrial
SDS	Sodium Dodecyl Sulfate
SHR	Hypertensive Rats
SNS	Sympathetic Nervous System
SPM	Semipermeable Membrane
USP	United States Pharmacopeia
vs	Versus
WHO	World Health Organization
XRPD	X-Ray Powder Diffraction
α	Alpha
β	Beta

1. Introduction

1.1 Hypertension

Hypertension, often known as high blood pressure (HBP), has been recognised as a serious independent risk factor for numerous diseases, especially cardiovascular disease[1]. According to the guidelines published by World Health Organization (WHO) in 2021, there are approximately 1.4 billion individuals worldwide affected by hypertension, but just 14 % have it under control[2]. Hypertension has been also called a "silent killer" as most patients normally cannot notice any symptoms or warning signs, so it may cause stroke suddenly and damage to the heart and blood vessels in vital organs such as the brain and kidneys[3]. Every year, hypertension complications kill 9.4 million people globally[4]. For this reason, it is essential to have regular blood pressure measurements.

1.1.1 Types and diagnosis

Clinically, hypertension is categorised into two types, which include primary and secondary hypertension[5]. Primary hypertension that also called essential hypertension is the most prevalent hypertension type, which is an independent disease with elevated blood pressure as the main clinical manifestation. The disease accounts for 90-95 % of the total number of patients with hypertension. Although the causes of primary hypertension remain unknown, factors such as age, excess salt intake, obesity, smoking, alcohol consumption, sedentary lifestyle, and gene have all been linked to primary hypertension[6].

Far less commonly, the remaining 5–10 % of cases of HBP are classified as secondary hypertension, which generally occur at an earlier age and no family history. The etiology of

secondary hypertension is clear, and it is associated with another medical condition or a side effect of treatment. Such conditions include the chronic renal disease, renovascular disease, pregnancy, and endocrine disorder. Drugs that may cause hypertension include non-steroidal anti-inflammatory drugs (NSAIDs), oral contraceptive, and corticosteroid[7, 8].

In the United Kingdom, as per the National Institute for Health and Care Excellence (NICE), the cut-off point between normal blood pressure and hypertension is defined as a sustained systolic blood pressure (SBP) ≥140 mm Hg or diastolic blood pressure (DBP) ≥90 mm Hg following repeated tests. If the clinic blood pressure is between 140/90 mm Hg and 180/120 mm Hg, ambulatory blood pressure monitoring (ABPM) or home blood pressure monitoring (HBPM) with an average of 135/85 mmHg or higher can also be utilised for hypertension diagnosis[9, 10].

1.1.2 Regulation of blood pressure

Blood pressure can provide information on the functioning of the heart and blood arteries in the body. Maintaining the blood pressure within normal limits can help prevent cardiovascular disease and death[11]. Numerous physiological systems are required to maintain normal blood pressure, and their disruption may contribute to the development of essential hypertension.

The renin-angiotensin-aldosterone system (RAAS) may be the most critical of the endocrine systems involved in blood pressure regulation. Renin, an aspartyl protease enzyme, is produced and released from kidney juxtaglomerular cells in response to a variety of stimuli. These stimuli contain generate increased sympathetic nervous system (SNS)

activity, decreased perfusion pressure in the renal afferent arterioles, decreased sodium chloride content in the macula densa segment of the renal distal tubules, and local actions of nitric oxide and prostanoids[12]. Once renin has been released into the blood, it can act on its target, angiotensinogen. Angiotensinogen is a precursor protein that is synthesised in the liver and cleaved by renin to create angiotensin I (Ang I). The angiotensin converting enzyme (ACE) then converts angiotensin I to angiotensin II. Angiotensin II (ang II) is the primary effector peptide of the RAAS, and it plays a key role in the maintenance of normal blood pressure levels[13]. The high level of Ang II levels are known to cause hypertension[14]. Angiotensin II also acts on the adrenal cortex to stimulate the release of aldosterone. Aldosterone is a mineralocorticoid, a steroid hormone secreted from the zona glomerulosa of the adrenal cortex. Aldosterone can affect renal and vascular directly by activating mineralocorticoid receptors (MR)[15]. The activation of MR in the nucleus can promote sodium retention and elevate blood pressure. If the cell surface MR is activated, it will result in vasoconstriction[16].

1.1.3 Pharmacological treatment of hypertension

Hypertension is a chronic illness that can lead to significant complications if the patient's blood pressure is not under control. Pharmacological medication is the main hypertension management[17].

Preferred antihypertensive drugs include ACE inhibitors (ACEI), angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs) and diuretics, although no specific class of first-line drugs has shown superiority[2]. Other types of antihypertensive medications

like alpha (α) and beta (β) blockers should be reserved for patients who still have hypertension on two or more of the preferred medications.

1.1.3.1 Agents that block production or action of angiotensin

For individuals who are not African-American but with type 2 diabetes or who are younger than 55 years of age, ACEI and ARB are the first choice of antihypertensive medications[9]. ACEI inhibits the activity of ACE, preventing it from converting Ang I to Ang II. Thus, block the formation of the vasoconstrictor Ang II, resulting in a lowering of BP. Additionally, ACE inhibitors reduce the breakdown of bradykinin, a peptide associated with vasodilation to achieve vascular protective actions[15]. Frequently prescribed ACEI include captopril, enalapril, lisinopril, perindopril and ramipril. ACEIs, on the other hand, have some negative effects like dry cough. ACEIs cause a dry cough in roughly 1.5-11 % of patients[18], and this dry cough goes away quickly after the ACEI is discontinued. The most widely accepted explanation is that bradykinin and substance P are degraded by ACE and then accumulated in the upper and lower respiratory tracts by ACEIs. Bradykinin increases the sensitivity of the airway sensory nerves by releasing neurokinin A and substance P from stretch receptors that adapt quickly and C-fiber receptors. As a result, the smooth muscle in the airways tightens, causing bronchospasm and a cough[19].

If an ACEI is not tolerated, for example, if the dry cough does not improve, an ARB should be considered as another type of antihypertensive drugs. ARB is a kind of antihypertensive drugs acting on RAAS after ACEI, which also has good curative effect on hypertension and cardiovascular diseases. Although there are many similarities between

ARB and ACEI in antihypertensive and cardiovascular protection, ARB directly acts on Ang II receptor, blocking RAAS more fully and directly, avoiding "Ang II escape"[18], and has a good antihypertensive effect. ARB can be divided into 3 categories: i) dibenzimidazole, such as losartan, irbesartan; ii) non-dibenzimidazole, such as ibesartan; iii) non-heterocyclic, such as valsartan. ARB is suitable for mild, moderate and severe hypertension patients. In addition to antihypertensive effect, ARB also has the effect of protecting the cardiovascular system and improving glucose metabolism [20-22].

1.1.3.2 Calcium Channel Blockers

CCBs are also first-line antihypertensive drugs for black patients and people at age ≥ 55 as well as no type 2 diabetes[9]. CCBs restrict the flow of extracellular calcium across the cell wall via ion-specific channels. Although various forms of similar channels have been identified, the currently available CCBs inhibit the L-type channels in humans[23]. When the inward calcium flux is stopped, the smooth muscle cells of the blood vessels relax, resulting in vasodilation and a decrease in blood pressure. Contractility is decreased in heart muscle, and sinus pacemaker and atrioventricular conduction velocities are retarded[24]. Calcium channel antagonists are classified into two broad categories depending on their primary physiologic effects. They are non-dihydropyridines and dihydropyridines, respectively. These two subclasses of drugs bind to separate locations on the L-type calcium channel[25].

The non-dihydropyridine CCB, such as verapamil and diltiazem, have inhibitory effects on the sinoatrial (SA) and atrioventricular (AV) nodes, resulting in a slowing of cardiac conduction and contractility[26]. The patients with heart failure, low ejection fraction,

second or third degree AV blockage, or sick sinus syndrome should not take non-dihydropyridines since they can produce bradycardia and worsen cardiac output[27].

The dihydropyridine CCBs, such as amlodipine and felodipine, have greater vascular selectivity and fewer cardiac side effects. They can be used to treat high blood pressure and angina[27]. Sustained release formulations of diltiazem, and verapamil have been developed to reduce their side effects associated with their quick onset and short duration of action[28]. According to NICE guidelines, CCBs are licensed for use in conjunction with a number of other antihypertensive drugs in Step 2 treatment, including ACEI and ARB[9, 29, 30].

1.1.3.3 Diuretics

Diuretics are frequently utilised as first-line antihypertensive medications in patients who are intolerant to CCB or who have heart failure[9]. Diuretics aid in the removal of excess salt (sodium) and water from the body. The majority of these medications assist the kidneys in excreting more sodium into your urine. Sodium helps in the removal of water from the blood, thereby decreasing the amount of fluid that flows through veins and arteries. This results in a decrease in blood pressure[31]. The three types of diuretic medications are called thiazide, loop, and potassium-sparing diuretics. While thiazide diuretics are the most commonly prescribed, loop diuretics are also effective, and when combined with a potassium sparing diuretic, the risk of hypokalaemia and hypomagnesaemia is reduced[32].

1.1.3.4 Beta-blockers and alpha-blockers

β blockers are another class of antihypertensive drugs, although they are no longer the

first choice for initial treatment of most patients. They work by preventing the hormone adrenaline from binding to β receptors. But β receptors are located throughout the body and are involved in a wide variety of physiological processes, so β blocker drugs can have a variety of unwanted effects when they are used to block these receptors[33]. Bradycardia and hypotension are two common side effects. β-blockers can be divided into: 1) non-selective β receptor blockers; 2) Selective β_1 receptor blockers. β blockers selectively bind to β_1 receptors to produce better antihypertensive effects and less side effects than non-selective β blockers, such as reduced cardiac output, renin release, and central sympathetic impulses[34]. The 2016 Canadian guidelines do not recommend β blockers as the first choice for elderly patients with hypertension[35].

As for α blockers, according to their selectivity to receptor subtypes, α blockers can be divided into three categories: non-selective α-blockers, selective α_1 blockers, and selective α_2 blockers, which have different therapeutic effects[36]. At present, the peripheral alpha-blockers commonly used in clinic to treat hypertension include terazosin, prazosin, doxazosin.

However, α_1 adrenergic antagonists are only advised as supplementary therapy, not as monotherapy, for hypertension. Postural hypotension is especially prevalent following the first dose of the α_1 adrenergic antagonist[37]. In the European Guidelines for Hypertension in 2013, α blockers have not been used as first-line antihypertensive drugs. In general, combined use of α blockers can be considered for patients who still cannot control blood pressure satisfactorily after sufficient application of diuretics, CCB, ACEI and ARB[38, 39].

Although α blockers are generally not the first choice, it can effectively help reduce

blood pressure when the combined use of first-line antihypertensive drugs is invalid[40]. Besides, α receptor tissue agents can also be combined with β blockers for the antihypertensive treatment of pheochromocytoma patients[41]. The administration of alpha-blockers in the weeks prior to surgery counteracts the effects of the excess hormones secreted by the pheochromocytoma, thereby lowering the frequency and severity of dangerous intra-operative and post-operative blood pressure fluctuations[42]. Furthermore, α-blockers have great potential in the clinical treatment of patients with hypertension and prostatic hyperplasia in addition to the treatment of patients with refractory hypertension and oncocytoma[43]. Studies have shown that there are a lot of α receptors in the mucosal smooth muscle of hyperplastic prostate and prostatic urethra. α receptor blockers can effectively relax the smooth muscle without affecting urination[44]. However, relevant clinical studies are few at present, and more basic and clinical studies are needed to find corresponding evidence and more effective therapeutic methods. α blockers should be administered before sleep to prevent postural hypotension. During use, pay attention to measuring blood pressure in sitting and standing position, and it is best to use controlled release preparations[45].

1.2 Sustained Release Drug Delivery

The term "hypertension" refers to a group of chronic disorders. Chronic disease therapy requires the administration of medications on a repeated basis. For drugs with a very short half-life, it is necessary for them to be administered multiple times a day. To overcome this, the development of a sustained release dosage form is required, which aids in the maintenance of the drug's therapeutic effective concentration over time[46]. After a single dose of a medicine, a portion of the drug dose incorporated into the sustained release dosage form is released immediately and the rest of the amount is released at a predetermined rate for an extended period of time (usually 12 to 18 h)[47]. The ultimate goal of each therapy is to achieve a therapeutically effective and non-toxic steady state tissue level. The creation of the appropriate dosage form is a critical component of achieving, providing, and completing this goal.

1.2.1 Limitation of conventional drug therapy

Conventional drug delivery systems are designed to provide an immediate release of drug. Such immediate-release products lead to relatively rapid drug absorption and onset of accompanying pharmacodynamic effects. However, the duration of action of conventional drug therapy is usually short. This is because of the inability of the conventional dosage form to control drug delivery[48]. The limitations related with the conventional drug release systems are:

1. Poor patient compliance. For some drugs that have a very short half-life, the frequency of administration increases which could increase the chances of the patient forgetting to take the drug.
2. Hard to keep at a uniform drug level. A characteristic plasma concentration profile of the peak-valley (Figure 1) is achieved, which makes it challenging to achieve a constant condition.
3. The precipitation of adverse effects. The inevitable fluctuations in drug concentration may lead to a lack of therapeutic effect or an overdose, especially in a drug with a small therapeutic index.

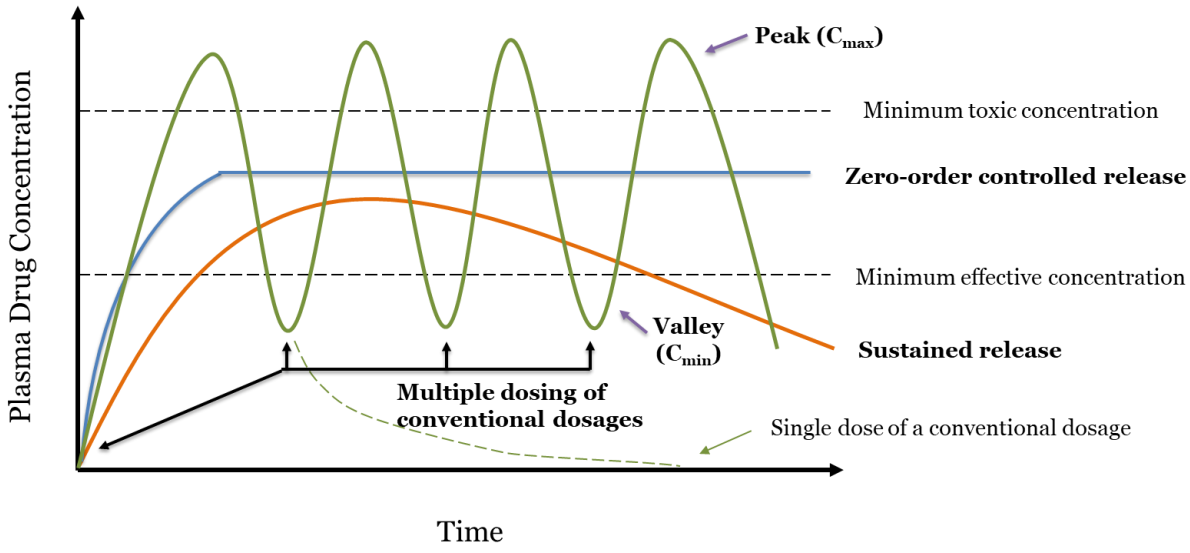


Figure 1: Drug levels in the plasma released from conventional release system (green curve), sustained release system (orange curve), and controlled release system (blue curve)[49].

1.2.2 Advantage of sustained release drug delivery system:

There are advantages linked to the use of sustained-release systems for drug delivery[50, 51]:

1. Decrease in frequency of dosing: With a well-designed sustained release drug delivery system, dosing frequency can be reduced significantly while drug concentrations in the blood and target tissue cells are maintained.
2. Enhanced patient compliance and convenience: Long-term therapy for chronic illnesses often shows a lack of compliance, as successful pharmacological therapy depends on the patient's ability to comply. With decreased dosage frequency the patient is less likely to neglect a dose. Day and night management is also more convenient
3. Less fluctuation in drug blood levels: Controlling drug release and rate of absorption results in smaller peak plasma concentration values and less fluctuations in the drug plasma levels.
4. Reduction in adverse side effects and improved efficiency in the treatment: local effects in the stomach can be diminished by presenting the drug in an enteric-coated form.
5. Overall reduction in health care expenses: In contrast to conventional dosage forms, the cost of the initial sustained release form may be higher, but, on average, over an extended period, treatment costs may be lower. This is due to increased therapeutic benefit, fewer side effects, and less time required by health-care professionals to supply pharmaceuticals, as well as to monitor patients[52].

1.3.2 Disadvantage of sustained release drug delivery system:

Oral sustained-release formulations, like all other formulations, also come with disadvantages. In most circumstances, a single dose of sustained release preparation entails a greater amount of drug than that in conventional dosage forms. If the drug reservoir of a sustained release preparation is damaged and the drug is released all at once, the drug concentration may rise above the therapeutic window, resulting in toxicity. Therefore, when developing sustained-release formulations, it is necessary to take the likelihood of dose dumping into consideration. Aside from that, once the medication begins to be released, stopping it even if essential can be a challenging task. Furthermore, the cost of manufacturing sustained-release formulations is higher than the cost of manufacturing typical dosage forms[47].

1.2.3 Criteria for choosing drugs for sustained release dosage forms

After administration of a sustained-release product, the formulation should remain in the upper gastrointestinal tract long enough for the drug to be released from the dosage form at a predetermined rate, dissolve in the gastrointestinal fluids and be absorbed at a rate that will replace the amount of drug being metabolised and excreted[53]. In general, the drugs best suited for incorporation into a sustained-release product have the following characteristics:

A. Desirable half-life

Drugs with a half-life of 2-8 hours are the best choice for sustained-release formulations because they can effectively reduce the frequency of drug use. In order to

maintain the effect of pharmaceuticals with a half-life of less than 2 hours, an excessive amount of drugs must be administered in the dosage unit. As a result, the dosage form itself becomes too large and unusable. Drugs with a half-life greater than 8 hours, on the other hand, are generally not recommended for use in sustained-release dosage forms since they already have a sustained-release action[54]. Besides, medicines that act by interfering with enzyme systems may have a longer duration of action than suggested by their quantitative half-lives due to lingering effects and the restoration of the compromised biosystem[55].

B. Small dose:

In general, a dose of 0.5-1.0 g is considered the maximum single dose for conventional dosage forms. The same applies to the configuration of extended-release dosage forms. If the dose of the drug is too high, its suitability as a controlled-release candidate is seriously uncertain. The main reason for this is that the size of the unit dose becomes too large for it to be administered without difficulty. Another explanation is that the more the dose delivered, the greater the fluctuation between Cmax and Cmin throughout each interval of dosing time. A medication with a narrow therapeutic range should be concerned about the extent of the fluctuations between the highest and minimum steady state plasma levels[56].

C. High therapeutic index:

They have a sufficient margin of safety. The most frequently cited indicator of a drug's safety margin is its therapeutic index, which is defined as the median toxic dose divided by the median effective dose. The therapeutic index of highly strong medicines may be narrow

or extremely tiny. The higher the therapeutic index, the safer the medication. Owing to the technological limits of precise control over release rates and the possibility of dose dumping due to a product defect, drugs that are delivered in extremely small doses or have extremely narrow therapeutic indices are poor candidates for formulation into sustained release formulations e.g. digitoxin. Patient abuse (for example, chewing a dose unit) may also result in toxic medication levels[57].

D. Desirable absorption window:

In some cases, oral medications only reach a special area of the gastrointestinal tract and are only partially absorbed. The 'absorption window' refers to this area. It is not recommended to use controlled release dose forms of drugs with an absorption window, such as fluorouracil and thiazide diuretics.

E. First pass clearance:

If a drug is provided in a controlled release form and undergoes severe hepatic first pass processing, the delivery of the drug to the body is impeded significantly[56].

1.3 Classification of Oral Sustained Release Systems

Oral drug delivery system is the most popular and convenient route of drug administration because of its ease of administration, flexibility of formulation and patient compliance[58]. The predominant drug delivery systems available in the market are oral drug delivery systems. It accounts for approximately half of all drug delivery systems available on the market today[59, 60].

While many oral pharmaceutical products are available on the market for sustained release, there are relatively few mechanisms that enable extending release of drugs from the formulation: diffusion, dissolution, water penetration and ion exchange. Currently, the majority of sustained release formulations are based on one of these processes or a combination of them[61].

1.3.1 Diffusion controlled system

Essentially, diffusion process describes the migration of drug molecules from a high-concentration zone to a low-concentration region[62]. In diffusion-controlled formulations, drug molecules are released over a long period of time by diffusion through a water-insoluble polymeric membranes (reservoir systems) or a polymer matrix (monolithic system)[63]. Figure 2 illustrates the process of drug release from diffusion-based reservoir and monolithic systems.

1.3.1.1 Reservoir diffusion system

In reservoir diffusion devices, drug molecules are contained within a membrane that is

not soluble in water. One or more hydrophobic polymers are used to construct this membrane. The rate-limiting membrane allows the active pharmaceutical ingredients (API) to diffuse into the surrounding media environment[64]. The drug release rate remains constant in the steady state, resulting in a zero-order release.

1.3.1.2 Matrix diffusion system

Matrix system is a different form of solid preparations, consisting of a matrix made of one or several polymer materials mixed with drugs and other components. Even in water or body fluid, such drug preparation can be maintained its skeleton structure, and the drug is evenly distributed in the skeleton, which acts as a drug reservoir. The drug particles located on the surface of the formulation unit will first be dissolved and rapidly release the drug. Thereafter, drug particles located further away from the surface of the release unit will be dissolved and released to the outside of the release unit by diffusion in the pores[65]. Matrix systems are commonly used to maintain release rate. The release rate modulates and prolongs the release of the drug. These systems are further classified into hydrophobic and hydrophilic matrices.

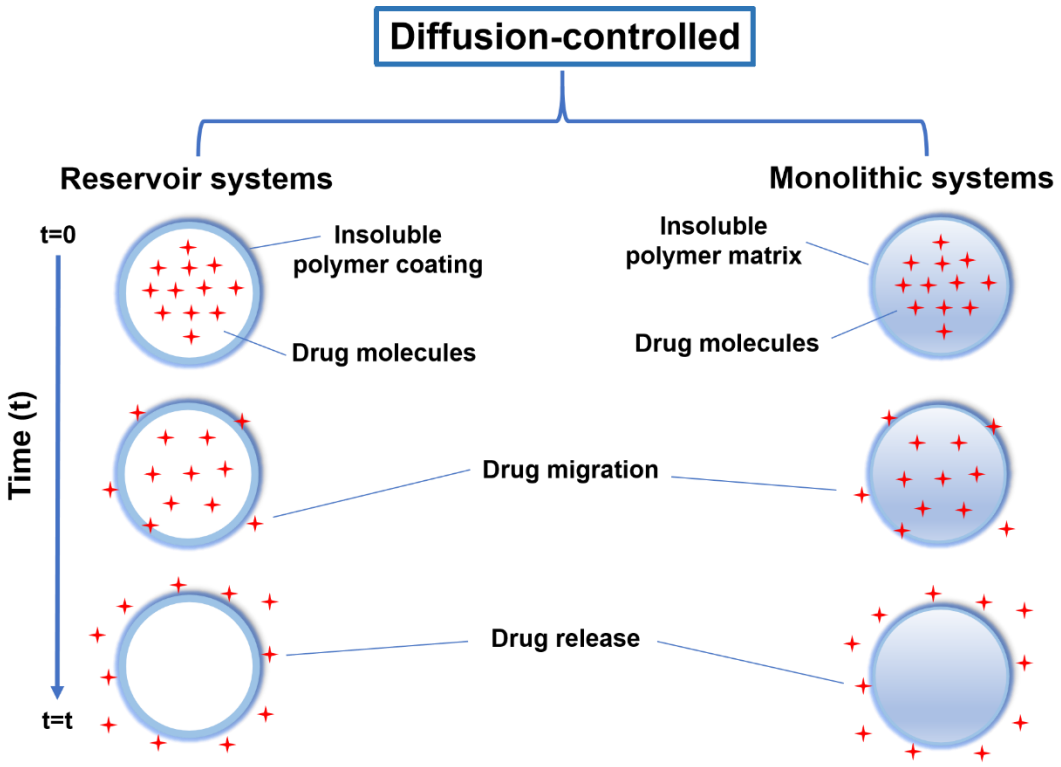


Figure 2: Schematic diagram of drug release from diffusion-based reservoir and monolithic systems.

A. Hydrophobic Matrix

Hydrophobic matrix tablets are the most common and frequently used oral sustained release dosage form, consisting of insoluble or inert polymers as the backbone material. Theses backbone materials include ethyl cellulose, hydrogenated cottonseed oil and methacrylic acid copolymers[66-68]. The mechanism of drug release from the tablet matrix is most likely diffusion. For example, isosorbide mononitrate extended-release tablets are insoluble framework tablets used for the treatment of coronary heart disease[69]. Based on the characteristics of sustained-release formulations where the blood concentration is maintained at a low level for a long time, antipyretic, analgesic and anti-inflammatory drugs with adverse effects such as gastrointestinal bleeding and ulcers can be made into sustained-

release dosage forms to reduce the occurrence of such reactions. Currently, the NSAIDs diclofenac sodium extended-release capsules and ibuprofen extended-release capsules are made into insoluble matrix formulations[70].

Hydrophobic matrix systems are not suitable for insoluble drugs as the concentration gradient is too low for complete drug release. Incomplete release of the drug in the gastrointestinal tract has proven to be a potential risk when considering formulation design and this needs to be looked at and considered during the development process[64]. A hydrophobic polymer used in the matrix tablets is Eudragit®, in the development of novel drug delivery systems, there are studies demonstrating that Eudragit® polymers have been widely used in thin film coatings to improve the quality of products[71]. Eudragit® polymer is a synthesis of acrylic acid and methacrylate. Over the past few years, their formulations of pH-triggered and novel drug delivery systems have shown great potential in combining a wide range of therapeutic areas, including proteins, vitamins, hormones, vaccines and genes[72]. Different grades of Eudragit® have been used to design and deliver treatments at specific sites via the oral route, e.g., stomach-specific administration, intestine-specific administration, colon-specific administration, and mucosal specific administration[72]. Additionally, Kollidon® SR is spray-dried polyvinyl acetate containing soluble polyvinylpyrrolidone (povidone) in the ratio 8:2, which can be used for the production of sustained-release matrix preparations, including tablets, pellets, and granules[73]. Based on the application potential of hydrophobic polymers in gastroretentive drug delivery matrix systems, double-layer gastroretentive tablets containing insoluble drugs have been developed[74].

1.3.2 Dissolution controlled system

The key factor of controlling drug release in the dissolution-controlled system is the solubility of the polymer. The limited solubility form is obtained by combining the drug with the slow soluble polymer, so that the drug leaves the formulation immediately after the polymer begins to dissolve[75]. For example, the drug is made into appropriate salts or other derivatives, coated with slow-dissolving materials, mixed with slow-dissolving materials as a carrier to make matrix tablets. Coating includes tablet coating and small pill coating, the package of different thickness, you can get different speed of the product. The dissolution process obeys the Noyes-Whitney equation. This kind of pharmaceutical preparations fall into two categories, one is the coating product of slow dissolving material, the other is the matrix product of slow dissolving material (Figure 3).

1.4.2.1 Encapsulation dissolution systems

The thickness and dissolution rate of the polymer membrane that surrounds the drug core control drug release in the encapsulated dissolution system (reservoir system). When the coated polymer membrane dissolves, the drug is delivered in its entirety, much like in an immediate-release formulation[47]. Such as Spansule®, SODAS®, Procanbid® are controlled by this mechanism.

1.3.2.2 Matrix dissolution systems

For the matrix type, the drug is distributed in a water-soluble polymer matrix and is

released when the polymer matrix dissolves. Matrix porosity, wettability and permeability are the factors that determine the drug release rate in the overall preparation. Extentabs®, Timespan®, Repetab®, Chronotab®, Adalat® are examples of this category of sustained-release systems. The solubility of polymers may depend on pH depending on the polymer properties, therefore pH could also affect the rate of dissolution.

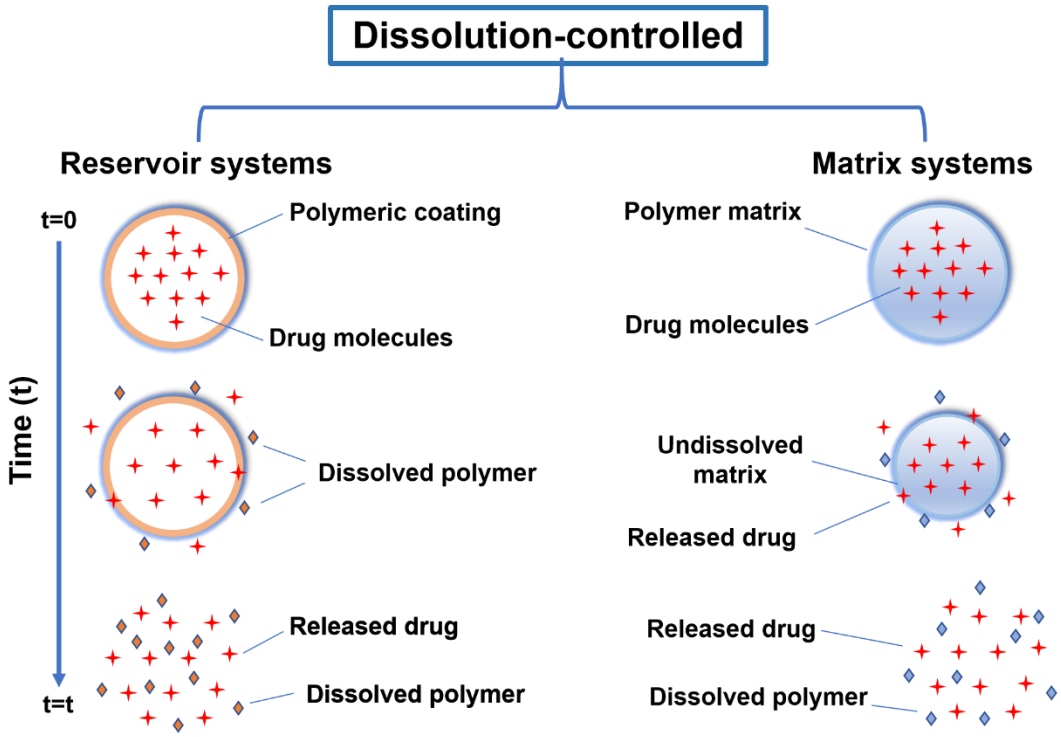


Figure 3: Schematic diagram of drug release from dissolution-based reservoir and matrix systems.

A. Hydrophilic Matrix

Hydrophilic matrixes are frequently employed to extend drug release rates. Activation of the release mechanism happens when a hydrophilic matrix comes in contact with water. Their application comes with different advantages, including the ease of production and the good homogeneity of the matrix sheet. Upon contact with aqueous media, a gel is formed

which restricts drug release. In a hydrophilic matrix tablet formulation, the matrix building material with the fastest polymer hydration capability is the optimum choice. Due to the rapid entry of water, an insufficient polymer hydration rate may result in early drug diffusion and tablet disintegration. Hydrophilic matrix is made of cellulose derivatives, non-cellulose polysaccharides, acrylic polymers, vinyl polymers and natural gums. Hydroxypropylmethylcellulose (HPMC) is a widely used polymer which belongs to non-ionic cellulose ethers. HPMC is a hydrophilic, biodegradable, and biocompatible polymer having a wide range of applications in drug delivery, dyes and paints, cosmetics, adhesives, coatings, agriculture, and textiles. It has unique solubility in both hot and cold organic solvents. HPMC has higher organic solubility and thermo-plasticity than other methyl cellulose[76]. Polyvinylpyrrolidone (PVP) is soluble in water, soluble in most organic solvents, low toxicity, good physiological compatibility is rare, especially in medicine, food, cosmetics and other fields closely related to people's health[77]. PVP has excellent physiological inertia, which means no participation in human metabolism; it has excellent biocompatibility, which means no stimulation to skin, mucous membrane, eyes and so on. Pharmaceutical grade PVP is one of the three new pharmaceutical excipients advocated internationally, which can be used as a binder for tablets and granules[78].

1.3.3 Diffusion and dissolution controlled system

Many hydrophilic polymers are water-soluble and expandable in controlled-release forms. Among them, chitosan, alginic acid and pectin are natural polymers with these properties, while, HPMC is most commonly used in synthetic or semi-synthetic polymer

matrix systems. In swelling systems, the balance between swelling dynamics, drug diffusion, and polymer dissolution/erosion determines the polymer release rate. When diffusion and dissolution erosion have similar rates, the global dynamics follows a zero-order model[79] . Thus, sustained release is most likely to be more linked to drug diffusion if drug solubility is high and more likely to be linked to polymer erosion if drug solubility is low. Uceris® is an example of a sustained-release product that embodies a dissolution/erosion-controlled oral formulation. It contains budesonide, a low-solubility, high-permeability BCS class II glucocorticoid, used in the form of oral sustained-release tablets for the treatment of ulcerative colitis[80].

1.3.4 Osmotically controlled system

Osmotic systems control the release of a drug by using osmotic pressure as a driving factor. The simplest osmotic system is called elementary osmotic pump (EOP). It consists of a semipermeable membrane (SPM) enclosing a core containing one or more osmotic agents, with a delivery orifice into the membrane (Figure 4(a))[81]. Upon contact with aqueous fluids, water penetrates into the EOP at a rate depending on the membrane fluid permeability and the osmotic pressure of the core. The drug solution is delivered at a rate proportional to the rate of water entering the tablet[82]. Drugs with low water solubilities are not fully released through the EOP due to the lack of sufficient driving force[83].

For this reason, push- pull osmotic pump (PPOP) was developed to overcome the water solubility limitations of EOP. The core of PPOP is made up of two layers: an osmotic drug reservoir and a polymeric push compartment (Figure 4(b)). Like in EOPs, drug release

occurs through an orifice. When the expandable agent swells, it puts pressure on the drug layer, causing the drug to be released at a constant speed via the hole[84].

In contrast to other drug delivery systems, osmotic system release is not affected by the gastrointestinal tract (GIT) environment, such as PH, hydrodynamics, and the action of food[85]. Drug is delivered at a steady rate within a limit range of concentrations, which produces a relatively constant plasma concentration of the drug[86]. As an expansion agent in PPOPs, many hydrophilic polymers have been utilised, with high-molecular-weight (PEO) polymers being the most common choice[87].

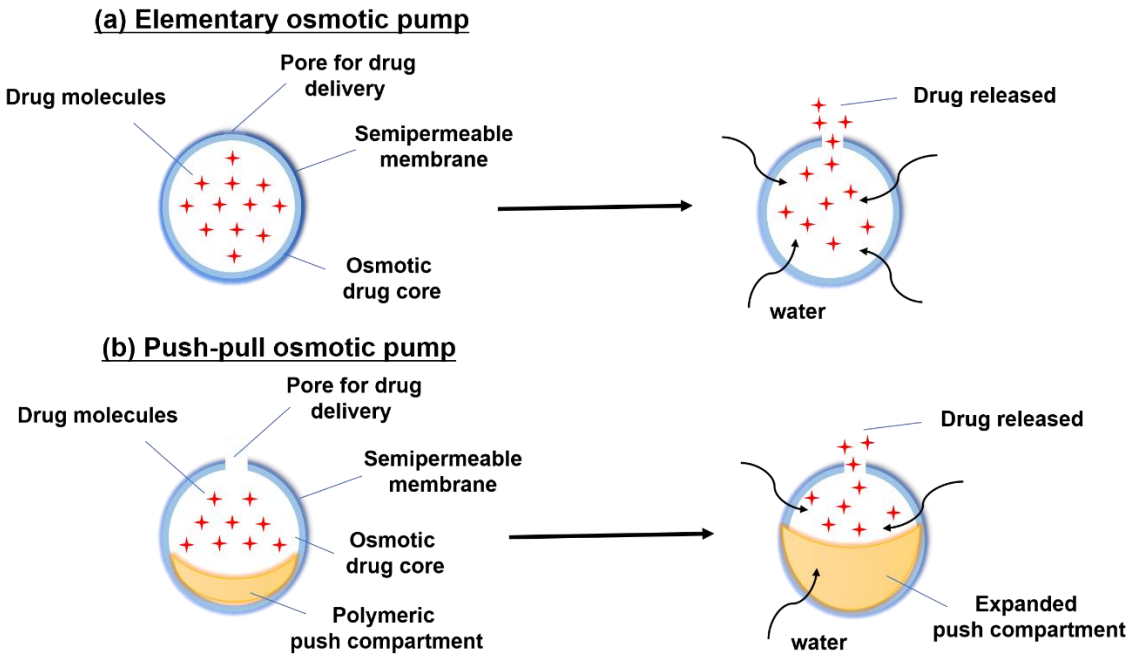


Figure 4: Schematic diagram of drug release from (a) Elementary osmotic pump (b) Push-pull osmotic pump.

1.3.5 Ion-exchange controlled systems

Ion-exchange resins, ionic polymer materials that are insoluble in water, can be employed as controlled release drug carriers. They consist of two major components: a

structural portion composed of a polymer matrix, often styrene crosslinked with divinylbenzene (DVB), and a functional portion composed of the ion-active group. Because the resins may include either positively or negatively charged ionic groups, they may be classified as cation- or anion-exchange resins[88]. Ionic drugs could be released via ion-exchange controlled release systems. These ionic drugs link to the resins through electrostatic interactions and are released when they come into contact with similarly charged ions in the release environment[89]. Numerous parameters influence the rate of release, including the pH and ionic strength of the release environment, the molecular weight and charge density of both the resin and the drugs, as well as the particle size and cross linking density of the resin[90]. An oral suspension for morphine sulphate (MST CONTINUS®) is an example for ion-exchange release system[91].

1.4 MT-1207 as a novel drug for hypertension treatment

MT-1207 is a novel multitarget antihypertensive agent, which is being tested in terms of efficacy in phase II clinical trials. The mechanism underlying the hypotensive effect of MT-1207 is the inhibition of simultaneously adrenergic α_1 receptor, and serotonin (5-Hydroxytryptamine) 5-HT_{2A} receptor[92]. In hypertension animal models, MT-1207 was found to be able to reduce hypertension without causing an increase in heart rate. Moreover, long-term administration of MT-1207 can reduce organ damage, particularly in the kidneys, as well as stroke mortality[92]. In healthy subjects experiments, MT-1207 was well tolerated and indicated an antihypertensive impact[93]. As a result, it is believed to be promising and effective in the treatment of hypertension.

1.4.1 Physicochemical properties of MT-1207

MT-1207 is a weak basic agent with a pK_a value of 7.31. Its molecular weight is 428.98 g/mol. MT-1207 is a white or off-white crystalline powder that is slightly soluble in methanol solution and dimethylformamide, almost insoluble in water, acetonitrile or absolute ethanol. The melting point of MT-1207 anhydrous crystal is approximately203-208 ˚C. The LogP for MT-1207 is 4.16 which indicates that the drug is hydrophobic. The compound is unstable under light and oxidation conditions, and the product should avoid illumination and contact with oxidants during storage.

1.4.2 Pharmacological mechanism of MT-1207

MT-1207, as a multi-targeted antihypertensive agent, is an antagonist of α_1 receptor and 5-HT_{2A} receptor.

α_1 receptors are primarily located in blood vessel smooth muscle cells. The activation of α_1 receptors causes smooth muscle contraction as well as vasoconstriction of the peripheral arterial and venous systems. Within the heart, activation of α_1 receptors results in an increase in left ventricle contractile force and mediates coronary artery vasoconstriction[94]. The α_1 adrenergic receptor antagonists are a class of drugs that bind to and block type 1 α-adrenergic receptors, inhibiting smooth muscle contraction in the process. Their use in hypertension therapy is predicated on the fact that α-adrenergic blocking inhibits vascular resistance in arterioles, resulting in a rise in venous capacitance and a decrease in blood pressure[37].

The 5-HT_{2A} receptor is a subtype of the 5-HT_2 receptor that belongs to the serotonin receptor family. Serotonin (5-HT) is a monoamine neurotransmitter found in the gastrointestinal tract, platelets, and the central nervous system of animals (including humans). 5-HT is recognised to play a role in heart regulation. It controls vasoconstriction and relaxation via the 5-HT_{2A} and 5-HT_{2B} receptors, respectively[95]. Additionally, 5-HT_{2A} receptors are also involved in a number of critical physiological activities occurring in the peripheral and central nervous systems. After a vascular injury, released 5-HT induces vasoconstriction, platelet aggregation, enhanced vascular permeability, and cell proliferation. This is probably activated by the $5HT_{2A}$ receptor[96]. As a result, antagonists of 5-HT_{2A} have been utilised to treat cardiovascular disorders[97].

1.4.3 Pre-clinical and clinical studies of MT-1207

In pre-clinical experiments, different animal models of hypertension were used to validate the pharmacodynamic of MT-1207. Spontaneously hypertensive rats (SHR) were given 1.25-20 mg/kg of MT-1207 by intravenous injection as a single dosage and dogs with one clip of both kidneys (2K1C) were given 0.25-6 mg/kg by intravenous injection. Results concluded that MT-1207 rapidly lowered blood pressure within 5 minutes of administration and demonstrated a dose-effect relationship. The hypotensive effect lasted for 8 h and12 h in SHR and 2K1C dogs, respectively, and there was no sudden increase in heart rate. Multiple doses of MT-1207 were administered over a 7-day period in 2K1C dogs and SHR. A significant reduction in blood pressure was observed along with a slight decrease in heart rate and a recovery of blood pressure after discontinuation of the dose. Long-term

administration of MT-1207 (10 mg/kg/d for more than 40 days) to SHR was found to have a stable hypotensive effect, reduced renal and cardiovascular damage and improved baroreflex sensitivity. It also had the additional effect of delaying the onset of stroke and death in susceptible SHR[92].

In clinical trials, 40 healthy Chinese subjects participated a randomized, double-blind, placebo-controlled, ascending-dose study to validate the pharmacokinetics, pharmacodynamics, and tolerability of a single dose of MT-1207. The doses of the single dosage tests were set at 5 mg, 10 mg, 20 mg, and 40 mg per dose, respectively. The pharmacokinetic effects of food on the absorption of MT-1207 also was conducted with another 16 participants.

The results indicated both systolic and diastolic blood pressure, as well as heart rate, were reduced following administration. The MT-1207 group showed a higher decrease in blood pressure between 4 and 24 hours after dosing than the placebo group, demonstrating an anti-hypertensive effect, particularly in the 10-40 mg dose group. The drop in blood pressure in the MT-1207 group showed a dose-dependent trend in the 5-20 mg group. Food has been demonstrated that delayed the absorption of MT-1207 which showed as prolonged the T_{max} of MT-1207 compared to the fasted condition (3.0 hours vs. 1.5 hours). However, $t_{1/2}$ was similar between the fasting and food-receiving groups[93]. No serious adverse reactions or deaths occurred in all participants, indicating that MT-1207 was well tolerated by Chinese test subjects. Dizziness, postural hypotension and vomiting were the most common adverse reactions in this study.

1.4.4 A previous study for once-a-day sustained release of MT-1207

A once-daily sustained-release tablet formulation containing HPMC K15M as the release retardant was successfully developed by Vrettos et al. and characterised *in vitro* and *in vivo* [98]. *In vitro* release testing demonstrated that formulation containing 31% w/w of HPMC K15M released MT-1207 over 24 hours at a nearly consistent release rate for up to 20 hours. The optimised formulation (A1) was forwarded to an *in vivo* study in Beagle dogs and the tablets were compared to 10 mg immediate-release tablets. The results of the pharmacokinetic investigation indicated that the optimised formulation offered a sustained release of the drug *in vivo*, with a $t_{1/2}$ approximately 2–2.5 times that of the immediate-release tablets. The $AUC_{0\text{-}24h}$ values for sustained-release tablets were the same as those for immediate-release tablets at 30 mg, showing that the same quantity of the drug was absorbed in each case, therefore bioequivalence between the two formulations had been demonstrated. However, plasma levels were found to be less than 1 ng/mL at the 24-hour time point and, as a result, did not fulfill expectations for a once-a-day tablet formulation because the drug plasma concentration was deemed to be outside of therapeutic levels at that time point.

1.5 Objective

MT-1207 is a multi-targeted antihypertensive drug with half-life in human of about 4.8–7.4 hours and requires two daily doses to maintain adequate plasma concentrations. This increased dosing frequency necessitates the administration of MT-1207 in the form of

a controlled-release delivery system. Even though Vrettos et al. reported the development of a once-a-day formulation, it was decided, based on the *in vivo* animal testing results of that study, to conduct further optimisation.

The objective of this present study was to optimise a once-a-day oral sustained release tablet for MT-1207 with better release properties than the one prepared by Vrettos et al. Different polymers and polymer combinations were tried out as release retardants in the tablets and their effects on drug release were investigated.

2. Materials and methods

2.1 Materials

Micronised MT-1207 hydrochloride:colloidal silicon dioxide mixture with a mass ratio of 5:1 and MT-1207 hydrochloride reference standard were gifted from Shenyang Haiwang Biotechnology Co., Ltd (Shenyang, China). Sodium acetate anhydrous, hydroxypropyl methylcellulose average molecular weight 90000 (HPMC K15M), hydroxypropyl methylcellulose average molecular weight 120000 (HPMC K100M), Kollidon® SR, polyvinylpyrrolidone K30 (PVP K30), and magnesium stearate were purchased from Sigma-Aldrich (Gillingham, United Kingdom). Eudragit® RSPO was donated by Evonik Industries (Kirschenallee, Germany). Alpha-D-lactose monohydrate, acetic acid glacial, methanol HPLC grade, and propan-2-ol HPLC grade were purchased from Fisher Scientific (Loughborough, United Kingdom).

2.2 Methods

2.2.1 High-performance liquid chromatography-ultraviolet (HPLC-UV) method for the quantitation of MT-1207 samples

The quantitation of MT-1207 samples were conducted using a previously reported HPLC-UV method (Vrettos, et al., 2021). In brief, a HPLC system (Dionex Ultimate 3000 HPLC system, Thermo Fisher Scientific®, United Kingdom) was utilised to determine the MT-1207 content. Chromatographic separation was performed using an ACE® Generix 3 C18 column (150 × 4.6 mm, 3 μm). The mobile phase was composed of 40% v/v of 0.1 M

sodium acetate buffer pH 4.0 and 60% v/v methanol. The pump flow rate was maintained at 0.7 mL/min. The column temperature was set at 40 ± 0.5 °C and the injection volume was 20 µL. The wavelength for UV detection was 230 nm. The MT-1207 content was each time calculated using a calibration curve which covered a concentration range of 1 - 100 µg/mL.

2.2.2 Standard calibration curve for MT-1207

Five miligram of MT-1207 was accurately weighed and dissolved in 50 mL of 0.1 M acetate buffer pH 4.0 to prepare a100 µg/mL stock solution. The appropriate volume of stock solution was each time diluted with 0.1 M acetate pH 4.0 to prepare the 7, 10, 20, 50 and 70 µg/mL working standard solutions. Furthermore, the appropriate volume of the 10 µg/mL working standard solution was each time diluted with 0.1 M acetate pH 4.0 to prepare the 1, 2 and 5 µg/mL working standard solutions. Then, 20 µL from each working standard solution were injected into the HPLC system and the peak area was recorded at 230 nm. The calibration curve was plotted (Peak Area vs Concentration).

2.2.3 Preparation of tablets

To manufacture the sustained release matrix tablets, the micronised MT-1207: silicon dioxide mixture was used with various types of polymers as release retardants (HPMC, Eudragit RSPO, Kollidon SR) in different ratios. The polymer matrix consisted of HPMC K15M and HPMC K100M for batch series “A”, HPMC K15M and Eudragit® RSPO for batch series “B”, and HPMC K15M, Eudragit® RSPO and Kollidon® SR or HPMC K15M

and Kollidon® SR for batch series “C”. Tablet formulations were shown in Tables 1-3.

Table 1: Tablet formulation design of Series “A”.

Components (mg)	D1	D2	D3	D4	D5	D6	D7	D8	D9
MT-1207 Hydrochloride	30	30	30	30	30	30	30	30	30
Colloidal silicon dioxide	6	6	6	6	6	6	6	6	6
Alpha-D-lactose monohydrate	100	100	100	80	80	80	60	60	60
Polyvinylpyrrolidone K30	20	20	20	20	20	20	20	20	20
HPMC K15M	52.5	35	17.5	67.5	45	22.5	82.5	55	27.5
HPMC K100M	17.5	35	52.5	22.5	45	67.5	27.5	55	82.5
Magnesium stearate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 2: Tablet formulation design of Series “B”.

Components (mg)	E1	E2	E3
MT-1207 Hydrochloride	30	30	30
Colloidal silicon dioxide	6	6	6
Alpha-D-lactose monohydrate	80	65	20
Polyvinylpyrrolidone K30	20	10	10
HPMC K15M	70	70	70
Eudragit RSPO	20	45	90
Magnesium stearate	0.5	0.5	0.5

Table 3: Tablet formulation design of Series “C”.

Components (mg)	K1	K2	K3	K4	K5
MT-1207 Hydrochloride	30	30	30	30	30
Colloidal silicon dioxide	6	6	6	6	6
Alpha-D-lactose monohydrate	30	52	55	55	75
Polyvinylpyrrolidone K30	0	0	20	20	0
HPMC K15M	70	70	70	70	70
Eudragit RSPO	45	45	0	0	0
Kollidon SR	45	23	45	45	45
Magnesium stearate	0.5	0.5	0.5	0.5	0.5

The tablet preparation was conducted using a previously reported procedure by Vrettos, et al.[98]. Different matrix tablet formulations were manufactured via wet granulation or partial direct compression, according to the flow chart depicted in the Figures 5, respectively. In brief, the micronised MT-1207: silicon dioxide mixture was geometrically diluted with alpha-D-lactose monohydrate before adding the remaining excipients. The mixture was manually blended for 15 minutes. Then, the powder mixtures were transferred into a beaker and a sufficient volume of isopropyl alcohol (IPA) was added as droplets under manual mixing using a spatula to form a wet mass. The formed wet mass was manually sieved through a 12-mesh sieve followed by drying at 60 °C for 3 hours. Afterwards, the dried granules were collected and sieved through a 25-45-170-mesh sieve array, and the fines (particle size < 90 μm) were disposed of. After adding magnesium stearate as a

lubricant, the granulated mixture was thoroughly mixed for 5 minutes. Finally, the granules were compressed into tablets using flat, round punches and dies with a diameter of 8 mm in a Piccola rotary tablet press machine (Riva®, Argentina). To investigate the influence of manufacturing method on drug release in series "C," an additional step was included to the preparation process for K3 and K4. After wet granulation and sieving, Kollidon® SR was added as an extra granular component and mixing was conducted for 15 minutes. Additionally, to investigate the potential effect of particle size between Kollidon® SR and dried granules, the particle size of K3 was kept as close as possible to between 315 µm and 90 µm.

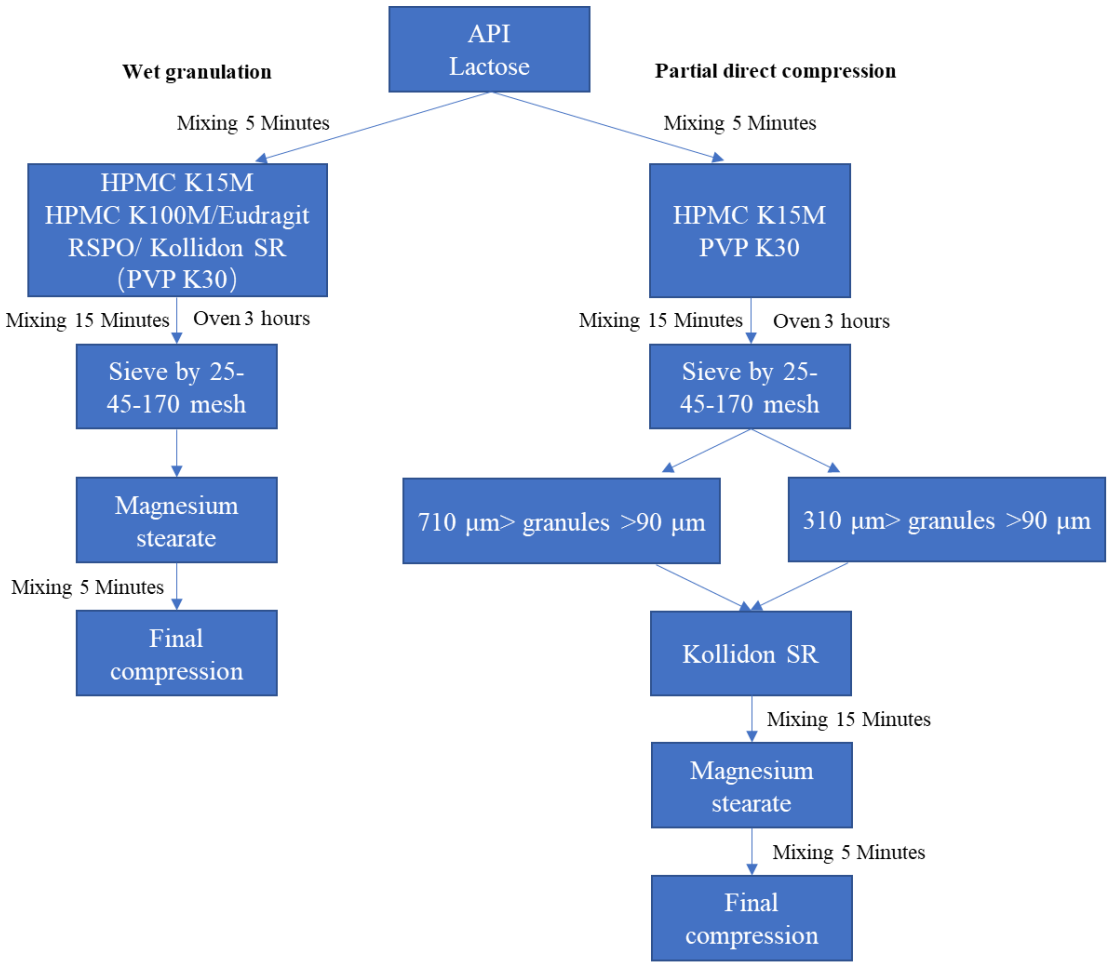


Figure 5: Process flow chart for manufactured by wet granulation and partial direct compression.

2.2.4 Flowability characterisation of the granules

The flowability of the granules prepared for each of the different formulations was characterised via bulk/tapped density testing, as per United States Pharmacopeia (USP) <616>[99], using a tapped density tester (Copley®, Nottingham, United Kingdom). Each time, a specified amount of granulated mixture was weighed (M_0) and introduced into a 100-mL measuring cylinder. After the initial volume (bulk volume, V_0) was recorded, the bulk/tapped density tester was set for 10, 500, and 1250 taps. The corresponding powder volumes V_{10}, V_{500}, and V_{1250} were recorded. The V_{1250} was considered the final tapped volume (V_t), if the difference from V_{500} to V_{1250} was less than or equal to 1 mL. The bulk and tapped density values were calculated using the Equations 1-2 below:

$$Bulk\ density(\rho_{bulk}) = M_0 / V_0 \qquad \text{(Equation 1)}$$

$$Tapped\ density(\rho_{tapped}) = M_0 / V_t \qquad \text{(Equation 2)}$$

The flowability of the different granulated mixtures was characterised via calculating the compressibility (Carr) index and Hausner ratio values, as per USP[100]. These values were calculated as per the Equations 3 and 4. The compressibility index and Hausner ratio are two measures which used to predict powder flow characteristics. In a free-flowing powder, the inter-particle interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poor flowable materials, more inter-particle interaction is usually observed, and a wider disparity between bulk and tapped densities could be found.

These differences are reflected in the compressibility index and the Hausner Ratio[99].

$$Compressibility\ Index = 100 \times [(\rho_{tapped} - \rho_{bulk})/\rho_{tapped}] \quad \text{(Equation 3)}$$

$$Hausner\ ratio = \rho_{tapped}/\rho_{bulk} \quad \text{(Equation 4)}$$

2.2.5 Evaluation of tablets

2.2.5.1 Tablet weight variation

The uniformity of tablet weight was determined as per USP <905> [101]. Ten tablets were randomly selected from each batch and individually weighed to assess the tablet weight variation. The mean tablet weight was calculated and expressed as % of the label claim. After that, the standard deviation and acceptance value were calculated. The tablet weight variation was considered to be within the acceptable range set in USP if the acceptance value was less than 15 % [101].

2.2.5.2 Hardness and friability of tablets

Hardness shows the capacity of a tablet to resist mechanical shocks during handling. The hardness of the tablets was determined using a C50 Tablet Hardness tester (Engineering Systems (Nottm)®, Nottingham, United Kingdom). The test was carried out in accordance with USP <1217>[102] . For each batch, 10 tablets were tested. The tablet was positioned in the hardness tester and the hardness value was recorded in kg units. A vernier calliper was used to check the dimensions of the tablet (diameter and thickness).

Friability testing is carried out for analysis of the effects of friction and shocks,

typically causing chip, cap, or breakage of the tablet. The friability of the tablets was evaluated in accordance with USP <1216>[103]. Ten tablets ($m_{initial}$) were weighed together and were transferred into the friability tester (Erweka®, Heusenstamm, Germany). The machine was set to a revolution rate of 25 rpm for 4 minutes. After that, the tablets were collected and carefully wiped down using a brush. The weight of these ten tablets (m_{final}) was recorded again and the percent friability was determined using Equation 5 below. The maximum weight loss should be less than 1.0 %, according to USP to ensure an acceptable tablet quality[103].

$$\% \ Friability = (m_{initial} - m_{final}) \times 100 / m_{initial} \qquad (Equation\ 5)$$

2.2.6 Differential scanning calorimetry (DSC) analysis

The thermal properties of MT-1207 hydrochloride and pulverised optimised tablets were evaluated using DSC Q2000 (TA® Instruments, Centennial Park, United Kingdom). Each time, 1-5 mg of sample were weighed and sealed inside an aluminum pan with pierced aluminum lid. The analysis was conducted at a heating ramp rate of 5 °C/min in an atmosphere of nitrogen over the temperature range of 40 °C to 240 °C. The nitrogen flow rate was set at 50 mL/min .

2.2.7 Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectrophotometric studies

ATR-FTIR analysis was conducted for the MT-1207 hydrochloride reference standard

and the pulverised K5 tablets using a Cary 630 FTIR spectrophotometer (Agilent Technologies®, Craven Arms, United Kingdom). In IR spectral research, the interactions between drug and excipients could be observed by detecting any changes in drug peaks within the physical drug mixture. 1-2 mg of MT-1207 hydrochloride reference standard or optimised sustained-release tablet formulation solid sample was placed on the lid. These amounts are generally enough to give a disc of 10-15 mm diameter and a spectrum of suitable intensity . The scanning range was set at 4000-650 cm^{-1}.

2.2.8 X-ray Powder Diffraction (XRPD) Analysis

The diffractograms of the MT-1207 hydrochloride reference standard and pulverised optimised tablet solid samples were obtained using an X'Pert Pro MPD X-ray diffractometer (Malvern Panalytical®, Malvern, United Kingdom) using Cu Kα radiation (λ=1.5406Å). A voltage of 40 mV and a current of 40 mA were applied. The scanning angle (2θ) ranged from 4 ° to 50 ° with a sample rotation speed of 15 rpm.

2.2.9 In vitro release testing

A USP II dissolution apparatus (Copley®, Nottingham, United Kingdom) equipped with paddles was used for the *in vitro* release testing of the different tablet formulations. The experiments were conducted in 500 mL 0.1 M acetate buffer pH 4.0 at 37 ± 0.5 °C. The equipment was configured at a constant paddle rotation speed of 75 rpm. Stainless steel sinkers were employed to keep the tablets from floating and sticking to the bottom of the vessels. Sample collection was carried out at predetermined timepoints (1, 2, 4, 6, 8, 12, 16,

and 24 hours). The samples were then centrifuged at 2300 g for 10 minutes. After that, 1 mL of supernatant was transferred into an HPLC vial and 20 μL was injected into the HPLC for analysis. For each formulation, *in vitro* release testing was performed in triplicate. The content of MT-1207 was calculated at each timepoint using the equation derived from the calibration curve. Additionally, for the optimised formulation, an *in vitro* release experiment was also carried out using a paddle rotation speed of 200 rpm.

2.2.10 Data analysis

2.2.10.1 Model independent method

The dissolution profiles of the different tablet formulations was compared to A1 tablets that were prepared during a previous study (Vrettos, et al., 2021)[98]. For this purpose, the difference (f_1) and similarity factors (f_2) were applied as a simple model-independent approach approved by the Food and Drug Administration (FDA) [104]. The factor f_1 calculates the percentage of the difference between the two curves all over time points, as shown in Equation 6. The factor f_2 measures the closeness between the two profiles as seen in Equation 7:

$$f_1 = \left\{\frac{\sum_{t=1}^{n}|R_t - T_t|}{\sum_{t=1}^{n} R_t}\right\} \times 100 \qquad \text{(Equation 6)}$$

$$f_2 = 50 \times \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \qquad \text{(Equation 7)}$$

where n is the number of sampling time points, R_t is the dissolution value at time point

t of the reference product and T_t is the dissolution value of the test product at time t. Two *in vitro* release profiles are considered similar when the f_1 value is within the range 0 to 15 and the f_2 value ranges between 50 and 100.

2.2.10.2 Model dependent methods

The kinetics of drug release from *in vitro* dissolution testing were studied by fitting the release data to the zero-order (Equation 8), first-order (Equation 9) and Higuchi (Equation 10) kinetic models. Furthermore, the data were plotted utilising the Hixson-Crowell cube root rule to assess the drug release in relation to changes in particle/tablet surface area and diameter (Equation 11)[105]. Finally, the release mechanism was elucidated by fitting the data to Korsmeyer-Peppas model (Equation 12).

$$Q=Q_0 - K_0 t \qquad \textit{(Equation 8)}$$

Where Q is the amount of drug released at time t, Q_0 is the initial amount of drug released and K_0 is the zero-order rate constant.

$$Log\ Q = Log\ Q_0 - K_1 / 2.303 \qquad \textit{(Equation 9)}$$

Where Q is the amount of drug remained at time t, Q_0 is the initial amount of drug released and K_1 is the first-order rate constant.

$$Q = K_H \bullet t^{1/2} \qquad \textit{(Equation 10)}$$

Where Q is the percentage cumulative drug released at time t; K_H is the Higuchi-type release rate[106].

$$\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = K_{HC} \bullet t \qquad \textit{(Equation 11)}$$

where Q_0 is the initial amount of the drug in the system; Q_t is the amount remaining in the system on time t; K_{HC} is the rate constant for the Hixson-Crowell equation, which relates surface and volume.

$$Q_t / Q_\infty = K \bullet t^n \qquad \textit{(Equation 12)}$$

Where Q_t / Q_∞ is the fraction of drug released at time t, K is a constant incorporating the structural and geometric features of the drug/polymer and n is the release exponent associated with the release mechanism. The model is often used to describe the drug release behaviour from polymeric systems[107]. For a cylinder tablet, n=0.45 refers to Fickian diffusion mechanism; $0.45<n<0.89$ refers to non-Fickian (anomalous) release; n=0.89 refers to Case II (Zero order) release; $n>0.89$ refers to super case II type of release[108, 109]

3. Results and Discussion

3.1 Calibration curve of MT-1207

The calibration curve was obtained by plotting Peak Area vs Concentration. The MT-1207 calibration curve was established in a concentration range from 1 to 100 µg/mL in 0.1 M acetate buffer pH 4.0 (Figure 6). The coefficient of determination (R^2) was measured to be 0.99998 which corresponded to a good linearity of the curve.

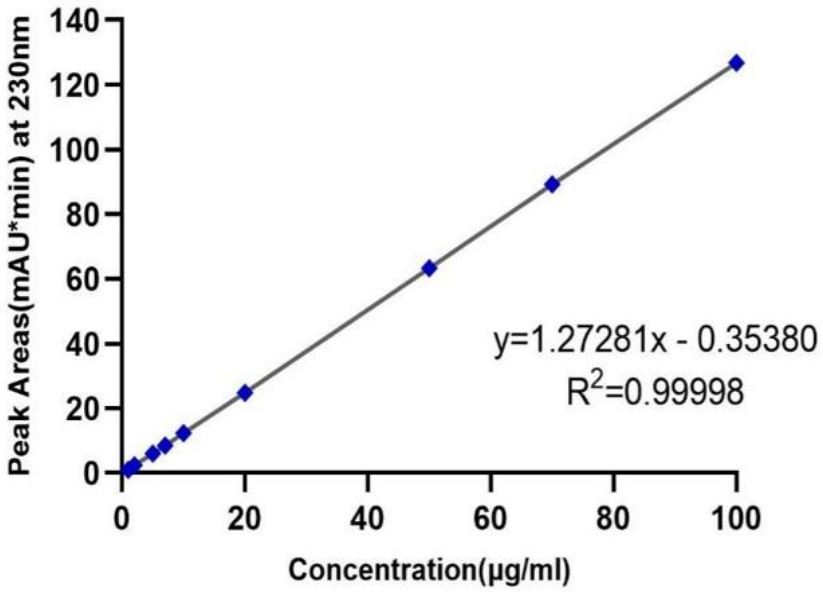


Figure 6: Standard curve of MT-1207 in 0.1 M acetate buffer pH 4.0.

3.2 Characterisation of flowability of granules

Before compression, the granules of MT-1207 were evaluated in terms of compressibility and flowability through bulk density, tapped density, compressibility (Carr) index, and Hausner ratio measurements. Bulk density and tapped density values were found to range from 0.255 to 0.374 g/mL and 0.280 to 0.418 g/mL, respectively. The Carr index and Hausner ratio values were calculated using the above bulk and tapped density values.

The Carr index is a good indicator of the compressibility of a powder[110]. The Carr index would be small in a free-flowing powder due to the close value in the bulk and tapped densities. Normally, a Carr index value below 15 % is desirable to achieve both good flowability and compressibility[111]. Another indicator correlated to the flowability of a powder blend is Hausner ratio, which reflects the particle friction[112]. Generally, Hausner ratio values less than 1.25 result in acceptable flow properties[113]. The results of pre-compression parameter characterisation are shown in Table 4.

As can be seen in Table 4, the Carr index values reported for D1-D4, D6, D8, E1, and K1-K3 granules indicated good to excellent flow properties, as per USP[100] . This was further supported by the respective Hausner ratio values seen in the same table. On the other hand, the powder blends of D5, D7, D9, E2-E3, and K4-K5 had relatively high Carr compressibility index values. However, since their flowability was still characterised as fair, based on the respective Carr index and Hausner ratio values, as per USP[100] , the granular blends were deemed suitable for tableting.

3.3 Post-Compression Parameters of Tablet Formulations

Tablets from the different formulations were subjected to different post-compression evaluation tests, including thickness, hardness, weight variation, and friability. The results of the tablet post-parameter characterisation are presented in Table 5.

The average thickness of all tablets ranged from (3.70 ± 0.02) to (4.06 ± 0.01) mm which indicated their uniform thickness. Additionally, all formulations had average hardness values that ranged between 11.79 and 24.21 kg. This demonstrated a sufficiently

high mechanical strength of the tablets which, in turn, could ensure their integrity during handling. This was further confirmed by friability testing, where all tablet batches demonstrated friability values that were well below the permitted limit of 1.0 % established by USP[103]. This validated their high quality and resistance to abrasion throughout packing, handling, and transportation[114]. Finally, the average weight of the prepared tablets of all formulations was found to be between 226.3 and 227.6 mg, while the calculated acceptance values were below 15.0. This demonstrated a uniform tablet weight for the different batches, as per USP [101].

Table 4: Characterisation of granules of all formulations.

Formulations Number	Bulk Density (g/mL)	Tapped Density (g/mL)	Carr Index (%)	Hausner Ratio
D1	0.348	0.401	13.0	1.15
D2	0.347	0.389	10.6	1.12
D3	0.359	0.398	10.0	1.11
D4	0.304	0.351	13.5	1.16
D5	0.318	0.388	18.0	1.22
D6	0.293	0.344	13.1	1.17
D7	0.291	0.355	18.1	1.22
D8	0.304	0.338	10.1	1.11
D9	0.288	0.348	17.2	1.21
E1	0.327	0.371	11.9	1.13
E2	0.3	0.357	15.8	1.19
E3	0.324	0.394	17.7	1.22
K1	0.333	0.388	14.2	1.17
K2	0.255	0.28	8.9	1.1
K3	0.374	0.418	10.4	1.12
K4	0.336	0.409	17.9	1.22
K5	0.315	0.376	16.1	1.19

Table 5: Physical Parameters of Tablets of all formulations. Results are reported as mean ± standard deviation.

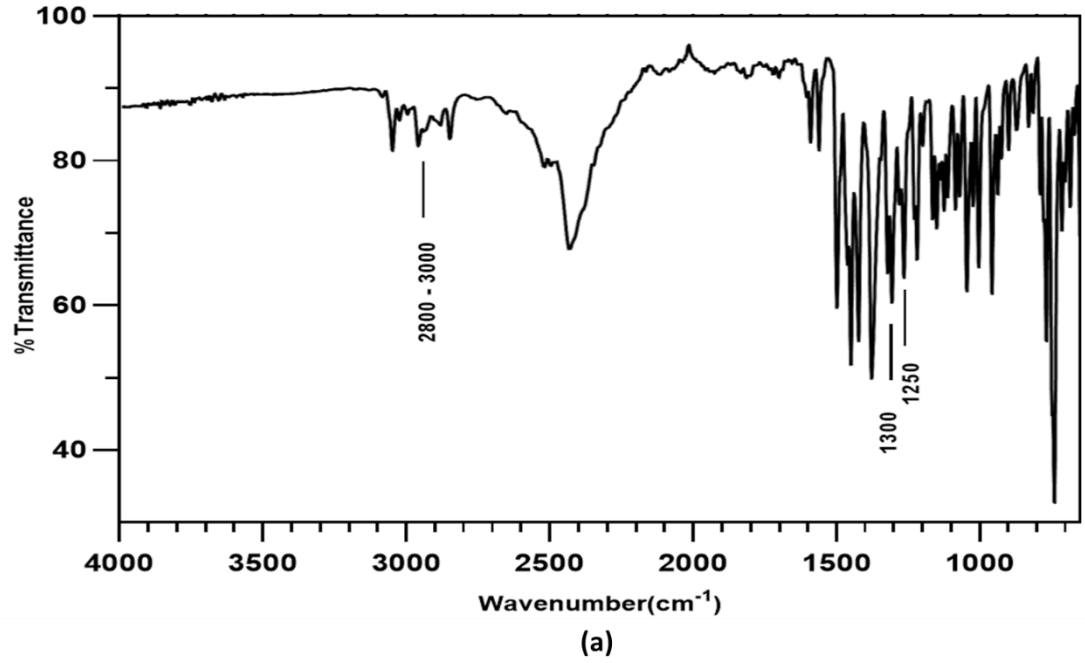
Formulations Number	Thickness (mm)	Weight variation (mg)	Acceptance values	Hardness (Kg/cm3)	Friability (%)
D1	3.75 ± 0.01	227.0 ± 0.49	1.17	12.90 ± 0.77	0.1
D2	3.70 ± 0.01	227.2 ± 0.70	1.67	14.34 ± 1.34	0.02
D3	3.70 ± 0.02	227.6 ± 0.44	1.06	13.66 ± 0.54	0.04
D4	3.72 ± 0.02	227.1 ± 0.55	1.32	15.89 ± 0.88	0.03
D5	3.87 ± 0.02	227.3 ± 0.31	0.74	14.58 ± 0.54	0.06
D6	3.74 ± 0.02	227.3 ± 0.53	1.28	17.26 ± 0.85	0.05
D7	3.83 ± 0.01	226.7 ± 0.62	1.49	16.00 ± 0.62	0.1
D8	3.83 ± 0.02	226.3 ± 0.62	1.49	16.08 ± 0.48	0.04
D9	3.83 ± 0.01	226.5 ± 0.76	1.83	16.93 ± 0.68	0.03
E1	3.82 ± 0.02	227.1 ± 0.47	1.13	14.39 ± 0.67	0.09
E2	3.74 ± 0.01	226.9 ± 0.43	1.08	16.43 ± 0.46	0.06
E3	4.06 ± 0.01	227.3 ± 0.54	1.29	11.79 ± 0.74	0.1
K1	3.88 ± 0.01	227.0 ± 0.66	1.57	22.69 ± 0.87	0.02
K2	3.89 ± 0.01	227.2 ± 0.70	1.67	17.38 ± 0.48	0.04
K3	3.83 ± 0.01	227.6 ± 0.21	0.49	20.62 ± 1.23	0.03
K4	3.74 ± 0.01	227.1 ± 0.47	1.13	24.21 ± 1.29	0.05
K5	3.76 ± 0.02	227.0 ± 0.59	1.42	18.02 ± 1.15	0.04

3.4 Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) Analysis

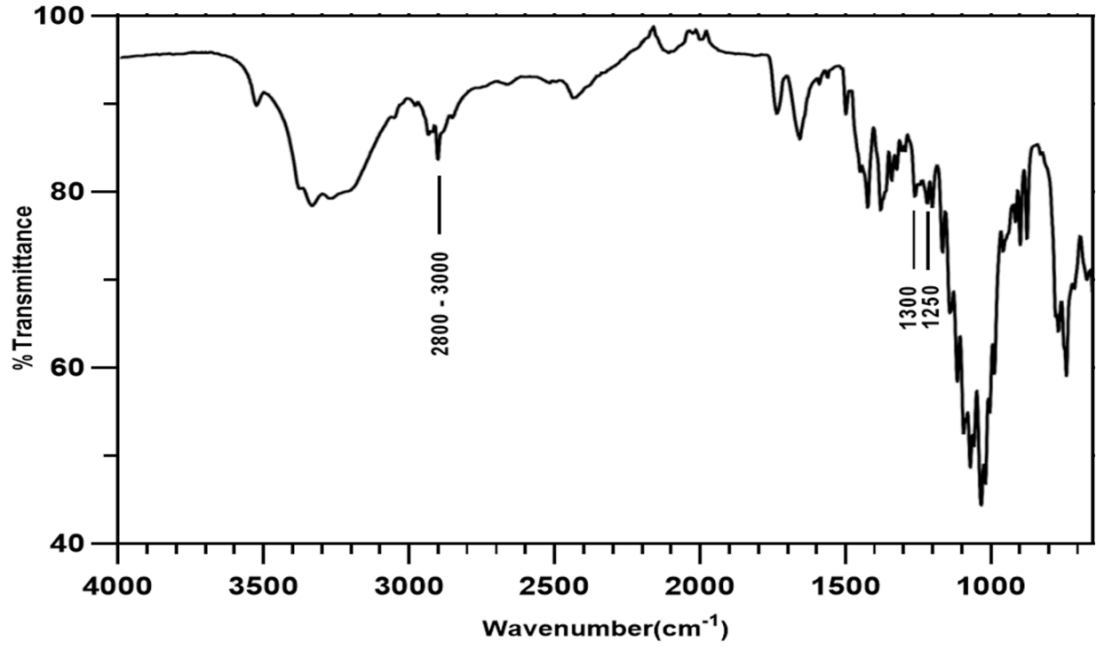
The chemical compatibility of MT-1207 with the excipients was examined using ATR-FTIR spectrometer. The FTIR spectra of MT-1207 hydrochloride reference standard, pulverised K5 (optimised) tablet, HPMC K15M, Kollidon® SR, and colloidal silicon dioxide can be seen in Figure 7 (a-e).

All the characteristic peaks of MT-1207 were present in the spectra of the drug and the

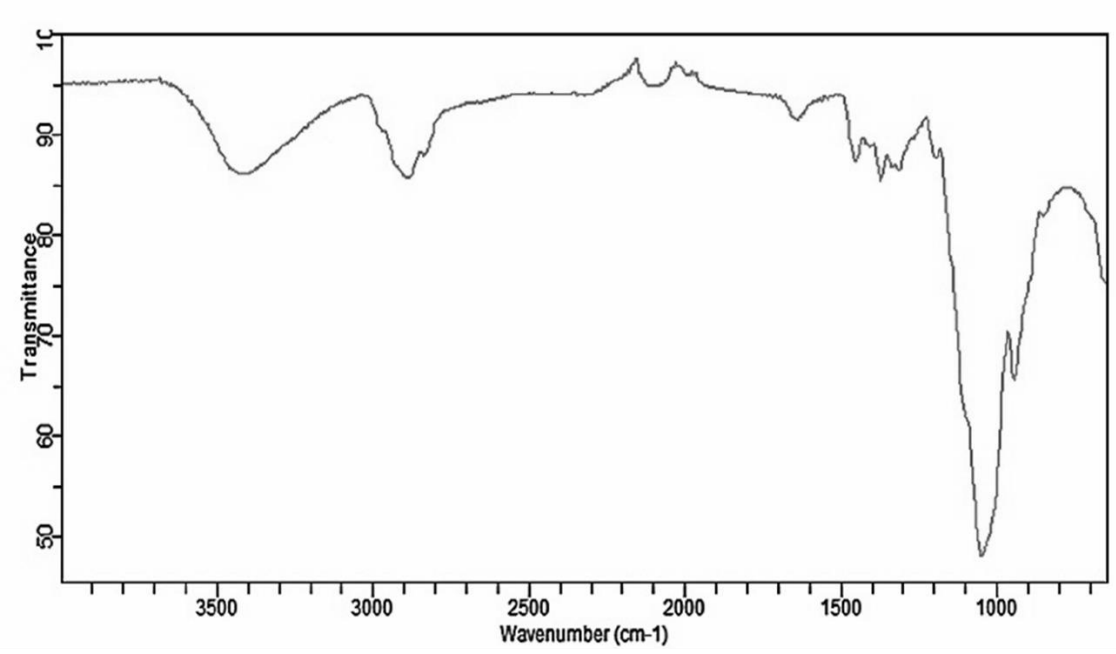
pulverised K5 mixture. The aliphatic and aromatic C-H stretch peaks in the 2800-3000 cm^{-1} range (Figure 6(a)) can be seen intact in the pulverised tablet spectra (Figure 6(b)). Additionally, peaks corresponding to C-N stretch can be observed in the 1250–1300 cm^{-1} range in the spectra of drug reference (Figure 6(a)) and pulverised K5 tablets (Figure 6(b)). Finally, no additional peaks can be seen in the fingerprint region of the pulverised K5 tablets, which looked like the result of a simple mixing of MT-1207 hydrochloride with HPMC K15M, Kollidon® SR and silicon dioxide. Based on these findings, it was determined that there was no significant chemical interaction between the API and the excipients since the main peaks of the MT-1207 were found to be unaffected in the pulverised mixtures. This demonstrated that MT-1207 was chemically compatible with the different excipients.



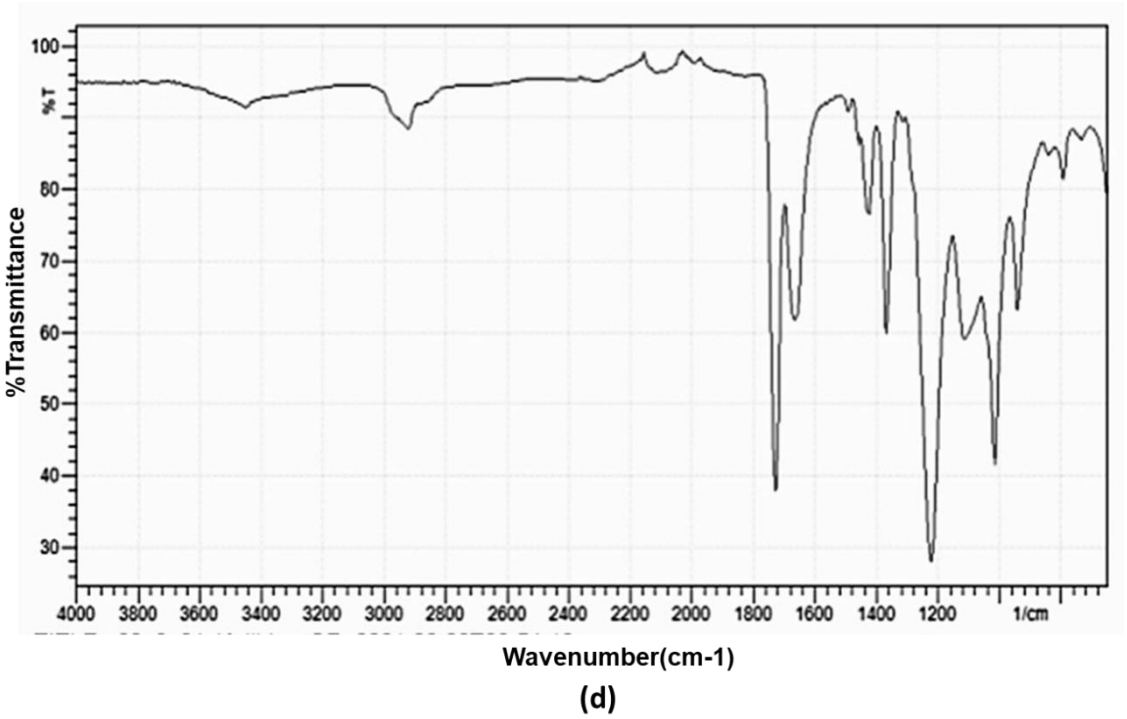
(a)


100
80
60
40
% Transmittance
2800 - 3000
1300
1250
4000
3500
3000
2500
2000
1500
1000
Wavenumber(cm-1)

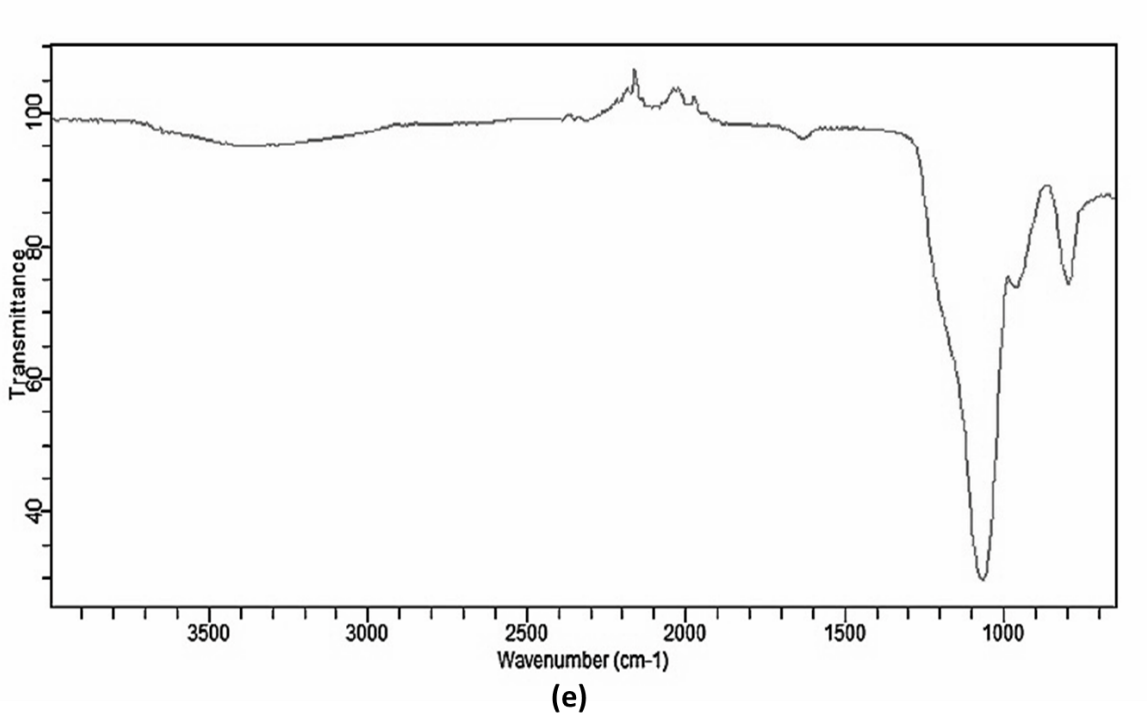
(b)


90
80
70
60
50
Transmittance
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

(c)



(d)



(e)

Figure 7: FTIR spectra of (a) MT-1207 hydrochloride reference standard, (b) pulverised K5, (c) HPMC K15M, (D) Kollidon® SR and (E) colloidal silicon dioxide.

3.5 Differential Scanning Calorimetry studies

The thermal behaviour of MT-1207 hydrochloride reference standard and optimised K5 tablets was evaluated using DSC. Figure 8(a) shows the DSC thermogram of MT-1207 hydrochloride. The sharp endothermic peak obtained at around 210 °C corresponded to the melting point of pure anhydrous MT-1207 hydrochloride crystal. In the thermogram of the optimised K5 formulation (Figure 8(b)), different peaks were recorded. The peak at around 140-150 °C potentially corresponded to the dehydration of alpha-D-lactose monohydrate towards beta-lactose. Then the peak at approximately 210 °C was linked to the melting point of beta-lactose. The temperature values that were recorded in the present study and corresponded to the phase transition of alpha-D-lactose monohydrate and melting point of beta-lactose were similar to the results reported in the literature[115, 116]. However, an endothermic peak was also noted at temperature values ranging from 155 to 165 °C. This thermal event was possibly related to the fusion point of HPMC[117, 118]. Finally, a relatively broad endothermic peak was recorded at around 190 °C. This peak potentially corresponded to the melting point of MT-1207 hydrochloride crystal. However, compared to that of the reference standard, the DSC profile of K5 demonstrated a broadening of MT-1207 hydrochloride crystal endothermic peak along with a shift to a lower temperature. This could be related to the mixing procedure. The purity of each component was reduced in the mixture and may have resulted in a lower melting point and broader peak of the drug crystal[119, 120]. Furthermore, the oven drying and the use of different granulating fluid such as IPA may also lead to the enthalpy decreased[121]. Afterward, the intense physical interactions that may have occurred between the drug and its excipients during the process

of mixing and compressing the powder into tablets could have significantly resulted in the brodening of the MT-1207 endothermic peak[122].

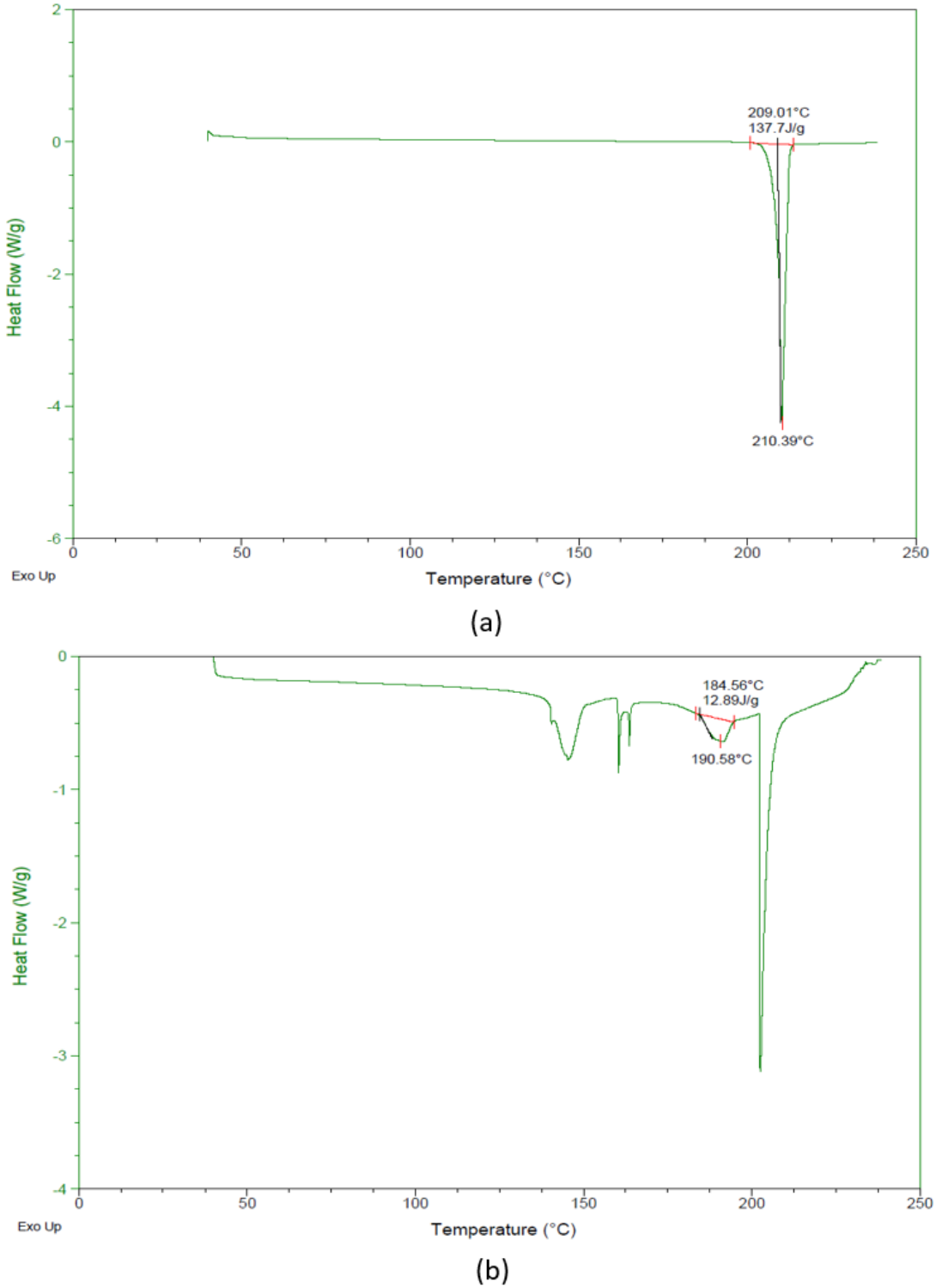


Figure 8: DSC thermograms of (a) MT-1207 hydrochloride reference standard (b) Formulation K5.

3.6 X-ray powder diffraction Analysis

To assess the physical state of the drug in the optimised tablets, the crystalline behaviour of MT-1207 hydrochloride reference standard and pulverised K5 tablet mixture was examined using XRPD (Figure 9). The crystalline nature of the drug was illustrated by the XRPD pattern. The peaks over the angle (˚2theta) values of 11.5, 15, and 18 in the drug layer diffractogram were attributed to the MT-1207 hydrochloride reference standard, whereas the remaining peaks were assigned to alpha-D-lactose monohydrate. Alpha-D-lactose monohydrate was the only crystalline excipient used in the formualtion. The reduced height of the drug peaks in the K5 diffractogram can be attributed to the dilution effect of the MT-1207 with the excipients[123]. The majority of excipients were amorphous and produced no XRPD signal, while their presence resulted in a decreased proportion of the crystalline API in the mixture, compared to the reference standard. The diffractogram of the crystalline materials exhibit very minor variations in peak intensity and no significant changes in peak position. Therefore, it can be inferred that the drug is only present in the tablet in the form of its anhydrous crystals within the sensitivity of the analysis.

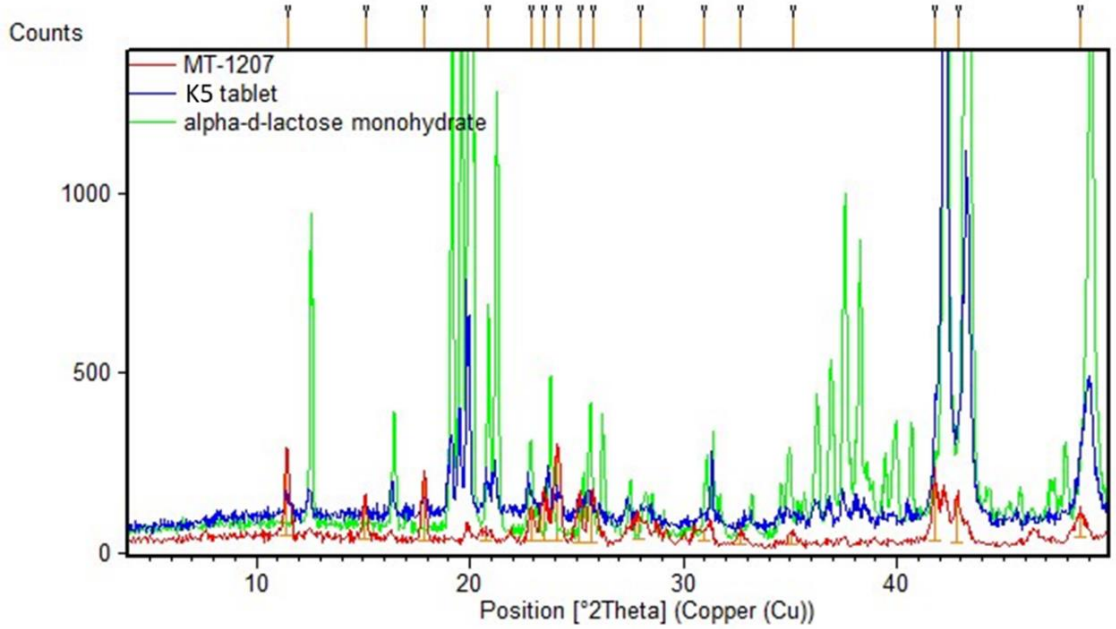


Figure 9: Diffractograms of MT-1207 hydrochloride reference standard [tallest peak at 11.5 angle(°2Theta)], K5 [tallest peak at 20 angle (°2Theta)] and alpha-D-lactose monohydrate [tallest peak at 19 angle (°2Theta)] combined [Intensity (Counts) vs Position(°2Theta)].

3.7 In Vitro *Drug Release Studies*

3.7.1 Drug release from A1 in new buffer

The dissolution medium for in vitro release tests in the previous study was 0.1 M phosphate pH 6.8 with 0.2% w/v sodium dodecyl sulfate (SDS)[98]. However, the findings of the *in vivo* study in Beagle dogs indicated that the release of the API was much faster *in vivo* than *in vitro*[98]. There are different factors that may have contributed to this discrepancy. It could be due to the poor wet strength of the tablets which may have resulted in their premature disintegration *in vivo*[124, 125]. Also, the medium that was selected for that study may not have ensured sink conditions during the *in vitro* release testing. Furthermore, another possible explanation is that the gastric pH in beagle dogs is lower than 6.8, which may have contributed towards the faster release of the drug in vivo. In the previous literature,

there is a wide variety in the reported gastric pH of dogs. A past study was reported by Akimoto et al., and the finding indicated that the pH of the fasting dog stomach ranged between 2.7 and 8.3 (mean at 6.8)[126]. However, recent investigations by Sagawa et al. and Mahar et al. demonstrated that fasting dog gastric pH levels were still in a relatively acidic condition but slightly higher than human gastric pH[127, 128]. Since the solubility of MT-1207 in the medium was 0.06 mg/mL, it was regarded as a potential drug release limiting factor[129]. Based on this consideration, the phosphate dissolution medium was substituted with 0.1 M acetate buffer pH 4.0 in which the solubility value of MT-1207 was 6.5 mg/mL.

A1 was a previously optimised formulation which contained HPMC K15M as the release retardant and gave a 24-hour sustained-release in phosphate medium. The same tablets were tested in 0.1 M acetate buffer pH 4.0. The results of the *in vitro* drug release testing of A1 tablets in the new medium can be seen in Figure 10. 94% release of MT-1207 was achieved within 12 hours which was much faster than that the release of the API in 0.1 M phosphate pH 6.8 with 0.2% w/v SDS[98]. This indicated that drug solubility was potentially a limiting factor for drug release in phosphate buffer since its solubility in acetate buffer was much higher than that in the previous medium. Furthermore, the release profile in acetate buffer appears to be closer to the actual situation *in vivo* since, after oral administration of A1 tablets, the peak plasma concentration in the *in vivo* study conducted by Vrettos et al. was recorded within approximately 4 hours[98]. Therefore, the acetate dissolution medium was deemed fit for purpose and was used for the *in vitro* release testing of the different formulations that were prepared and characterised in the present study.

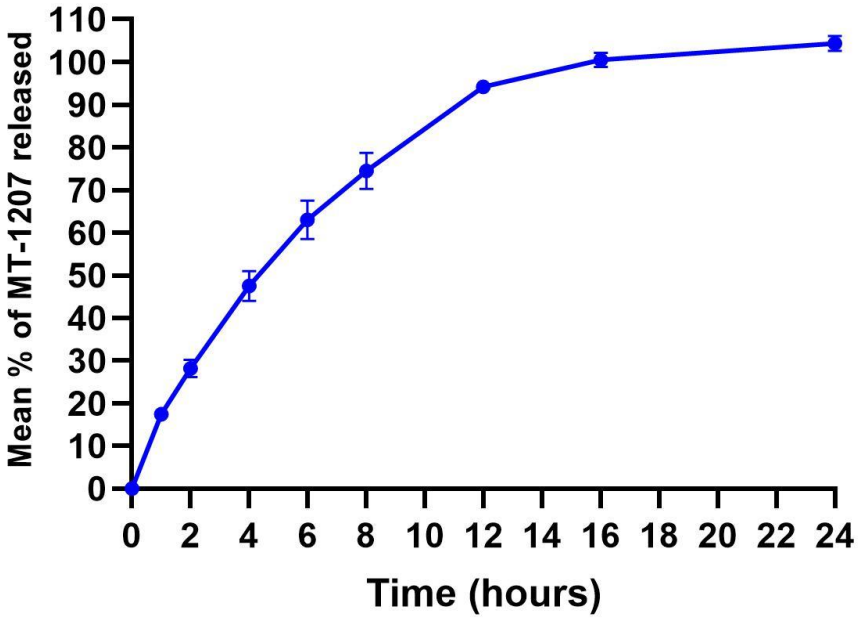


Figure 10: Release profiles of MT-1207 from A1 tablets in 0.1 M acetate buffer pH 4.0 (n = 3 tablets). The results are presented as mean ± standard deviation.

3.7.2 Drug release from tablet formulations containing combinations of HPMC K15M and HPMC K100M

As part of the formulation optimisation, HPMC K100M, a higher viscosity grade polymer, was included in the formulation as an additional hydrophilic release retarding polymer alongside HPMC K15M. The use of higher viscosity grades of HPMC can result in lower drug release rates as the pores of high-viscosity HPMC block up quickly and inhibit further liquid uptake[130]. In the optimised A1 tablets from the previous study, the concentration of HPMC K15M was 31% w/w. Two series of experiments were designed and conducted to find the optimum percentage of HPMC K100M in the formulations. The first series of experiments entailed the preparation and characterisation of MT-1207 sustained-release tablets that contained different drug:polymer ratios (3:7, 3:9, 3:11). The second series of experiments was concerned with the manufacturing and characterisation

of MT-1207 sustained-release tablets containing different HPMC K15M:HPMC K100M ratios (% w/w) (75:25, 50:50, 25:75). The quantity of MT-1207 was the same (30 mg) in all formulations. The *in vitro* release profiles of MT-1207 from the different formulations prepared using varying ratios of HPMC K15M and HPMC K100M are illustrated in Figure 11 (a-c).

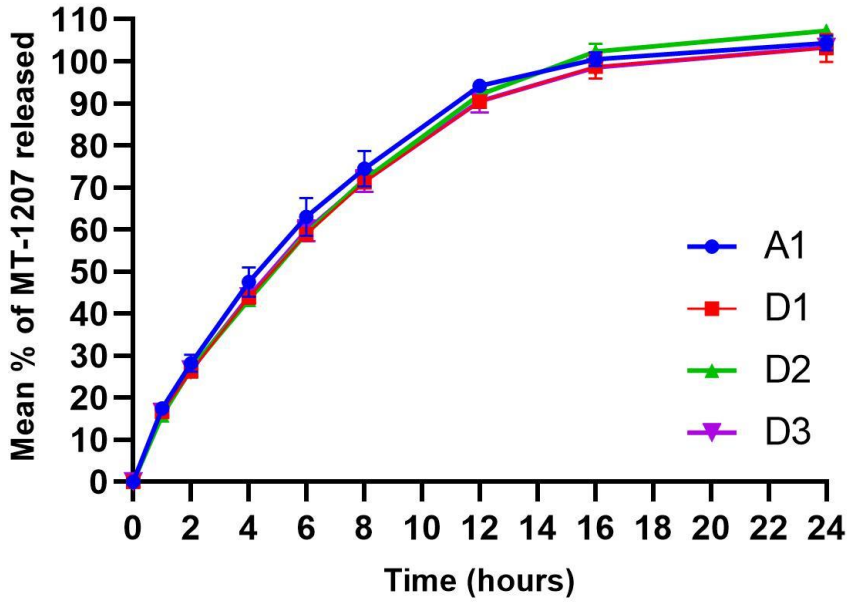
Formulations D1-D3 composed of drug:polymer ratio of 3:7 failed to provide a sustained release of MT-1207 beyond 12 h. D1-D3 released 90.4%, 92.1% and 90.5% of the drug at 12 h, respectively, which was similar to the release profile as A1 tablets. Similarity factor analysis between D1-D3 and A1 showed that the f_2 factor was greater than 50 (A1 vs D1, D2, D3 = 75, 77 and 77, respectively) and f_1 factor was 5. These values were within the similarity limits of the respective factors, as per FDA guidelines ($0<f_1<15$, $50<f_2<100$)[131]. Therefore, this analysis indicated there was no statistically significant difference between the *in vitro* drug release profiles of A1 and D1-D3.

The formulations with drug: polymer ratio of 3:9 (D4-D6) and 3:11 (D7-D9) extended the drug release to 16 h. However only D7-D9 was statistically different from A1 in terms of their *in vitro* release profiles, with f_2 values of 49, 48, and 48 respectively. On the basis of all formulations containing these three various drug:polymer ratios, it was clear that the rate of drug release was reduced gradually as the concentration of polymers increased. In the literature, it has been indicated that drug release rate of matrix tablets decreases with increasing HPMC concentrations. That is probably due to the fact that as the concentration of HPMC increases, the degree of physical cross-linking of the polymer chains increases significantly. This in turn can increase the tortuosity of the release pathway of the matrix

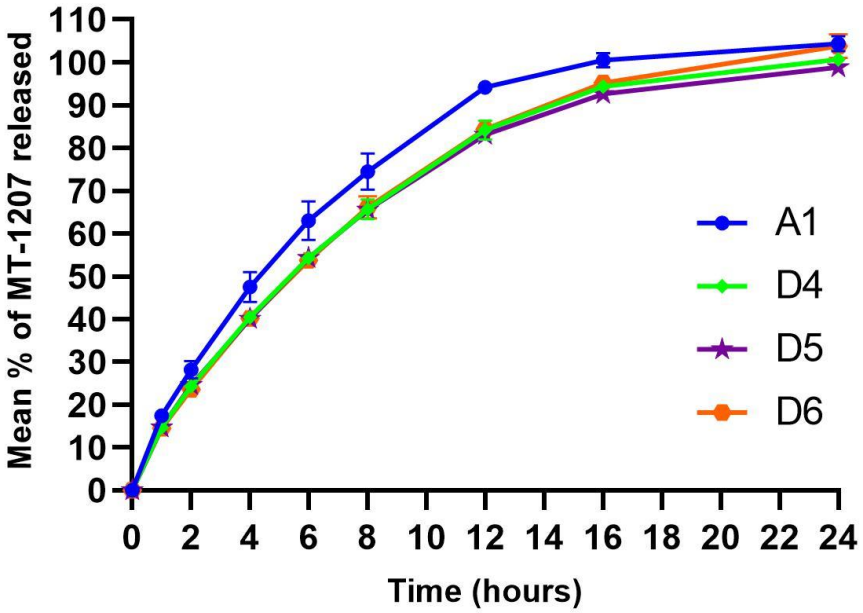
tablets and result in a slower release of the drug[132, 133].

Furthermore, no statistically significant changes in the drug release profile were observed among formulations containing different HPMC K15M: HPMC K100M ratios with f_1 values varying between 0.4 and 2.4 and f_2 values varying between 82 and 99. This phenomenon was also described in the literature. Ramu et al. employed five different HPMC K15M:HPMC K100M ratios to control drug release rates and reported that there was no statistically significant difference in drug release between the different formulations[134].

Therefore, it seemed that matrix tablets containing only hydrophilic polymers were not able to provide a 24-hour sustained release of MT-1207 in acetate buffer pH 4.0. This may be associated with drug solubility in the medium. In the previous study conducted by Vrettos et al., drug release from the optimised formulation was found to be mainly controlled by matrix erosion, which was consistent with the release pattern of poorly soluble drugs in the literature[98, 135]. However, as solubility increases, the drug might be released by diffusion as well. This could potentially result in the drug also acting as a pore-former, making the gel structure weak and more porous, thereby further enhancing the rates of drug release[136, 137].


Mean % of MT-1207 released
110
100
90
80
70
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (hours)
A1
D1
D2
D3

(a)


Mean % of MT-1207 released
110
100
90
80
70
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (hours)
A1
D4
D5
D6

(b)

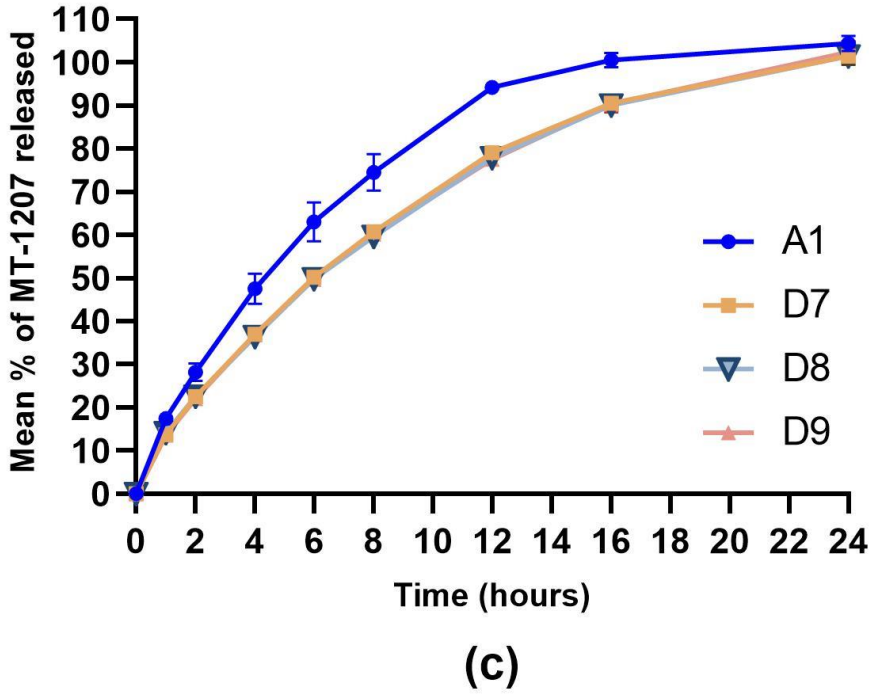


Figure 11: Release Profiles of MT-1207 from formulations containing combinations of HPMC K15M and HPMC K100M polymers at drug:polymer ratio of (a) 3:7 (b) 3:9 (c) 3:11 (n=3 tablets). The results are presented as mean ± standard deviation.

3.7.3 Drug release from tablet formulations containing combinations of HPMC K15M and Eudragit® RSPO

Since hydrophilic matrix tablets did not seem to be capable of providing a 24-hour sustained release of MT-1207 in acetate buffer, it was decided to incorporate hydrophobic polymers into the HPMC K15M matrix. Eudragit® RSPO is a hydrophobic polymer that can be used as a matrix former for the formulation of sustained-release dosage forms. Due to the hydrophobicity of the polymer, Eudragit® RSPO can be effective at retarding drug release by reducing the rate at which solvent molecules penetrate the polymer matrix[138, 139]. Different tablet formulations were prepared containing various percentage values of Eudragit® RSPO. The *in vitro* release testing results for those sustained-release tablets can

be seen in Figure 12.

It seemed that the drug release was sustained up to only 12 hours for tablet formulations E1 which contained 9% Eudragit® RSPO. E2 and E3 containing 20% and 40% w/w Eudragit® RSPO, respectively, prolonged the release of the drug up to 16 hours. No statistically significant difference in the drug release profiles was observed between tablets containing 20% and 40% Eudragit® RSPO (f_2=63, f_1=10). However, statistically significant difference in drug release was recorded between tablet formulations containing 10% and 40% w/w Eudragit RSPO (f_2=49, f_1=23). These results indicated that when the percentage of Eudragit® RSPO in the formulation is increased, its hydrophobic properties could slow down the penetration of the dissolving medium into the matrix, thus resulting in a reduction in the dissolution and diffusion rate of the drug[140].

When similarity factor analysis was conducted for the *in vitro* release profiles of these formulations and A1, it was realised that only the *in vitro* release profile of E3 tablets was statistically different to that of A1 formulation (f_2=42, f_1=25). Therefore, matrix tablets comprised of HPMC K15M and Eudragit® RSPO did not appear to be capable of providing a 24-hour sustained release of MT-1207 in acetate buffer pH 4.0. This could be due to the presence of quaternary ammonium groups in Eudragit® RSPO. Although Eudragit® RSPO is a pH-independent polymer, in acidic pH, the breakdown of these quaternary ammonium groups results in the formation of pores in the matrix, allowing for the release of more drugs[141, 142].

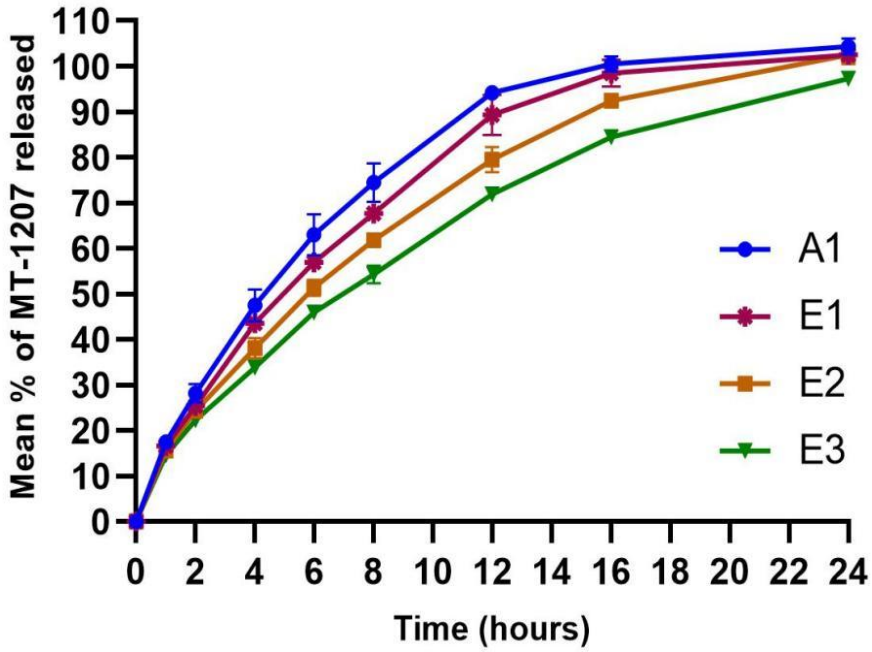


Figure 12: Release Profiles of MT-1207 from formulations containing combinations of HPMC K15M and Eudragit® RSPO (n=3 tablets). The results are presented as mean ± standard deviation.

3.7.4 Drug release from tablet formulations containing combination of HPMC K15M, Eudragit® RSPO, and Kollidon® SR

According to past literature, when sustained release cannot be achieved to the desirable extent with Eudragit® RSPO alone, additional hydrophobic excipients can be incorporated into the formulation to further decrease the drug release rate and extent[143]. Kollidon® SR was added as another release retardant into formulation E2. Kollidon® SR consists of polyvinyl acetate (PVA) (80% w/w) and polyvinyl pyrrolidone (PVP) (20% w/w) in a physical mixture. PVA is a very plastic material that maintains the geometric shape of tablets during dissolution. PVP, on the other hand, is a water-soluble agent that can cause pore formation and consequently contribute towards diffusion-controlled drug release[144].

The incorporation of Kollidon® SR at a percentage of 20% w/w (formulation K1) resulted in a significant prolongation of drug release with about 80% of MT-1207 released at the 24-hour timepoint (f_2=42, f_1=22)(Figure 13). Therefore, the incorporation of this polymer into the tablets provided a promising *in vitro* release profile that could result in a 24-hour sustained release of MT-1207 *in vitro*. However, since the release of MT-1207 from these tablets was marginally around 80%, it raised concerns regarding the potentially low bioavailability of the API due to an incomplete release *in vivo*. As per the EMA guidelines, the bioavailability of a drug from a controlled-release formulation under development should fall within 80.00-125.00% of the bioavailability of the drug from the reference (immediate-release) formulation to ensure bioequivalence between the different formulations[145].

Finally, decreasing the drug:polymer ratio (formulation K2) did not confer any statistically significant changes to drug release (f_2=90, f_1=2). This finding was in contrast with previous literature reports. Mulani et al. demonstrated that, as the formulation content of Kollidon® SR was reduced, the rate and extent of drug release were significantly decreased[146]. A possible explanation for this phenomenon was given in the study by Hussain et al.[147]. This could be the combination of the two hydrophobic polymers reduced the effective area between the drug and the dissolving media, hence decreasing the rate of drug diffusion from the matrix. In their experiments, two hydrophobic polymers, glyceryl monostearate and stearic acid, were combined to prolong the duration of drug release of gliclazide. Adjusting the ratio of the two excipients, however, has no significant effect within a particular range[147].

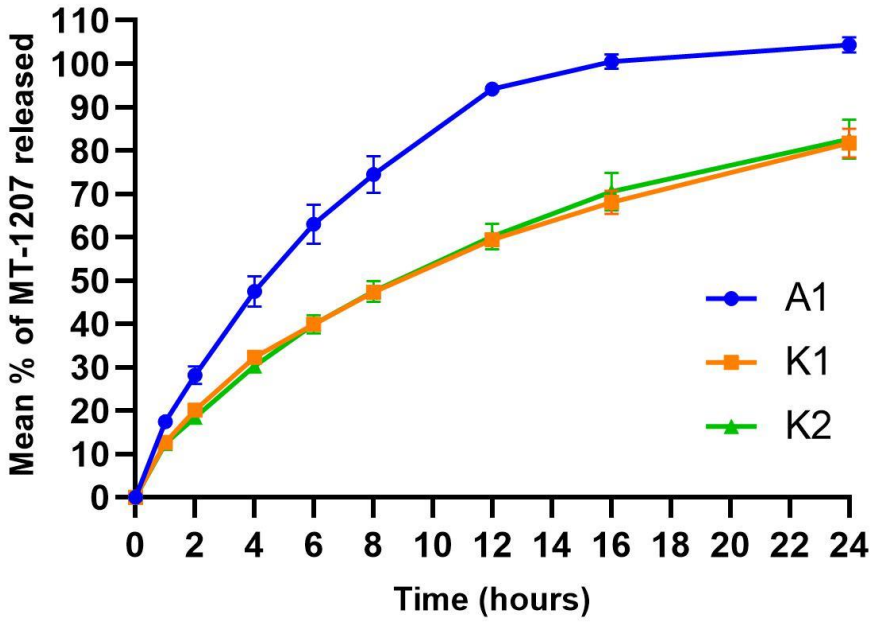


Figure 13: Release Profiles of MT-1207 from formulations containing combinations of HPMC K15M, Eudragit® RSPO, and Kollidon® SR (n=3 tablets). The results are presented as mean ± standard deviation.

3.7.5 Drug release from tablet formulations containing combination of HPMC K15M and Kollidon® SR

Based on the promising *in vitro* release data acquired from the incorporation of Kollidon® SR into the matrix it was decided for Eudragit® RSPO to be eliminated from the tablet formulations. The rationale for this decision was the fact that Kollidon® SR seemed to have strong release retarding properties and could potentially be successful in providing the desired 24-hour sustained release in combination with HPMC K15M. Mulani et al. used the same amounts of Kollidon® SR and Eudragit® RSPO to test the release of propranolol hydrochloride, and they found that compared to Kollidon® SR, Eudragit® RSPO-based formulations released the API much faster[146]. Furthermore, an *in vitro* drug release higher than 80% at the 24-hour timepoint was desirable to ensure a complete release of the API

from the tablets, as per the EMA specifications[148]. Therefore, tablets containing 20% w/w of Kollidon® SR were prepared and characterised in terms of their *in vitro* drug release to see whether a simpler tablet formula could provide a complete 24-hour sustained release of MT-1207. The *in vitro* release profiles from K3 to K5 containing HPMC K15M and Kollidon® SR can be seen in Figure 14.

According to previous reports, the recommended compression method for Kollidon® SR is direct compression[146, 149]. This is because previous investigations have shown that wet granulation in the preparation of Kollidon® SR-based formulations may result in faster drug release, compared to formulations prepared using direct compression methods. One possible reason to explain this phenomenon is that, during granulation, water-soluble PVP is deposited on the PVA particles, resulting in the formation of discrete particles positioned between the PVA particles. Consequently, this could contribute to a faster hydration of tablet formulations and the generation of more porous structures in matrix tablet[150, 151]. However, due to the small particle size and poor flowability of MT-1207 powder, direct compression was not possible. Considering that the particle size of Kollidon® SR was around 100 μm, a comparison between K3 and K4 in terms of their *in vitro* release profiles could provide information on a potential impact of the particle size of Kollidon® SR on drug release, in addition to the necessity of partial direct compression. Additionally, *in vitr*o release profile comparisons between K3 and K5, and between K4 and K5 provided information on the effect of the preparation procedure.

Formulation K3 released 83.5% in 24 hours and formulation K4 released 83.7% in 16 hours. However, the results of similarity factor showed that there was no statistically

significant difference between K3 and K4 regarding their *in vitro* drug release profiles (f_2=58, f_1=14). Interestingly, K5 which was produced using wet granulation exhibited extended release for up to 24 hours. 77.7% of the drug was released over 16 hours, and 88.8% of the drug was released by the 24-hour timepoint. The value of f_2 for comparison between K3 and K5, and between K4 and K5 was 75 and 68, respectively, which meant that there was not statistically difference between the *in vitro* release profiles of these three formulations. The similarity factor analysis results showed that both preparation method and particle size had no effect on drug release. Although the results of this study contradicted the hypotheses mentioned earlier, they were consistent with findings reported by Buler et al.[73]. Although direct compression is still the preferred approach for the preparation of Kollidon® SR-based formulations, wet granulation, roller compaction, and melt extrusion are other acceptable methods.

When these three formulations were compared to A1 for their *in vitro* release profiles, the values obtained for f_2 were 37, 44, 39 for comparisons between A1 and K3, K4 and K5, respectively, and the respective f_1 values of were all higher than 15. Therefore, the *in vitro* drug release profiles of each of the three formulations were statistically different to that of A1. Considering the ease of preparation, K5 was selected as the optimal formulation. Furthermore, these results confirmed that elimination of Eudragit® RSPO from the formulation had a minimum effect on the rate and extent of drug release from the tablets containing Kollidon® SR. When K5 was compared to K1 and K2, it was realised that f_2 was higher than 50 and, therefore, there was no statistically significant difference between the *in vitro* drug release profiles of those formulations.

In the previous studies, Kollidon® SR was frequently compared to HPMC K15M for drug releasing profile. However, it is rare for Kollidon® SR to be taken in conjunction with HPMC K15M, perhaps because previous reports have demonstrated that there may be no significant difference in the rates of release when comparing these two polymers separately. In a previous study conducted by Sahoo et al., the use of either HPMC K15M or Kollidon® SR as release retardants was effective in prolonging the release of propranolol hydrochloride. At around 12 h, formulations containing 60% w/w HPMC K15M and 60% w/w Kollidon® SR released 91% and 97% of the drug, respectively[152]. Furthermore, similar findings were reported by Sakr et al. who prepared tablets containing 30% w/w Kollidon® SR and 30% w/w HPMC K15M. Both of these tablet formulations prolonged the release of albuterol sulphate up to 8 hours[153]. In contract with the above, Kumar et al. successfully extended the release of ondansetron hydrochloride from a formulation containing HPMC K100M and Kollidon® SR at a ratio of 1:1 up to 24 hours using a factorial design[154].

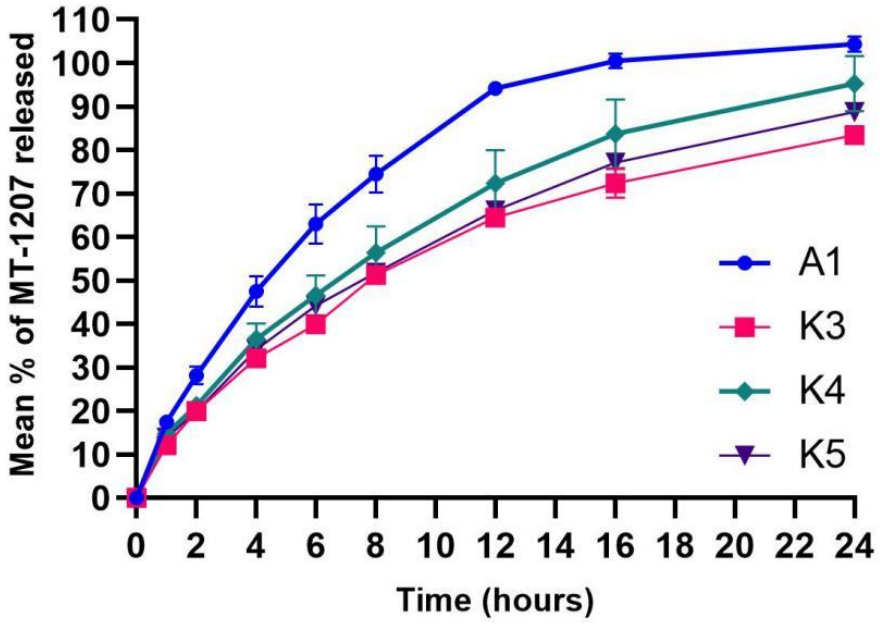


Figure 14: Release Profiles of MT-1207 from formulations containing combinations of

HPMC K15M and Kollidon® SR (n=3 tablets). The results are presented as mean ± standard deviation.

3.7.6 In vitro *release at 200 rpm for optimised formulation*

The correlation between *in vitro* release behavior and *in vivo* performance of sustained release drug products is complicated and highly dependent on several drug-related factors, such as formulation, the property of the drug[155]. A 200-rpm paddle rotation speed was used in an *in vitro release* experiment for K5. It has been previously reported that the *in vivo* conditions in fasted Beagle dogs may be better simulated using 200 rpm rotation speed, compared to usual rotation speed[124]. Despite the high-shear forces, the K5 tablets successfully sustained the release of MT-1207 over 24 hours (Figure 15). The *in vitro* drug release profile was similar to that recorded at 75 rpm ($f_2 = 72$, $f_1 = 6$). These findings further demonstrated the robustness of the sustained release of MT-1207 from the K5 tablets. Furthermore, since the drug release was unaffected by the magnitude of shear forces applied, it was probably largely independent of matrix erosion[156, 157].

Furthermore, animals are commonly employed to evaluate drugs and dosage formulations for oral bioavailability prior to human testing. The canine is a particularly valuable animal model to predict the ingestion capacity and is convenient for feeding oral dose forms. However, the bioavailability of dogs following the oral route differs from that of humans in many circumstances. Preclinical animal studies only give estimates of human bioavailability. This is mostly due to the numerous physiological differences between animals and humans such as motility and pH[158]. In dogs, the rate of meal emptying and subsequent recovery of the fasting motility pattern is substantially slower. In trials of various dosage forms designed to prolong

gastric retention time, canine data may lead to overoptimistic estimations for human performance[159].

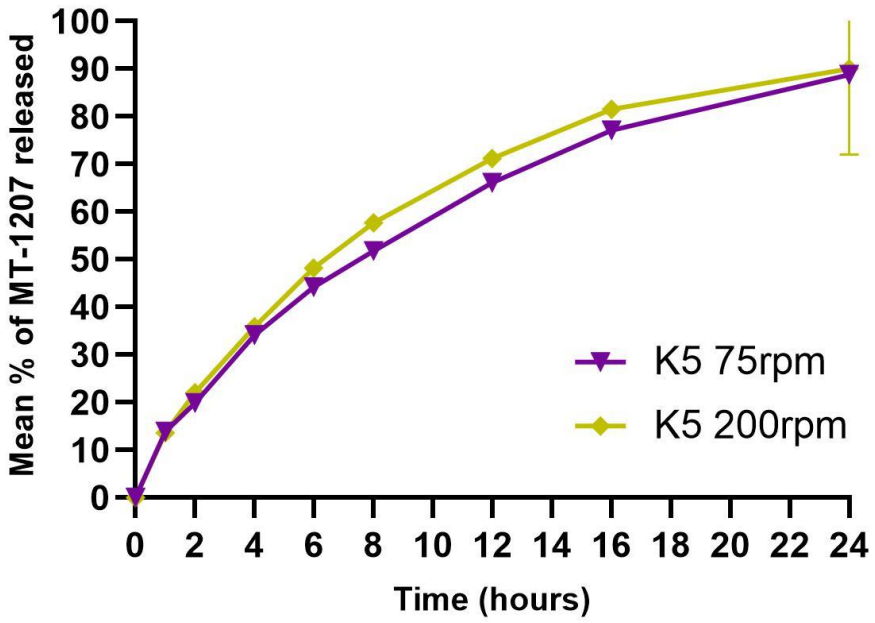


Figure 15: *In vitro* release profiles of MT-1207 from K5 tablets at paddle rotation speeds of 75 rpm and 200 rpm (n=3 tablets).

3.8 Kinetics and mechanism of MT-1207 release

To elucidate the kinetics of drug release from these formulations, the dissolution data were fitted to zero-order (cumulative amount of drug released vs time), first-order (log cumulative quantity of drug remaining vs time), Higuchi (cumulative drug release vs square root of time) and Hixson – Crowell (the cube root of the percentage of drug remaining vs time) models. The first 60% drug release data were also fitted to the Korsmeyer-Peppas model which is often used to describe the drug release behaviour from the polymeric systems[108,160]. When the data is plotted as a log of drug amount released versus time, a linear curve is generated with a slope equal to n and the K can be obtained from the Y-intercept. Drug release kinetics data from MT-1207 matrix tablets described by various mathematical

models and equations are listed in Table 6. The highest R^2 can indicate which model best describes the drug release kinetics from different formulations.

As indicated in Table 6, the drug release data obtained from all different formulations except K1 seemed to best fit at the first-order model, with R^2 values ranging between 0.9806 and 0.9978. This suggested that the release followed first-order kinetics and was controlled by reactant concentration from the formulation. For formulation K1, the best fitting model was the Higuchi model, with R^2 value of 0.9879.

When the data were plotted against the Korsmeyer-Peppas model, R^2 ranged between 0.9984 and 0.9999 and the value of diffusional coefficient (n) for all the formulations were in the range of 0.596 to 0.733. The high R^2 values demonstrated a good fit of the release data to the model, while the n values indicated that the mechanism of drug release was non-Fickian diffusion (anomalous transport). This suggested that the drug release mechanism was potentially controlled by a combination of drug diffusion and matrix erosion.

For the formulations containing HPMC K15M and K100M, the hydrophilic matrix system controls drug release by drug diffusion and erosion of the gel layer. When the gel layer is properly generated, drug release is mostly determined by the rate of drug molecule diffusion of the gel. However, as more water penetrates into the matrix, the outer polymer layer hydrates, accompanied by polymeric chain relaxation, resulting in erosion[136]. For the formulations containing HPMC K15M and Eudragit® RSPO, the most likely case is that HPMC swelling may produce a gel through which the drug diffuses, but also the drug can be released through the eroding Eudragit matrix[140]. According to a previous report, for formulations containing a high content of Kollidon® SR, the drug release mechanism was

Fickian diffusion. This could be the fact that the drug is mainly released by diffusion through the matrix pores. However, in formulations containing Kollidon® SR (K1-K5), the content of the polymer only ranged between 10 and 20% w/w. Additionally, its combination with HPMC allowed for swelling and erosion, resulting in a non-Fickian release model[161, 162].

Table 6: Kinetic analysis results for MT-1207 *in vitro* release from plots of all formulations

Batch number	Zero order		First order		Higuchi		Hixon-crowell		Korsmeyer-Peppas		
	K_0	R^2	K_1	R^2	K_H	R^2	K_{HC}	R^2	K_{KP}	n	R^2
D1	10.486	0.9594	0.149	0.9957	21.972	0.9635	0.044	0.9897	16.192	0.719	0.9998
D2	10.475	0.9625	0.149	0.9965	21.927	0.9607	0.044	0.9911	15.947	0.729	0.9998
D3	10.595	0.9617	0.151	0.996	22.185	0.9618	0.045	0.9907	16.202	0.726	0.9999
D4	8.894	0.9577	0.133	0.9978	21.364	0.9621	0.039	0.9926	14.902	0.716	0.9998
D5	8.88	0.9571	0.133	0.9973	21.334	0.9626	0.039	0.9921	14.923	0.715	0.9998
D6	8.873	0.9638	0.132	0.9977	21.278	0.9576	0.039	0.9942	14.431	0.733	0.9999
D7	8.211	0.958	0.118	0.9963	19.722	0.9622	0.035	0.9901	13.75	0.717	0.9998
D8	8.104	0.9512	0.116	0.9938	19.501	0.9668	0.034	0.9861	13.997	0.699	0.9996
D9	8.14	0.9588	0.117	0.9965	19.549	0.9614	0.035	0.9904	13.573	0.719	0.9998
E1	9.301	0.9431	0.144	0.9965	22.42	0.9702	0.042	0.9887	16.536	0.683	0.999
E2	8.425	0.9403	0.123	0.9909	20.327	0.9737	0.036	0.9814	15.237	0.674	0.9997
E3	7.481	0.9255	0.104	0.9806	18.105	0.98	0.031	0.9679	14.23	0.645	0.9992
K1	5.708	0.8708	0.084	0.97	16.455	0.9879	0.025	0.948	13.634	0.596	0.9989
K2	5.707	0.9041	0.083	0.9835	16.357	0.9786	0.025	0.9674	12.481	0.637	0.9992
K3	6.053	0.9192	0.09	0.9892	17.294	0.9716	0.027	0.9772	12.598	0.661	0.9984
K4	6.803	0.9134	0.108	0.9923	19.462	0.975	0.031	0.9817	14.464	0.651	0.9991
K5	6.275	0.899	0.096	0.9869	18.000	0.9793	0.028	0.971	13.899	0.631	0.9985

4.Conclusion and future work

4.1 Conclusion

MT-1207 is a multi-targeted anti-hypertensive drug that has indicated to be promising in treating hypertension and is currently undergoing phase II clinical trials in the form of immediate-release tablets. However, the requirement for increased frequency of oral uptake of immediate-release tablets to treat hypertension necessitated the development of a once-a-day sustained-release tablet formulation.

The aim of this study was to optimise the sustained release formulation of MT-207 to maintain an effective level of the drug over a 24-hour period. Different matrix tablet formulations of MT-1207 were prepared using HPMC K15M in combination with different polymers.

Tablets containing a combination of HPMC K15M and HPMC K100M were unable to provide a sustained drug release beyond 16 hours *in vitro*, irrespective of the polymer or drug:polymer ratios applied. The same *in vitro* release behaviour was recorded for tablets that were prepared containing HPMC K15M and Eudragit® RSPO as release retardants. However, tablets containing 31% w/w HPMC K15M and 20% w/w Kollidon® SR were able to provide a complete 24-hour sustained release of MT-1207 *in vitro* at both 75 and 200 rpm paddle rotation speeds. The robust and desirable properties and release behaviour of this MT-1207 formulation rendered it promising as a once-a-day treatment for hypertension.

4.2 Future Work

A stability study will be conducted for the optimised formulation of MT-1207 to assess its stability under extreme temperature, humidity and light conditions. This study is a

necessary part of formulation development and quality control of each pharmaceutical product and should, therefore, be conducted for the optimised formulation of the present study. Furthermore, the tablets will be forwarded to a pharmacokinetic study in Beagle dogs to ascertain the effectiveness of the formulation and conclude whether this formulation provided a more sustained pharmacokinetic profile of MT-1207, compared to A1 formulation that was previously developed by Vrettos et al. The optimised tablets are currently being tested in Beagle dogs for its *in vivo* pharmacokinetic studies .

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