Chronopharmaceutical drug delivery system of Anti-Hypertensive drugs: Process optimization and biopharmaceutical evaluation

Thesis Submitted by

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PROFORMA-1

"STATEMENT OF ORIGINALITY"

I, Apollo James, Registered on 09 November 2015 do hereby declare that this thesis entitled "Chronopharmaceutical drug delivery system of Anti-Hypertensive drugs: Process optimization and biopharmaceutical evaluation" contains literature survey and original research work done by undersigned candidate as part of Doctoral Studies.

All Information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I have fully cited and referred all material and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iThenticate Software is **5%**.

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I do hereby declare that the work incorporated in the thesis entitled, "Chronopharmaceutical drug delivery system of Anti-Hypertensive drugs: Process optimization and biopharmaceutical evaluation" submitted by Apollo James, has been carried out by me in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, and Nandha College of Pharmacy, Erode, Tamilnadu under the supervision of Prof.JasminaKhanam and Prof.(Dr.)Mohanraj, Department of Pharmaceutics, Nandha College of Pharmacy, Erode-638032. Neither the thesis nor any part thereof has been submitted for any other degree.

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Dedicated to Almighty,
My Family and
All Well Wishers

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Preface

The current study entitled “chronopharmaceutical drug delivery system of anti- hypertensive drugs: process optimization and bio pharmaceutical evaluation” involves the design, formulation, in vitro and in vivo evaluation of a pulsatile core in cup drug delivery system. Chronopharmcological delivery of antihypertensive drugs is necessary to manage an increase in blood pressure in the early morning by consuming the drug at bedtime. The drug delivery system was aptly designed to release the drug adequately when required, which helps to avoid the presence of a high dose of metoprolol and atenolol throughout the day in the blood circulation and to reduce adverse reactions. The drugs chosen for the study were the cardioselective beta-blockers metoprolol and atenolol. The designed formulation was with a sustained drug release polymeric plug, an immediate release core, and a plug/mucoadhesive plug. The formulation provides the drug a lag period before the sudden burst delivery from the inner core. That helps in the delivery of drugs at a predetermined time. Preformulation studies were carried out to characterize the drugs and the excipients and identify any incompatibilities. The formulation was optimized using the least square method in JMP software. An optimised formulation was prepared by the direct compression method. Furthermore, the developed formulations were evaluated using in vitro and in vivo studies.

The introduction, chapter 1, describes chronobiology, chrono pharmacology, chronotherapeutics, and chronopharmaceutics. The chapter also gives an idea about the influence of chronobiology in various diseases and the necessity of adopting chronotherapy for the best therapeutic outcomes. Chronopharmaceutical drug delivery systems play a significant role in chronotherapy. Various chronokinetic

drugs and different pharmaceutical delivery systems and their use in various diseases are specified in the chapter.

Chapter 2 included the updated research work and reviews that support the design and completion of the research work.

Chapter 3 specifies the aim and objective of the work.

Chapter 4 (materials and methods) discusses the preformulation studies, the formulation design and development, optimization of the formulation, preparation of the core in cup tablets, and their evaluation methods in detail.

Chapter 5 encompasses the results of the entire work, starting from the preformulation studies. All results are written in detail with the support of the study reports and justified with scientific evidence to prove their acceptability in pulsatile drug delivery.

Chapter 6 contains the conclusion of the study in detail.

Chapter 7 is the list of references used in the entire research work.

CONTENT

LIST OF ABBREVIATIONS

Acronyms	Abbreviations
ACTH	Adrenocorticotropic hormone
TSH	Thyroid Stimulating Hormone
TNF	Tumor Necrosis Factor
INF	Interferon
ICS	Inhaled corticosteroid
NSAID	Non steroidal anti-inflammatory drug
5-FU	5-Fluorouracil
MBPS	Morning blood pressure surge
ABPM	Ambient blood pressure monitoring
RCT	Randomised controlled trials
HMG-CoA	3-hydroxy-3methylglutaryl-coenzyme A
C_{max}	Maximum concentration of drug reaches systemic circulation
T_{max}	Time to reach maximum concentration
MIC	Minimum Inhibitory Concentration
VPA	Valporic acid
AUC	Area under the curve
AUMC	Area under the first movement curve
PDDS	Pulsatile drug delivery system
HPMC	Hydroxy propyl methyl cellulose
EV Ac	Ethylene and vinyl acetate
IR	Immediate release
SR	Sustained Release
CDDS	Chronotherapeuticdrug delvery system
DALY	Disability-adjusted life year
BP	Blood Pressure
3DP	3D Printing
L-HPC	L-hydroxypropyl cellulose

HCO	Hydrogenated Castor Oil
BCS	Biopharmaceutical Classification system
FDA	Food and Drug Administration
DSC	Differential scanning calorimeter
FTIR	Fourier Transform Infrared spectroscopy
PVP	Polyvinyl pyrrolidine
MCC	Microcrystalline cellulose
API	Active Pharmaceutical Ingredient
ICH	International Council of Harmonization
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
HPLC	High performance liquid chromatography
DDW	Double distilled water
USP	Untied States Pharmacopeia
DF	Degree of freedom

LIST OF TABLES

Table No. **Page No.**

LIST OF FIGURES

Figure No. **Page No.**

CHAPTER-1

1 INTRODUCTION

Oral administration of drugs is the most effective method of delivery to achieve systemic effects for the disease, such as hypertension. Oral drug administration techniques account for over half of all drug delivery systems now on the market, and these techniques have distinct value in terms of patient compliance and simplicity of delivery. Chronotherapeutics, a type of therapeutic delivery strategy, has been essential in the treatment of chronic diseases in recent years. Traditional drug delivery strategies sought to enhance therapeutic efficacy while avoiding side effects by delivering medication in a constant or continuous manner. The drug is released in a regulated or variable manner using these dosage forms. Diseases are treated by delivering individuals drugs in a variety of standard dosing regimens. All of these dose patterns should be taken in a consistent manner to keep the drug concentration within a therapeutically suitable level. Controlled distribution drug maintains a constant level of the drug rather than administering it as and when it is required. Conventional dose formulations are insufficient to address the demands of conditions that manifest during a certain time of day or night. Modified-release formulations are necessary to reduce dose frequency and improve patient adherence. Some of the concerns related with modified dosage form formulations include drug resistance, tolerance, and activation of physiological systems due to sustained constant drug concentration in the body. Pulsative dosage formulations can assist with this issue. Pulsatile dosage forms are useful when constant Plasma drug levels are neither desired, nor a perfect therapeutic regimen impact is achieved from a regularly fluctuating drug concentration. (Bussemer T, et al. 2008, Manuntikumar B Mehta et al. 2004)

The study of biological rhythms is known as chronobiology. It focuses at how time influences biological activity and internal biological clocks. Chronobiology has evolved into a multidisciplinary subject of interest in general medicine and psychiatry during the last few decades. Chronobiology research is constantly evolving, and it has aided in the creation of an interdisciplinary field of study. The daily light-dark or day-night cycle, as well as the earth's rotation, have had long-term and consistent biological effects on living organisms. These biological clocks aid organisms in completing their tasks on a 24-hour cycle. This adaptive capacity

allows behavioural and physiological harmony by predicting cyclic changes. These endogenous clocks are beneficial since they can act autonomously and retain time without relying on external light-dark transitions to keep track of time. The endogenous circadian rhythms that are controlled by the suprachiasmatic nucleus, the body's primary circadian clock.Figure 1.1 represents the variation in the body function according to the time variance. The circadian clock influences the entire reaction of the body. It also influences the disease pathology and the effectiveness of a drug. It is becoming viable to target circadian rhythms for illness prevention and therapy as our understanding of the molecular and cellular mechanisms that underlie circadian physiology and pathology grows. The notion of synchronizing disease treatment with a patient's body clock or circadian rhythm, the more technical word for the biological processes is known as chronotherapy. (Shidhaye SS et al. 2010, Richards Grayson AC et al. 2003, Santini JTJ et al., 1999). Circadian rhythms differ amongst organ systems. Circadian rhythm affects when the appearance of the most severe symptoms of a disease. Hypercholesterolemia, allergic rhinitis, asthma, rheumatoid arthritis, peptic ulcer, mental disorders, diabetes, cancer and cardiovascular disease are some of the chronic diseases that have been examined. Some of the diseases and the influence of circadian rhythm are discussed.

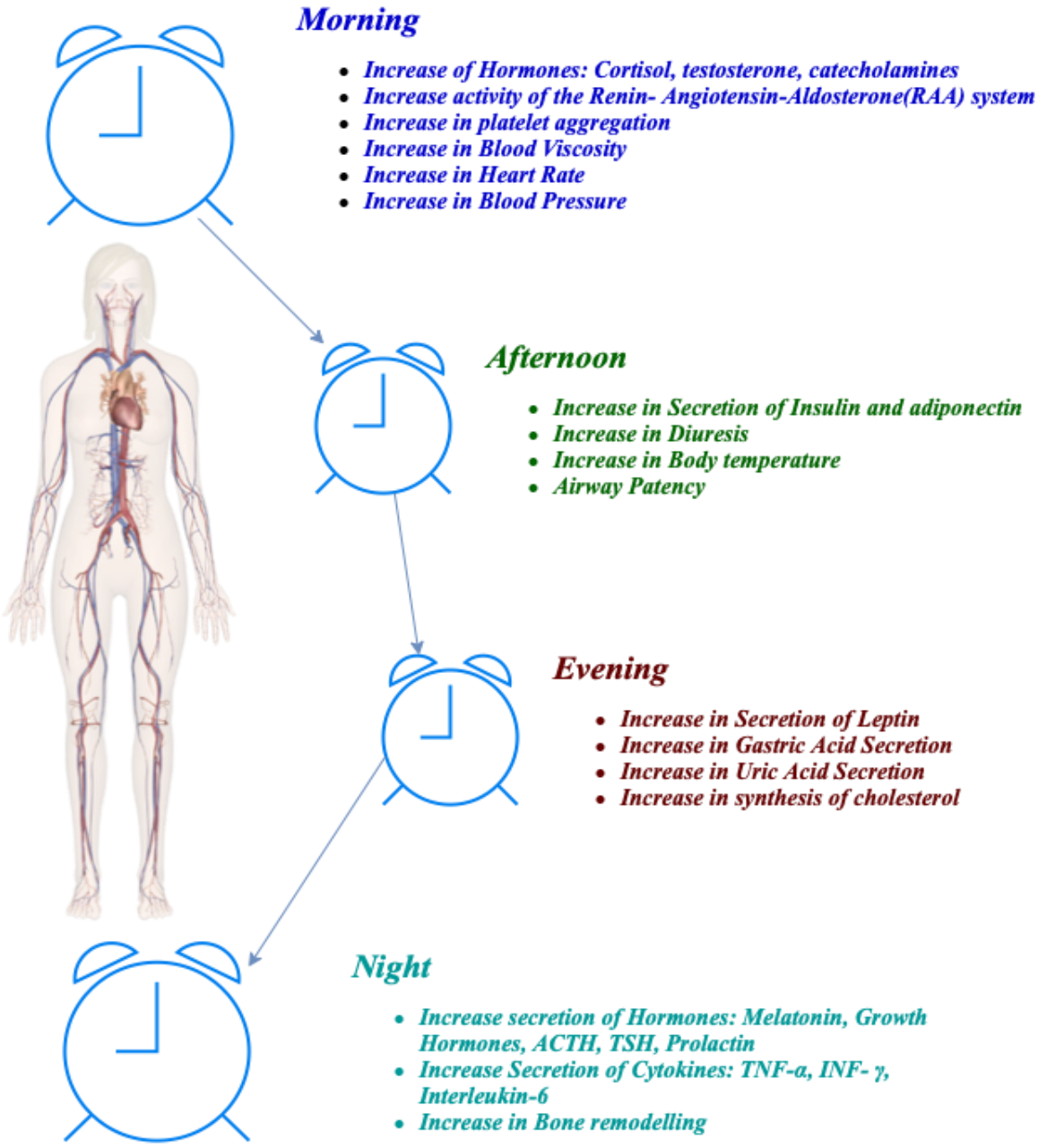


Figure 1.1. Influence of time on body functions

1.1 Diseases with existing circadian rhythm

The disorders targeted for pulsatile delivery have quite enough empirical support to justify the use of a timed pharmaceutical drug delivery system over traditional drug administration. Night time asthma, arthritis, duodenal ulcer, cancer, cardiovascular disease, diabetes, hyperlipidaemia, and neurological disorders are among these diseases (Yang SY et al. 2010).

1.1.1 Bronchial asthma

It is defined by airway inflammation, which makes the lower respiratory tract hypersensitive to a variety of environmental stimuli. (Smolensky MH et al., 2007) Airway resistance gradually increases during the night in asthmatic patients. This kind of asthma is distinguished by an increase in symptoms, airway reactivity, and/or lung function. During the day, antigen stimulates mast and eosinophil cells, causing them to produce pro-inflammatory mediators, which causes edema, smooth muscle bronchospasm, contraction, and overactivation of mucus glands, culminating in mucus hypersecretion of the lung's limited airways. Because bronchoconstriction and clinical symptoms worsening have a diurnal rhythm, it is an excellent candidate for chronotherapy. (Smolensky MH et al., 2007)

Bronchial asthma has a diurnal cycle, with symptoms peaking at night and in the early morning. Dosing anti-asthmatic medications at night has been demonstrated to be effective in bronchial asthma studies. A single dose of the bulk of the drugs presently used for asthma chronotherapy at night aids patients' adherence to their prescriptions and improves asthma management. Because of the day-night cycle and circadian rhythm dependency, chronotherapy is both essential and beneficial for Nocturnal Bronchial Asthma. (Martin RJ et al., 1998, Waldman-Wagner c. 1990, Lewis SM et al. 1996) Chronotherapy is increasing in popularity because it allows clinicians to administer drugs at certain times based on the pathophysiology of the disease, improving patient treatment efficacy and compliance. The goal of bronchial asthma chronotherapy is to achieve the best possible long-term outcomes: fewer asthma symptoms, optimal peak flow levels, the fewest number of medication side effects, and the maintenance of regular or slightly elevated pulmonary function, lifestyle, exercise, and sleep in asthmatic patients (Smolensky MH et al. 1997. Darzen JM et al. 1997, Mandal AS et al., 2010). Some of the pharmaceuticals often used for asthma chemotherapy include anti-inflammatory medications to regulate airway inflammation, bronchodilator 2 agonists to relieve bronchospasm and bronchoconstriction, and corticosteroid treatment for very severe types of BA. (Alessandra Maroni et al. 2010, Zuzana Diamant et al. 2018, Vianna EO 1998). Synthetic glucocorticoids have been employed as a short-term "burst" to treat an asthmatic exacerbation, as well as long-term avoidance of symptoms in severe

chronic asthma; inhibition, regulation, and inversion of inflammation (Harter JG et al. 1963). Inhaled corticosteroids (ICS) are a highly effective anti-inflammatory medication for the long-term management of persistent asthma. Inhaled corticosteroids can reduce or eliminate the need for oral steroids (McFadden ER et al. 1991).

Adult bronchial asthma is most commonly treated with first-generation aerosol corticosteroids (Dyer Mj et al. 2006). These drugs reduce airway hyperresponsiveness to antigenic and other asthma stimuli by reducing the synthesis of cytokines that promote airway inflammation (Barnes PJ et al. 1990, Barnes PJ et al. 2004, Horiguchi T et al. 2004)

1.1.2 Allergic rhinitis

Allergy rhinitis symptoms include sneezing, sinus rhinorrhea, itchy red eyes, nasal irritation, and trouble breathing (Buriko N et al. 2005). Each symptom was shown to occur more frequently before breakfast and in the morning, and less frequently in the afternoon.

1.1.3 Pain

Pain control is one of the most important therapies aims (Bruguerolle B et al., 2009). The highest threshold was observed towards the end of the resting period, whereas the lowest was obtained at the end of the engagement phase. Individuals with rheumatoid arthritis showed increased levels of interleukin-6 and C- reactive protein in their plasma, which follow a diurnal pattern (Saitohl T et al. 2000). Opioid peptides such as 5-hydroxytryptamine, bradykinin, glutamate, NO, substance P, cytokines, and prostanoids also activate nociceptors (Bruguerolle B et al. 2009). In a rat model, the fraction of substance P in the brain is higher at night than during the day. Endogenous opioid peptide levels in newborns and human adult participants are higher in the morning and much lower in the late afternoon, according to studies. Patients with osteoarthritis have less pain during the day and increased pain at night. Patients with rheumatoid arthritis have discomfort that worsens in the morning and progressively improves in the afternoon. Two of the symptoms include finger swelling and joint pain (Reddy RK et al. 2009). Patients with gastroesophageal reflux

disease have night time pain (Bruguerolle B et al., 2009). Renal colic, on the other hand, occurs in the morning regardless of gender or the presence or absence of visible kidney stones. The kind and duration of the pain influence the type of analgesics used and how they are delivered. Nociceptive pain is treated with antiepileptics, local anaesthetics, and tricyclic antidepressants, whereas neurogenic pain is managed with anticonvulsants, paracetamol, NSAIDs, and morphinomimetics.

1.1.4 Duodenal ulcer

In most duodenal ulcer patients, stomach acid output is greatest in the late afternoon and reduces largely in the early hours (Saitohl T et al. 2000, Moore JG et al. 1986) One set of researchers assessed the consequences of ulcer rupture on a daily (circadian), weekly (circaseptan), and annual (circannual) basis (Svancs C et al. 1998). Across seasons, decades, and days of the week, a regular and very steady circadian rhythm has been identified. Perforations in the intestines increased initially about lunchtime and then again around evening, with duodenal perforations increasing the most in the afternoon.

1.1.5 Cancer

Several clock genetic features are linked to the stimulation of transcription and post-transcription processes, as well as the presence of regulatory loops in mammalian cells that cause circadian oscillation (30). CLOCK: BMAL1 protein dimers, in particular, are required for the transcription of the clock genes Per and Cry. However, the clock genes Per1, Per2, Bmal1, and Rev-erb have been shown to be expressed in a few mouse models. The desynchronization of certain tumour cells that form a solid tumour may result in tissue-level clock gene-related rhythm alterations.

A deviation of minutes or hours in each cell's intrinsic rhythm from its neighbours causes this condition. Reduced expression of Per1, Per2, or Per3 genes at a single time point in comparison to reference tissue implies a shift in the molecular clock of human cancers. Furthermore, cancer patients have greater blood flow to the affected area than the rest of the body (8). Animal studies have also demonstrated that the rate

of survival varies according to the circadian dose schedule of anticancer drugs (Levi F et al. 2007). The percentage of patients who lived approximately doubled when 6-mercaptopurine and methotrexate were administered in the evening rather than the morning (Reddy RK et al. 2009). Another set of researchers compared the impact of continuous 5-flourouracil (5-FU) infusion to 5-FU delivery regimens with peaks at 4 a.m., 10 a.m., 4 p.m., or 10 p.m. (Altinok A et al. 2009). According to the result findings, 5-FU has a negligible cytotoxic effect on circadian delivery. On a daily basis, blood flow variation at the rat's subcutaneous tumour site was also observed (Hori K et al. 1992).

The findings revealed that tumour arterial flow was much higher at night than during the day. There were no significant differences in mean arterial pressure, cancer development, or body mass among the day and night time groups of rats. Normal tissue blood flows were measured in the subcutaneous tissue, liver, kidney, cortex, bone marrow, and tumour tissues (SLC) both during the day and at night (Hori K t al. 1996). Rats were used as test subjects. In all normal tissues, there were no significant changes in average blood circulation across two separate time zones. Blood flow in malignant cells, on the other side, was much higher late at night than early in the morning. These findings suggest that blood circulation at the tumour location follows a 24-hour cycle.

1.1.6 Cardiovascular diseases

In cardiovascular disease, vascular responsiveness and capillary resistance remained higher in the morning and gradually decreased over the day. Platelet aggregability rises in the morning, whereas fibrinolytic activity falls, resulting in blood hypercoagulability. As a result, between both the hours of 10 am and 12 pm, the risk of myocardial injury and sudden cardiac arrest is increased (Tofler GH et al. 1987). The stated blood pressure in ambulatory blood monitoring fluctuates significantly during the day. This variance is influenced by peripheral factors such as race, sexuality, autonomic nervous system tone, vasoactive chemicals, haematological, and renal variables. Heart rate, blood pressure, autonomic tone imbalances, and circulating levels of catecholamines that govern cardiac arrhythmias all show considerable diurnal change, contributing to the formation of the circadian rhythm

(Portaluppi F et al. 2007). Atrial arrhythmias tend to be diurnal, with high frequencies during the day and decreased frequency at night, and the abnormal foci appear to be subject to same long-term autonomic control as normal pacemaker tissue. According to the study, ventricular tachyarrhythmias show a late morning peak in those who have had a previous myocardial infarction and an afternoon peak in people who've had a recent heart attack. Coronary ischemia, angina pectoris, acute myocardial, and sudden cardiac death also are unevenly distributed throughout a 24-hour period, with reach high in the late afternoon or early evening. The pharmacokinetics and pharmacodynamics of various oral nitrates, calcium channel blockers, and -adrenoceptor antagonist drugs have been shown to be affected by the circadian time of administration. ZiyanXie et al., performed thea systematic review and meta-analysis for Morning blood pressure surge (MBPS) and cardiovascular diseases and they sated that MBPS are linked to a higher risk of cardiovascular disease and death. With more focus on diurnal blood pressure changes and widespread use of ambient blood pressure monitoring (ABPM), chronotherapy, or drug delivery based on biological rhythm, has been shown to enhance cardiovascular outcomes. The goal of this research is to see how antihypertensive medication chronotherapy affects MBPS in patients with hypertension.

Adult patients having primary hypertension have been included in randomised controlled trials (RCTs) evaluated the effectiveness of evening and morning delivery of the same drugs. According to the findings, antihypertensive drugs administered in the evening had a superior blood pressure-lowering influence on MBPS than those administered in the morning. The night time regimen did not raise the risk of adverse events, according to the safety evaluation. The details on these studies are explained using figure 1.2. (Xie Z et al. 2021)

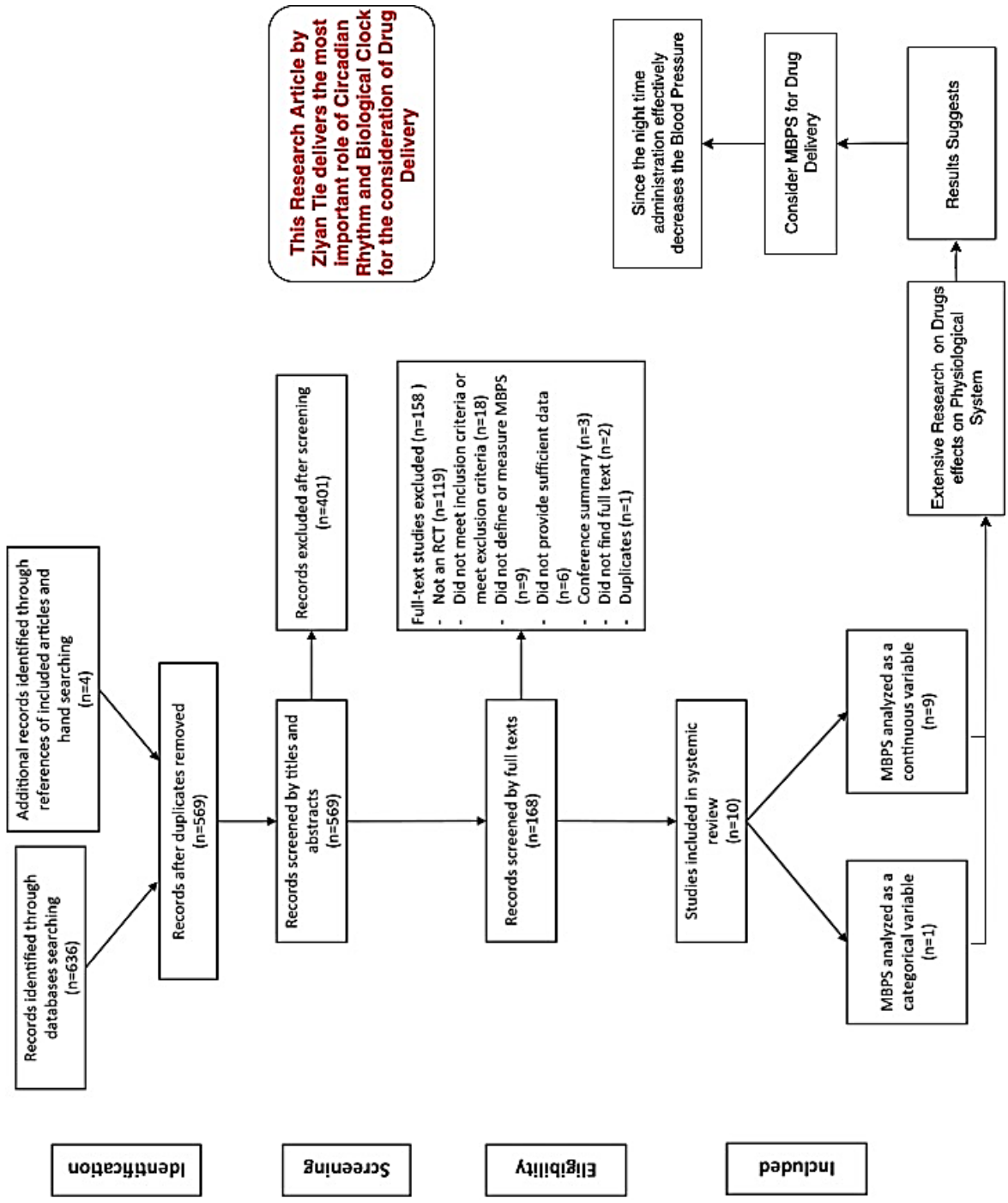


Figure 1.2. Study details

1.1.7 Diabetes

Circadian cycle of insulin needs and its actions are frequently questioned in the context of type I diabetes, both physiologically and clinically (Waldhausl W et al. 1989). Insulin is often supplied in a pulsatile rhythm, although it can also be injected irregularly. Insulin could have a cyclic circadian rhythm of 8-30 minutes, suggesting

that it is at its peak performance. Basal insulin release also has positive inotropic and distorted on B cells. Stress hormones such as cortisol, epinephrine, and growth hormone may impair target cell sensitivity to insulin action and hyperglycemia, whereas intrinsic rhythmicity, dehydration, and prolonged insulin cessation may stimulate a secondary feedback signal on insulin release, which may aim to increase blood sugar levels. Circadian modulators of insulin action and action are separated, and the manner of insulin release is regulated subsequently. As a result, any shift in plasma insulin concentrations between a daily peak and lowest must be regarded a complicated secondary circadian rhythm, separate from its short-term rhythmicity. It is due to the fluctuating second insulin resistance that develops in the early morning and late afternoon.

1.1.8 Hypercholesterolemia

A circadian pattern is seen during cholesterol manufacturing. The production of cholesterol is frequently higher at night time than during the day. It varies based on the individual. Maximum output occurs 12 hours after last meal, in the early morning. In analyses, the evening dosage of 3-hydroxy-3methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor was found to be more efficacious than the morning dose. The rate-limiting enzyme HMG-CoA is more active at night (Jones PJH et al. 1990). The diurnal variations, on the other side, are induced by the periodicity or degradation of the regulatory enzyme.

1.1.9 Sleep disorder

Most biological signalling system, including such central and autonomous nervous system's sleep disorders, have a complex temporal structure that includes rhythmic and pulsatile variations at many frequencies. The amount of sleep required by each individual is typically constant. Regardless of the fact there is a great deal of variation between persons (Kumar VM et al. 2008). Sleep is characterised by a rhythmic (circadian) combination of changes in body biochemical and physiological systems. Whenever a circadian rhythm gets interrupted or specific processes throughout sleep are abnormal, a variety of ailments can arise. For example, delayed sleep phase syndrome is characterised with serious sleep insomnia (Ritschel WA et al. 1998). Sleep is usually not possible before 3 a.m. or until getting up at the usual

hour becomes incredibly tough. Individuals differ in their capacity to acclimatise to the circadian cycle. Identifying individual variance is half the battle when dealing with various sleep problems.

1.1.10 Epilepsy

The circadian cycle may also impact some forms of epileptic seizures (Hofstra WA et al. 2008). The influence of a circadian rhythm in epilepsy of certain partial seizures has been identified in numerous experimental animal models. The method for monitoring the human circadian cycle is also investigated. Behavioural chronobiology allows for the detection of potentially novel regulatory systems related to the basic causes of epilepsy (Poirel C et al. 1991). As a result, the circadian psychophysiological patterns of epilepsy expose dynamic biological systems, implying endogenous mechanisms of intermodulation among observation and seizure susceptibility. Furthermore, applying chronobiologic concepts to epileptic behaviour implies the formation of new heuristic features in comparative psychophysiology.

1.1.11 Alzheimer's disease

Changes in the circadian rhythm occur in Alzheimer's disease patients (Volicer L et al. 2001). Individuals having Alzheimer's disease had lower diurnal motor behaviour, a higher proportion of nocturnal activity, poorer inter-day motor activity stability, and a later action acrophase (peak period) than healthy adults. Alzheimer's disease induces neurodegeneration in the suprachiasmatic nucleus, disrupting the brain's circadian rhythms. This disorder causes patients to have a higher core body temperature. Circadian abnormalities are visible in this disorder, along with cognitive and functional deterioration. There are still no additional changes evaluated.

1.1.12 Parkinson's disease

Parkinson's disease produces autonomic dysfunction, which results in several alterations to the circadian rhythm in blood pressure, and also increased diurnal blood pressure variation and postprandial hypotension (Bruguerolle B et al. 2002). The occurrence of a circadian rhythm in this situation, however, has yet to be confirmed. Clinical data show daily fluctuations in motor activity pattern, but it is hard to forecast the influence on illness progression and the subsequent functions of drugs.

1.1.13 Coagulation disorder and thrombosis

The mobility and preservation of blood within the circulatory system are critical to life (Haus E et al. 2007). The haemostatic system is generated by the actions and interactions of several factors that work together to provide these dual functions. Many circulatory and haemostatic system components, including muscle cells, the aorta, peripheral vascular muscle, and endothelium, were shown to have a circadian rhythm. Circadian rhythm time structure variations can result in hypercoagulability and thrombosis, or hypocoagulability and haemorrhage. Haemostasis is affected by peripheral resistance, blood circulation, blood viscosity, blood pressure, and heart rate. Peripheral vascular resistance decreased in the afternoon, leading to an increase in blood circulation in diurnally active individuals. The vasomotor tone of the coronary and peripheral artery, and the vasoconstrictor responses to adrenaline, are greater in the morning than in the afternoon. Thromboglobulin concentrations are highest at 6 a.m. and lowest around noon and midnight. Factor VII fluctuates significantly between 8 a.m. and noon, although its antigen level doesn't.

1.1.14 Infectious disease

The incidence of infectious illnesses has changed over time (Beauchamp D et al. 2007). Fever caused by bacterial illnesses is more common in the evening, but fever caused by viral infections is considerably more common during the day, and influenza is prevalent through-out winter season. Morbidity and mortality were found to be the highest in the winters and lowest in the summer in both the northern and southern hemispheres. The weight of nasal discharges in cold patients' peaks in the morning, then falls throughout the day until rising slightly in the evening time. Though the cause of individual infectious disease seasonal patterns is complicated due to the involvement of several factors, seasonal cycles in infectious diseases are commonly attributed to seasonal variations in weather/atmospheric conditions, pathogenic or incidence of casual pathogens, and/or variations in host actions.

1.2 Chronokinetic drugs

Antihypertensive drugs: Almost all physiological activities and pathological processes, including the pulmonary circulation, exhibit repeated rhythmic variations within 24 hours of the day. As per clinical chronopharmacological studies, the effects

of antihypertensive drugs on blood pressure or heart rate rhythms vary with the time of day. Chronopharmacokinetic investigations revealed daily fluctuations in the pharmacokinetics of propranolol, oxprenolol, nifedipine, verapamil, and other medications. Following morning administration of certain lipophilic medications, C_{max} was higher and/or T_{max} was shorter than after overnight treatment. Not whether daily changes in the pharmacokinetics were found, the dose-response relationship was generally time-dependent.

1.2.1 Antibiotics

Many studies have revealed that the kinetics of antimicrobial medications change with time. As per experimental animal models, the interval during which antibiotic levels may likely be higher than the MIC (T>MIC) is the most crucial factor in determining the in vivo efficiency of antibiotics such as beta-lactams, which have deadly effects irrespective of concentration in vitro. As a result, frequent alterations in pharmacokinetics could be to blame for decreased chemotherapeutic action. This is crucial when low-susceptibility bacteria are involved in the infectious process (Rebuelto M et al. 2003). Another important aspect of antibiotic chronokinetics is that, as we observed with aminoglycosides, not only the effectiveness but also the toxicity of the treatment can fluctuate depending on the daytime. The below given drugs are the most noteworthy antibiotic chronokinetics findings:

1.2.1.1 Aminoglycosides

When aminoglycosides were supplied during the experimental animals' rest period, the toxicity was at its highest, but when they were treated during the active process, the toxicity was at its minimum. According to multiple studies and data in the current literature, the toxicities of aminoglycosides on renal function can be reduced by administering the medication in a single injection each day while patients are active (at day time). The mechanisms behind the temporal variation in aminoglycoside kidney damage are yet unclear (Michel lebrun et al. 1999).

1.2.1.2 Gentamicin

Gentamicin's effectiveness and toxicology changed throughout a 24-hour period, with efficacy being greatest whenever the drug's toxicity was least.

Thus, administering gentamicin early or late in the day in people may reduce renal toxicity while increasing the efficiency of such antibiotics (Roy p et al. 2004).

Tobramycin: Tobramycin taken at 200 h (night time) had a substantially higher CLT and AUC than tobramycin administered at 1400 h. (day period) (Rebuelto M et al. 2003).

1.2.1.3 Amikacin

In humans, amikacin had higher kel values during the day than at night.

1.2.1.4 Ceftriaxone

Total clearance of ceftriaxone varies throughout the day in rats, peaking during the dark (active) time and decreasing during the daylight (relaxation) period (Rebuelto M et al. 2003).

1.2.1.5 Ciprofloxacin

The fraction of ciprofloxacin eliminated in urine in humans was greater when the antibiotics were provided at 1000 hours as opposed to 2200 hours.

1.2.1.6 Valporic acid

During the absorption phase following oral therapy, mean absolute VPA concentrations in plasma was significantly higher during the day than at night. Despite no variations in other pharmacokinetic characteristics between the morning and evening trials, C_{max} was higher, t_{max} remained shorter, and the absorption rate constant (ka) significantly higher in the morning.

1.2.1.7 Sumatriptan

Sumatriptan is the drug of choice for migraine treatment, because migraine is an illness with symptoms that occur at regular intervals, chronotherapy may be helpful in addressing the problem. Following the 7h injection, the mean peak serum concentration. Following the administration at 7:00 a.m. and 01:00 pm, the mean area under serum concentration time curve from zero to the last time point (AUC0-t), the area under the serum concentration time curve from zero to infinity (AUC0-infinity), and the area under the first movement curve (AUMC) were considerably

larger after the 1900 h treatment. The mean oral clearance and apparent volume of distribution were considerably lower after the 700h administration than after the 1900 h administration. The differences could be attributable to changes in the degree of absorption over time and/or changes in hepatic flow on a daily basis (Poondru S et al. 2000).

1.2.1.8 Cyclosporine

The pharmacokinetics of cyclosporine were studied in five pancreas transplant patients in one research. In these cases, the lower apparent clearance between night and day results in a somewhat expanded area under the concentration-time curve. There was a significant delay in mean residence time after the dusk treatment, and the night time area under the moment curve is higher than the dawn value. We propose three chrono pharmacokinetics dosing ways to obtain equal cyclosporine exposure throughout activity and resting times by changing the dusk dose delivery timing or transferring the normal amount. The necessity for a more sophisticated time-dependent cyclosporine dose approach to balance dawn and dusk drug exposure and improve immune suppression is highlighted by these trends and disparities (Cipolle RJ et al. 1988).

1.2.1.9 Methotrexate

In one trial, six children with leukaemia were given a methotrexate dosage at 10 am and 9 pm As a result, plasma clearance at night decreased considerably. Differences in passive tubular reabsorption produced by the urine pH cycle would be the primary source of these variations (among others). In another animal (4 Pigs), a high diurnal regularity of methotrexate serum levels was detected in two pigs at 01:00 in theafternoon (Aurelie Premaud et al. 2002).

1.2.1.10 NSAID

Ketoprofen: When ketoprofen was administered in the morning, the rate of absorption also was enhanced. Indomethacin: The peak concentrations was significantly higher and sooner when the drug was given at 07:00 or 11:00 h rather than 15:00, 19:00, or 23:00 h (MarikkiHalsas et al. 1999).

With reference to the above-mentioned challenges in achieving the drug delivery based on the circadian rhythm and biological clock, the following drug delivery approaches found to be more promising to administer the drug by understanding the chrono pharmacology of any drug substance.

1.3 Chronopharmaceutics

Chronopharmaceutics has been defined as a discipline of pharmaceutics dedicated to the development and evaluation of drug delivery systems that release a bioactive agent at a frequency that ideally meets the biological requirement of a certain disease therapy. Chronotherapeutics considers the pharmacokinetics of medications as well as the vulnerability of target tissues due to the temporal structure of physiochemical processes and activities of the body, such as circadian and other rhythms. The administration of drugs at periods when they are most efficacious and well tolerated is one way to improve pharmacotherapy efficiency. A medication's chronotherapy can be achieved by the proper timing of conventionally prepared tablets and capsules, as well as a customised drug delivery system that synchronises drug concentrations with disease activity rhythms (figure 1.3). Various drug delivery systems have been designed to synchronize the drug release with the circadian rhythm.

1.3.1 Pulsatile drug delivery system (PDDS)

Pulsatile devices are gaining popularity because they deliver drug to the ideal targeted spot at the proper time and dose, allowing for spatial and temporal administration while also increasing patient compliance. A pulsatile delivery system was devised to address the demand of rapid drug release after a lag period. The fundamental aspect to consider when establishing pulsatile drug release is the circadian rhythm. When constant medication release, such as zero order drug release, is not desired, the pulsatile administration approach can be quite effective. The pulsatile delivery system has been recognized as a time-controlled release mechanism since it is unaffected by pH, enzymes, or gastrointestinal motility.

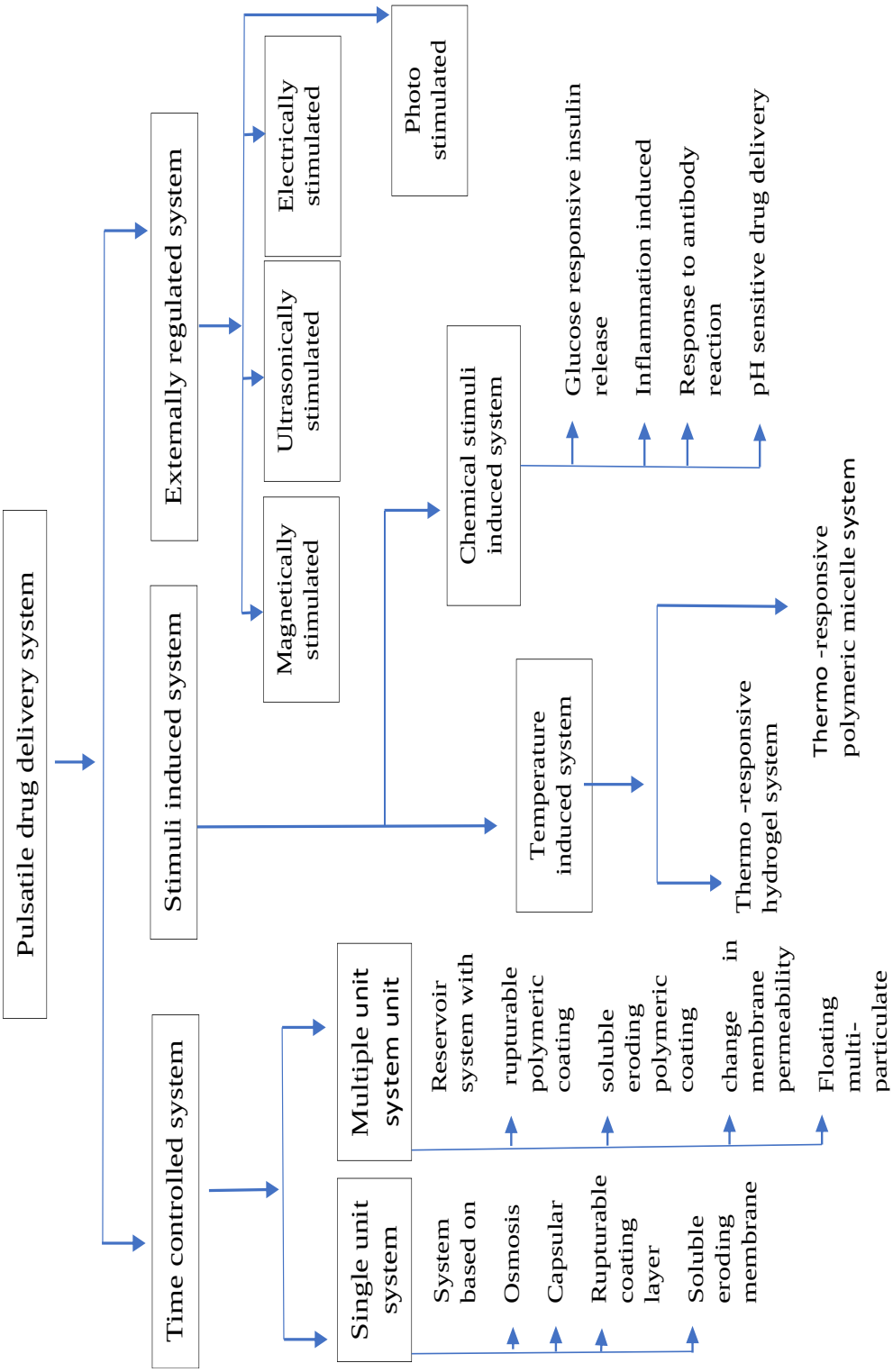


Figure 1.3. Chronopharmaceutical drug delivery systems

PDDS can be utilised to treat disorders with a circadian rhythm at the root of their pathophysiology. It has been revealed that illnesses have distinct cyclic rhythms, and that appropriately pacing therapy regimens can enhance therapeutic results in some chronic ailments. A chronotherapeutic therapy method is one in which medication availability in vivo is timed to coincide with the cyclic rhythms of

drug-related biological processes in order to optimise benefit while minimising risk (figure 1.4) (Barzegar-Jalali et al. 2006).

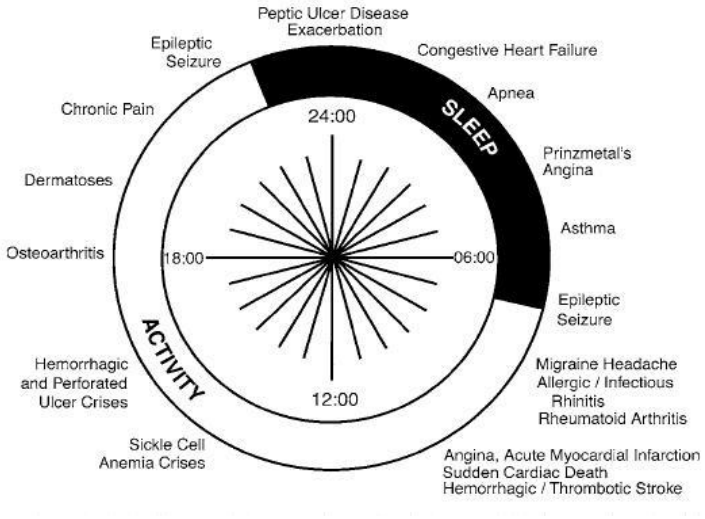


Figure 1.4. Diseases that necessitate PDDS are depicted in a circadian rhythm diagram.

The three basic types of PDDS based on the pulse regulation of drug release include time-controlled pulsatile release (regime with a single or many components), intrinsic stimulation driven release, and pulsatile release systems driven by external stimuli. PDDS is also classified into three types based on dose form: tablets, capsules, and pellets. The cup and core tablets are both compatible with the chronotherapeutics system.

There are two types of pulsatile delivery methods: time-controlled and site-specific. The release of the first group is predominantly regulated by the system, whereas the release of the second group is mostly influenced by physiological variables in the gastro intestinal tract, such as pH or enzymes. (Shidhaye SS et al. 2010)

Classification of Pulsatile Drug Delivery Systems:

Three Categories:

1. Time-controlled pulsatile release systems

2. Stimuli-induced pulsatile release system

3. Externally regulated pulsatile release system

1.3.1.1 Time-controlled pulsatile release systems

With erodible coating layer and system for bulk erosion

1.3.1.1.1 Bulk erosion

Bulk erosion occurs when the rate of water intrusion exceeds the rate of deterioration. Degradation occurs throughout the polymer sample in this example and continues until a threshold weight is achieved. Degradation products become tiny enough to be dissolved at this time, and the structure begins to become much more porous and hydrated. As a result, there is a time lag before the drug may be delivered that corresponds to the time necessary to attain critical molecular weight. Several research groups have used this approach to examine purposed formulation.

1.3.1.1.2 System for surface erosion

In this system, the reservoir device is coated with a soluble or erodible layer that dissolves over time and releases the drug after a predetermined lag period, similar to how the drug is entrapped in the core layer of hydroxyl propyl methyl cellulose (HPMC) and an additional layer of enteric-coated film outside it in a chronotropic system. Another example of a surface eroding system is the time clock system, which consists of a solid dosage form coated with lipid barriers such as carnauba wax and beeswax, as well as surfactants. When one of these systems comes into contact with an aqueous media, the coat emulsifies or erodes after a certain amount of time. It is unaffected by gastrointestinal motility, pH, enzymes, or gastric residence.

System of delivery with a rupturable covering layer

For the release of a drug, these methods rely on the breakdown of the coating layer.

Effervescent excipients, swelling agents, or osmotic pressure can be used to provide the pressure required for coating rupture.

It has been reported that an effervescent combination of citric acid and sodium bicarbonate was incorporated in a tablet core covered with ethyl cellulose. After the coating ruptured, the carbon dioxide produced by water penetration into the core resulted in a pulsatile release of the drug. The mechanical qualities of the coating layer may influence the release. When compared to more flexible films, the weak and non-flexible ethyl cellulose film broke properly. The lag time grows as the coating thickness and hardness of the core table increase. Superdisintegrants, which are highly swellable agents, were employed to create a capsule-based system that included a drug, swelling agent, and rupturable polymer layer.

1.3.1.1.3 Capsule-shaped system with a release control plug

The Pulsincap method is a popular capsule-based pulsatile device. It was created by the Michigan-based R.P. Scherer International Corporation (Arora S et al. 2008). It is made up of a drug reservoir encased in an insoluble shell. Swellable hydrogel plugs enclose the medication material inside the capsule body. Such plugs are made from hydrogels of various viscosity classes, including hydroxypropyl methyl cellulose, poly methyl methylacrylates, poly vinyl acetate, and poly ethylene oxide. The size of the plug determines the lag time. The soluble cap of the capsule shell, which dissolves in the presence of dissolving or gastric juice, holds the entire system together. After eating gastric media, the plugs swells and forces itself even beyond capsule, allowing the drug to be released.

Bussemer et al. developed and tested a drug-filled hard gelatine capsules with a swollen layer and an outer water-insoluble yet porous covering (Bussemer T et al. 2003). As the width of the exterior coating layer rises, so does the lag time. It can be decreased to a bare minimum by including a hydrophilic pore developing and increasing the size of the bulging layer (figure 1.5).

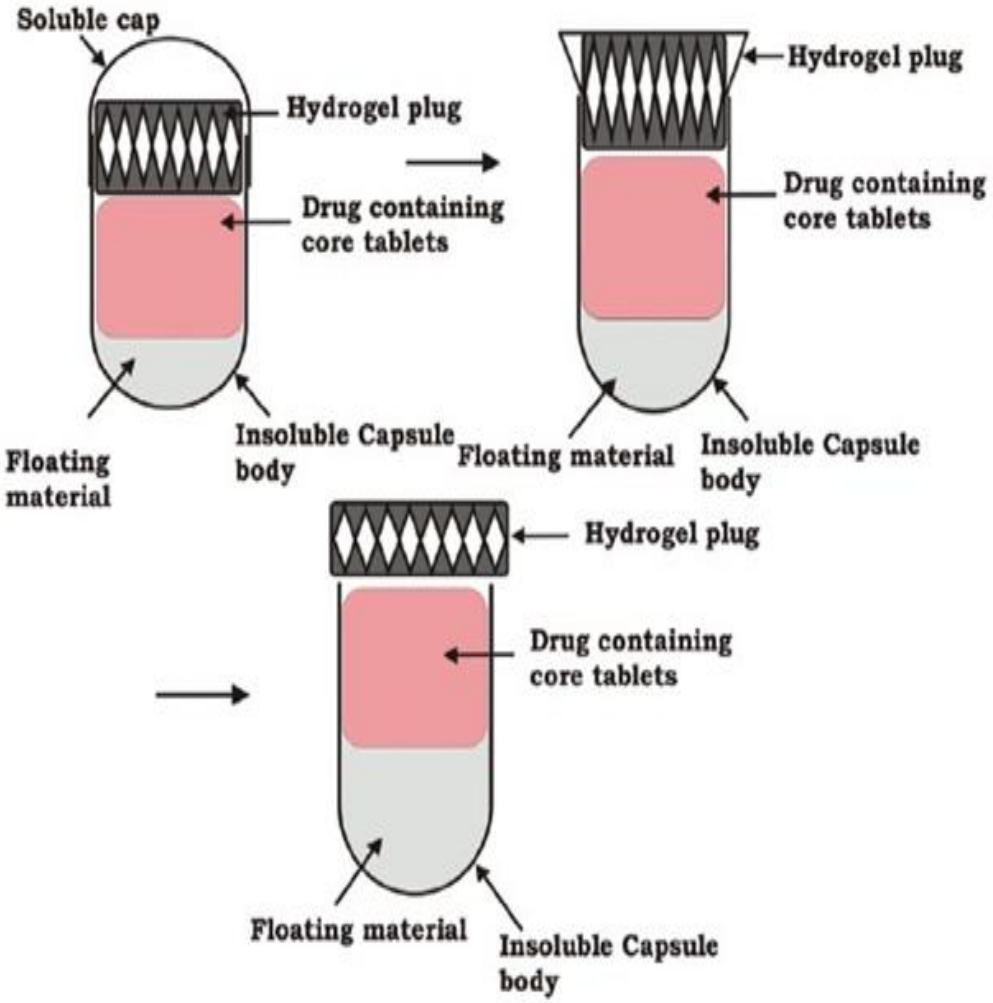


Figure 1.5. System based on capsule

Krogel and Bodmeier put a pulsatile delivery method based on an impermeable capsule shell with an erodible plug to the test (Krogel I et al. 1998). First, the drug and excipients were put into the insoluble capsule body. The Plug was created by either simultaneous interpretation or coagulating a meltable plugs inside the capsule aperture. The disintegration or erosion period of the plug determines the lag time. They later modified the plug's composition (Krogel I et al. 1999). This plug is made from pectin and the pectinase enzyme. The enzyme destroys the plug because it is present. Super disintegrants were used in the development of capsule-based systems including a drug, a swelling substance, and a rupturable polymer matrices (Mohamad A et al. 2006). The swelling chemical expands to the point when the polymeric film completely ruptures. This approach may be used to distribute any solid or liquid pharmaceutical composition.

1.3.1.2 Stimuli-induced pulsatile release system

Stimuli-based drug delivery systems distribute the drug in response to biological stimuli. The drug is released as a result of stimulation-induced changes in the gels or micelles, which may deswell, swell, or erode in response to the relevant stimuli. The drug is released in these systems upon activation by any biological component, such as temperature or other chemical stimuli. Drug release processes include drug ejection from the gel as the fluid phase syneresis out, concentration gradient, electrophoresis electrode, and drug liberation when the gel or micelle complex erodes. There has been a lot of interest in developing a stimuli-sensitive delivery system that releases a drug when a certain enzyme or protein is present (Maroni A et al. 2005).

1.3.1.2.1 Osmosis-based system

In these systems, the capsule is protected by a selectively permeable membrane. An insoluble plug, an osmotic substance, and a drug formulation are all included in the capsule. Gastric fluid can flow through when the slightly permeable capsules shell comes into contact with GI fluid. As a response, the plug expands, resulting in osmotic pressure (figure 1.6).

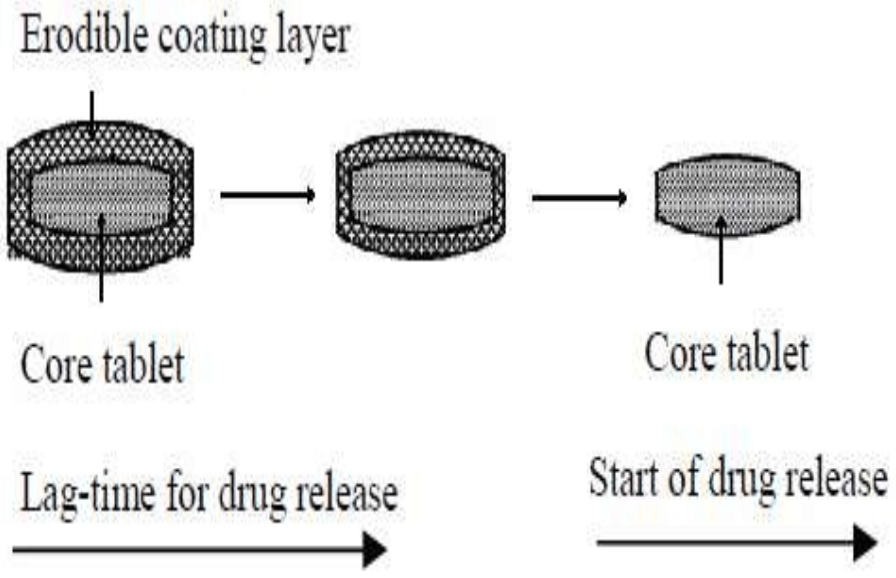


Figure 1.6. Drug delivery with an erodible coating layer in a pulsatile drug delivery system is depicted schematically.

When such pressure exceeds the tensile characteristics of the membrane, it explodes, and the time taken for the outer layer to rupture is referred to as the lag period. After a set period of time, the plug is expelled, allowing the medication to be released. Barzegar-Jalali and associates developed an osmotic capsule, hard gelatin capsules with acetaminophen, sorbitol as an osmotic agent, and Na. dodecyl sulphate as a releasing promoter were produced. The capsules shell was closed with white bees' wax and overlaid with lipophilic plasticizer-containing slightly permeable cellulose acetate (castor oil) (Linkwitz A et al. 1994) when a semipermeable membrane reacts with water, it enables water to pass through while also increasing osmotic pressure inside the shell. It causes the plugs to fall from outside shell and the medication to be discharged by increasing the hydrostatic pressure.

1.3.1.2.2 OROS technology

Chronset is currently a patented OROS delivery device that provides a bolus medication dosage to the gastrointestinal system based on time or location. It is entirely dependent on osmosis. The active drug is kept in a reservoir surrounded by a semipermeable membrane perforated with a distribution aperture and then shaped into a tablet. This tablet has two layers: one drug surface and one osmotically active component. When the osmotic agent came in contact with GI fluid, it changes from non-dispensable to dispensable viscosity. Active drug is forced out of the channel as a function of the osmotic agent's pump action. It is often used in the production of extended-release tablets (AsimSattwa Mandal et al. 2006).

1.3.1.2.3 System with erodible, soluble or rupturable membrane

In this instance, a different concept was employed. A drug reservoir is protected by a dissolving or erodible barrier. Whenever the barrier dissolves or erodes, the drug is released from the reservoir. The time clock ® system consists of a solid dosage form protected by lipid barriers comprised of carnuba wax and bees' wax, and also surfactants such as polyoxyethylenesorbitanmonolate (Pozzi F et al. 1994, Wilding Ir et al. 1994). The layer erodes or emulsifies in the aqueous environment in a timeframe proportional to the radius of the coating, exposing the center for dispersion. In a study with human volunteers, the presence of intestinal enzymes, mechanical activity of the stomach, or gastro-intestinal pH didn't even appear to

affect lipophilic film redispersion, and the lag time appeared to be independent of gastric residency duration (Niwa K et al. 1995). Because of lag time grows with layer thickness, such methods are appropriate for water-soluble drugs.

1.3.1.2.4 System with change in membrane permeability

Pellet cores coated using amino-methylacrylate copolymer and comprising drug and succinic acid exhibited sigmoid release patterns (Narisawa S et al. 1996, Narisawa S et al. 1994). The water inside the media dissolves the succinic acid. The drug and acid environment within improve the permeability of the polymer coating. In fact, the presence of different counter-ions in the media can affect the porosity and water absorption of acrylic polymers containing quaternary ammonium groups (Bodmeier R et al. 1996). Eudragit RS 30D's polymer side chain has a positively polarized quaternary ammonium group that is always accompanied by negatively HCl counterions. The ammonium group's hydrophilic nature increases the polymer's interface with water, resulting in a shift in permeability and controlled water penetration of the active core. A little amount of sodium acetate in the pellet core also influences the drug permeability of the Eudragit film. This results in the entire dosage being released in a couple of minutes. This technology is used to manufacture an acid-containing core.

1.3.1.3 Externally regulated pulsatile release system

An electric field provides benefits as an external stimulus, such as the availability of equipment that enables precise control over the size of the current, duration of electric pulses, interval between pulses, and so on. Polyelectrolytes are used to make electrically responsive delivery systems, which are both pH and electro-responsive. Electro-responsive hydrogels usually deswell, swell, or erode when subjected to an electric field. Magnetic stimulation is also used in drug delivery.

1.3.1.3.1 Ultrasonically stimulated

Ultrasound is primarily utilised to promote medication absorption through biological barriers such as the skin, lungs, intestinal wall, and blood vessels. Several studies have been published that describe the influence of ultrasonography on regulated medication delivery.

Kost and colleagues illustrated an ultrasound-enhanced polymer. Miyazaki et al. employed ultrasound to boost the release of 5-fluorouracil from an ethylene and vinyl acetate (EVAc) matrix by up to 27-fold. The amount of 5-fluorouracil release increased inrespective to the power of the ultrasound.

1.3.1.3.2 Pulsatile release triggered by magnetic fields

One of the earliest approaches studied to construct an externally controlled drug delivery system was the use of an oscillating magnetic to govern drug release from a polymer matrix. Magnetic carriers respond to magnetic fields via integrated minerals such as magnetite, iron, nickel, cobalt, and so on. Magnetic carriers for biomedical applications must be water-based, biocompatible, non-toxic, and non-immunogenic. The strategy's mechanistic approach is based on magnetic attraction, which slows the flow of oral drugs in the gastrointestinal system (figure 1.7). This is accomplished by inserting a second magnetic component inside capsules or tablets.

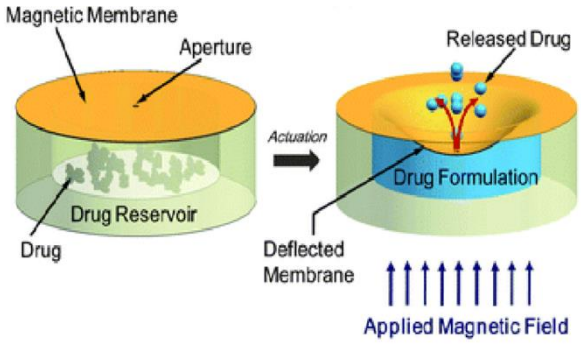


Figure 1.7.Pulsatile release triggered by magnetic fields

When do we need chronokinetic studies?

There are some instants in which chronokinetic study is needed:

Some instances necessitate chronokinetic investigation:

- Whenever possible, daily pharmacokinetic oscillations may be to blame for time-dependent changes in drug effects (For example, some antimicrobials are much more efficient at certain times of the day),
- When the therapeutic range of the drug is restricted, or when illness symptoms are clearly tied to the 24-hour clock. (Asthma, angina pectoris, myocardial infractions, and ulcer disease are a few examples.)

- A drug's therapeutic effect is closely related to its plasma levels, although the latter is phase dependent.
- Whenever a drug has a significant adverse impact that can be avoided or mitigated by adjusting the time of administration (e.g. aminoglycosides, nephrotoxicity).

The primary goal of drug delivery system design is to determine when and where the drug will be released. For such understanding, the episode of numerous biological occurrences is quite crucial. For this reason, the metoprolol pulsatile drug delivery system was created, which may release the drug when blood pressure has to be controlled in the morning. This system was built using the Cup and core approaches, which comprised immediate release (IR), sustained-release (SR), and a polycaprolactone plug layer. Various preformulation experiments aided in the formulation of the components. The IR and SR tablets were bilayered, with the IR layer completely coated in polycaprolactone. For the F5 batch, the IR and SR tablet release profiles were optimised, and then a pulsatile drug delivery system was built. The manufactured tablet was used in clinical trials, which included the use of BaSO4 tagged tablets for X-ray exams. All of the findings pointed to the best time for metoprolol to be released, which can be helpful for people who are more prone to blood pressure irregularities in the morning. (James, Apollo et al. 2022)

A high risk of haemorrhagic stroke and cardiac mortality has been linked to an early morning spike in blood pressure. This can be avoided by using a planned lag time and constructing chronotherapeutic drug delivery systems (CDDS), which can help administer Propranolol hydrochloride at the right time. The coating on the capsule body was adjusted to keep it intact for more than 12 hours. The formulation displays 6.946 percent cumulative drug release after 5 hours, and it takes 12 hours to release 98 percent of Propranolol hydrochloride. A chronotherapeutic system necessitates a lag time of at least 5–6 hours, followed by complete medication release within 12 hours. The propranolol HCl will be released at the early morning from the formulation which prevents the haemorrhagic stroke and cardiac death (Singh, D. K et al. 2015)

Chronopharmaceutics drug delivery system intended for antihypertensive agents

Blood pressure increases are considered to be responsible for 7.5 million fatalities globally, accounting for around 12.8 percent of all deaths. This equates to 57 million DALYs (disability-adjusted life years), or approximately 3.7% of all DALYs. As a result, high blood pressure is a substantial risk factor for coronary heart disease, including ischemic and haemorrhagic stroke. Blood pressure levels have regularly and beneficially been linked to an increased risk of stroke and coronary heart disease. The risk of cardiovascular disease increases with each 20/10 mmHg increase in blood pressure in certain age groups, beginning at 115/75 mmHg. High blood pressure causes heart disease, peripheral arterial disease, renal illness, ocular bleeding, and visual degeneration in addition to coronary heart disease and stroke. The management of diastolic and systolic blood pressure till it is less than 140/90 mmHg is connected to the reduction of cardiovascular diseases. According to the World Health Organization, hypertension affects 1.13 billion people worldwide, with around two-thirds of them living in low- and middle-income economies. In 2015, one out of every four males and one out of every five women were diagnosed with hypertension. Only around one in every five hypertensive people gets their health under control. High blood pressure is the leading cause of mortality worldwide. One of the global noncommunicable disease objectives is to reduce hypertension incidence by 25% by 2025.

In order to reduce mortality rates, today's environment needs Chrono pharmacological formulations that promote patient compliance, maximize drug distribution at the target site, and limit adverse effects. Pulsatile drug release refers to a process in which a medication is delivered rapidly after a specific lag period or time gap in accordance with the circadian rhythm of disease circumstances. (Bussemer T et al. 2008) Long-term therapy can result in drug resistance, which can be hazardous. Because the right drug concentration for certain periods is accessible, the choices are restricted. (Smolensky MH et al. 2007) This strategy is advantageous because to the drug's large metabolic first-pass and is directed to a particular location in the gastrointestinal stream to achieve the therapeutic goal.

These days, pulsatile drug administration is becoming increasingly common. The key advantage of this drug administration technology is that the chemical is only released when it is needed. As a result, the danger of developing drug resistance, which is frequent in both conventional and sustained release formulations, is reduced. Furthermore, certain anticancer drugs are quite dangerous. These drugs create substantial problems in both standard and sustained release treatment. On the market there are a slew of FDA-approved Chrono therapy drug. When a long-term impact is not required and drugs are dangerous, this approach is most effective. The most crucial component of this formulation's creation is defining the circadian rhythm, or even a suitable parameter for when the drug should be released. Another problem is a shortage of suitable rhythmic biomaterial, which has to be degradable, appropriate, compatible, and rhythmically responsive to certain biomarkers. Regulatory issues are also important. During the preapproval process, demonstrating Chrono therapeutic effects in clinical settings might be difficult. To be authorised, the FDA currently relies heavily on the creation and implementation of risk mitigation systems as a method for permitting pulsatile drug delivery to expose circadian rhythm with such an appropriate system anywhere else in the universe.

Hence, formulation and design of these kind of PDDS for the treatment of hypertension will provide the way to deliver the antihypertensive drug based on circadian rhythm (figure1.8). Addition to this, plethora of applications can be extended in terms of patient compliance, safest drug deliver, effective treatment of hypertension, and cost-effective dosage form etc.,

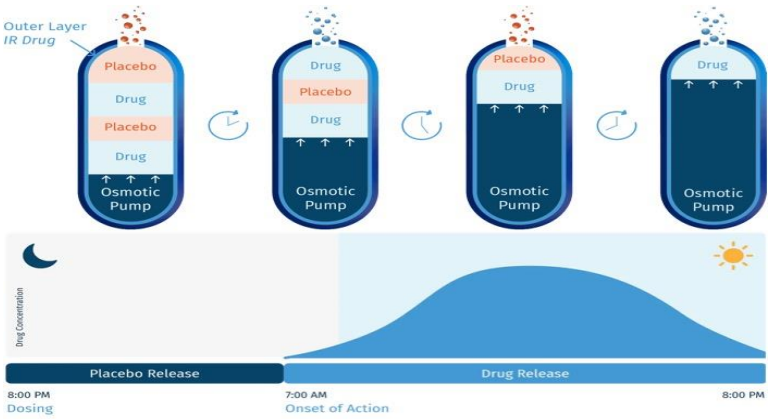


Figure 1.8. DDS based on osmosis

CHAPTER-2

2 LITERATURE SURVEY

ElMeshad AN at al., in 2020, studied core in cup tablets. Ethylmorphine hydrochloride (EtM) is a morphine derivative used as an analgesic to relieve severe pain in cancer and bone damage patients. The goal of this study was to develop and test a core in cup tablet that included two doses of EtM, with the cup being formulated as a lyophilized oro-dispersible tablet for IR and the core being made as a directly compressed tablet for SR. For the optimization of tablets made using the lyophilized form and direct compression procedures, a factorial design of 41.22 was utilized for the former and a 32 for the latter. All of the produced tablets had acceptable physical qualities that met pharmacopeial requirements. The cup for immediate release was chosen from two lyophilized ODTs formulations. At the same time, one directly compressed tablet formula was chosen to represent the sustained core based on the in vitro release profile. Two core in cup tablets, B1 and B2, which were evaluated for in vivo absorption and showed maximum plasma concentrations (Cp_{max})

Madhavi AV et al., in 2020, studied the pulsatile drug delivery system of zafirlukast. In today's pharmaceutical research environment, great emphasis has been placed on patient health in terms of therapeutic efficacy and cost-effectiveness (price factor). The formulation is made up of core tablets that were created using the direct compression process. A naturally occurring swelling agent was used to cover the core pills (carbopol& Karaya gum). The hardness of prepared pulsatile tablets was studied in depth. In a six-hour in vitro release profile, the first five hours exhibit minimal drug release, whereas the latter six hours show rapid and transient release. The influence of temperature and moisture on the degradation of Zafirlukast appears to be reduced when tablets are coated, according to stability tests. Over a 7-8-hour period, the pulsatile release was obtained from the pill.

In the year of **2019, Heon-Jeong Lee** detailed the impact of circadian rhythm in the field of disease diagnosis and therapy. He described that the circadian rhythm could play a key role in disease development and treatment. The Circadian clock is an internal clock device that regulates the majority of bodily

operations. Because disturbance of the biological clock is linked to a variety of human disorders, resetting the clock can aid in the treatment or prevention of a number of illnesses. The clinical signs of oxidative stress and inflammation have been demonstrated to improve when the circadian clock is restored.

Potucek P et al., 2019, described circadian patterns and their relationship to the cardiovascular system. Circadian patterns in the cardiovascular system (CVS) physiological functions are regulated by a complex set of endogenous variables. Its normal operation depends on maintaining this rhythmicity, whereas disrupting the synchronization with the natural day-night cycle can raise the risk of cardiovascular disease. Cardiovascular pathology follows a cyclic pattern as well; temporal susceptibility and the peak risk period linked with high blood pressure (BP) can be predicted. Because of its rhythmic character, considerable differences in efficacy between morning and evening drug delivery may occur; proper timing of pharmacological intervention in hypertension management may alter treatment efficacy.

Kumar PJ et al., in 2018, formulated and evaluated a pulsatile drug delivery of lovastatin. The goal of this study was to develop pulsatile Lovastatin drug administration in order to lower plasma cholesterol levels and prevent cardiovascular disease. Pulsincaps were made from Capsule bodies that had been treated with formaldehyde. It was sealed using the capsule's unhardened cap. Emulsion solvent evaporation was used to make the microspheres. The hydrogel plug (karaya gum and lactose in a 1:1 ratio) with a hardness of 4.5kg/cm2 and a weight of 100 mg was placed in the capsule opening and found to be effective in delaying drug release in small intestinal fluid. The formulation demonstrated a lag period of 5 hours. To avoid variable gastric emptying, the sealed capsules were thoroughly coated with 5 percent cellulose acetate phthalate using a dip-coating process. Based on dissolution tests, optimized microsphere formulations were chosen. Drug release in the colon might be regulated by optimizing the concentration of polymers in the microspheres, according to dissolution studies of the pulsatile capsule device in liquids with varied pH (1.2, 7.4, and 6.8).

Ramón C. Hermida et al., 2018, evaluated the effect of changing the time of therapy without adjusting the dose of prescribed medicines on the circadian rhythm of blood pressure was studied. Two hundred fifty hypertension patients were evaluated who were given three antihypertensive medications in a single morning dose. When all of the medicines were taken a right after waking up, there was no effect on ambulatory blood pressure. It was found that one tablet at bedtime significantly reduced the blood pressure spike in the morning.

In **2018, Bowles, N P et al.,** described the ned of chronotherapy in hypertension. Blood pressure has a well-documented 24-hour rhythm with a morning surge, which could explain the morning increase in unfavorable cardiovascular events. Morning doses of antihypertensive medication have traditionally been used to reduce daytime blood pressure rises, but the absence of nocturnal dropping blood pressure has also been linked to an increased risk of cardiovascular disease. Effectively lowered nocturnal blood pressure can counteract the morning spike in BP. Antihypertensive therapy given at night improved overall 24h BP profiles despite illness comorbidity.

In 2018, ShigehiroOhdo described the importance of chronopharmacology and chronotherapy. The drug delivery time effect not only pharmacodynamics but also affect the pharmacokinetics of a drug. Synchronizing the drug release to the rhythm of the disease activity. One way to improve pharmacotherapy efficiency is to administer medications at a time when they are very well tolerated. The use of biological rhythm in pharmacotherapy can be achieved by the proper timing of typically prepared tablets and capsules, as well as the use of a specific drug delivery method to synchronize drug concentrations with disease activity rhythms. He has discussed the alterations in dosing time to enhance the therapeutic outcome.

Siddam, H et al., in 2016., prepared and evaluated a sustained release gastro retentive oral dosage form of atenolol to attain atenolol absorption from the upper GIT and improve its absorption window. The flow characteristics of the polymeric blend(s) were assessed prior to compression. The tablets were made using bioadhesive polymers like Carbopol 934P and hydrophilic polymers such

as HPMC in a direct compression process. Physical properties, swelling index, buoyancy lag time, bioadhesive strength, and in vitro drug release tests were assessed on the produced. The average bioadhesive strength observed was between 16.2 and 52.1 gm. After 24 h, the optimized blend had 92.3 percent drug release. Slow-release boosted bioadhesive strength and swelling index while increasing carbopol 934P content. The improved formulations' n values ranged from 0.631 to 0.719, demonstrating a non-fickian irregular mass transfer. The research contributed to the development of an optimal once-daily gastro retentive floating drug delivery system with increased floating, swelling, and bioadhesive properties, as well as improved bioavailability.

Jadhav et al., 2016, described the core in cup pulsatile drug delivery systems. Because of the obvious advantages of the oral route of drug administration, oral controlled drug delivery systems are the most used type of controlled drug delivery system. The drug is released at a constant or variable rate in these systems. A pulsatile drug delivery system (PDDS) is a system in which medicine is released quickly after a well-defined lag time in accordance with the disease's circadian rhythm. Time-controlled pulsatile release, internal stimuli-induced release, and external stimuli-induced pulsatile release systems are the three primary classes of PDDS based on the pulse-regulation of drug release. Pulsatile delivery refers to an active ingredient release that is delayed to a predetermined period of time to satisfy specific chronotherapeutic needs. Oral drug delivery also targets distal intestinal regions like the colon. Most oral pulsatile delivery systems are coated formulations with a polymer added as a release-controlling agent. When exposed to aqueous fluids, the coating acts as a protective barrier for a short time before failing due to a variety of reasons that vary depending on the coating's Physico-chemical and formulation qualities.

Hermida RC et al., in 2016, discussed the chronopharmacotherapy of hypertension. There is growing interest in the potential benefits of chronotherapy for many disorders, given the accumulating knowledge that circadian rhythmicity resides in every cell and all organ systems. Blood pressure has a well-documented 24-hour rhythm with a morning surge, which could explain the morning increase in unfavorable cardiovascular events.

Morning doses of antihypertensive medication have traditionally been used to reduce daytime blood pressure rises. However, the absence of nocturnal dropping blood pressure has also been linked to an increased risk of cardiovascular disease.

Gunda RK et al., in 2015, conducted a study on developing atenolol gastro retentive floating tablets. The primary objective of the study was to use a 32 factorial design to create floating atenolol pills. Class-III drug atenolol was used in the formulation. By adopting a direct compression approach and a 32-factorial design, floating tablets of atenolol were made using various amounts of HPMC and sodium bicarbonate in various combinations. The concentration of HPMC K15M and sodium bicarbonate is necessary to accomplish desired drug release. These factors were chosen as independent factors, while the dissolution was taken as the dependent variable. Nine formulations were created in all, and their hardness, friability, thickness, percent drug content, floating lag time, and in vitro drug release were tested. The chosen formulation follows Higuchi's kinetics, with non-Fickian diffusion as the mechanism of drug release.

Chaudhary SS et al., in 2015, studied the urapidil Chronomodulated drug delivery system for the treatment of hypertension. The chronomodulated system used was a dual approach, with immediate-release granules and pulsatile release mini-tablets being placed into a hard gelatin capsule. Eudragit S-100 was used to coat the mini-tablets, which supplied the lag time. Various parameters such as coating duration and coat thickness were investigated to get the desired release. Mini-tablets were examined for hardness, thickness, friability, weight fluctuation, drug content, disintegration time, and in-vitro drug release, while immediate release granules were evaluated for micrometrical qualities and drug release. Fourier transform infrared spectroscopy and differential scanning calorimetry investigations were used to verify drug-excipient compatibility while scanning electron microscopy studies were used to examine pellet morphology.

Basu VR et al., in 2014, studied the design and development of metoprolol core in cup delivery system. The goal of the study was to create a pulsatile

metoprolol succinate drug delivery system employing a core-in cup tablet design. Metoprolol is a beta blocker that is used in the treatment of hypertension. Because the drug has high solubility and is released fast after oral administration, it is necessary to limit the drug's release from the formulation and minimize dose frequency. HPMC K 100M, Sodium Alginate, and Polyox WSR 303 polymers were used to prepare the core of the cup tablet. A total of nine formulations were created and tested for different tablet qualities. All of the formulations met all of the requirements. In-vitro drug release tests were performed, and it was observed that a 1 h lag time was attained by the tablet due to the formulation design.

In 2014, Suresh Rewar reviewed the chronopharmaceutical drug delivery systems. A chronopharmaceutical drug delivery system is a unique system that delivers a pattern of real-time drug input at varied release rates, which can be accomplished using stimuli-sensitive and pulsatile drug delivery systems. Pulsatile drug delivery systems are gaining popularity because they deliver the medicine to the specific site of action at the right time and in the right amount, allowing for spatial and temporal administration while also boosting patient compliance. Pulsatile Drug Delivery Systems are time-controlled drug delivery systems in which the system manages the lag time regardless of environmental parameters such as pH, enzymes, GIT motility, and so on. These systems are intended for chronopharmacotherapy, which is based on the body's natural circadian cycle. Drug administration is synchronized with rhythms in chronopharmacotherapy to create maximum therapeutic efficacy while causing the least harm to the patient. Chronic disorders with circadian cycles, such as cardiovascular ailments, may benefit from chronopharmaceutical drug administration.

B Bonthagarala et al., in 2014, studied pulsatile delivery system of atenolol. The purpose of this study was to develop and test a pulsatile medication delivery system for atenolol. The prepared pulsatile delivery system is made up of two parts: an active ingredient-containing core tablet and an erodible cup. Superdisintegrants and the active component Atenolol were used to make the immediate-release core pill. Various ratios of hydroxy propyl methyl cellulose

(HPMC K100) and ethyl cellulose 5Cps were used in the press coating of optimize formula. Physical properties, in vitro disintegration time, and in vitro drug release profile of developed formulations were assessed (lag time). Based on these characteristics, it was discovered that the optimised pulsatile release system had a delay time of 2 h and an in-vitro drug release time of 8 h, with 97.8% of the medication released. The developed formulation exhibited suitability in chronotherapeutic delivery in hypertension.

Amol M. et al., in 2012, studied the chrono-modulated therapy of atenolol. The study was focused on investigating and developing an oral dosage form that releases the medicine over a predetermined time period after ingestion. Pulsatile release tablets include an atenolol-containing core and a pH-sensitive polymeric covering to delay drug release and enhance gastric resistance time. The formulation allows colon administration to be pursued in a time-dependent manner. The goal of this study is to compare different pH-sensitive polymers (sodium alginate, Eudragit S-100, ethyl Cellulose) at different ratios in order to develop a suitable dosage form with no drug release in the upper gastrointestinal tract (GIT), allowing for site-specificity and time-controlled delivery of atenolol.

In 2011, 24. Salunkhe AK et al., developed and evaluated a pulsatile delivery system of metoprolol. The goal of the study was to develop and test a floating pulsatile drug delivery system for metoprolol tartrate. A core tablet was carrying the active component, an erodible outer layer, and a top cap buoyant layer made up the prepared floating pulsatile delivery system. Superdisintegrants were used in conjunction with the active component to create a rapid-release core tablet. Different grades of hydroxy propyl methyl cellulose (HPMC), HPMC K15M and sodium bicarbonate were used in the formulation. Physical properties, drug loading, in vitro disintegration time, in vitro drug release profile (lag time), floating lag time, floating time, and in vivo, X-ray studies were all tested for the developed formulations. The optimized floating pulsatile release formulation had a floating lag time of 4 min, a floating time of 12 hours, and a release lag time of 6 hours, according to these assessment parameters.

Jain D et al., in 2011, studied the technologies in pulsatile drug delivery systems. In a pulsatile drug delivery system, the drug is released fast and completely at a pulse after a lag time, and these systems are designed to work with the body's circadian rhythm. These products have a sigmoid release profile, which is defined by a time frame. These methods are useful for drugs that have a chronopharmacological behavior and require nightly administration, as well as those that have a first-pass influence. The approaches and commercialized technologies that have been created to provide pulsatile delivery are covered in this article. PulsincapTM, Diffucaps®, CODAS®, OROS®, and PULSYSTM are examples of commercialized products that use the aforesaid technique to provide a sigmoidal drug release profile. Asthma, peptic ulcers, cardiovascular disease, arthritis, attention deficit syndrome in children and hypercholesterolemia are among the diseases where PDDS has shown promise.

Shidhaye S et al., in 2010, described pulsatile drug delivery systems and their impact on chronotherapy. Pulsatile delivery systems are gaining popularity for the development of medications for which traditional controlled-release methods with a prolonged release pattern are ineffective. Most physiological, biochemical, and molecular activities in healthy organisms alter on a 24-hour cycle. Drug administration can be synchronized with circadian cycles to improve efficacy and reduce the negative effects of chronotherapeutic drugs. These products have a sigmoidal release profile, including a period of no release (lag time) before the medication is released quickly and completely. The utilization of soluble/erodible polymer coatings, rupturable membranes, and membrane permeabilities is used in various capsular, osmotic, single, and multiple unit systems. PULSYSTM, CODAS®, TIMERx®, and DIFFUCAPS® are examples of commercialized technologies that use one of the following methods to give a drug release profile with a sigmoidal curve.e As a result, these systems are set to the body's circadian clock.

Youan BB, in 2010, discussed the details of chronopharmaceutical drug delivery systems and their status. Chrono modulating infusion pumps such as Melodie™, Panomat™ V5, controlled-release microchip, 3DP technologies, floating pulsatile systems, a combination of floating and pulsatile drug delivery

systems, etc. He also discussed the advanced nanotechnology approaches in chronotherapy. Transdermal chronotherapeutic system ChronoDose™ and the use of various polymers that can be used in the development of chronopharmaceutical systems were included in the review.

Ali j et al., in 2010, described the features of pulsatile drug delivery systems. Pulsatile medication delivery is frequently used interchangeably with chronotherapeutic drug administration. This needs to be considered because both drug delivery systems are addressing completely distinct patient demands, as well as different formulators' intents. Chronotherapeutic systems are totally dependent on the body's circadian needs and responses, as well as the necessity for a drug's maximum concentrations at a specific time of day, as evidenced by an unending list of ailments that evoke the related symptoms at a specific time of day. When it comes to the formulation approach, there aren't many differences between site-specific chronotherapeutic systems and basic and more conventional intestinal or colon targeted systems because the mechanism and site of drug landing are nearly identical in both, despite the formulator's intent being different. A perfect pulsatile system delivers the medicine in several pulses with multiple troughs in the release profile. The article examines the key distinctions between the two systems and emphasizes the importance of adopting the correct terminology for these two distinct systems that cater to different purposes.

In 2010, B. R. Gandhi et al., discussed about various chronopharmaceutical drug delivery systems. It is critical to design innovative drug delivery devices that can release a specific amount of medicine in a pulsed manner, simulating the operation of biological systems while limiting unwanted side effects. Pulsatile delivery, defined as the release of medications after predetermined lag periods, is gaining popularity, particularly in light of new chronotherapeutic techniques. The review article studied several design techniques, such as reservoir, capsular, and osmotic delivery systems, as well as drug delivery systems that produce the pulsed or triggered release of bioactive chemicals driven by thermal, electrical, or magnetic stimuli.

In 2009, Nagaraju R et al., designed and developed a core in cup system of metoprolol to achieve a chronotherapeutic outcome. The wet granulation process was used to make Metoprolol succinate core-in-cup matrix tablets. The formulation followed zero-order drug release kinetics out of all the formulations tested. The formulation was created with 7.5 percent hydrogenated castor oil (HCO) and 4% hydroxyl propyl methylcellulose with the goal of achieving a 24 h linear release profile. There is no first burst release; 16.17 percent of the medication is released in the first hour. The release could last up to 24 hours. The dissolution data study in various kinetic equations such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas was used to investigate drug release kinetics. Based on the R2 value (0.9975), drug release kinetics was found to follow zero-order kinetics. The formulation followed both erosion and diffusion as release mechanisms.

In 2009, Yogendeanayak et al., designed a programmable drug delivery system to prevent the morning surge in BP. The study's goal was to create a pulsatile valsartan capsule dosage form for tailored distribution. Blood pressure rises in most people in the early morning hours, which can lead to significant cardiovascular issues. In chronopharmacokinetic research, formulations with constant/programmable delivery rates make it feasible to release the medication at a particular time or a regulated pace. In a pre-coated capsule, the produced system included a swellable polymer (l-hydroxypropyl cellulose (L-HPC), xanthan gum, polyethylene oxide, or sodium alginate) as well as a drug tablet and an erodible tablet (L-HPC or guar gum). Various tests such as in vitro dissolution and ex vivo continuous dissolution-absorption investigations were used to explore various formulation parameters. We discovered that the kind of polymer, number of polymers, and erodible tablet all impacted drug release. The formulation using 200 mg sodium alginate and an erodible tablet (150 mg) containing 50% guar gum and 46% lactose had a 5-6 hour lag time and 10+/-2.1 percent drug release in the first 6 hours after fast release (99+/-1.7 percent release in 12 hours). The medication absorption was delayed in continuous dissolution-absorption research utilizing an everted rat intestinal segment. As a result, this method can give a convenient way to time the release of valsartan,

which may be beneficial for patients with morning surge in BP.

J Sajan et al., in 2009, described chronotherapeutics and chronotherapeutic drug delivery systems. The study describes in detail chronotherapy and various diseases and the role of chronotherapy in those diseases. The chronophermacokinetics of Antihypertensive drugs and anti-inflammatory drugs are also described. The review elaborated on enteric-coated systems, layered systems, time-controlled explosion systems, sigmoidal release systems, press-coated systems and the osmotic delivery systems and their role in chronopharmaceutical drug delivery.

In 2007, Smolensky MH et al., discussed the circadian rhythm and its influence on the practice of medicine and therapy in patients. Circadian rhythms play a role in severe and life-threatening medical events, such as myocardial infarction and stroke, and the manifestation and severity of chronic disease symptoms, such as allergic rhinitis, asthma, and arthritis. Furthermore, body rhythms can have a substantial impact on how patients respond to diagnostic testing and, more importantly, treatments, which is the focus of this particular issue. One of the fundamentals of chromotherapeutics is the planned dosing of the drug in synchrony with the biological rhythm that influences the pathophysiology of a disease.

In the year **2007, Michael H Smolensky et al.,** specified that almost all bodily activities, including those that influence pharmacokinetic characteristics including drug absorption and distribution, drug metabolism, and renal elimination, have diurnal fluctuations. The circadian phase influences the onset and symptoms of disorders such as myocardial infarction, angina pectoris, stroke, and ventricular tachycardia. In normotensives and primary hypertensive individuals, blood pressure and heart rate are highest during the day, with an overnight decline and an early morning rise. This regular pattern is interrupted or even reversed in around 70% of cases of secondary hypertension, resulting in overnight peaks in blood pressure. They suggest a medical therapy that is timed according to the circadian rhythm. They concluded that there is compelling evidence that the dose/concentration-response connection of drugs is influenced

by the time of day.

In 2001, Pankaj Mathur et al., discussed the details of chronotherapy in hypertension. Circadian blood pressure fluctuations are relevant in several pathophysiological diseases. Typical diurnal variation has been widely reported, with greater BP in the early hours of the morning and a fall in BP in the evening hours, especially during sleep time. Several physiological mechanisms govern BP and induce circadian variation, including circadian rhythm in monoaminergic systems. Based on the results of the research, it suggests that antihypertensive drug delivery at bedtime is superior to daytime administration in order to improve cardiovascular outcomes and renal function.

Srinija k et al., prepared and evaluated the core in cup tablets of lamotrigine. When used orally, lamotrigine, a BCS class II drug has numerous drawbacks (first-pass metabolism and higher Cmax). The study's goal is to create core in cup mucoadhesive tablets with the regulated, unidirectional release, improved patient compliance, and fewer side effects. In this work, release retarding polymers such as sodium alginate, xanthan gum, and HPMC E 15LV in the core and HPMC K 15M in the cup are used to make core in cup tablets for mucoadhesion. For the investigation, a L9 orthogonal array Taguchi design was created. For L9 orthogonal array Taguchi runs, the dependent variable evaluated was percent drug release, from which the formulation with the highest S/N ratio was optimized.

CHAPTER-3

3 AIMS AND OBJECTIVE

Synchronizing the drug release to the rhythm of the disease activity is the basic principle of chronotherapy. One way to improve pharmacotherapy efficiency is to administer medications when they are very well tolerated. Blood pressure has a well-documented 24-hour rhythm with a morning surge, which could explain the morning increase in unfavorable cardiovascular events. Morning doses of antihypertensive medication have traditionally been used to reduce daytime blood pressure rises. However, the absence of nocturnal dropping blood pressure has also been linked to an increased risk of cardiovascular disease. Effectively lowered nocturnal blood pressure can counteract the morning spike in BP. Chronotherapy in hypertension reduces the risks of early morning sudden increase in blood pressure, drug associated side effects and increases drug efficacy and patient compliance.

The current research aims to design, develop and evaluate the acceptability of chronotherapeutic delivery of a core in cup metoprolol tablet and a mucoadhesive core in cup atenolol tablet.

The objectives of the research work are

- To design a core in cup delivery system of metoprolol and a mucoadhesive core in cup formulation of atenolol.
- Preparation and evaluation of the designed formulation to identify the feasibility of the designed formulation to achieve chronotherapeutic delivery of the drug

Working plans for the study

- Preformulation studies: characterization of drugs by analyzing solubility and melting point. Identification and compatibility studies using DSC and FTIR
- Formula development and optimization using statistical methods

- Formulation development of metoprolol core in cup tablet and atenolol mucoadhesive core in cup tablet
- Evaluation of the developed formulation: hardness, friability, weight variation, disintegration time, in vitro and in vivo drug release.

CHAPTER-4

4. MATERIALS AND METHODS

4.1 Introduction

The goal of chronopharmaceutical medication delivery is to synchronize the illness' inherent chronological time and therapeutic administration (Bonthagarala B et al. 2014). When a disease exhibits chronological behavior, it is not required to maintain a constant drug concentration throughout the day. However, delivering the optimum dose at a specific moment. Heart rate and blood pressure have a distinct diurnal pattern in both normotensive and hypertensive patients. The heart rate and blood pressure are higher in the early morning and lower at night while sleeping (Bowles NP et al. 2018, Rehman B et al. 2020, Sah ML et al. 2012). According to previous studies, during the hours 6:00 am and 12:00 pm, patients had a 29 %, 40 %, and 49 % greater risk of cardiac mortality, heart attack, and stroke, respectively, in the period of 6.00 am and 12 pm (Latha K et al. 2010). Conventional drug delivery systems release the drug instantly or possess a particular drug release pattern throughout the day, to maintain the optimum drug concentration at the site of action. To overcome these, chronopharmaceutical drug delivery methods are used (Krishnaswamy R et al. 2017). In the present study, time-adjustable pulsatile release systems of metoprolol (core in cup tablet) was designed and evaluated for bedtime dosage administration and early morning release of the medicine to manage blood pressure. Metoprolol is cardio-selective beta-blockers used to normalize the heart rate and reduce the strain on the heart and blood pressure. Metoprolol is a BCS class II drug with an elimination half-life of 3.5 h. The drug has a pKa value of 9.6 and shows 50% bioavailability (Chaudhary SS et al. 2015). The drug delivery systems are aptly designed to release the drug adequately when required, to avoid the presence of a high dose of metoprolol throughout the day in blood circulation, and to reduce the adverse reactions (Pizzuto M et al. 2010, Saleem U et al. 2019). The study involves designing of ‘core in cup’ drug delivery system in which the core tablet is an immediate-release (IR) tablet and the plug is a sustained drug release (SR) polymeric layer. The direct compression method was used to make the core and the cup tablet. The immediate-release layer was composed of metoprolol, croscarmellose sodium, microcrystalline cellulose (MCC), sorbitol, talc, and magnesium stearate. The SR plug was composed of hydroxyl propyl methylcellulose (HPMC), polyvinyl pyrrolidine (PVP), MCC, lactose, talc, and magnesium stearate. The compression force, disintegrant

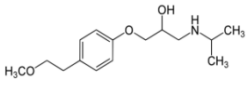
concentration in the immediate release core, and HPMC concentration in the plug layer were optimized using the least square method. The formulations were evaluated for primary parameters such as hardness, friability, disintegration, and weight variation. In vitro and in vivo studies were conducted to evaluate drug release from the immediate release core, sustained-release plug, and the core in cup tablet.

4.2 Materials and Methodology

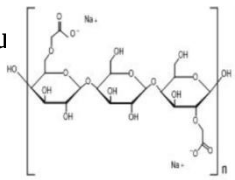
4.2.1 Materials

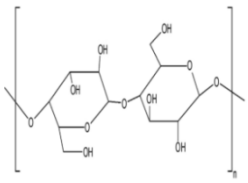
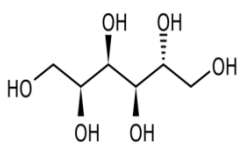
The chemicals, reagents, active pharmaceutical ingredients, cell culture media and other materials required for the study were enlisted in the following table. Detail information about the materials was mentioned for future reference.

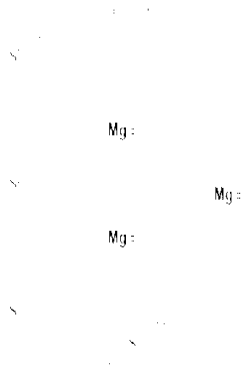
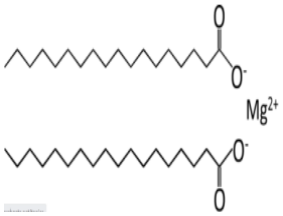
Table 4.1 List of chemicals

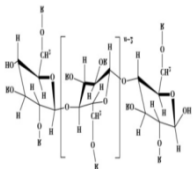
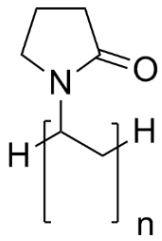
Metoprolol	**Manufactured by:** Carbaniopvt ltd
	Chemical Formula: $C_{15}H_{25}NO_3$
	Molecular weight: 267.3639
	Molecular weight: 267.3639
	Boiling Point: 398ºC
	Melting Point: 120 °C
	Solubility (mg/ml) at 25 °C:water>1000; methanol >500; chloroform 496; acetone 1.1; acetonitrile 0.89; hexane 0.001
	Lambda max (λmax): 224 nm
	LogP: 1.88
	Caco2 Permeability: -4.59
	Dissociation Constants: Basic pKa: 9.56
	Indication: Angina, heart failure, myocardial infarction, atrial fibrillation, atrial flutter, and hypertension.
	**Description:** The drug metoprolol is a beta-blocker used to treat hypertension and angina, as well as to lessen myocardial infarction mortality. Metoprolol, a selective beta-1 blocker that is typically found as succinate and tartrate derivatives, depending on whether the formulation is intended for immediate or delayed release.

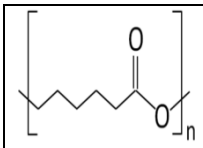
	The decreased systemic bioavailability of the succinate derivative allows for the development of these formulations. It is still one of the most commonly given beta-blockers in general clinical recommendations in the Netherlands, New Zealand, and the United States.
	Pharmacodynamics: Metoprolol is generally reported to cause a dose-dependent drop-in heart rate and cardiac output in normal subjects. A decrease in cardiac excitability, cardiac output, and myocardial oxygen demand causes this effect. 6 Metoprolol works to treat arrhythmias by decreasing the slope of the pacemaker potential and inhibiting the rate of atrioventricular conduction.
	Mechanism of action: Metoprolol is a beta-1-adrenergic receptor inhibitor that has little effect on beta-2 receptors and is only found in cardiac cells. Without exhibiting activity towards membrane stability or intrinsic sympathomimetics, this inhibition reduces cardiac output by causing negative chronotropic and inotropic effects.
	Absorption: Metoprolol is virtually entirely absorbed in the gastrointestinal tract when taken orally. After 20 minutes of intravenous treatment and 1-2 hours of oral administration, the maximal serum concentration is reached. When given intravenously, metoprolol has a 100% bioavailability; when given orally, the tartrate derivative has a 50% bioavailability and the succinate derivative has a 40% bioavailability. Concomitant delivery of meals increases the absorption of metoprolol in the form of the tartrate derivative.
	Volume of distribution: Metoprolol has a reported volume of distribution of 4.2 L/kg. 5 Metoprolol is able to permeate the

	blood-brain barrier due to its properties, and up to 78 percent of the drug delivered can be found in CSF fluid.
	Protein binding: Only around 11% of the injected dose is detected bound to plasma proteins, indicating that metoprolol is not significantly bound to plasma proteins. The majority of it is bound to serum albumin.
	Metabolism: Metoprolol undergoes extensive first-pass hepatic metabolism, which accounts for approximately half of the administered dose. The activity of CYP2D63, and to a lesser extent, the activity of CYP3A4, drives the metabolism of metoprolol. Metoprolol metabolism is mostly represented by hydroxylation and O-demethylation processes.
	Route of elimination: Metoprolol is primarily eliminated by the kidneys. Less than 5% of the dose is retrieved intact from the discarded dose.
	Clearance: Patients with normal renal function have a clearance rate of 0.8 L/min. The clearance rate drops to 0.61 L/min in cirrhotic patients.
	Drug interactions: Epinephrine, an antidepressant, ergot medicine and MAO inhibitor
	Dose: Take 25 to 100 mg once a day at the beginning
Cross Carmellose sodiu 	**Manufactured by:** Lobachemie
	Chemical Name: 2,3,4,5,6-Pentahydroxyhexanal
	Molecular weight: Average molecular weight of the compound is 240.208
	Use: Disintegrant
	Description: Croscarmellose sodium, often known as sodium CMC, is a carboxymethylcellulose sodium cross-linked polymer. It's a white, fibrous, free-flowing powder that's extensively employed in pharmaceutical manufacture as an FDA-approved disintegrant.
	Melting Point: 148.889°C

	Density (bulk): 0.53 g/ml
	Refractive index: 37.35 $m^3 \cdot mol^{-1}$.
	Solubility: When combined with water, it produces a thick colloidal solution. It is ethanol insoluble.
	Stability and storage conditions: Keep the container firmly closed in a dry, well-ventilated location. Keep the original container at room temperature. Keep incompatible materials out of reach.
MCC 	**Manufactured by:** Lobachemie
	Chemical Name: 4-O-[(1S)-hexopyranosyl]-D-glycero-hexopyranose
	Molecular weight: Average molecular weight of the compound is 160.255.
	Description: Microcrystalline cellulose is a refined, partially depolymerized cellulose made by combining mineral acids with alpha-cellulose derived as a pulp from fibrous plant material. Micro Crystalline sub micron size colloidal particles have been purified and depolymerized. It has good water absorption, swelling, and dispersion properties and is metabolically inert.
	Melting Point: 260-270°C
	Density (bulk): 1.76 g/cm3
	Refractive index:1.652 n/D
	Solubility: Insoluble in water.
	Stability and storage conditions: Stable under ordinary condition of use and storage.
Sorbitol 	**Manufactured by:** SD fine chemicals
	Chemical Name: (2S,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol
	Uses: Disintegrant
	Description: Sorbitol is utilised as a tablet diluent in wet granulation or dry compression formulations in pharmaceuticals. Because of its pleasant taste, it's often utilised in chewable tablets, and it's also used as a gelatin plasticizer in capsule formulations.
	Melting Point: 110°C
	Density (bulk):1.489 g/cu cm 20 °C

	Refractive index:1.3330 at 20 °C
	Solubility: Very soluble in water slightly soluble in ethanol
	Stability and storage conditions: Keep the container firmly closed in a dry, well-ventilated location.
	Stability/Shelf Life: In strongly acidic and alkaline conditions, sorbitol forms water-soluble chelates with several divalent and trivalent metal ions. With rapid agitation, liquid polyethylene glycols are added to a sorbitol solution to generate a waxy, water-soluble gel with a melting temperature of 35-40 °C. Sorbitol solutions can also discolour when they come into contact with iron oxide. Sorbitol accelerates the breakdown of penicillin in both neutral and aqueous solutions.
Talc 	**Manufactured by:** SD fine chemicals
	Description: Talc is a magnesium (Mg), silicon and oxygen (SiO2, silica), and water-based hydrous silicate mineral. $Mg_3Si_4O_{10}(OH)2$ is its chemical formula. Talc is a generally pure mineral with minor quantities of aluminium, iron, manganese, and titanium.
	Molecular weight:379.27
	Uses: Disintegrant
	Description: In the pharmaceutical industry, talc is used primarily as a glidant to improve powder flow in tablet compression.
	Melting Point:900-1000 °C
	Density (bulk):2.7 g/cm³
	Refractive index:1.58
	Solubility: Insoluble in water and ethanol
	Stability and storage conditions: Keep the container in a well-ventilated area. Well closed
Magnesium stearate 	**Manufactured by:** SD fine chemicals
	Description: Magnesium stearate is a metallic salt boundary lubricant that has two fatty acid equivalents (typically stearic and palmitic acid) and a charged magnesium. It is reasonably priced, chemically stable, and has a high melting point and lubricating property. In the formulation development, magnesium stearate was used at a concentration of 0.25 percent –5.0 percent w/w
	Molecular weight:591.2
	Uses: It's used in infant powders, as a lubricant for plastics, as an emulsifying agent in cosmetics, as a drier in paints and varnishes, as a flatting agent, as an anti-caking agent in foods and fire extinguishers, and as a lubricant in the production of pharmaceutical tablets.
	Melting Point:88.5 °C

	Density (bulk):1.02 g/cm³
	Refractive index:1.58
	Solubility: soluble in water (10 mg/ml).
	Stability and storage conditions: Keep the container in a well-ventilated area.
HPMC 	**Manufactured by:** SD fine chemicals
	Description: Polymeric compounds containing hydroxypropyl methylcellulose repeating units. The molecular weight, percentage of hydroxyl groups, percentage of hydroxypropyl groups, and viscosity measurements are all used to define the properties of Hypromellose polymers. Food Additives, Excipients, and Lubricants Are only few of the commercial items that contain them.
	Molecular formula: $C_{56}H_{108}O_{30}$
	Molecular Weight:1261.4
	Melting Point: 225 to 254°C
	Uses: It can be used in a variety of dosage forms as a film-former, thickener, sustained-release agent, emulsifying agent, and suspending agent, making all kinds of pharmaceutical formulations more evenly dispersed, tough without being broken, or with sustained release effects or stable emulsions
PVP 	**Manufactured by:** SD fine chemicals
	Chemical name:1-ethenylpyrrolidin-2-one
	Density:1.144g/cm3
	Molecular Formula:$(C_6H_9NO)n$
	Molecular Weight:111.1418 (monomer)
	Boiling Point:217.6ºC at 760 mmHg
	Melting Point: 130ºC
	Melting Point: 130ºC
	Solubility: Soluble in water giving a colloidal solution; practically insoluble in ether; soluble in alcohol, chloroform
	Viscosity:2.07 cP at 25 °C
	Uses: It can be used as a tablet and capsule binder, a film forming for ophthalmic solutions, a flavoring agent for liquids and chewable tablets, and an adhesive for transdermal systems, among other things.
	Storage: Store in original containers. Keep containers securely sealed.
Polycaprolactone	**Manufactured by:** SD fine chemicals

	**Description:** Polycaprolactone is a biodegradable, semi-crystalline thermoplastic polyester made by ring-opening polymerization of -caprolactone at high temperatures (200°C) in the presence of a suitable catalyst such as dibutyltin dilaurate. It is a white to light yellow, hygroscopic, amorphous powder.
	Molecular Formula: $C_{16}H_{30}O_7$
	Molecular Weight: 334.40
	Chemical name: 2-[2-(6-hydroxyhexanoyloxy)ethoxy]ethyl 6-hydroxyhexanoate
	Density: 1.145 g/cm3
	Flash point: 60°C
	Solubility: Water soluble
	Storage condition: Stable under normal conditions. Store in in a well closed container.

4.2.2 Preformulation studies

Preformulation is the primary step of product development in which the physicochemical properties of the active pharmaceutical ingredient and other excipients are studied before product development. Preformulation studies help to check the drugs' and excipients' physiochemical effects on the quality of product formulation and performance (Trevor M. Jones et al. 2018). Any physicochemical interactions between an API and excipients in a formulation has the potential to change the physical, chemical, therapeutic, organoleptic, pharmacological, and stability aspects of a dosage form, affecting the dosing accuracy, side effects, and patient adherence (Aboud MK et al. 2017). The preformulation studies conducted for metoprolol are as follows.

4.2.2.1: Preparation of calibration curve

The spectrophotometric method was used to assay the drug. A standard calibration curve of pure drug metoprolol was plotted with the concentration vs. absorbance values measured at λmax of 224 nm. A series of diluted solutions were prepared with solvent methanol and diluent 0.2M phosphate buffer (Aboud MK et al. 2017). The linear regression curve was used to measure the unknown

4.2.2.2 Solubility studies

A saturation solubility study of metoprolol was carried out according to BCS (biopharmaceutical classification system) guidelines. In 500 ml conical flasks, 25mg of each drug was added to 250ml of each medium of 0.1N HCl pH 1.2, distilled water pH 7.0, and phosphate buffer pH 6.8. Flasks were shaken in an orbital shaker at 37°C for 48 hours. The samples were then filtered and diluted before being analyzed with a UV spectrophotometer (Hamidi S et al. 2017).

4.2.2.3 Melting point determination

The determination of the melting point aids in the identification of the material and its purity. The melting point device was used to determine the melting point (Silva AC et al. 2014).

4.2.2.4. Drug-excipient compatibility studies using differential scanning Calorimeter (DSC)

DSC is a thermoanalytical technique that measures the amount of heat required to raise the temperature of a sample. Using a DSC, the thermograms of the drug and excipients were recorded. As a baseline, an empty pan was used. About 1 g of drug/excipients/drug and excipient mixture was weighed and sealed in a small aluminum pan and was heated to 20-400^{0}C at a rate of 10^{0}C/min with constant purging of dry nitrogen 50 ml/min. A blank pan was sealed and used as a reference. DSC thermogram was obtained using automatic thermal analyzer equipment. The thermograms were analyzed to check the compatibility of the drugs and the excipients (Dourado D et al. 2019).

4.2.2.5 Fourier transform infrared spectroscopy (FTIR)

The drug-excipient interactions were analyzed using FTIR tests (FTIR-8400S, Shimadzu). FTIR spectra of metoprolol, cross carmellose sodium, Sodium starch glycolate, MCC, sorbitol, talc, polycaprolactone, HPMC, PVP, lactose, magnesium stearate and the physical mixtures were obtained using the standard KBr Disc/Pellet method. The samples were compacted into pellets after being pulverized with anhydrous KBr. With a resolution of 4 cm^{-1}, an analysis was carried out over the range 4000–400 cm^{-1} (Lau E. 2001).

4.2.3 Preparation of pulsatile tablets

4.2.3.1 Formulation of core in cup tablet

A ‘core in cup’ tablet with a SR plug layer, IR core, and a cup was prepared by the direct compression method. The pulsatile core in the cup bilayer tablet was made with a 12 mm punch. The IR layer was compressed in a 6 mm punch with a low compression force. The cup material, polycaprolactone, was filled into a 12 mm punch die and slightly pressed. The core tablet was placed in the slightly pressed polycaprolactone bed and subsequently filled the gap between the die wall and tablet with polycaprolactone (110 mg). The top of the core tablet was then filled with the SR mixture and compressed to form a tablet (Wang H et al. 2017, Sokar MS et al. 2013).

4.2.4 Design of Experiment

The software JMP was used to generate the experimental design. JMP software is the data analysis tool that helps tackle statistical problems by accessing data from various sources. It utilizes the input (factors) and output (response) variables for generating and analyzing the experimental design. In this experimental design, we have used Least Square Method for the optimization. A least-squares method is a form of mathematical regression analysis used to determine the line of best fit for a set of data, providing a visual demonstration of the relationship between the data points. The relationship between a known independent variable and an unknown dependent variable is represented by each point of data. The input variables for immediate release tablet were compression force and concentration of disintegrant used, whereas for sustained release, the

input variables are compression force and concentration of HPMC. The analytical measurements representing response factors for immediate release were tablet hardness, disintegration, and dissolution, whereas sustained-release responses were hardness and dissolution.

4.2.4.1 Study of flow property, Study of flow property (angle of repose, bulk and tap densities, Carr index, Hausner's ratio) of optimized formula powder material

Powder qualities (particle size, size distribution, density, shape, and surface properties), apparatus features (shoe and die design), and operating conditions influence powder flow behavior during die filling. Understanding powder flow properties is essential for selecting and optimizing operating parameters and controlling the powder filling process. The angle of repose, compressibility index (Carr's index), and Hausner ratio were used to measure the flow properties of powders.

4.2.4.2 Angle of repose

The angle of repose was used to determine the frictional force in a loose powder, and the angle of repose was measured using the fixed funnel method. As a function of the powder flow property, the maximum angle (θ) permitted between the surface of the powder pile and the horizontal plane was determined (Lau E 2001) using the formula,

$$\theta = \tan^{-1}(h/r),$$

Here, h is the height of the cone and r is the radius of the cone base.

4.2.4.3 Bulk density, ρ_b

Bulk density is the ratio of the mass of an untapped powder sample to its volume, including the inter particulate void volume, which is the bulk density of a powder. As a result, both the density of powder particles and the spatial arrangement of particles in the powder bed affect bulk density. The bulk density is measured in g/mL. It is the ratio of the mass of the powder (M) to the volume, V (including the inter-particle void volume) of an untapped powder sample. $\rho_b = M/V_o$

4.2.4.4 Tapped density (ρ_t)

Tapped density is higher than bulk density; it is obtained after mechanically tapping a cylinder containing the sample. It is calculated by the ratio of the powder mass (M) and tapped volume; Vt attained after tapping 100 times using a mechanical tapped density tester ($\rho t = M/ Vt$).

4.2.4.5 Measure of powder compressibility

4.2.4.5.1 Carr's Index

The Compressibility index (Carr's Index) is a measure of a powder's propensity to be compressed based on bulk and tapped densities. In free-flowing powders, inter-particle interactions are minor, and bulk and tapped densities will be closer in value. Poor flowing materials are cohesive, resulting in a wider gap between bulk and tapped densities (Lau E 2001). The Compressibility Index, which is determined using the following formula,

$$Carr'sindex = \left[\frac{(\rho_t - \rho_b)}{\rho_t}\right] \times 100$$

Where, $\rho_t$is the tapped density ρ_b is the bulk density

4.2.4.5.2 Hausner's Ratio

The flow ability of powders or granules is expressed by Hausner's ratio, where V_o is the untapped apparent volume (cm^3) and V_t is the final tapped volume (cm^3) (Lau E 2001).

Hausners ratio = V_o/V_t

4.2.5 Evaluation of core in cup tablet

4.2.5.1 Hardness

The Monsanto hardness tester was used to determine the hardness of the tablets (force necessary to break the tablet). Hardness was measured by placing the tablet between the moving and fixed jaws of the apparatus and applying pressure. Six tablets of metoprolol were used in the experiment (Lau E 2001).

4.2.5.2 Friability

Friability refers to a tablet's ability to endure mechanical stress during handling. It aids in determining the tablet's vulnerability to fragmentation, chipping, and other forms of damage. The tablets were placed in a friabilator and spun at 25 rpm for four minutes. The initial weight and final weight of the tablets were measured to determine friability.

$$\% \text{ friability} = \frac{W_f - W_i}{W_i} \times 100$$

4.2.5.3 Weight variation

The average weight of 20 tablets was obtained after each tablet's weight was measured individually. The tablet passes the weight variation tests if not more than two tablets are beyond the prescribed limits, according to the USP specification (Jain D et al. 2011, Chaudhary SS et al. 2015).

$$Averageweight = \frac{W_{total}}{n_{tab}}$$

Where $W_{total}$is the total weight of tablets and n_{tab} is the number of tablets.

4.2.6 Disintegration time of the IR core tablet

The disintegration test apparatus was used to test the tablets' disintegration time. Water, 0.1 N HCl and pH 6.8 phosphate buffer were three distinct mediums used. The tablets were inserted in the apparatus's basket's six tubes. The basket was pushed up and down in the media at a rate of 29 to 32 cycles per minute (Patil S et al. 2015). The time required for the tablets to disintegrate completely was recorded.

4.2.6.1 In vitro drug release studies

Each drug layer (IR core, the SR plug, and the core in a cup tablet), was evaluated separately to study the drug release profile. The primary factor for selecting the optimal formulation among trials in each layer preparation was in vitro drug release. Each data is the average of three repetitions.

4.2.6.2 *In vitro* drug release from IR core tablet and SR plug

In vitro drug release experiments for metoprolol, the dissolution media for the SR plug was 0.1 N HCl and for the IR core tablet was pH 6.8 phosphate buffer. The dissolution medium was agitated at 50 rpm (Shao Y et al. 2015, Cazorla-Luna R et al. 2019, Chisty SJ Chisty SJ et al. 2016, Ashwini MS et al. 2013). At predetermined time intervals, a predetermined number of aliquots were removed for analysis and replaced with an equal amount of fresh dissolution medium. A spectrophotometer set to 224 nm (metoprolol) wavelength was used to examine the aliquots. Similarly, the metoprolol (Metatus 50 mg) was investigated and compared with the prepared IR tablets (dissimilarity/similarity studies).

4.2.6.3 Evaluation of cup layer

4.2.6.3.1 Effect of the SR cup on drug release

The influence of the SR cup layer on drug release from the IR core tablet was studied by analyzing the 'core in cup' tablet without the plug layer and the IR core tablet alone. The dissolution media used in the test was maintained the same as that of the IR core tablet drug release studies.

4.2.7 Drug release from core in cup tablets of metoprolol

In USP dissolution apparatus II, a dissolution test for the bilayer tablet was performed. The dissolution medium used for the first 300 min was 730 mL of 0.1M HCl solution. The tablet was then subjected to a 6.8 pH phosphate buffer solution by the addition of 0.2 M $Na_3PO_4.12H_2O$ into the existing media (Patel VD et al. 2017). A spectrophotometer set to 224nm wavelength was used to examine the withdrawn samples. The dissolution media for core in cup tablet formulation was 0.1M HCl throughout the dissolution study. The spectrometric analysis for metoprolol was conducted at 224nm.

4.3 Modeling of drug release

To examine the drug release kinetics, the dissolution study data were fitted into zero order, first order, Korsmeyer-Peppas, Higuchi, and Hixson-Crowell models (Craciun AM et al. 2019).

4.4 Water uptake and erosion studies

Water absorption and erosion were studied using the USP dissolution apparatus type I. The weight of the empty basket and the tablets were recorded before starting the experiment. The tests were carried out in the same prescribed conditions as the dissolution test. At predetermined times, individual tablets were taken out of the medium. The tablets were weighed following the completion of the test. The tablets were dried at 80^0C until they attained a constant weight. After they had cooled to room temperature, the tablets were weighed again. To compute the % erosion, the below formula was used (Patil AS et al. 2011, Chauhan BS et al. 2014, Sonani NG et al. 2010).

$$\%\ erosion = \frac{W_i - W_f}{W_i} \times 100$$

Where W_i represents the initial weight of the tablet and W_f represents the final weight of the dried tablet.

The excess weight gain of the tablet at each time interval was used to calculate water uptake by the tablet.

$$Weightincreased = \frac{W_{wet} - W_f}{W_f} \times 100$$

Where W_{wet}represents the weight of the wet tablet

4.5 Stability study

The stability tests on the tablets were carried out in compliance with ICH guidelines. The tablets were maintained in two different study conditions. One batch of tablets was kept in a desiccator for three months at 25°C and 60% RH in the presence of anhydrous calcium chloride in polypropylene containers. Another pair was kept in a stability chamber at 40°C and 75 RH for three months. Three months later, dissolution tests and the physical appearance of the tablets were evaluated (Lachman L et al. 1965, Chaurasia G et al. 2016, Nagaraju R et al. 2009).

4.6 In-vivo Studies

The goal of the study was to investigate the bioavailability of metoprolol core in cup tablets in a rabbit model, and it was carried out according to CPCSEA criteria. The Institutional Animal Ethical Committee authorized pro forma B, which included protocols for animal studies Nandha college of Pharmacy, Erode. Registration no: 688/ PO /Re /S / 02 /CPCSEA. Six healthy male rabbits weighing approximately 1 to 5 Kilograms were selected and divided into two groups. Each rabbit was housed for three weeks under standard conditions with a 12 h light/dark cycle with free access to water and a basal diet for one week. Further, they were subjected to fasting for 12 hours with access to drinking water. After 12 hours, the prepared formulations were administered (16mg/kg) through the oral route by keeping them upright. Blood samples of 1 ml have been collected from the tail vein using a needle at 15, 30, 60, 120, 180, 240, 300, 360, 420, 480, and 540 minutes after administration. All blood samples were immediately put into heparin and subjected to centrifugation at 4000 rpm for 10 min to separate the plasma. Then the plasma was frozen and stored at -20ºC until the analysis.

The HPLC method was established for the analysis of the drugs. Phosphate buffer and acetonitrile in an 80:20 ratio were used as a mobile phase. The flow rate was maintained at 1 ml/min. The linearity range was determined between 5 and 100 ng/ml, with a correlation coefficient of 0.9991. Before HPLC analysis, plasma was filtered using a 0.25 m membrane filter. The supernatant was

obtained by centrifuging 0.2 mL of filtered plasma and diluting it with 1 mL of acetonitrile. After evaporating the supernatant under nitrogen, the residue was combined with 0.3 ml of HPLC mobile phase. The sample was injected into the HPLC column with a UV detector set to wave length of 224 nm. The kinetic parameters including C_{max}, T_{max}, AUC were analysed (Jain D et al. 2011, Mohanty C et al. 2021, Sultana A et al. 2018). The results of this study were depicted as plot of plasma concentration vs time, and AUC was used to calculate bioavailability.

4.7 Result and Discussion

4.7.1 Preparation of calibration curve

The graphical plots of absorbance (y) vs concentration (x) of metoprolol (Table 4.2 and Figure 4.1) yielded the linear calibration curve equations $y = 0.0363x + 0.0186$, $R^2 = 0.9997$, which was used to calculate the amount of drug released in dissolution studies of different batches of products.

Table 4.2. Absorbance data for calibration curve of metoprolol

Concentration (µg/ml)	**Absorbance**
2	0.089
4	0.166
6	0.237
8	0.31
10	0.38
Regression equation	y=0.0185x + 0.0951, $R^2 = 0.9996$

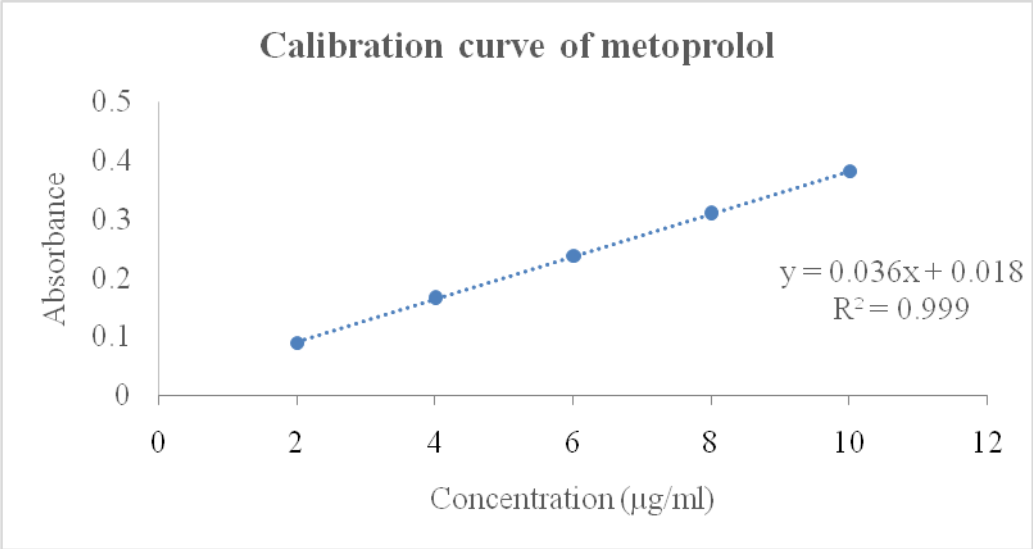


Figure 4.1. Calibration curve of metoprolol

4.7.2 Solubility studies

Saturation solubility of metoprolol was observed as 12.6±0.24, 8± 0.43, 35.7± 0.18 mg/mL at 25°C in double-distilled water (DDW), 0.1 N HCl, and pH 6.8 phosphate buffer. The drug was found to be sparingly soluble in DDW, HCl and pH 6.8 phosphate buffer. Metoprolol was found to be sparingly soluble in DDW, HCl and soluble in pH 6.8 phosphate buffer (Table 4.3).

Table 4.3. Solubility of metoprolol

Solvent	**Solubility (mg/ml)**
Distilled water pH 7.0	12.6 ± 0.24
0.1N HCl pH 1.2	8.24 ±0.43
Phosphate buffer pH 6.8	35.7 ± 0.18

4.7.3 Melting point determination

The melting point of metoprolol was found to be 120 ^{0}C. It was comparable to the melting point reference provided by the USP (figure 4.2 and 4.3).

Figure 4.2 and 4.3 melting point device

4.7.4 Drug excipients compatibility study using DSC

DSC was used to determine the compatibility between drug and excipients used in the formulations. Individual components, such as metoprolol, cross carmellose, sorbitol, MCC, HPMC, lactose, magnesium stearate, talc, polycaprolctone, and the physical mixture of drug and excipients, were investigated using DSC. The thermograms of the physical mixture revealed no temperature shift and so no interactions, implying that there were no incompatibilities between drugs and excipients (Figure 4.4-4.14).

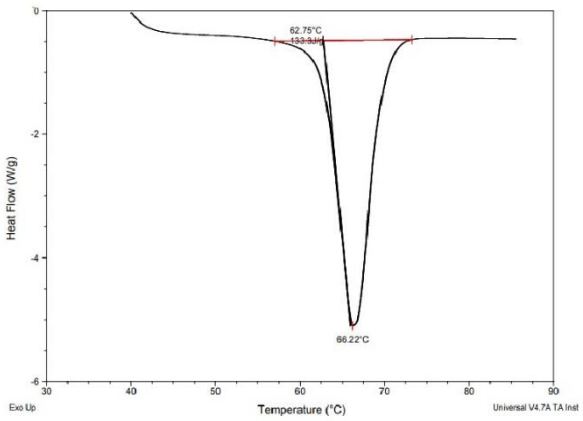


Figure 4.4. DSC thermogram of polycaprolactone

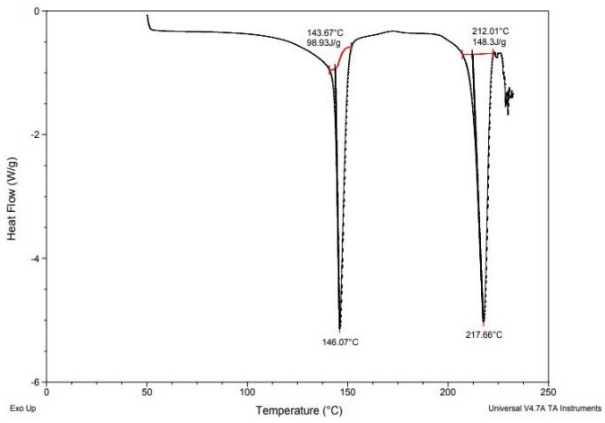


Figure 4.5. DSC thermogram of lactose

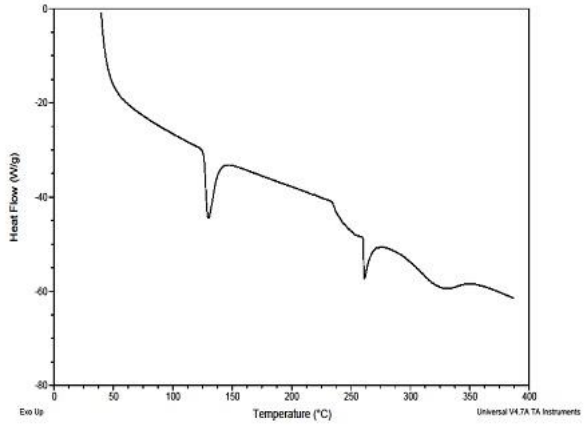


Figure 4.6.DSC thermogram of Metoprolol

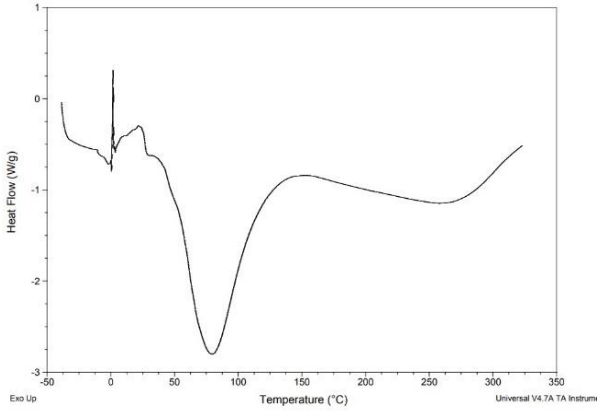


Figure 4.7. DSC thermogram of cross carmellosesodium

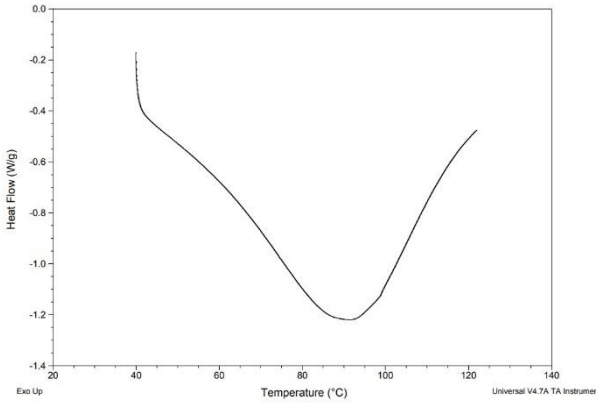


Figure 4.8. DSC thermogram of HPMC

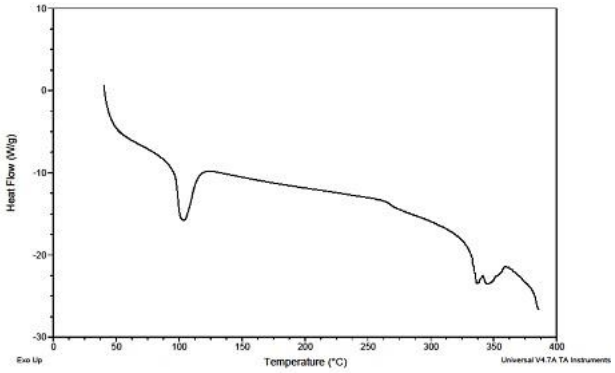


Figure 4.9. DSC thermogram of sorbitol

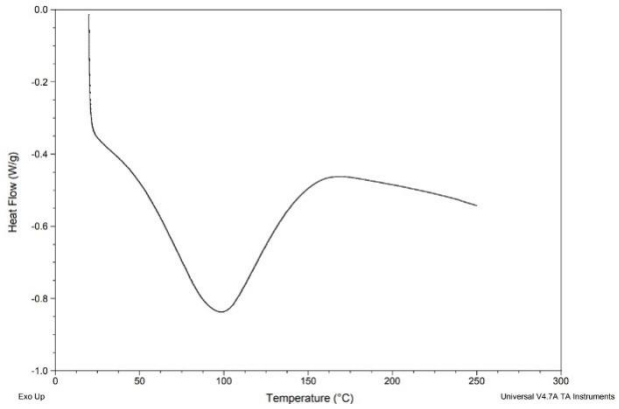


Figure 4.10. DSC thermogram of MCC

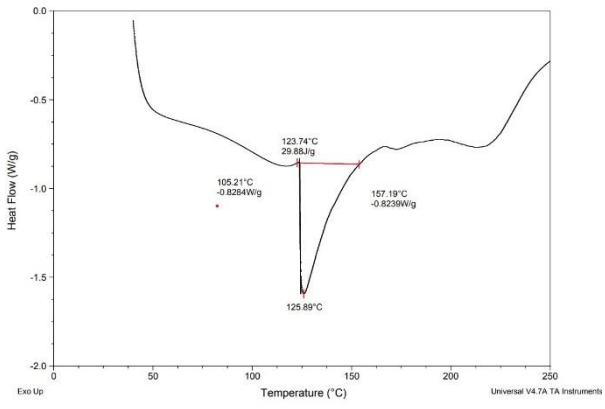


Figure 4.11. DSC thermogram of Talc

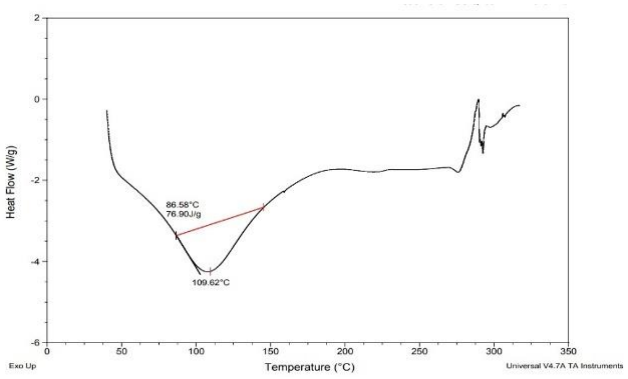


Figure 4.12.DSC thermogram of magnesium stearate

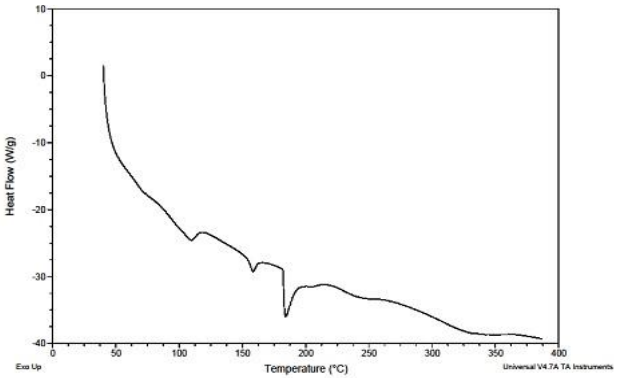


Figure 4.13. DSC thermogram of physical mixture of SR mixture of metoprolol

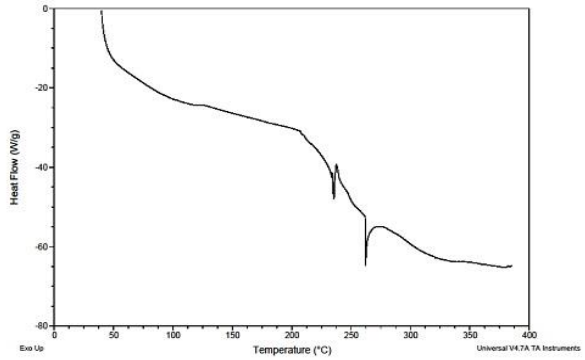


Figure 4.14. DSC thermogram of IR tablet mixture of metoprolol

4.7.5 FTIR

The IR spectra of the ingredients were analyzed to find the interaction between the drug and the excipients. The major functional groups of the ingredients were studied and compared with the IR spectra of the physical mixture (shown in Figure 4.15-4.16). IR spectra of the physical mixture did not show any incompatibilities.

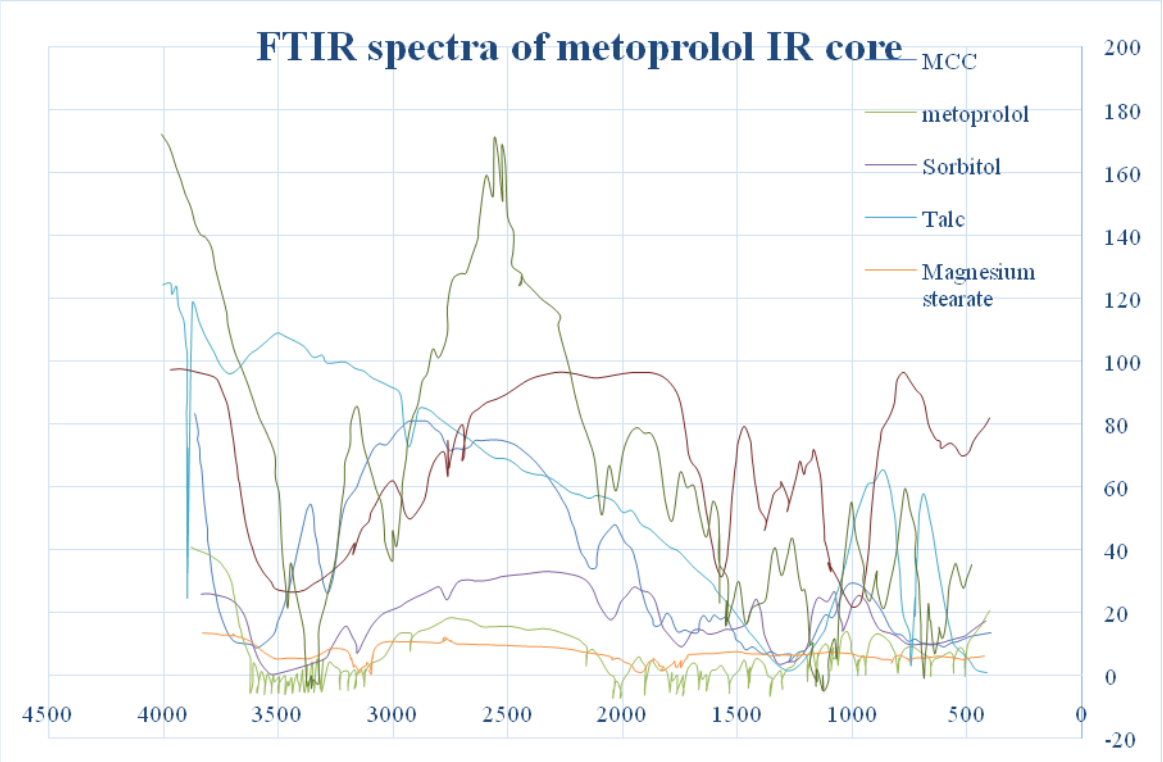


Figure 4.15. FTIR spectra of metoprolol IR core

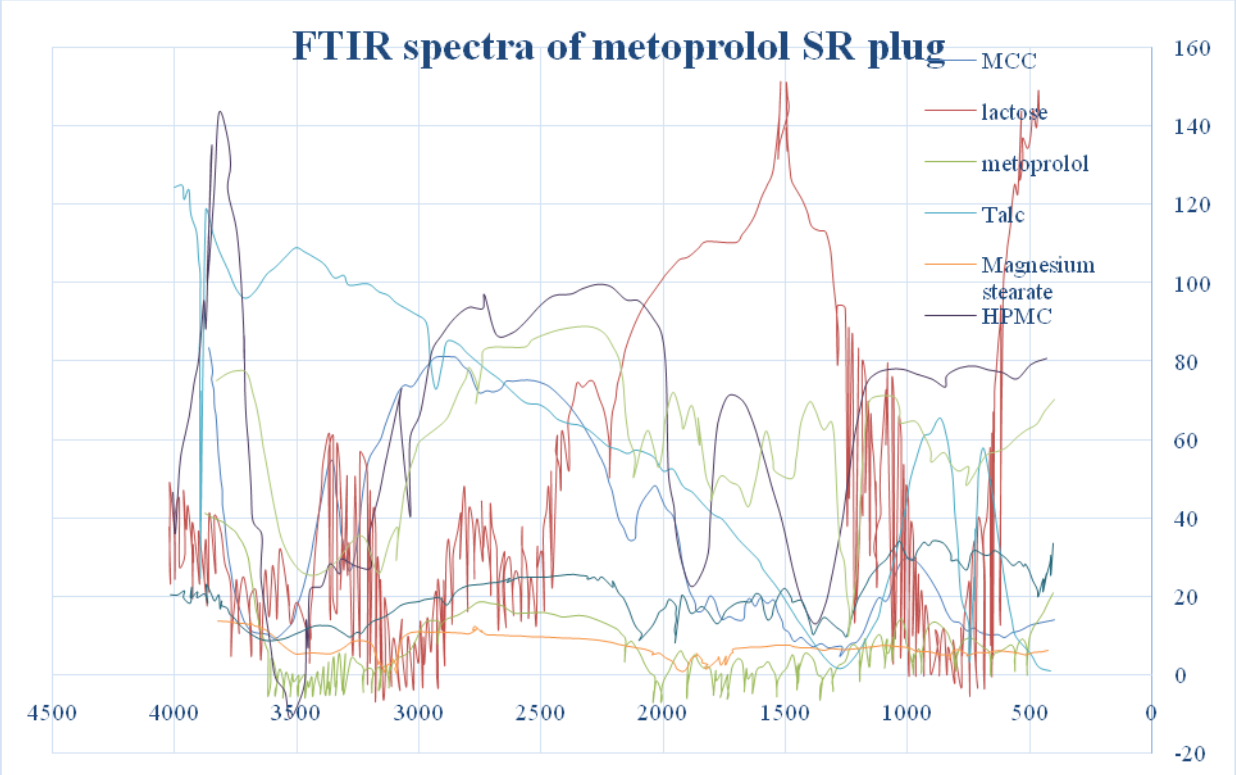


Figure 4.16. FTIR spectra of metoprolol SR plug

4.7.5.1 Preparation of Pulsatile Tablets

4.7.5.1.1 Design of experiment (JMP least square method)

The least-square approach minimizes the sum of the squares of the offsets (residual component) of the points from the curve to get the best-fitting curve or line of best fit for a group of data points. To forecast the behavior of dependent variables, least squares regression is employed. The formula for metoprolol SR and IR layer was optimized based on the experimental results obtained from the JMP least square method. The effect of independent variables % of HPMC and compression force on the % drug release and hardness of SR plug were identified from the predicted plots. Similarly, the effect of disintegrant concentration and compression force on drug release, disintegration time, and hardness were studied in the case of IR core of metoprolol.

4.7.5.1.2 Least Squares Fit applied to metoprolol IR tablet

The concentration of disintegrant directly influences the hardness, disintegration time and the drug release from the formulation. The suggested hardness for the 13 runs was 5, 5.5, 6, 6.5 and 7. The compression forces considered in the study were 6.51-9.41 kg/cm^3. The lack of fit F-value was 0.5539, indicated that the value was insignificant compared to the pure drug. There was a decrease in the drug release when the concentration of disintegrant was reduced. Also, the hardness was higher with the lowest concentration of disintegrant. The lowest disintegration time was observed with the highest concentration of disintegrant. Compression force and the drug release were inversely proportional. The compression force was fixed between 6.59 kg/cm^3 and 9.41 kg/cm^3. An increase in disintegration time was observed with the increase in compression force.

The lowest value for drug release was 97.19%, which was observed with the lowest concentration of disintegrant. There was also a decrease in % drug release when the compression force was maximum. The optimal formula suggested was with a 20% disintegrant, and the recommended compression force was 5 kg/cm^3 (Table 4.4-4.11 and figure 4.17-4.24).

Table 4.4. Optimization using least square method

Run	Disintegrant (mg)	Compression force (kg/cm3)	Hardness (kg/cm3)	disintegration time (min)	% drug release
1	10	8	6	6.91	99.34
2	2.93	8	6	10.41	101.13
3	10	8	6.5	7.08	100.56
4	10	8	6	6.83	99.76
5	17.07	8	6.5	4.46	99.75
6	5	9	6.5	8.83	100.34
7	15	7	5.5	4.83	100.74
8	10	9.41	7	7.58	99.45
9	10	6.59	5	6.33	100.45
10	10	8	6	6.83	98.88
11	15	9	6.5	5.16	99.56
12	5	7	5.5	8.25	97.19
13	10	8	6.5	6.91	100.76

Response Hardness (Kg/cm3)

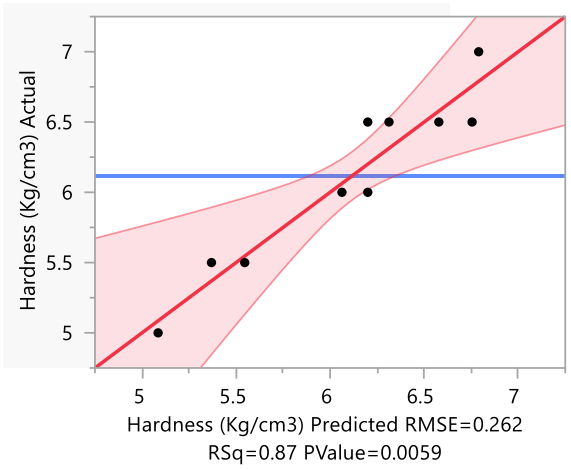


Figure 4.17. Actual by predicted plot of metoprolol IR core hardness

Table 4.5. Lack of fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack of Fit	3	0.18065189	0.060217	0.8029
Pure Error	4	0.30000000	0.075000	**Prob > F**
Total Error	7	0.48065189		0.5539
				Max RSq
				0.9161

Table 4.6. Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	6.2175899	0.116376	53.43	<.0001*
Disintegrant%(2,20)	0.1547117	0.181468	0.85	0.4221
Compression (Kg/cm3)(6,10)	1.2085956	0.192825	6.27	0.0004*
Disintegrant %*Disintegrant %	-0.019743	0.321906	-0.06	0.9528
Disintegrant %*Compression (Kg/cm3)	1.781e-16	0.471671	0.00	1.0000
Compression (Kg/cm3)*Compression (Kg/cm3)	-0.528215	0.399178	-1.32	0.2273

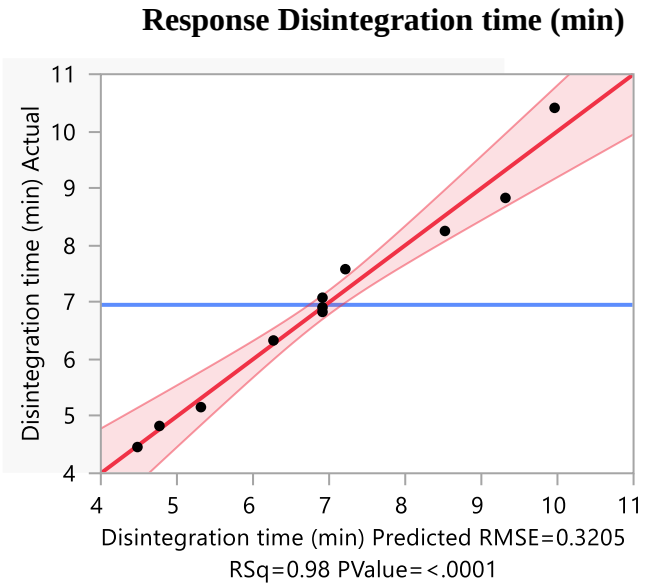


Figure 4.18. Actual by predicted plot of metoprolol IR core disintegration time

Table 4.7. Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack of Fit	3	0.18065189	0.060217	0.8029
Pure Error	4	0.30000000	0.075000	**Prob > F**
Total Error	7	0.48065189		0.5539
				Max RSq
				0.9161

Table 4.8. Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	6.5310814	0.142328	45.89	<.0001*
Disintegrant%(2,20)	-3.377417	0.221935	-15.22	<.0001*
Compression (Kg/cm3)(6,10)	0.6451186	0.235824	2.74	0.0291*
Disintegrant %*Disintegrant %	0.5010854	0.393691	1.27	0.2437
Disintegrant %*Compression (Kg/cm3)	-0.225	0.576853	-0.39	0.7081
Compression (Kg/cm3)*Compression (Kg/cm3)	-0.346045	0.488194	-0.71	0.5013

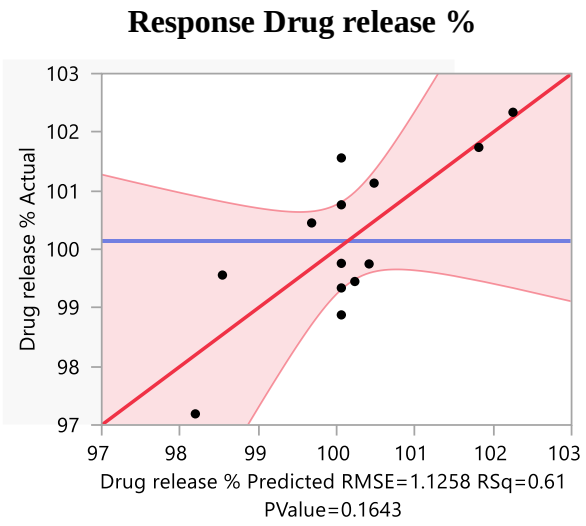


Figure 4.19. Actual by predicted plot of metoprolol IR core drug release %

Table 4.9. Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack of Fit	3	4.1317573	1.37725	1.1620
Pure Error	4	4.7408000	1.18520	**Prob > F**
Total Error	7	8.8725573		0.4275
				Max RSq
				0.7931

Table 4.10. Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	100.06318	0.500003	200.12	<.0001*
Disintegrant % (2,20)	0.098337	0.779666	0.13	0.9032
Compression (Kg/cm3) (6,10)	-0.341836	0.82846	-0.41	0.6922
Disintegrant %*Disintegrant %	0.6261308	1.383052	0.45	0.6645
Disintegrant %*Compression (Kg/cm3)	-6.597	2.026506	-3.26	0.0140*
Compression (Kg/cm3) *Compression (Kg/cm3)	-0.2084	1.715044	-0.12	0.9067

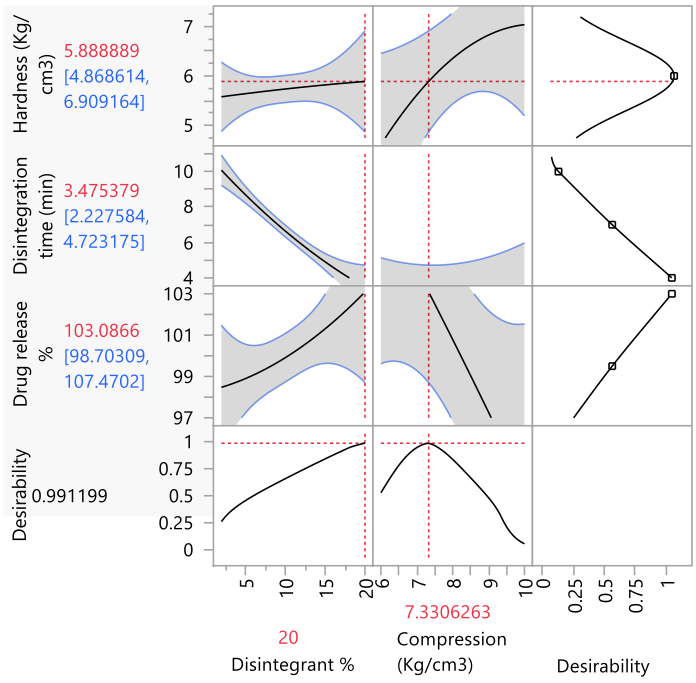


Figure 4.20. Prediction profile of metoprolol IR core

Table 4.11. Optimal formula for metoprololIR plug

Setting	Disintegrant %	Compression (Kg/cm3)	Hardness (Kg/cm3)	Hardness (Kg/cm3) Lower	Hardness (Kg/cm3) Upper	Disintegration time (min)	Disintegration time (min) Lower CI	Disintegration time (min) Upper CI	Drug release %	Drug release % Lower CI	Drug release % Upper CI	Desirability
Optimal	20	7.331	5.889	4.869	6.909	3.475	2.228	4.723	100.087	98.703	100.470	0.991

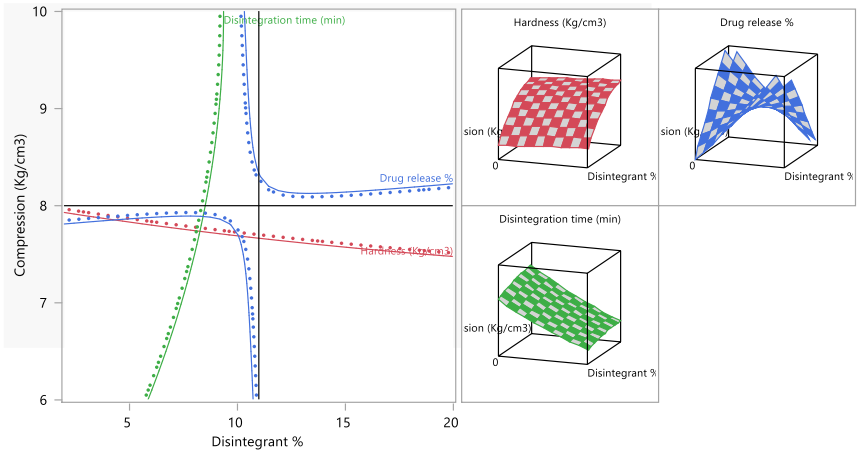


Figure 4.21. The plots of relationship between compression force (Kg/cm^3), disintegrant concentration with drug release %, disintegration time (min) , and hardness

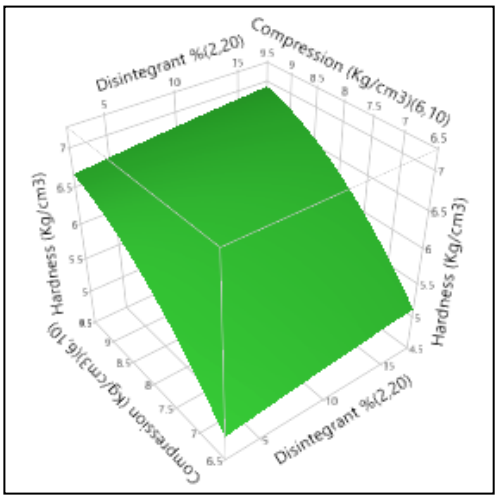


Figure 4.22. 3D plot of hardness (Kg/cm3)

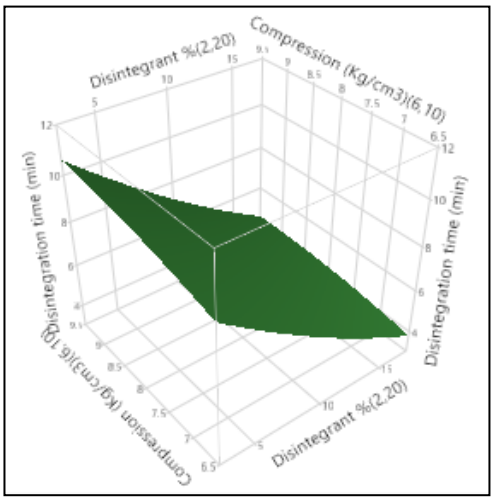


Figure 4.23. 3D plot of drug release

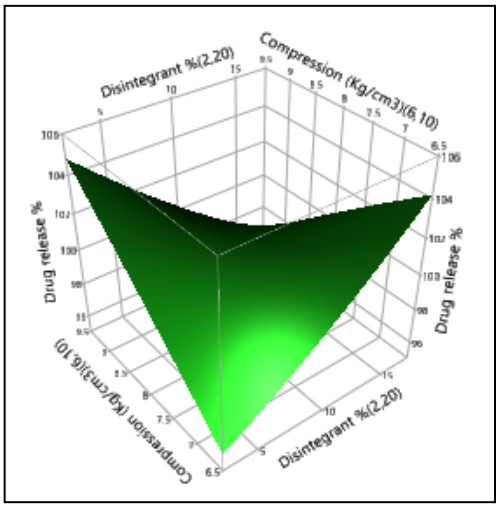


Figure 4.24. 3D plot of disintegration time (min)

4.7.5.1.3 Least Squares fit applied to metoprolol SR plug

The basic parameters influencing the hardness and percentage drug release were selected as the depended variables. Concentration of HPMC and the compression force were independent variables. The HPMC concentration was fixed between 5.857-34.1421. Lack of fit for % drug release was 0.0106. The compression forces used in 13 trials were 5, 6.5, 8, 8.621 and 4.3786.From the results, it was found that the increase in the concentration of HPMC decreased the % drug release. Highest compression force 34.1421kg/cm^3 demonstrated the least % of drug release i.e., 79.55%. Decrease and increase in compression force reduced the % drug release from the formulation. Compression force has a linear relationship with hardness. The optimal concentration of HPMC was predicted as 5 mg, and the compression force recommended was 6.633 kg/cm^3 (Table 4.12-4.16 and figure 4.25-4.30). The desirability of the optimized formula was 0.983.

Table 4.12. Optimization using least square method

Run	HPMC	Compression force (kg/cm^3)	% drug release	Hardness kg/cm3
1	10	5	97.82	5
2	10	8	98.12	6.5
3	30	6.5	82.16	6

4	20	5	83.13	5
5	20	6.5	90.21	6
6	20	4.3786	91.11	4.5
7	20	6.5	90.66	6
8	20	8.621	89.73	7
9	30	8	82.63	6.5
10	34.1421	6.5	79.55	6
11	20	6.5	92.75	6
12	20	6.5	93.21	6
13	5.857	6.5	100.23	6

Response drug release %

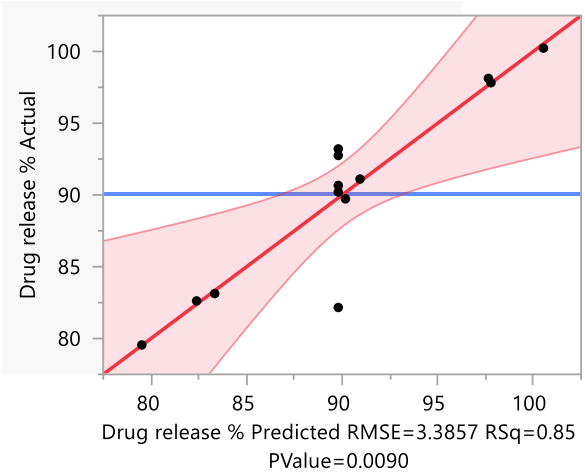


Figure 4.25. Actual by predicted plot of metoprolol SR plug drug release

Table 4.13. Lack of fit

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Lack Of Fit	3	0.633672	0.2112	0.0106
Pure Error	4	79.607880	19.9020	**Prob > F**

Source	DF	Sum of Squares	Mean Square	F Ratio
Total Error	7	80.241552		0.9983
				Max RSq
				0.8480

Table 4.14. Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	89.749919	1.489105	60.27	<.0001*
HPMC (5,35)	-11.24549	1.985052	-5.67	0.0008*
Compression (Kg/cm3)(4,10)	-0.03657	2.942956	-0.01	0.9904
HPMC *HPMC	0.2539687	2.888265	0.09	0.9324
HPMC *Compression (Kg/cm3)	-0.6075	5.078575	-0.12	0.9081
Compression (Kg/cm3)*Compression (Kg/cm3)	1.5115	5.134694	0.29	0.7770

Response Hardness (Kg/cm3)

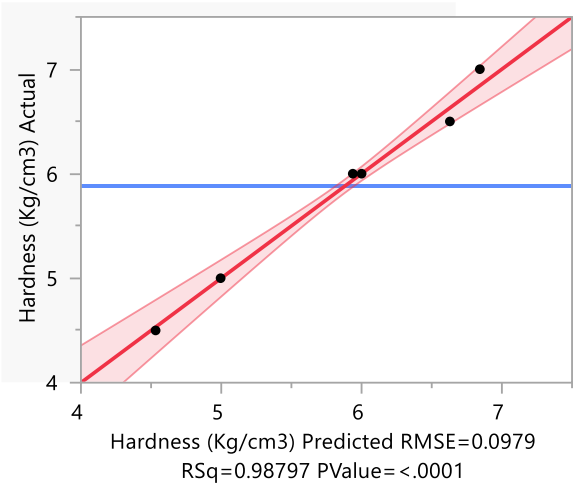


Figure 4.26. Actual by predicted plot of metoprolol SR plug

Table 4.15. Parameter estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	6.2549528	0.043061	145.26	<.0001*
HPMC (5,35)	-1.41e-11	0.057403	-0.00	1.0000
Compression (Kg/cm3) (4,10)	1.4255501	0.085103	16.75	<.0001*
HPMC *HPMC	-0.070313	0.083521	-0.84	0.4277
HPMC *Compression (Kg/cm3)	-2.91e-17	0.14686	-0.00	1.0000
Compression (Kg/cm3) *Compression (Kg/cm3)	-0.625	0.148482	-4.21	0.0040*

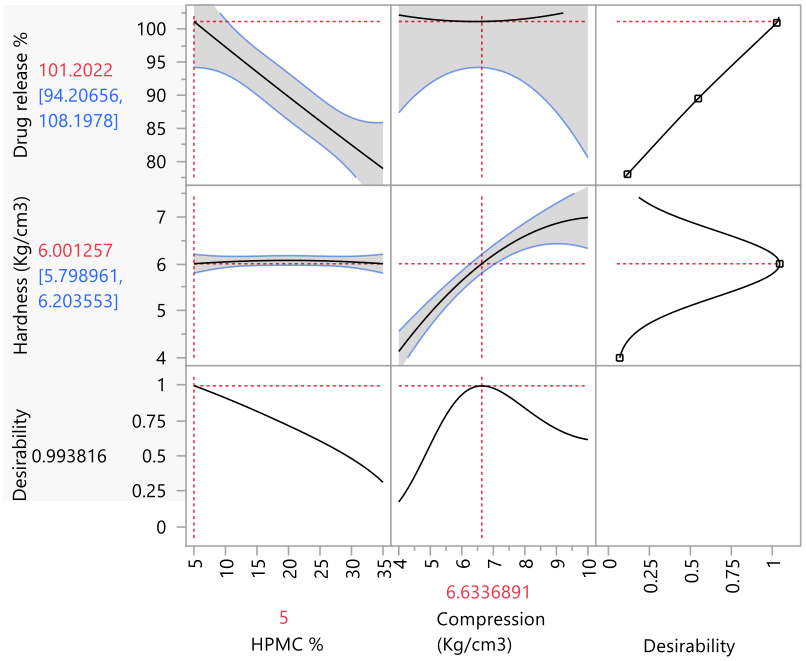


Figure 4.27. Prediction profile of metoprolol SR plug

Table 4.16. Optimal formula for metoprolol SR plug

Setting	HPMC	Compression (Kg/cm3)	Drug release %	Drug release % Lower CI	Drug release % Upper CI	Hardness (Kg/cm3)	Hardness (Kg/cm3) Lower CI	Hardness (Kg/cm3) Upper CI	Desirability
Optimal	5	6.994	100.719	94.294	100.145	5.951	5.331	6.571	0.983

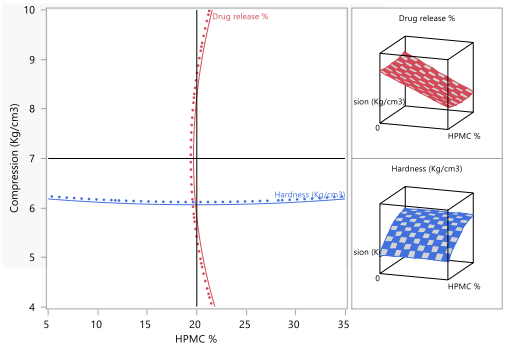


Figure 4.28. The plot of relationship between compression force (Kg/cm^3) and HPMC with drug release % and hardness (Kg/cm^3)

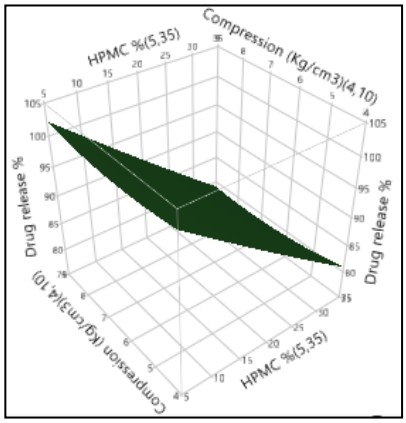


Figure 4.29. 3D plot for % drug release

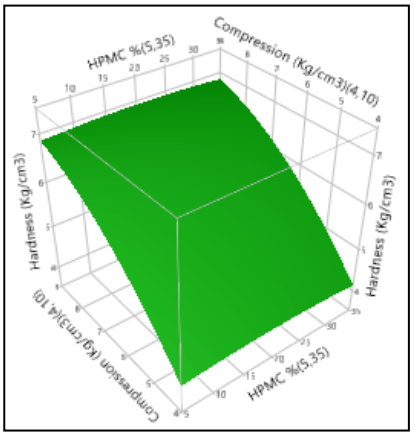


Figure 4.30. 3D plot for hardness

Optimized formula for core in cup tablet

The optimized formula for metoprolol core in cup tablet is as follows (Table 4.17-4.19).

Table 4.17. Composition of metoprolol IR core

Ingredients	**Quantity (%)**
Drug	13
Cross carmellose sodium (CCS)	20
MCC	10
Sorbitol	20.1
Talc	0.325

Magnesium stearate	0.325
Total weight	63.75
Punch size	6mm
Compression force	5 kg/cm^3

Table 4.18. Composition of metoprolol SR plug

Ingredients	**Quantity (%)**
Drug	12
HPMC	5
PVP in alcoholic solution	2.8
MCC	55
Lactose	32
Talc	0.5
Magnesium stearate	0.5
Total weight	107.8
Punch size	12 mm
Compression force	7 kg/cm^3

Table 4.19. Composition of metoprolol cup layer

Formulation	**Polycaprolctone (mg)**
M	110

4.7.6 Study of flow property (angle of repose, bulk and tap densities, Carr's index, Hausner's ratio) of powder material

4.7.6.1 Angle of repose

The flow characteristics and compressibility of powder mixes are critical in defining the quality and reproducibility of direct compression tablets. According to the USP, a powder sample with an angle of repose of 25–30^0 has good flow properties. All the powder mixes had an angle of repose ranging from 28-30º. The angle of repose of all the formulations is given in table 4.20.

4.7.6.2 Bulk density

The bulk density of the powder mix affects the flowability of the powder. Low bulk density associated with agglomerated particles negatively influences the flowability and of the powder. The observed bulk density of the formulations is given in table 4.20.

4.7.6.3 Tapped density

Tapped density is greater in the case of regularly shaped particles than irregularly shaped particles. Determined tapped density was mentioned in table 4.20. These values were used for the calculation of the Hausner's ratio and Carr's index.

4.7.6.4 Carr's index

Carr's compressibility index values have been used to define the flow properties of the powders. Carr's index for the studies was in the range of 10 - 14. These values indicate the excellent flowability of the powders (Table 4.20).

4.7.6.5 Hausner's ratio

Hausner's ratio is related to the interparticle friction between the particles. For a satisfactory flow property, Hausner's ratio should be less than 1.25. Table 4.20 lists the IR tablet's Hausner's ratio.

Table 4.20. Flow properties of immediate release formulations

Evaluation parameters	**Metoprolol**	
	IR	**SR**
Angle of repose	28.01	28.69
Bulk density	0.27	0.28
Tapped density	0.28	0.29
Carr's index	10.69	12
Hausner's ratio	1	1.11

4.7.7 Evaluation of core in cup tablet

All core in cup tablets passed the quality control tests performed. For metoprolol core in cup tablet thickness was 3.51±0.04mm, while its diameter was 6.08±0.01mm. Hardness (5.51±0.3 kg/cm^2) was within acceptable limits. The tested tablets had a friability of 0.25%. The average weight of 20 tablets was 284±0.9 mg [17] (Table 4.21).

Table 4.21. Quality control tests for Metoprolol core in cup tablets

Test	Result
Thickness	3.51±0.04mm
Diameter	6.08±0.01mm
Hardness	5.51±0.3 kg/cm^3
Friability	<0.25%
Average weight	284±0.9 mg

4.7.7.1 Formulation of core in cup tablet

The tablets were prepared as per the optimized formula. The images of the prepared tablets are provided below (Figure 4.31).

Figure 4.31. Prepared core in cup tablets

4.7.7.2 Disintegration time of the IR core tablet

The disintegration time for the immediate release core tablet was determined in water, 0.1 N HCl and pH 6.8 phosphate buffer. The disintegration of the core tablet was instantaneous in all three media. The disintegration time was less than 1 minute for metoprolol immediate release core (figure 4.32).

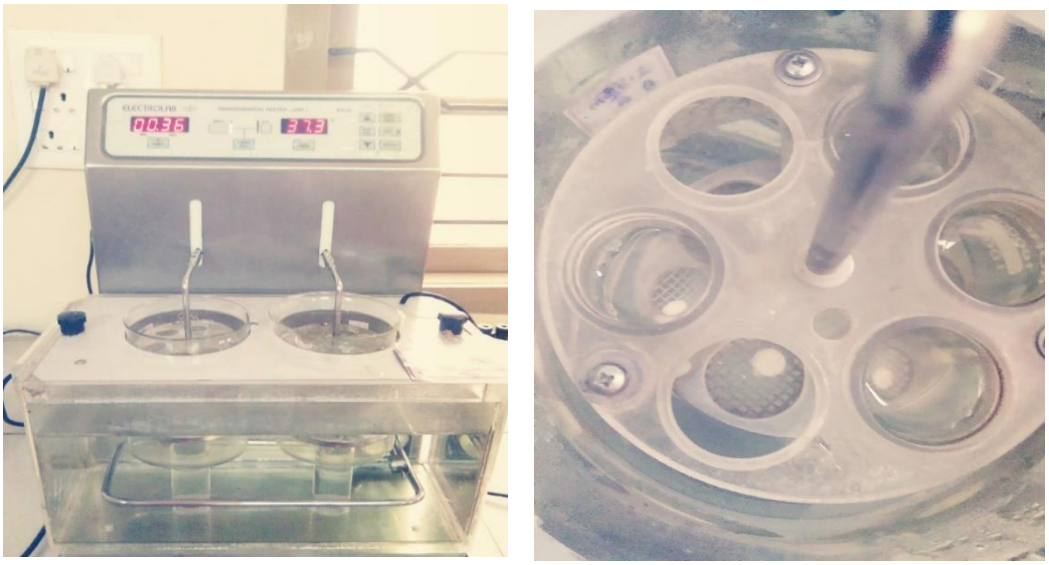


Figure 4.32. Disintegration studies

4.7.8 In vitro drug release from IR core tablet and SR plug

4.7.8.1 In vitro drug release from IR core tablet

In vitro cumulative release of metoprolol IR core tablet was studied in 900 mL 0.1N HCl at 37ºC and 50 rpm. To get an immediate release following the lag time, CCS was incorporated as a super disintegrant in the core tablet. Super disintegrants speed up the disintegration of direct compressible tablets and improve the solubility of medications that are difficult to dissolve. The drug release mechanism is dependent on the concentration of super disintegrants. The metoprolol IR core tablet released 98.379% metoprolol in 30 min (Table 4.23, 4.24 and Figure 4.33-4.35).

Figure 4.33. Dissolution

Table 4.22. % Cumulative metoprolol release from IR core tablet

Time (min)	Absorbance	Conc (mcg/ml)	Conc (mg/ml)	Concentration* dilution factor	Error	Bath Concentration	Drug release	% Cumulative drug release
0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.00
5	0.032	0.369	0.000	0.004	0.000	3.322	3.322	25.55
10	0.041	0.617	0.001	0.006	0.004	5.554	5.557	42.74
15	0.053	0.948	0.001	0.009	0.010	8.529	8.539	65.68
20	0.064	1.251	0.001	0.013	0.019	11.256	11.276	86.73
25	0.068	1.361	0.001	0.014	0.032	12.248	12.280	94.46
30	0.07	1.416	0.001	0.014	0.045	12.744	12.789	98.37

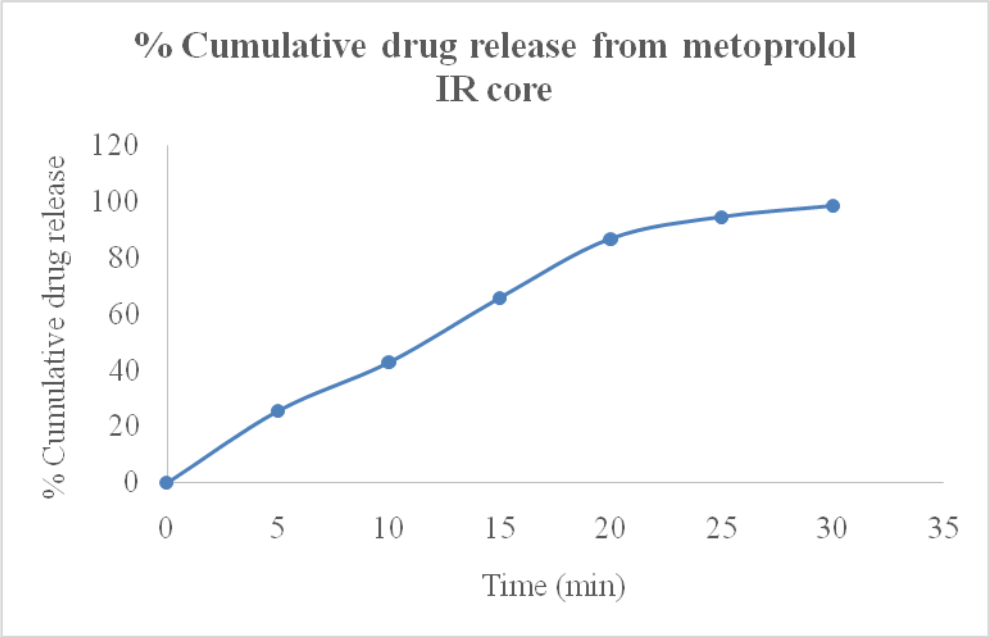


Figure 4.34. % Cumulative metoprolol release from IR core tablet

Drug release from the IR core of metoprolol was compared with the IR formulations available on the market. Metoprolol was compared with the marketed product Metatus 50 mg. The Metatus 50 mg tablet showed metoprolol release of 98.396% in 30 min, and that of the metoprolol core tablet was 98.379%. The calculated f2 value for the formulation compared to their marketed formulation was more than 50. Hence the drug release was concluded to be similar (Table 5.35, 5.36 and figure 5.56, 5.57).

Table 4.23. % Cumulative drug release from metoprolol marketed IR tablet (Metatus50mg)

Time (min)	Absorbance	Concentration (mcg/ml)	Conc (mg/ml)	Concentration* dilution factor	Error	Bath Concentration	Drug release	% drug release
0	0	0.000	0.00000	0.000	0.000	0.000	0.000	0.00
5	0.03	0.314	0.00031	0.003	0.000	2.826	2.826	21.74
10	0.043	0.672	0.00067	0.007	0.003	6.050	6.053	46.55

15	0.051	1.168	0.00117	0.012	0.010	10.512	10.522	61.94
20	0.064	1.251	0.00125	0.013	0.022	11.256	11.278	86.75
25	0.068	1.361	0.00136	0.014	0.034	12.248	12.282	94.47
30	0.07	1.416	0.00142	0.014	0.048	12.744	12.791	98.39

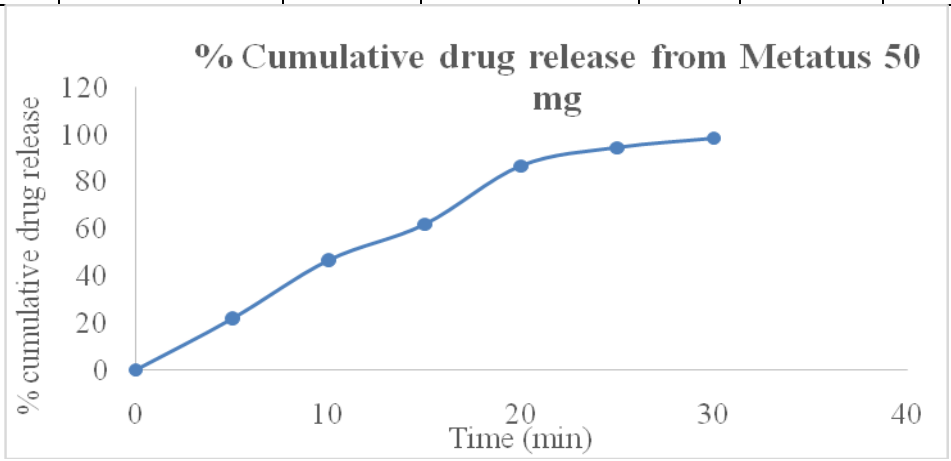


Figure 4.35. % Cumulative drug release from metoprolol marketed IR tablet (Metatus 50 mg)

4.7.8.2 In vitro drug release from SR plug

The sustained release polymeric plug contributes to the lag period prior to the onset of drug release from the IR core. The tablet plug was made with a SR polymer, HPMC, to maintain a low concentration of drug in the blood during the lag period and to reach the therapeutic concentration faster. When HPMC comes into contact with water, it produces a viscous gel layer that aids in the sustained release of the drug from the plug. The swelling expansion of the polymer prevents water from accessing the IR core, so HPMC produces a sustained drug release from the plug. As the swelling progresses, the plug's rigidity deteriorates, causing the plug layer to erode. Metoprolol SR plugs were able to release the drugs for up to 360 minutes. Metoprolol SR plug released 98.341% of the drug at 360 min (Table 4.24 and Figure 4.36).

Table 4.24. % Cumulative metoprolol release from SR core tablet

Time (min)	Absorbance	Conc (mcg/ml)	Conc (mg/ml)	Concentration* dilution factor	Error	Bath Concentration	Drug release	% Cumulative drug release
0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.00
60	0.025	0.176	0.000	0.002	0.000	1.587	1.587	12.20
120	0.041	0.617	0.001	0.006	0.002	5.554	5.555	42.73
180	0.053	0.948	0.001	0.009	0.008	8.529	8.537	65.66
240	0.058	1.085	0.001	0.011	0.017	9.769	9.786	75.27
300	0.063	1.223	0.001	0.012	0.028	11.008	11.037	84.89
360	0.07	1.416	0.001	0.014	0.040	12.744	12.784	98.34

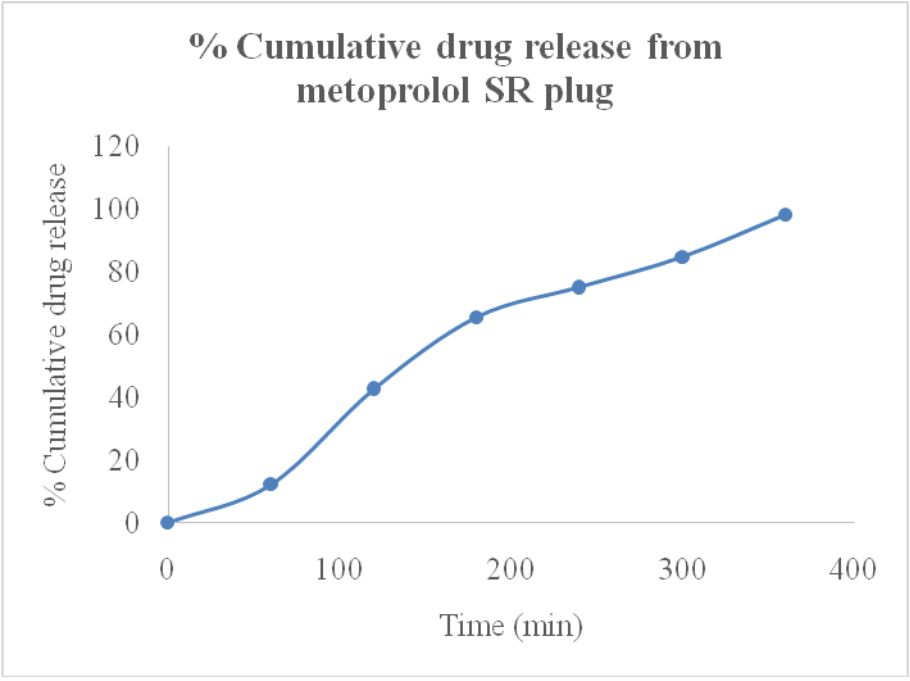


Figure 4.36. % Cumulative metoprolol release from SR core tablet

4.7.9 Evaluation of cup layer

4.7.9.1 Effect of the cup in drug release

To identify the effect of the cup on drug release from the core in cup tablets of metoprolol, drug release was studied with the 'core in cup' tablet without the plug layer (M1) and the IR core tablet (M2) alone. In both cases, the immediate release core was exposed to the dissolution media, which facilitates the leaching of water into the core and immediate disintegration of the IR tablets. Because the drug release profiles of M1and M2 were similar, the influence of the cup layer (polycaprolactone) on drug release was minimal (Figure 4.37).

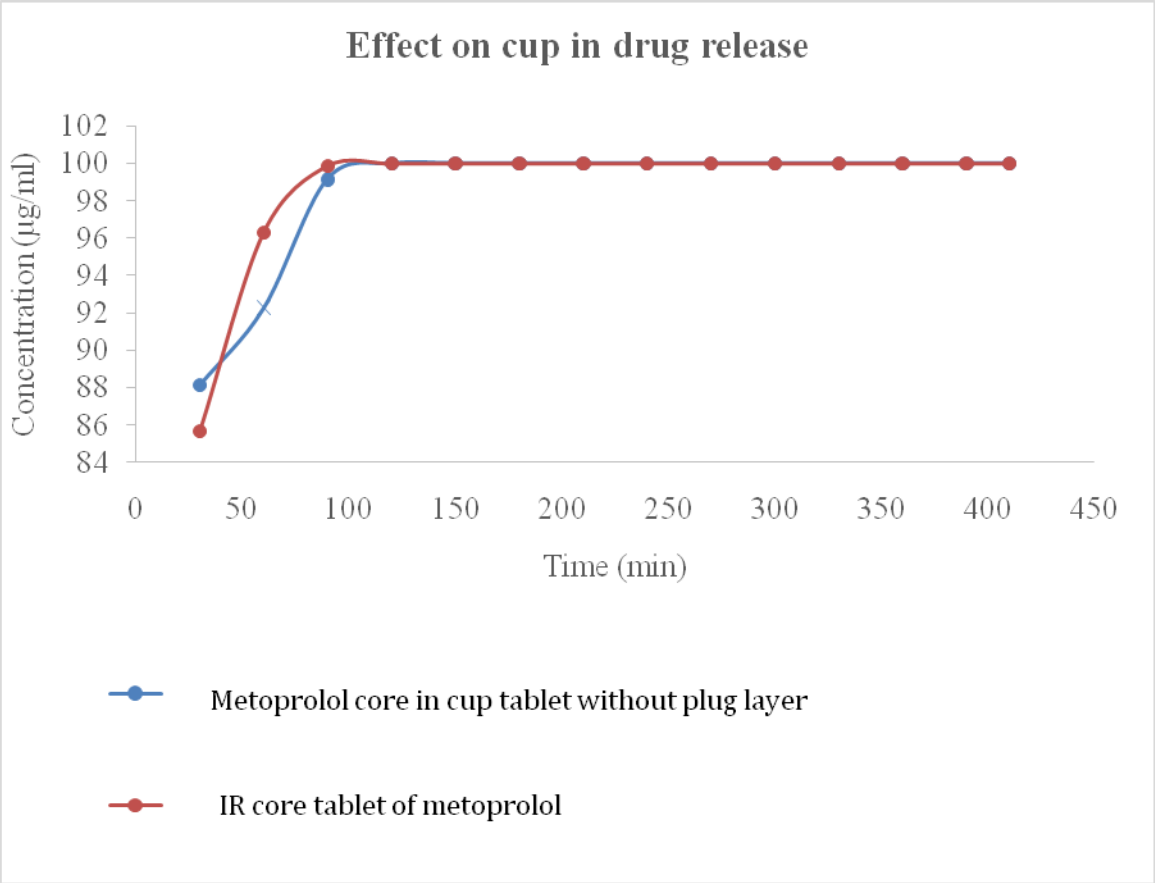


Figure 4.37. Effect of cup in drug release

4.7.9.1.1 Drug release from core in cup tablets of metoprolol

To make the final core in cup metoprolol pulsatile delivery system, optimized compositions of core tablet, plug, and polycaprolactone cup were used. It was

observed that 22.89 % of the drug was released from the formulation till 270 min when the plug was actively diffusing the drug. At 300 min, burst release of medication was observed, and there was a suddendrastic increase of drug release from 27.614-81.046%, and cumulative % release had reached 96.789 till 420 min, as drug release happened from the core formulation (IR) too. The drug release profile indicates that the created 'core in cup' tablet can be used as a pulsatile drug delivery system.

Table 4.25. % Cumulative drug release from metoprolol core in cup tablet

Time (min)	**Absorbance**	**Concentration (mcg/ml)**	**Concentration (mg/ml)**	**Concentration*dilution factor**	**Error**	**Bath Concentration**	**Drug release**	**% Cumulative drug release**
0	0	0.000	0.00000	0.000	0.000	0.000	0.000	0.00
30	0.023	0.121	0.00012	0.001	0.000	1.091	1.091	8.39
60	0.024	0.149	0.00015	0.001	0.001	1.339	1.340	10.30
90	0.026	0.204	0.00020	0.002	0.003	1.835	1.837	14.13
120	0.026	0.204	0.00020	0.002	0.005	1.835	1.839	14.15
150	0.028	0.259	0.00026	0.003	0.007	2.331	2.337	17.98
180	0.03	0.314	0.00031	0.003	0.009	2.826	2.836	21.81
210	0.031	0.342	0.00034	0.003	0.013	3.074	3.087	23.74
240	0.032	0.369	0.00037	0.004	0.016	3.322	3.338	25.67

270	0.033	0.397	0.00040	0.004	0.020	3.570	3.590	27.61
300	0.061	1.168	0.00117	0.012	0.024	10.512	10.536	81.04
330	0.063	1.223	0.00122	0.012	0.035	11.008	11.044	84.95
360	0.065	1.278	0.00128	0.013	0.047	11.504	11.552	88.85
390	0.066	1.306	0.00131	0.013	0.060	11.752	11.812	90.86
420	0.067	1.333	0.00133	0.013	0.073	12.000	12.073	92.87
450	0.069	1.388	0.00139	0.014	0.087	12.496	12.583	96.78
480	0.07	1.416	0.00142	0.014	0.101	12.744	12.844	98.80

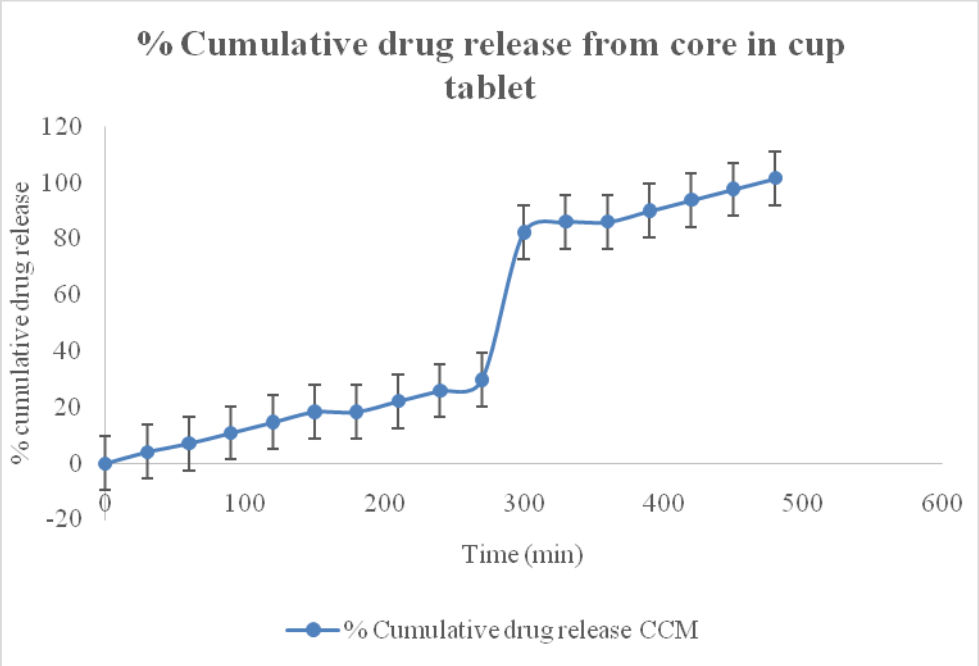


Figure 4.38 % Cumulative drug release from core in cup tablet

4.7.9.2 Modeling of drug release

The release of metoprolol from the core in cup fits well into the Korsmeyer-Peppas model, as indicated by the value R2 value of 0.962 for metoprolol and the profiles are linear (Table 4.26). It can be concluded that the mechanism of drug release from the core in cup tablets follows diffusion and swelling. The exponential coefficient (n) value was found to be > 0.5, indicating a non-Fickian diffusion mechanism. The release profiles exhibit biphasic stages as they involve the release of drug from the SR system initially at 0.1M HCl medium (in the case of metoprolol) up to 270 min, which is followed by a burst release of drug, and finally, the drug was released steadily in the last stage. In other models, linear profiles were not obtained (figure 4.39-4.44).

Table 4.26. The correlation coefficient values for dissolution kinetics data

Drug	Zero order R^2	Higuchi R^2	Peppa's-Korsmeyer		First order R^2	Hixon Crowell model R^2
			R^2	N		
Metoprolol	0.7007	0.5501	0.962	0.583	0.568	0.6086

Figure 4.39. Zero order kinetics of metoprolol

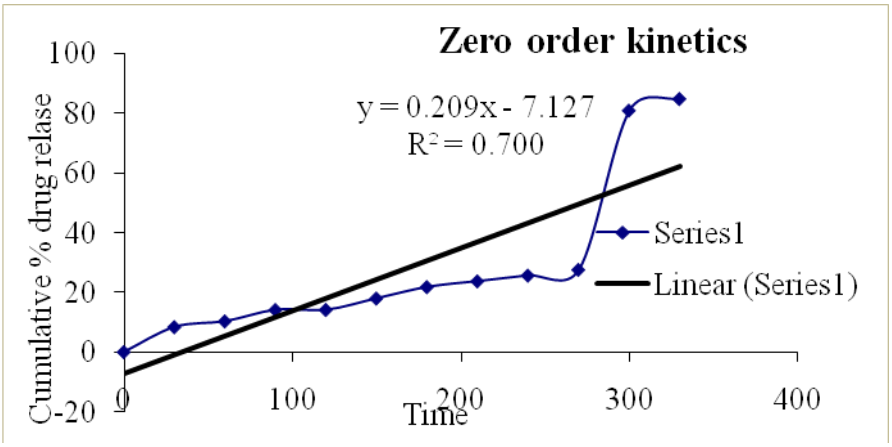


Figure 4.40. Higuchi model for metoprolol

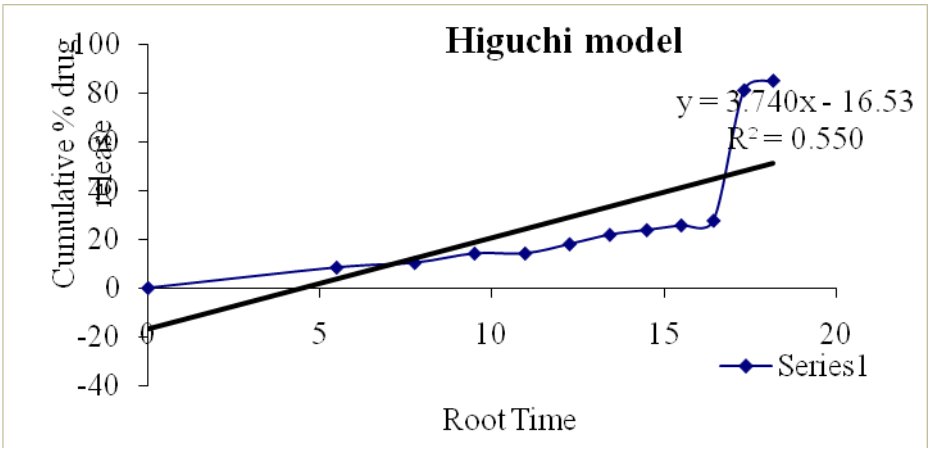


Figure 4.41. Peppa's-Korsmeyer model for metoprolol

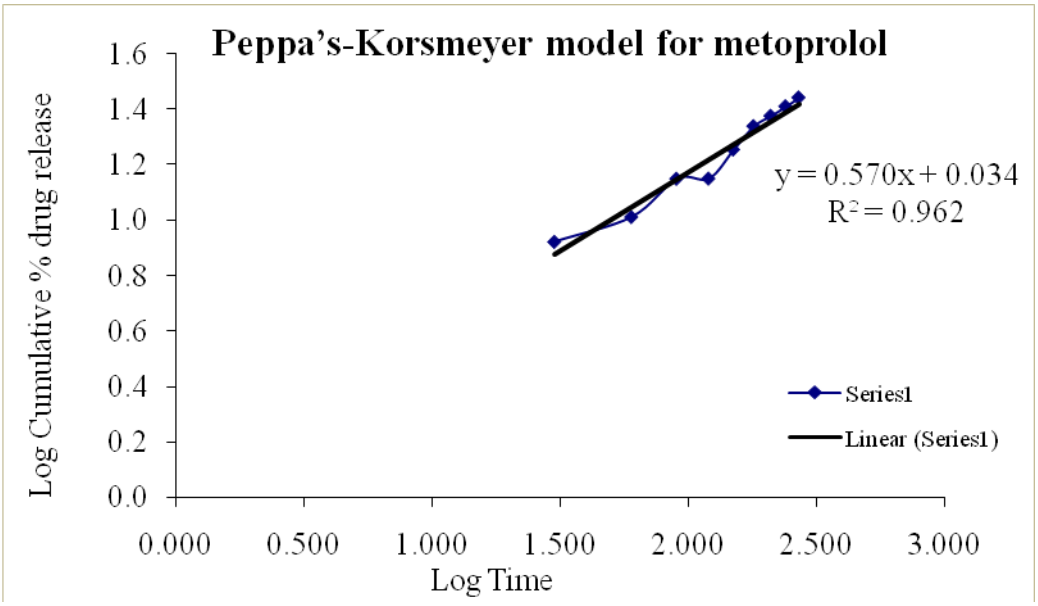


Figure 4.42. First order kinetic model for Metoprolol

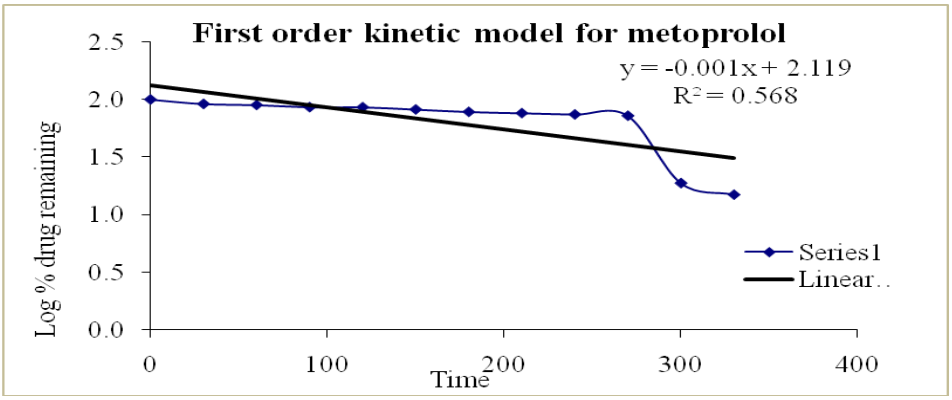
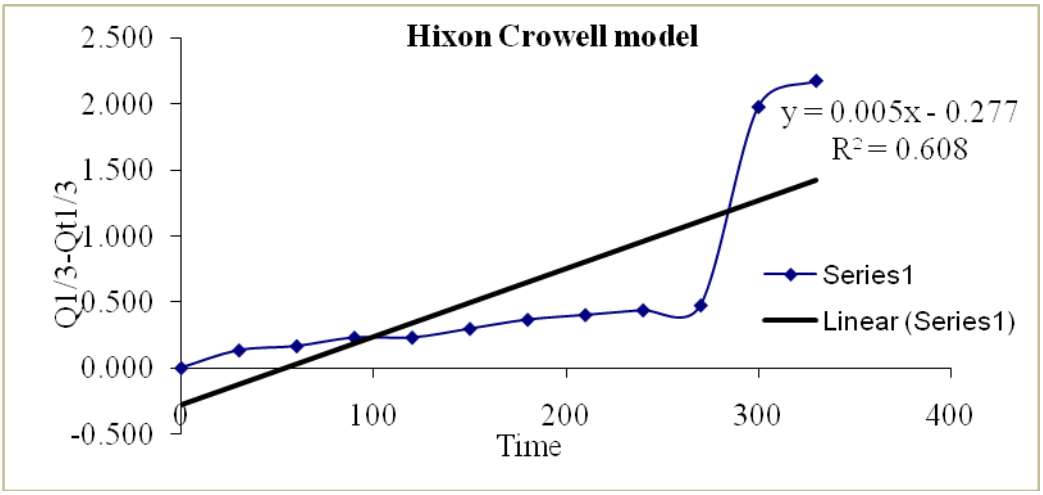


Figure 4.43. Hixon Crowell model for metoprolol



4.7.9.3 Water uptake and drug erosion studies

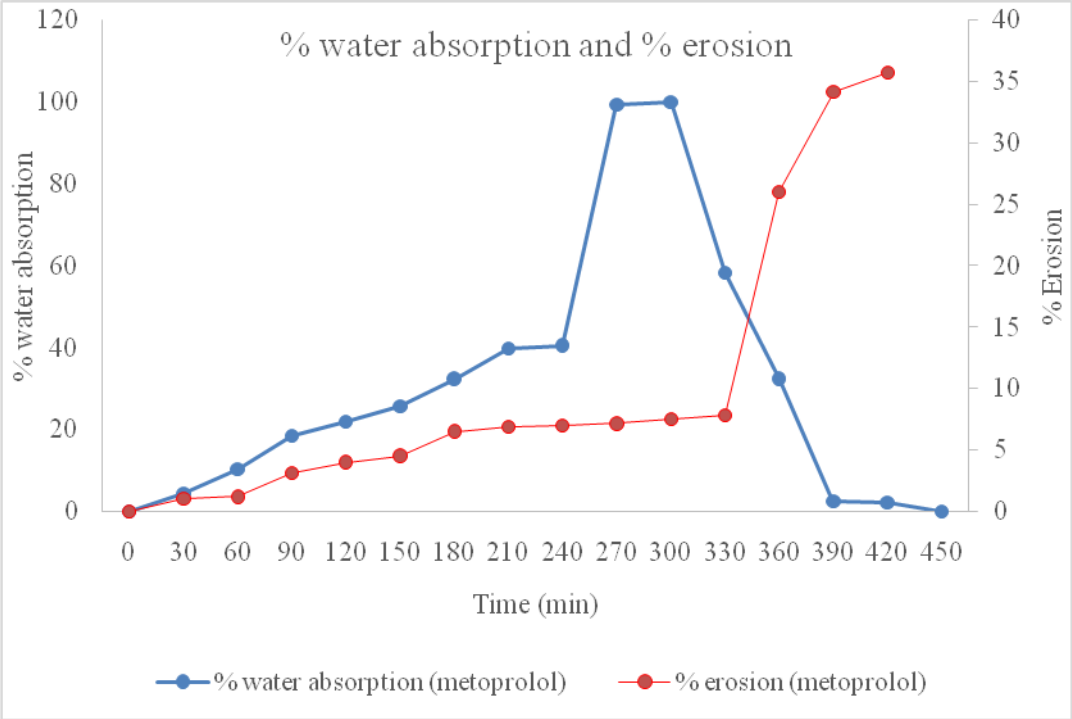
The metoprolol plug and core tablet were tested for water uptake and erosion in phosphate buffer pH 6.8 and 0.1 N HCl. When the tablet was placed in 0.1 N HCl, the HPMC plug layer absorbed the most water. The rate of water absorption increased gradually and reached a maximum at 300 min. There was a rapid drop in water absorption after 300 min, followed by the layer's quick disintegration. Figure 4.43 depicts the relationship between polymer water uptake and polymer breakdown (Table 4.27 and Figure 4.44).

Table 4.27.% of water absorption and % of erosion

Time (min)	% Water absorption (metoprolol)	% Erosion (metoprolol)
0	0	0
30	4.25	1
60	10.26	1.2
90	18.44	3.1
120	21.98	4
150	25.69	4.5
180	32.44	6.5
210	39.87	6.9
240	40.54	7

270	99.48	7.15
300	100	7.5
330	58.25	7.8
360	32.47	26
390	2.49	34.1
420	2.13	35.67
450	0	

Figure 4.44. % of water absorption and % of erosion



4.7.10 Stability study

Formulation F3 was kept for three months in both standard long-term storage (20°C, 60% RH) and accelerated stability (40°C, 75% RH). After the trial period, the tablets were examined for drug release, friability, hardness, and product look. The findings of the tablets' early tests showed no significant differences.

4.7.11 In-vivo Studies

The kinetic parameters, including Cmax, Tmax and AUC were analyzed. [34, 35] The in-vivo concentration of metoprolol with respect to time is shown in table 6, and their relative plasma drug concentration is illustrated in figure 5.75. (Table 4.28, 4.29 and Figure 4.45).

Table 4.28.*In-vivo* concentration of metoprolol

Time (min)	Concentration (ng/ml)
0	0
15	19.12
30	24.21
60	28.98
120	33.65
180	44.43
240	52.87
300	98.76
360	87.90
420	56.91
480	28.32
540	10.5

Figure 4.45. Plasma concentration profile of metoprolol core in cup tablet

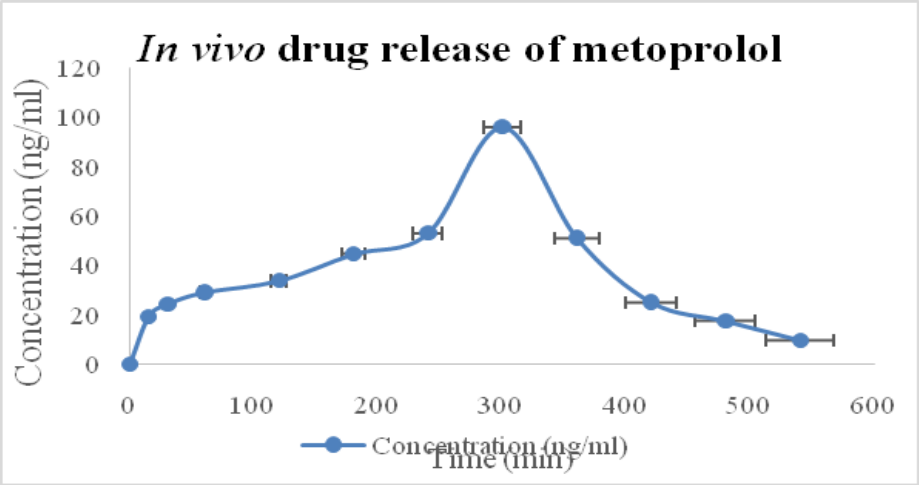


Table 4.29. Pharmacokinetic parameters

Parameters	**Metoprolol**
Tmax	300 mins
Cmax	98.76 ng/ml
AUC	19,628.6 min. ng/ml

CHAPTER-5

5. RESULTS AND DISCUSSIONS

5.1 Introduction

The goal of chronopharmaceutical medication delivery is to synchronize the illness inherent chronological time and therapeutic administration (Bonthagarala B et al. 2014). When a disease exhibits chronological behavior, it is not required to maintain a constant drug concentration throughout the day. However, del6ivering the optimum dose at a specific moment. Heart rate and blood pressure have a distinct diurnal pattern in both normotensive and hypertensive patients. The heart rate and blood pressure are higher in the early morning and lower at night while sleeping (Bowles NP et al. 2018, Rehman B et al. 2020, Sah ML et al. 2012). According to previous studies, during the hours 6:00 am and 12:00 pm, patients had a 29 %, 40 %, and 49 % greater risk of cardiac mortality, heart attack, and stroke, respectively, in the period of 6.00 am and 12 pm (Latha K et al. 2010). Conventional drug delivery systems release the drug instantly or possess a particular drug release pattern throughout the day, to maintain the optimum drug concentration at the site of action. To overcome these, chronopharmaceutical drug delivery methods are used (Krishnaswamy R et al. 2017). In the present study, time-adjustable pulsatile release systems of atenolol (mucoadhesive core in cup tablet) was designed and evaluated for bedtime dosage administration and early morning release of the medicine to manage blood pressure. Atenolol, a cardio-selective beta-blockers used to normalize the heart rate and reduce the strain on the heart and blood pressure. Atenolol is a BCS class III drug with an elimination half-life of 6-7h, pKa Value of 9.6, and bioavailability of 45-50% (Chaudhary SS et al. 2015). The drug delivery systems are aptly designed to release the drug adequately when required, to avoid the presence of a high dose of atenolol throughout the day in blood circulation, and to reduce the adverse reactions (Pizzuto M et al. 2010, Saleem U et al. 2019). Atenolol was designed as a mucoadhesive core in cup tablet to enhance its gastric retention time and to ensure the drug availability in the stomach at the programmed time of drug release since the drug has been found to be better absorbed from the stomach than in the lower gastrointestinal tract (GIT). The study involves designing of ‘core in cup’ drug delivery system in which the core tablet is an immediate-release (IR) tablet, the plug is a sustained drug release (SR) polymeric layer, and the cup is a mucoadhesive polymeric layer in the case of atenolol. The direct compression method was used to make the core

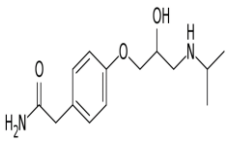
and the cup tablet. The immediate-release layer was composed of atenolol, croscarmellose sodium, microcrystalline cellulose (MCC), sorbitol, talc, and magnesium stearate. The SR plug was composed of hydroxyl propyl methylcellulose (HPMC), polyvinyl pyrrolidine (PVP), MCC, lactose, talc, and magnesium stearate. The compression force, disintegrant concentration in the immediate release core, and HPMC concentration in the plug layer were optimized using the least square method. The formulations were evaluated for primary parameters such as hardness, friability, disintegration, and weight variation. The mucoadhesive strength and mucoadhesive time were studied for atenolol mucoadhesive tablets. In vitro and in vivo studies were conducted to evaluate drug release from the immediate release core, sustained-release plug, and the core in cup tablet.

5.2 Materials and Methodology

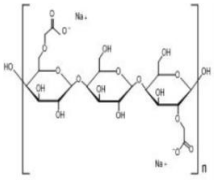
5.2.1 Materials

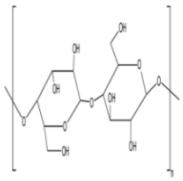
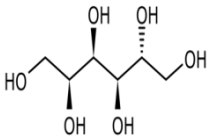
The chemicals, reagents, active pharmaceutical ingredients and other materials required for the study were enlisted in the following table. Detail information about the materials was mentioned for future reference.

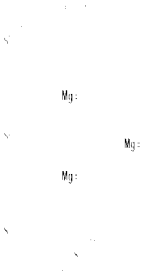
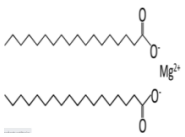
Table 5.1 List of chemicals

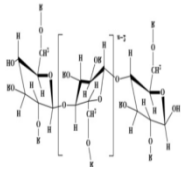
Atenolol 	**Manufactured by:** Carbaniopvt ltd
	Molecular formula: $C_{14}H_{22}N_2O_3$
	Molecular Weight: 266.34
	Chemical name: 2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide
	Physical description: Solid, White crystalline powder
	Melting Point: 146 - 148 °C
	Solubility (mg/ml) at 25 °C: Freely soluble in methanol; slightly soluble in isopropanol; very slightly soluble in acetone, dioxane; practically insoluble in acetonitrile, ethyl acetate; sparingly soluble in 96 percent ethanol; slightly soluble in isopropanol; very slightly soluble in acetone, dioxane; practically insoluble in acetonitrile, ethyl

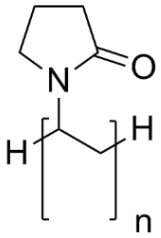
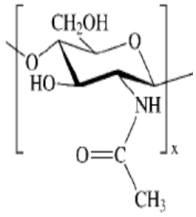
	acetate; chloroform.
	Lambda max (λmax): 276 nm
	LogP: 0.16
	Caco2 Permeability: -6.44
	Dissociation Constants: Basic pKa: 9.58
	Description: Atenolol is a synthetic beta-1 selective blocker used to treat hypertension and chronic angina, as well as to minimise mortality in hemodynamically stable individuals with a known or suspected myocardial infarction.
	Indication: Management of hypertension alone and in combination with other antihypertensives, management of angina pectoris associated with coronary atherosclerosis, management of acute myocardial infarction in hemodynamically stable patients with a heart rate greater than 50 beats per minutes and a systolic blood pressure above 100 mmHg.
	Absorption: Approximately half of an oral dose is absorbed in the gastrointestinal tract, with the rest excreted unaltered in the stool. Label The AUC can be reduced by roughly 20% when atenolol is given with food. Atenolol can pass the blood-brain barrier, although only slowly and to a limited extent.
	Volume of distribution: The gastrointestinal tract absorbs about half of an oral dose, with the rest eliminated unchanged in the faeces. Label When atenolol is administered with food, the AUC can be lowered by about 20%. Although atenolol can pass through the blood-brain barrier, it does so slowly and to a limited extent.
	Protein binding: In plasma, 6-16 percent is bound. Atenolol binds to two locations on human serum albumin.
	Metabolism: The liver has a very low metabolic rate. A hydroxylation event at the carbon between the amide and benzene groups produces the only non-conjugated metabolite. A glucuronide conjugate is the only other metabolite that has been confirmed. These metabolites account for 5-8 percent and 2% of the renally excreted dosage, respectively, with

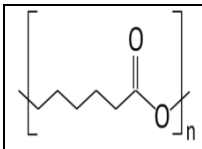
	the remaining 87-90 percent showing as unaltered medication. The hydroxylated metabolite has a beta-blocking action 1/10th that of atenolol.
	Route of elimination: Following IV injection, the kidneys remove 85 percent of the drug, with the remaining 10% ending up in the stool.
	Half-life: 6-7h
	Clearance: A renal clearance of 95-168 mL/min, total clearance is projected to be 97.3-176.3 mL/min.
	Toxicity: Mouse: 2 g/kg (Oral), 57 mg/kg (IV), 134 mg/kg (IP), 400 mg/kg (SC), Rat: 2 g/kg (Oral), 77 mg/kg (IV), 600 mg/kg (SC), Rabbit: 50 mg/kg (IV), Carcinogenicity & Mutagenicity, Reproductive Toxicity and toxicity during lactation.
	Contra indicated: Contra indicated in sinus bradycardia, heart block greater than first degree. It should be avoided in asthmatics. Calcium channel blockers, Prostaglandin synthase inhibiting drugs, Catecholamine-depleting drugs, Hypotension, Haloperidol, Theophylline and Cimetidine.
	Adverse Effect: Tiredness, bradycardia, postural hypotension, precipitation of heart block, intermittent claudication, Raynaud's phenomena, depression, dizziness, confusion, mood changes, nightmares, hallucinations, paresthesia, vertigo,bronchospasm, hypoglycaemia, purpura, thrombocytopenia.
Cross Carmellose sodium 	**Manufactured by:** Lobachemie
	Chemical Name: 2,3,4,5,6-Pentahydroxyhexanal
	Molecular weight: Average molecular weight of the compound is 240.208
	Use: Disintegrant
	Description: Croscarmellose sodium, often known as sodium CMC, is a carboxymethylcellulose sodium cross-linked polymer. It's a white, fibrous, free-flowing powder that's extensively employed in pharmaceutical manufacture as an FDA-approved disintegrant.
	Melting Point: 148.889°C

	Density (bulk): 0.53 g/ml
	Refractive index: 37.35 $m^3 \cdot mol^{-1}$.
	Solubility: When combined with water, it produces a thick colloidal solution. It is ethanol insoluble.
	Stability and storage conditions: Keep the container firmly closed in a dry, well-ventilated location. Keep the original container at room temperature. Keep incompatible materials out of reach.
MCC 	**Manufactured by:** Lobachemie
	Chemical Name: 4-O-[(1S)-hexopyranosyl]-D-glycero-hexopyranose
	Molecular weight: Average molecular weight of the compound is 160.255.
	Description: Microcrystalline cellulose is a refined, partially depolymerized cellulose made by combining mineral acids with alpha-cellulose derived as a pulp from fibrous plant material. Micro Crystalline sub micron size colloidal particles have been purified and depolymerized. It has good water absorption, swelling, and dispersion properties and is metabolically inert.
	Melting Point: 260-270°C
	Density (bulk): 1.76 g/cm3
	Refractive index:1.652 n/D
	Solubility: Insoluble in water.
	Stability and storage conditions: Stable under ordinary condition of use and storage.
Sorbitol 	**Manufactured by:** SD fine chemicals
	Chemical Name: (2S,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol
	Uses: Disintegrant
	Description: Sorbitol is utilised as a tablet diluent in wet granulation or dry compression formulations in pharmaceuticals. Because of its pleasant taste, it's often utilised in chewable tablets, and it's also used as a gelatin plasticizer in capsule formulations.
	Melting Point: 110°C
	Density (bulk):1.489 g/cu cm 20 °C

	Refractive index:1.3330 at 20 °C
	Solubility: Very soluble in water, slightly soluble in ethanol
	Stability and storage conditions: Keep the container firmly closed in a dry, well-ventilated location.
	Stability/Shelf Life: In strongly acidic and alkaline conditions, sorbitol forms water-soluble chelates with several divalent and trivalent metal ions. With rapid agitation, liquid polyethylene glycols are added to a sorbitol solution to generate a waxy, water-soluble gel with a melting temperature of 35-40 °C. Sorbitol solutions can also discolour when they come into contact with iron oxide. Sorbitol accelerates the breakdown of penicillin in both neutral and aqueous solutions.
Talc 	**Manufactured by:** SD fine chemicals
	Description: Talc is a magnesium (Mg), silicon and oxygen (SiO_2, silica), and water-based hydrous silicate mineral. $Mg_3Si_4O_{10}(OH)_2$ is its chemical formula. Talc is a generally pure mineral with minor quantities of aluminium, iron, manganese, and titanium.
	Molecular weight:379.27
	Uses: Disintegrant
	Description: In the pharmaceutical industry, talc is used primarily as a glidant to improve powder flow in tablet compression.
	Melting Point:900-1000 °C
	Density (bulk):2.7 g/cm³
	Refractive index:1.58
	Solubility: Insoluble in water and ethanol
	Stability and storage conditions: Keep the container in a well-ventilated area. Well closed
Magnesium stearate 	**Manufactured by:** SD fine chemicals
	Description: Magnesium stearate is a metallic salt boundary lubricant that has two fatty acid equivalents (typically stearic and palmitic acid) and a charged magnesium. It is reasonably priced, chemically stable, and has a high melting point and lubricating property. In the formulation development, magnesium stearate was used at a

	concentration of 0.25 percent –5.0 percent w/w
	Molecular weight:591.2
	Uses: It's used in infant powders, as a lubricant for plastics, as an emulsifying agent in cosmetics, as a drier in paints and varnishes, as a flatting agent, as an anti-caking agent in foods and fire extinguishers, and as a lubricant in the production of pharmaceutical tablets.
	Melting Point:88.5 °C
	Density (bulk):1.02 g/cm³
	Refractive index:1.58
	Solubility: soluble in water (10 mg/ml).
	Stability and storage conditions: Keep the container in a well-ventilated area.
HPMC 	**Manufactured by:** SD fine chemicals
	Description: Polymeric compounds containing hydroxypropyl methylcellulose repeating units. The molecular weight, percentage of hydroxyl groups, percentage of hydroxypropyl groups, and viscosity measurements are all used to define the properties of Hypromellose polymers. Food Additives, Excipients, and Lubricants Are only few of the commercial items that contain them.
	Molecular formula: $C_{56}H_{108}O_{30}$
	Molecular Weight:1261.4
	Melting Point: 225 to 254°C
	Uses: It can be used in a variety of dosage forms as a film-former, thickener, sustained-release agent, emulsifying agent, and suspending agent, making all kinds of pharmaceutical formulations more evenly dispersed, tough without being broken, or with sustained release effects or stable emulsions
PVP	**Manufactured by:** SD fine chemicals
	Chemical name:1-ethenylpyrrolidin-2-one
	Density:1.144g/cm3
	Molecular Formula:$(C_6H_9NO)n$
	Molecular Weight:111.1418 (monomer)

	**Boiling Point:**217.6ºC at 760 mmHg
	Melting Point : 130ºC
	Melting Point : 130ºC
	Solubility: Soluble in water giving a colloidal solution; practically insoluble in ether; soluble in alcohol, chloroform
	Viscosity:2.07 cP at 25 ℃
	Uses: It can be used as a tablet and capsule binder, a film forming for ophthalmic solutions, a flavoring agent for liquids and chewable tablets, and an adhesive for transdermal systems, among other things.
	Storage: Store in original containers. Keep containers securely sealed.
Chitosan 	**Manufactured by:** SD fine chemicals
	Description: Chitosan is a sugar that can be found in the shells of crabs, lobsters, and shrimp. It's used in the formulation development of pharmaceutical products. D-glucosamine and N-acetyl-D-glucosamine combine to form chitosan, a linear polysaccharide. Chitosan is found naturally in the cell walls of fungi, soil, and sediments, where it is formed by the degradation of chitin caused by bacteria that produce the deacetylase or chitosanase enzymes. Commercial chitosan, on the other hand, is made from the deacetylation of chitin found in the shells of various sea crustaceans, such as shrimps. Chitosan has protein aggregation capacity, emulsification capacity, film-forming ability, clarifying ability, and fatty acid absorption capability.
	Molecular Formula:$C_{56}H_{103}N_9O_{39}$
	Molecular Weight:1526.5
	Physical Description: Solid yellow powder
	Bulk density: 0.20- 0.38 g/mL
	Melting point: 250°C.
	Uses: Tablet disintegrant, controlled release polymer
	Storage: Store at low temperatures (2–8 °C) in sealed containers.
Polycaprolactone	**Manufactured by:** SD fine chemicals
	Description: Polycaprolactone is a biodegradable, semi-crystalline

	thermoplastic polyester made by ring-opening polymerization of -caprolactone at high temperatures (200°C) in the presence of a suitable catalyst such as dibutyltin dilaurate. It is a white to light yellow, hygroscopic, amorphous powder.
	Molecular Formula: $C_{16}H_{30}O_7$
	Molecular Weight: 334.40
	Chemical name: 2-[2-(6-hydroxyhexanoyloxy)ethoxy]ethyl 6-hydroxyhexanoate
	Density: 1.145 g/cm3
	Flash point: 60°C
	Solubility: Water soluble
	Storage condition: Stable under normal conditions. Store in in a well closed container.

5.2.2 Preformulation studies

Preformulation is the primary step of product development in which the physicochemical properties of the active pharmaceutical ingredient and other excipients are studied before product development. Preformulation studies help to check the drugs' and excipients' physiochemical effects on the quality of product formulation and performance (Trevor M. Jones et al. 2018). Any physicochemical interactions between an API and excipients in a formulation has the potential to change the physical, chemical, therapeutic, organoleptic, pharmacological, and stability aspects of a dosage form, affecting the dosing accuracy, side effects, and patient adherence (Aboud MK et al. 2017). The preformulation studies conducted for Atenolol are as follows.

5.2.2.1: Preparation of calibration curve

The spectrophotometric method was used to assay the drug. Standard calibration curves of pure drug atenolol were plotted with the concentration vs. absorbance values measured at λmax of 276nm, respectively. A series of diluted solutions were prepared with solvent methanol and diluent 0.2M phosphate buffer (Aboud MK et al. 2017). The linear regression curve was used to measure the unknown.

5.2.2.2 Solubility studies

A saturation solubility study of atenolol was carried out according to BCS (biopharmaceutical classification system) guidelines. In 500 ml conical flasks, 25mg of each drug was added to 250ml of each medium of 0.1N HCl pH 1.2, distilled water pH 7.0, and phosphate buffer pH 6.8. Flasks were shaken in an orbital shaker at 37°C for 48 hours. The samples were then filtered and diluted before being analyzed with a UV spectrophotometer (Hamidi S et al. 2017).

5.2.2.3 Melting point determination

The determination of the melting point aids in the identification of the material and its purity. The melting point device was used to determine the melting point (Silva AC et al. 2014).

5.2.2.4 Drug-excipient compatibility studies using differential scanning calorimeter (DSC)

DSC is a thermoanalytical technique that measures the amount of heat required to raise the temperature of a sample. Using a DSC, the thermograms of the drug and excipients were recorded. As a baseline, an empty pan was used. About 1 g of drug/excipients/drug and excipient mixture was weighed and sealed in a small aluminum pan and was heated to 20-400^{0}C at a rate of 10^{0}C/min with constant purging of dry nitrogen 50 ml/min. A blank pan was sealed and used as a reference. DSC thermogram was obtained using automatic thermal analyzer equipment. The thermograms were analyzed to check the compatibility of the drugs and the excipients (Dourado D et al. 2019).

5.2.2.5 Fourier transform infrared spectroscopy (FTIR)

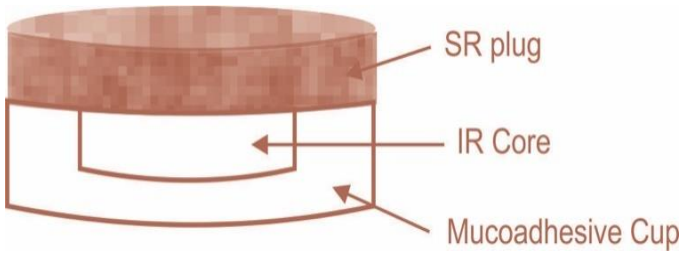
The drug-excipient interactions were analyzed using FTIR tests (FTIR-8400S, Shimadzu). FTIR spectra of atenolol, cross carmellose sodium, Sodium starch glycolate, MCC, sorbitol, talc, polycaprolactone, HPMC, PVP, lactose, magnesium stearate, chitosan and the physical mixtures were obtained using the standard KBr Disc/Pellet method. The samples were compacted into pellets after being pulverized with anhydrous KBr. With a resolution of 4 cm-1, an analysis was carried out over the range 4000–400 cm-1 (Lau E. 2001).

5.2.3 Preparation of pulsatile tablets

5.2.3.1 Formulation of core in cup tablet

A ‘core in cup’ tablet with a SR plug layer, IR core, and a cup (figure 5.1) was prepared by the direct compression method. The pulsatile core in the cup bilayer tablet was made with a 12 mm punch. The IR layer was compressed in a 6 mm punch with a low compression force. The cup material, polycaprolactone, was filled into a 12 mm punch die and slightly pressed. The core tablet was placed in the slightly pressed polycaprolactone bed and subsequently filled the gap between the die wall and tablet with polycaprolactone (110 mg). The top of the core tablet was then filled with the SR mixture and compressed to form a tablet (Wang H et al. 2017, Sokar MS et al. 2013). Similarly, the atenolol mucoadhesive core in cup system was also prepared by adding chitosan and polycaprolactone to the cup layer.

Figure: 5.1. Fabricated tablet for illustration



5.2.4 Design of Experiment

The software JMP was used to generate the experimental design. JMP software is the data analysis tool that helps tackle statistical problems by accessing data from various sources. It utilizes the input (factors) and output (response) variables for generating and analyzing the experimental design. In this experimental design, we have used Least Square Method for the optimization. A least-squares method is a form of mathematical regression analysis used to determine the line of best fit for a set of data, providing a visual demonstration of the relationship between the data points. The relationship between a known independent variable and an unknown dependent variable is represented by each point of data. The input variables for immediate release tablet were compression force and concentration of disintegrant used, whereas for sustained release, the input variables are compression force and concentration of HPMC. The analytical measurements representing response factors for immediate release were tablet hardness,

disintegration, and dissolution, whereas sustained-release responses were hardness and dissolution.

5.2.4.1 Study of flow property Study of flow property (angle of repose, bulk and tap densities, Carr index, Hausner's ratio) of optimized formula powder material

Powder qualities (particle size, size distribution, density, shape, and surface properties), apparatus features (shoe and die design), and operating conditions influence powder flow behavior during die filling. Understanding powder flow properties is essential for selecting and optimizing operating parameters and controlling the powder filling process. The angle of repose, compressibility index (Carr's index), and Hausner ratio were used to measure the flow properties of powders.

5.2.4.2 Angle of repose

The angle of repose was used to determine the frictional force in a loose powder, and the angle of repose was measured using the fixed funnel method. As a function of the powder flow property, the maximum angle (θ) permitted between the surface of the powder pile and the horizontal plane was determined (Lau E 2001) using the formula,

$$\theta = \tan^{-1}(h/r),$$

Here, h is the height of the cone and r is the radius of the cone base.

5.2.4.3 Bulk density, ρ_b

Bulk density is the ratio of the mass of an untapped powder sample to its volume, including inter particulate void volume, which is the bulk density of a powder. As a result, both the density of powder particles and the spatial arrangement of particles in the powder bed affect bulk density. The bulk density is measured in g/mL. It is the ratio of the mass of the powder (M) to the volume, V (including the inter-particle void volume) of an untapped powder sample.

$$\rho_b = M/V_o$$

5.2.4.4 Tapped density (ρ_t)

Tapped density is higher than bulk density; it is obtained after mechanically tapping a cylinder containing the sample. It is calculated by the ratio of the powder mass (M) and tapped volume; Vt attained after tapping 100 times using a mechanical tapped density tester ($\rho t = M/ Vt$).

5.2.4.5 Measure of powder compressibility

5.2.4.5.1 Carr's Index

The Compressibility index (Carr's Index) is a measure of a powder's propensity to be compressed based on bulk and tapped densities. In free-flowing powders, inter-particle interactions are minor, and bulk and tapped densities will be closer in value. Poor flowing materials are cohesive, resulting in a wider gap between bulk and tapped densities (Lau E 2001). The Compressibility Index, which is determined using the following formula,

$$Carr'sindex = \left[\frac{(\rho_t - \rho_b)}{\rho_t}\right] \times 100$$

Where, $\rho_t$is the tapped density ρ_b is the bulk density

5.2.4.5.2 Hausner's Ratio

The flow ability of powders or granules is expressed by Hausner's ratio, where V_o is the untapped apparent volume (cm^3) and V_t is the final tapped volume (cm^3) (Lau E 2001).

Hausners ratio = V_o/V_t

5.2.5 Evaluation of core in cup tablet

5.2.5.1 Hardness

The Monsanto hardness tester was used to determine the hardness of the tablets (force necessary to break the tablet). Hardness was measured by placing the tablet between the moving and fixed jaws of the apparatus and applying pressure. Six tablets of atenolol were used in the experiment (Lau E 2001).

5.2.5.2 Friability

Friability refers to a tablet's ability to endure mechanical stress during handling. It aids in determining the tablet's vulnerability to fragmentation, chipping, and other forms of damage.

The tablets were placed in a friabilator and spun at 25 rpm for four minutes. The initial weight and final weight of the tablets were measured to determine friability.

$$\% \text{ friability} = \frac{W_f - W_i}{W_i} \times 100$$

5.2.5.3 Weight variation

The average weight of 20 tablets was obtained after each tablet's weight was measured individually. The tablet passes the weight variation tests if not more than two tablets are beyond the prescribed limits, according to the USP specification (Jain D et al. 2011, Chaudhary SS et al. 2015).

$$Averageweight = \frac{W_{total}}{n_{tab}}$$

Where $W_{total}$is the total weight of tablets and n_{tab} is the number of tablets.

5.2.6 Disintegration time of the IR core tablet

The disintegration test apparatus was used to test the tablets' disintegration time. Water, 0.1 N HCl and pH 6.8 phosphate buffer were three distinct mediums used. The tablets were inserted in the apparatus's basket's six tubes. The basket was pushed up and down in the media at a rate of 29 to 32 cycles per minute (Patil S et al. 2015). The time required for the tablets to disintegrate completely was recorded.

5.2.6.1 In vitro drug release studies

Each drug layer (IR core, the SR plug, and the core in a cup tablet), was evaluated separately to study the drug release profile. The primary factor for selecting the optimal formulation among trials in each layer preparation was in vitro drug release. Each data is the average of three repetitions.

5.2.6.2 *In vitro* drug release from IR core tablet and SR plug

In vitro drug release experiments for the atenolol core tablets and the SR plug layer were conducted individually using the USP dissolution apparatus II. The temperature was kept at 37^0C. 0.1 N HCl was chosen as the dissolution medium for IR and SR tablets of atenolol. The dissolution medium was agitated at 50 rpm (Shao Y et al. 2015, Cazorla-Luna R et al. 2019, Chisty SJ Chisty SJ et al. 2016, Ashwini MS et al. 2013). At predetermined time intervals, a predetermined number of aliquots were removed for analysis and replaced with an equal amount of fresh dissolution medium. A spectrophotometer set to 224 276 nm (atenolol) wavelength was used to examine the aliquots. Similarly, the drug release profiles of the marketed IR tablets of atenolol (Devolol 50 mg) was investigated and compared with the prepared IR tablets (dissimilarity/similarity studies).

5.2.6.3 Evaluation of cup layer

5.2.6.3.1 Effect of the cup on drug release

The influence of the cup layer on drug release from the IR core was studied by analyzing the 'core in cup' tablet without the plug layer and the IR core tablet alone. The dissolution media used in the test was maintained the same as that of the IR core tablet drug release studies.

5.2.6.3.2 Mucoadhesive strength of the atenolol cup

The mucoadhesive strength of the three combinations of polycaprolactone and chitosan was measured. The force required to detach the drug from the attached membrane (porcine GI mucosa) was determined using the modified balance method. Porcine gastric mucosa with a thick layer of mucus was affixed to a rubber cork that had previously been attached to the vial in the bottom of a beaker with the relevant medium at a level slightly above the mucosa (figure 5.2). The weight was attached to the tablet through a rubber beaker and brought into touch with the porcine mucosa, and kept undisturbed for 5 minutes. The fastened thread is tied with the left arm of the balance. The mucoadhesive strength was measured by adding weights to the right-side pan in incremental increments and recording the weight at which the tablet disengaged from the mucosa (Shao Y et al. 2015).

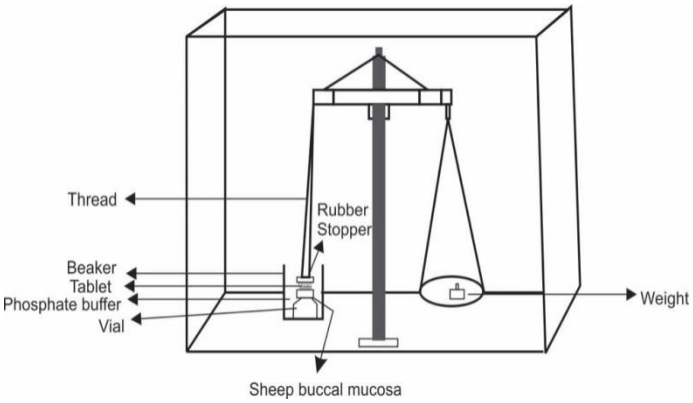


Figure 5.2. Modified Balance method for mucoadhesive strength calculation

5.2.6.4 Mucoadhesion residence time for atenolol cup

The tablets must remain adherent to the gastrointestinal mucosa to retain the drug in GIT for a certain period. Hence an ex vivo mucoadhesion test was used to determine the tablet's residence time in the gastrointestinal mucosa. Cyanoacrylate glue was used to adhere the sample of bovine GI mucosa on a stainless-steel plate measuring 8.5 cm x 5 cm. Further, each tablet was stuck to the mucosa with a specific pressure (500 g for 30 s). The prepared plate assembly was placed in a beaker containing 0.1N HCl at a 60° angle, then in a shaking water bath at 37°C and 15 rpm (Cazorla-Luna R et al. 2019).

5.2.7 Drug release from core in cup tablets of atenolol

In USP dissolution apparatus II, a dissolution test for the bilayer tablet was performed. For atenolol the dissolution medium used for the first 300 min was 730 mL of 0.1M HCl solution. The tablet was then subjected to a 6.8 pH phosphate buffer solution by the addition of 0.2 M $Na_3PO_4.12H_2O$ into the existing media (Patel VD et al. 2017). The spectrometric analysis for atenolol was conducted at 276nm.

5.3 Modeling of drug release

To examine the drug release kinetics, the dissolution study data were fitted into zero order, first order, Korsmeyer-Peppas, Higuchi, and Hixson-Crowell models (Craciun AM et al. 2019).

5.4 Water uptake and erosion studies

Water absorption and erosion were studied using the USP dissolution apparatus type I. The weight of the empty basket and the tablets were recorded before starting the experiment. The tests were carried out in the same prescribed conditions as the dissolution test. At predetermined times, individual tablets were taken out of the medium. The tablets were weighed following the completion of the test. The tablets were dried at 80^0C until they attained a constant weight. After they had cooled to room temperature, the tablets were weighed again. To compute the % erosion, the below formula was used (Patil AS et al. 2011, Chauhan BS et al. 2014, Sonani NG et al. 2010).

$$\%\ erosion = \frac{W_i - W_f}{W_i} \times 100$$

Where W_i represents the initial weight of the tablet and W_f represents the final weight of the dried tablet.

The excess weight gain of the tablet at each time interval was used to calculate water uptake by the tablet.

$$Weightincreased = \frac{W_{wet} - W_f}{W_f} \times 100$$

Where W_{wet}represents the weight of the wet tablet

5.5 Stability study

The stability tests on the tablets were carried out in compliance with ICH guidelines. The tablets were maintained in two different study conditions. One batch of tablets was kept in a desiccator for three months at 25°C and 60% RH in the presence of anhydrous calcium chloride in polypropylene containers. Another pair was kept in a stability chamber at 40°C and 75% RH for three months. Three months later, dissolution tests and the physical appearance of the tablets were evaluated (Lachman L et al. 1965, Chaurasia G et al. 2016, Nagaraju R et al. 2009).

5.6 In-vivo Studies

The goal of the study was to investigate the bioavailability of atenolol core in cup tablets in a rabbit model, and it was carried out according to CPCSEA criteria. The Institutional

Animal Ethical Committee authorized pro forma B, which included protocols for animal studies Nandha college of Pharmacy, Registration no: 688/PO/Re/S/02/CPCSEA. Six healthy male rabbits weighing approximately 1 to 5 Kilograms were selected and divided into two groups. Each rabbit was housed for three weeks under standard conditions with a 12 h light/dark cycle with free access to water and a basal diet for one week. Further, they were subjected to fasting for 12 hours with access to drinking water. After 12 hours, the prepared formulations were administered (16mg/kg) through the oral route by keeping them upright. Blood samples of 1 ml have been collected from the tail vein using a needle at 15, 30, 60, 120, 180, 240, 300, 360, 420, 480, and 540 minutes after administration. All blood samples were immediately put into heparin and subjected to centrifugation at 4000 rpm for 10 min to separate the plasma. Then the plasma was frozen and stored at -20ºC until the analysis.

The HPLC method was established for the analysis of the drugs. Phosphate buffer and acetonitrile in an 80:20 ratio were used as a mobile phase. The flow rate was maintained at 1 ml/min. The linearity range was determined between 5 and 100 ng/ml, with a correlation coefficient of 0.9991. Before HPLC analysis, plasma was filtered using a 0.25 m membrane filter. The supernatant was obtained by centrifuging 0.2 mL of filtered plasma and diluting it with 1 mL of acetonitrile. After evaporating the supernatant under nitrogen, the residue was combined with 0.3 ml of HPLC mobile phase. The sample was injected into the HPLC column with a UV detector set to wave length of 276 nm. The kinetic parameters including C_{max}, T_{max}, AUC were analysed (Jain D et al. 2011, Mohanty C et al. 2021, Sultana A et al. 2018). The results of this study were depicted as plot of plasma concentration vs time, and AUC was used to calculate bioavailability.

5.7 Results and Discussion

5.7.1 Preformulation study

5.7.1.1 Preparation of calibration curve

The graphical plots of absorbance (y) vs concentration (x) of atenolol (Figure 5.3) yielded the linear calibration curve equation $0.0185x + 0.0951$, $R^2 = 0.9996$, which were used to calculate the amount of drug released in dissolution studies of different batches of products.

Table 5.2Absorbance data for calibration curves of atenolol

Concentration (µg/ml)	Absorbance
2	0.1281
4	0.1761
6	0.2041
8	0.2421
10	0.2801
Regression equation	y = 0.0363x + 0.0186, R^2 = 0.9972

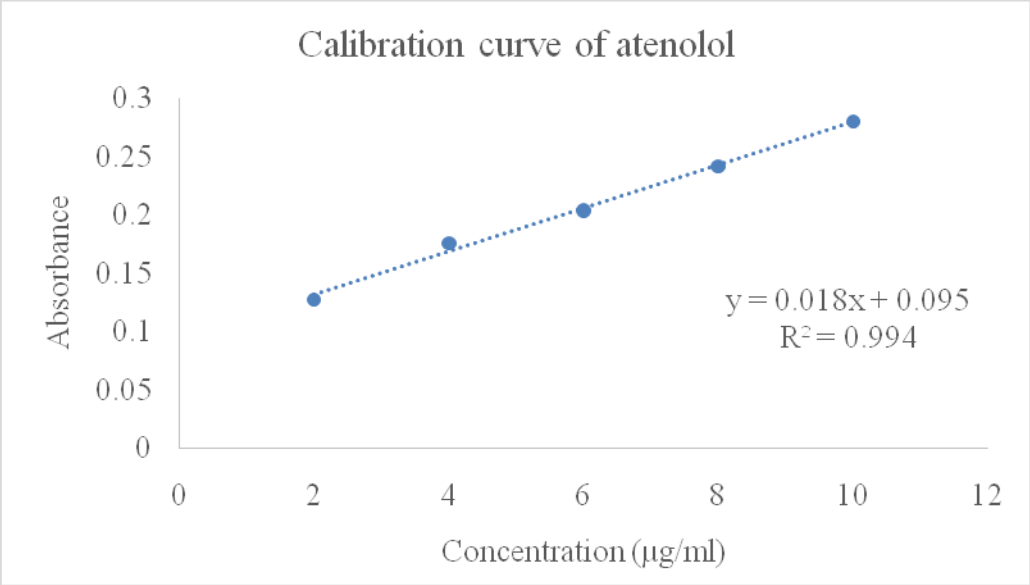


Figure 5.3. Calibration curve of atenolol

5.7.1.2 Solubility studies

Saturation solubility of atenolol was observed as 13.3±0.48, 12 ± 0.51, 10.12 ± 0.28 mg/mL at 25°C in double-distilled water (DDW), 0.1 N HCl, and pH 6.8 phosphate buffer. The drug was found to be sparingly soluble in DDW, HCl and pH 6.8 phosphate buffer. Atenolol was found to be sparingly soluble in DDW, HCl and pH 6.8 phosphate buffer (Table 5.3).

Table 5.3 Solubility of atenolol

Solvent	Solubility (mg/ml)
Distilled water pH 7.0	13.3 ± 0.48
Phosphate buffer pH 6.8	10.12 ± 0.28
0.1N HCl pH 1.2	12±0.51

5.7.2 Melting point determination

The melting point of atenolol was found to be 120 ^{0}C. It was comparable to the melting point reference provided by the USP (figure 5.4 and 5.5).

Figure 5.4 and 5.5 melting point device

5.7.3 Drug excipients compatibility study using DSC

DSC was used to determine the compatibility between drug and excipients used in the formulations. Individual components, such as atenolol, cross carmellose, sorbitol, MCC, HPMC, lactose, magnesium stearate, talc, chitosan, polycaprolctone, and the physical mixture of drug and excipients, were investigated using DSC. The thermograms of the physical mixture revealed no temperature shift and so no interactions, implying that there were no incompatibilities between drugs and excipients (Figure 5.6-5.17).

Figure 5.6. DSC thermogram of polycaprolactone

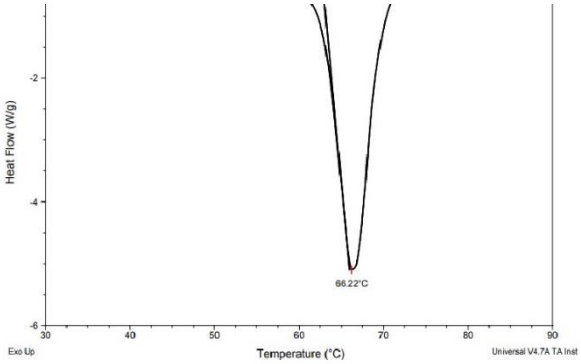


Figure 5.7. DSC thermogram of lactose

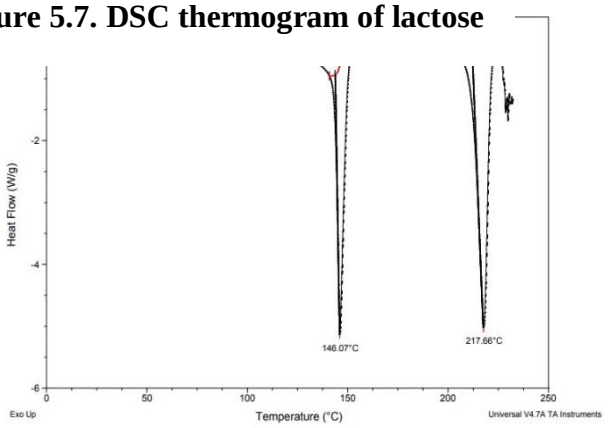


Figure 5.8. DSC thermogram of cross carmellosesodium

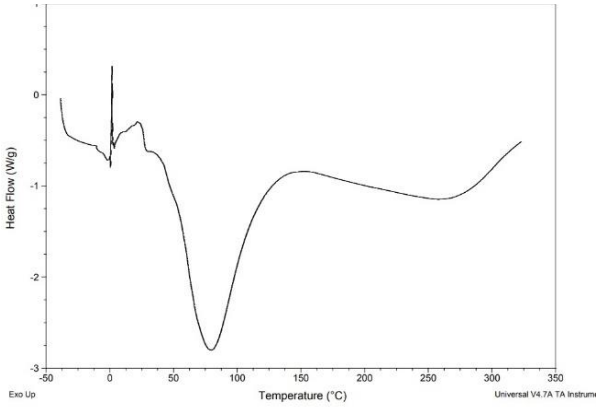


Figure 5.9. DSC thermogram of HPMC

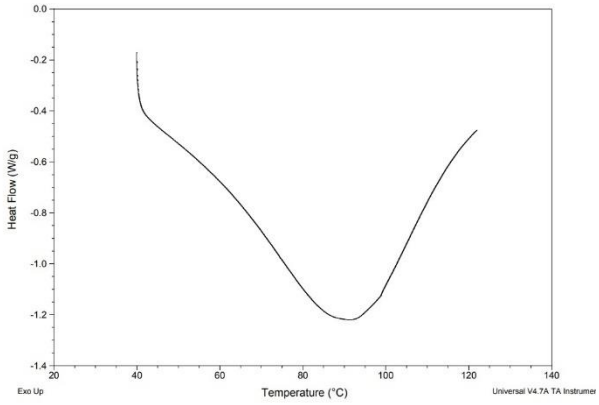


Figure 5.10. DSC thermogram of sorbitol

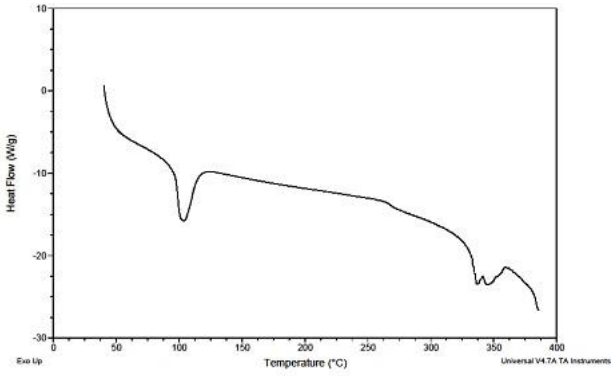


Figure 5.11.DSC thermogram of MCC

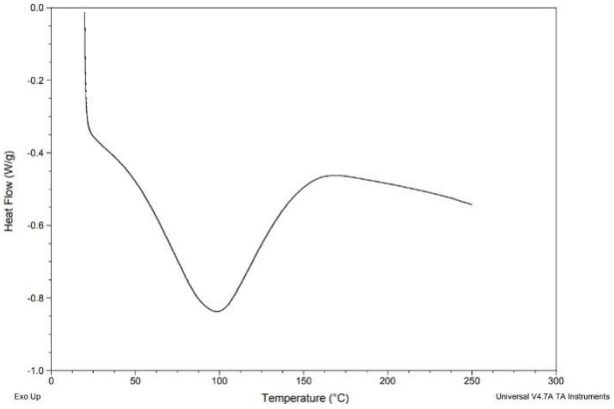


Figure 5.12.DSC Thermogram of chitosan

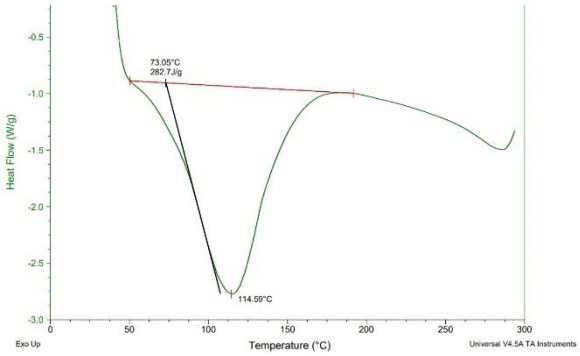


Figure 5.13.DSC Thermogram of Talc

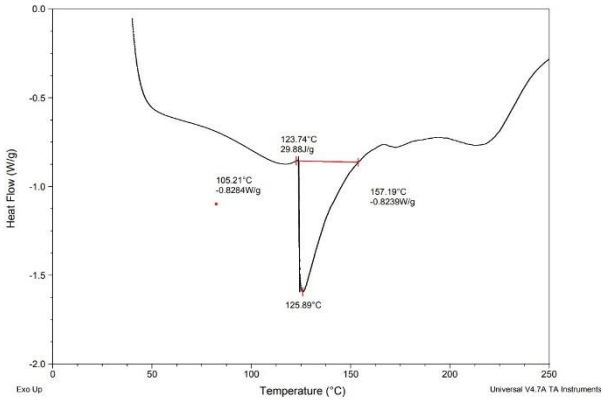


Figure 5.14.DSC thermogram magnesium stearate

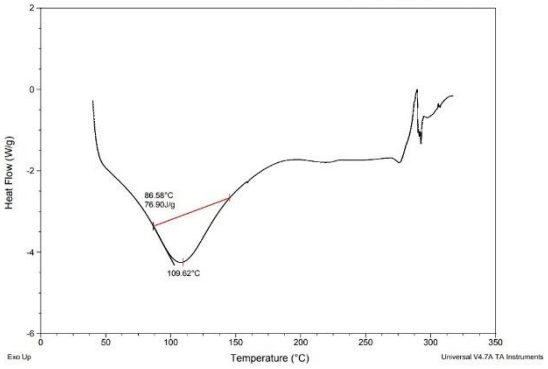


Figure 5.15. DSC thermogram of atenolol

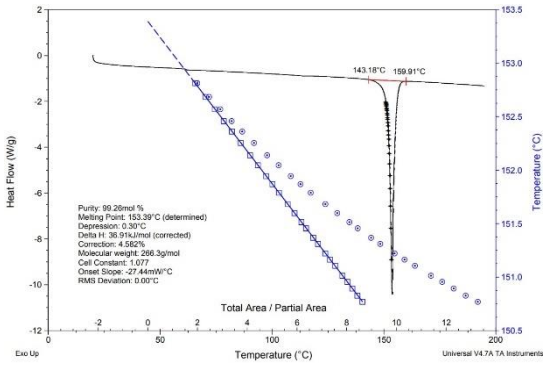


Figure 5.16. DSC thermogram of SR tablet mixture of atenolol

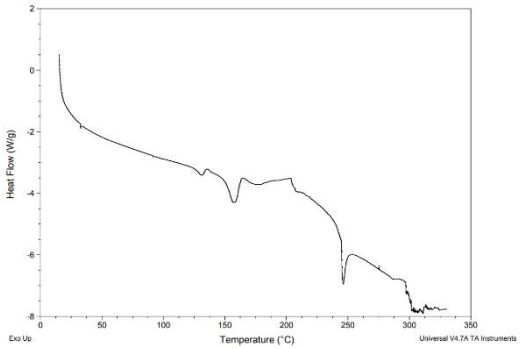
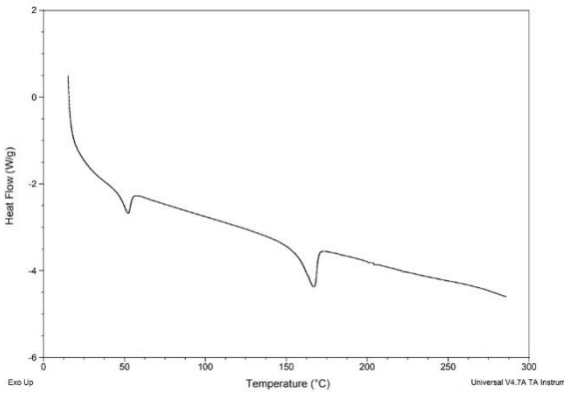


Figure 5.17. DSC thermogram of IR tablet mixture of atenolol



5.7.4 FTIR

The IR spectra of the ingredients were analyzed to find the interaction between the drug and the excipients. The major functional groups of the ingredients were studied and compared with the IR spectra of the physical mixture (shown in Figure 5.18 & 5.19). IR spectra of the physical mixture did not show any incompatibilities.

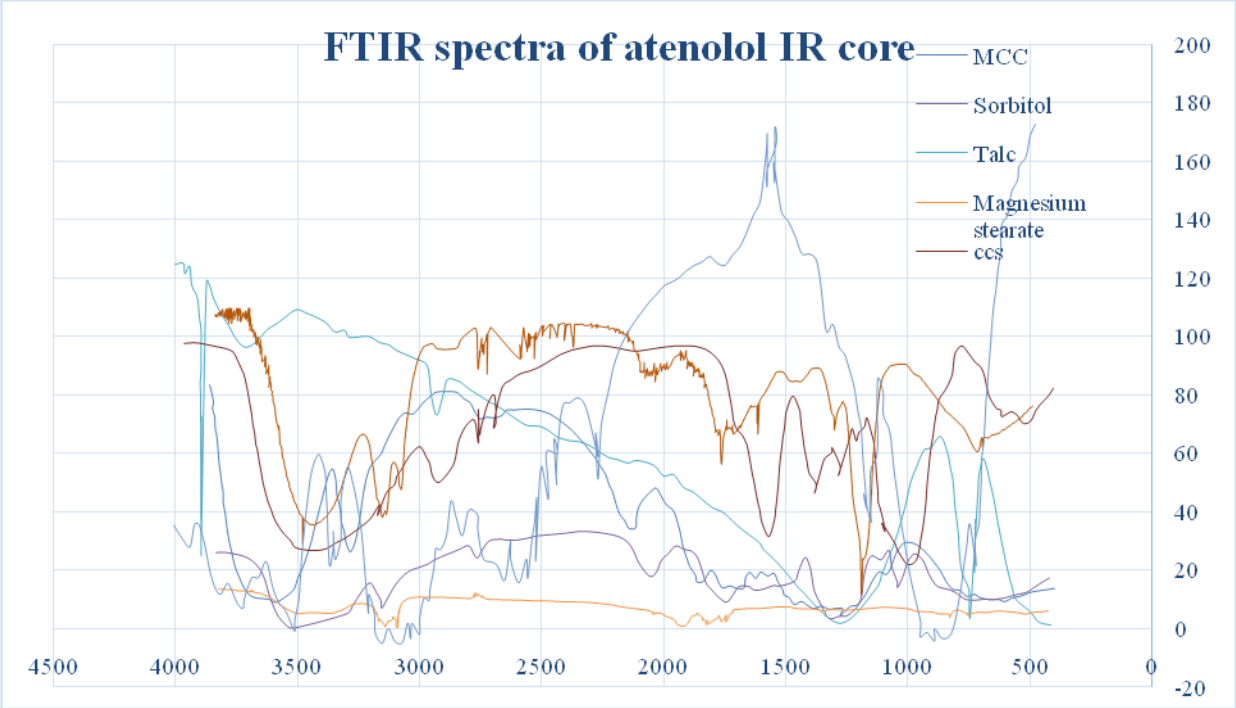


Figure 5.18. FTIR spectra of atenolol IR core

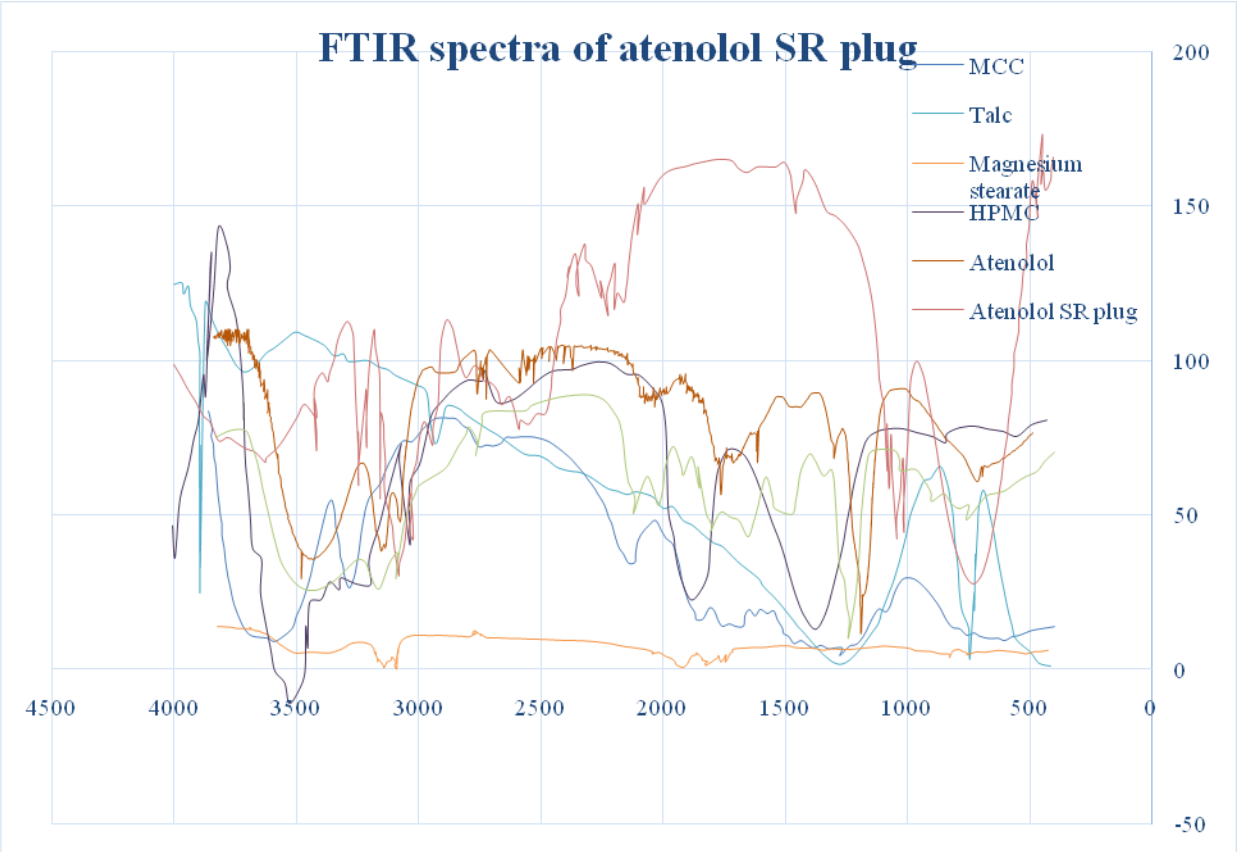


Figure 5.19. FTIR Spectra of atenolol SR plug

5.7.4.1 Preparation of Pulsatile Tablets

5.7.4.1.1 Design of experiment (JMP least square method)

The least-square approach minimizes the sum of the squares of the offsets (residual component) of the points from the curve to get the best-fitting curve or line of best fit for a group of data points. To forecast the behavior of dependent variables, least squares regression is employed. The formula for atenolol SR and IR layer was optimized based on the experimental results obtained from the JMP least square method. The effect of independent variables % of HPMC and compression force on the % drug release and hardness of SR plug were identified from the predicted plots. Similarly, the effect of disintegrant concentration and compression force on drug release, disintegration time, and hardness were studied in the case of IR core of atenolol.

5.7.4.1.2 Least squares fit applied to atenolol IR core

Two independent variables, disintegrant concentration and compression force were selected to optimize the hardness, disintegration time and % drug release in the case of

atenolol IR core. The disintegrant concentration was inversely proportional to the disintegration time. The disintegration time was found minimum when the disintegrant concentration was at its maximum (17.0711). Highest concentration of disintegrant and compression force exhibited the lowest % drug release compared to other combinations. No significant impact was found between the disintegrant concentration and the percentage of drug release. An increase in the concentration of disintegrant and compression force increased the disintegration time (Table 5.4-5.9 and Figure 5.20-5.26). The suggested formula was with 15% of disintegrant and 5 kg/cm^3 compression force. Desirability of the optimized formula was 0.6586.

Table 5.4 Optimization using least square method

Run	**Disintegrant mg**	**Compression force (kg/cm3)**	**Hardness (kg/cm3)**	**disintegration time**	**% drug release**
1	5	7	5.9	8.09	96.05
2	10	8	6.2	6.78	96.34
3	10	8	6	6.53	96.76
4	15	9	6.7	5.36	96.48
5	10	9.41	7.2	7.32	98.45
6	10	8	6.5	6.88	100.56
7	15	7	5.8	4.53	99.99
8	10	8	6.1	6.6	97.88
9	17.07	8	6.5	4.16	95.75
10	10	6.58	5.3	6.03	99.45
11	10	8	6.3	6.68	99.76
12	2.92	8	6	9.51	100.13
13	5	9	6.5	8.69	100.34

Response drug release %

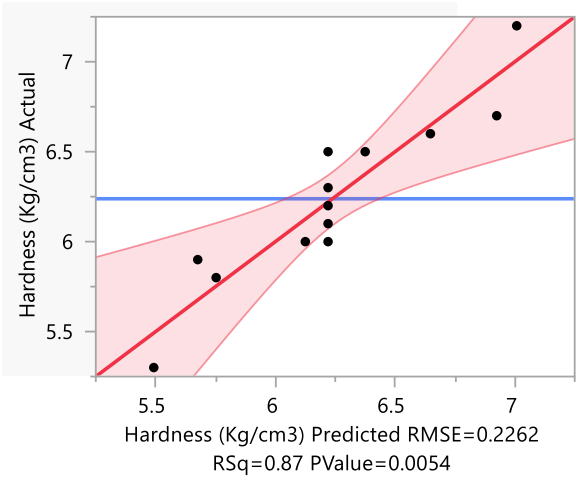


Figure 5.20 Actual by predicted plot of atenolol core

Table 5.5 Lack of fit of atenolol IR core drug release percentage

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Lack of Fit	3	0.21019894	0.070066	1.8937
Pure Error	4	0.14800000	0.037000	**Prob > F**
Total Error	7	0.35819894		0.2719
				Max RSq
				0.9458

Table 5.6 Parameter estimates of atenolol IR core drug release percentage

Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	6.1948333	0.099742	62.11	<.0001*
Disintegrant % (2,15)	0.1032047	0.123634	0.83	0.4314
Compression (Kg/cm3) (6,10)	1.0417524	0.173756	6.00	0.0005*
Disintegrant %*Disintegrant %	0.0253502	0.144945	0.17	0.8661
Disintegrant %*Compression (Kg/cm3)	0.13	0.294074	0.44	0.6718
Compression (Kg/cm3) * Compression (Kg/cm3)	0.0600006	0.343067	0.17	0.8661

Response disintegration time (min)

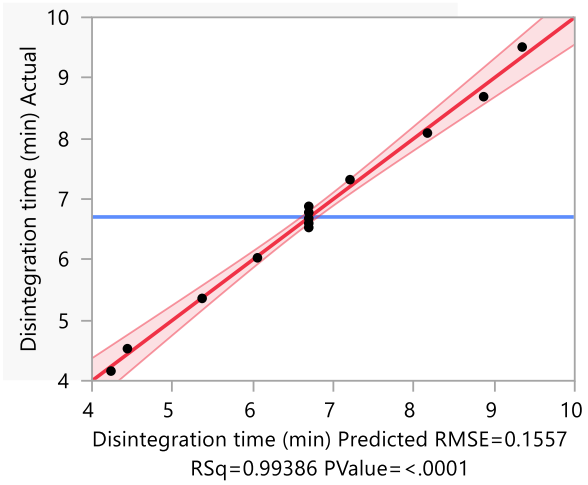


Figure 5.21. Actual by Predicted Plot of atenolol IR core disintegration time

Table 5.7 Parameter estimates of atenolol IR core disintegration time

Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	7.2404776	0.068672	105.44	<.0001*
Disintegrant % (2,15)	-2.387033	0.085122	-28.04	<.0001*
Compression (Kg/cm3) (6,10)	0.7790848	0.119631	6.51	0.0003*
Disintegrant %*Disintegrant %	0.0821791	0.099794	0.82	0.4374
Disintegrant %*Compression (Kg/cm3)	0.1495	0.20247	0.74	0.4843
Compression (Kg/cm3) * Compression (Kg/cm3)	-0.125504	0.236201	-0.53	0.6116

Table 5.8 Lack of fit

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Lack of Fit	3	0.09187770	0.030626	1.5722
Pure Error	4	0.07792000	0.019480	**Prob > F**
Total Error	7	0.16979770		0.3280
				Max RSq
				0.9972

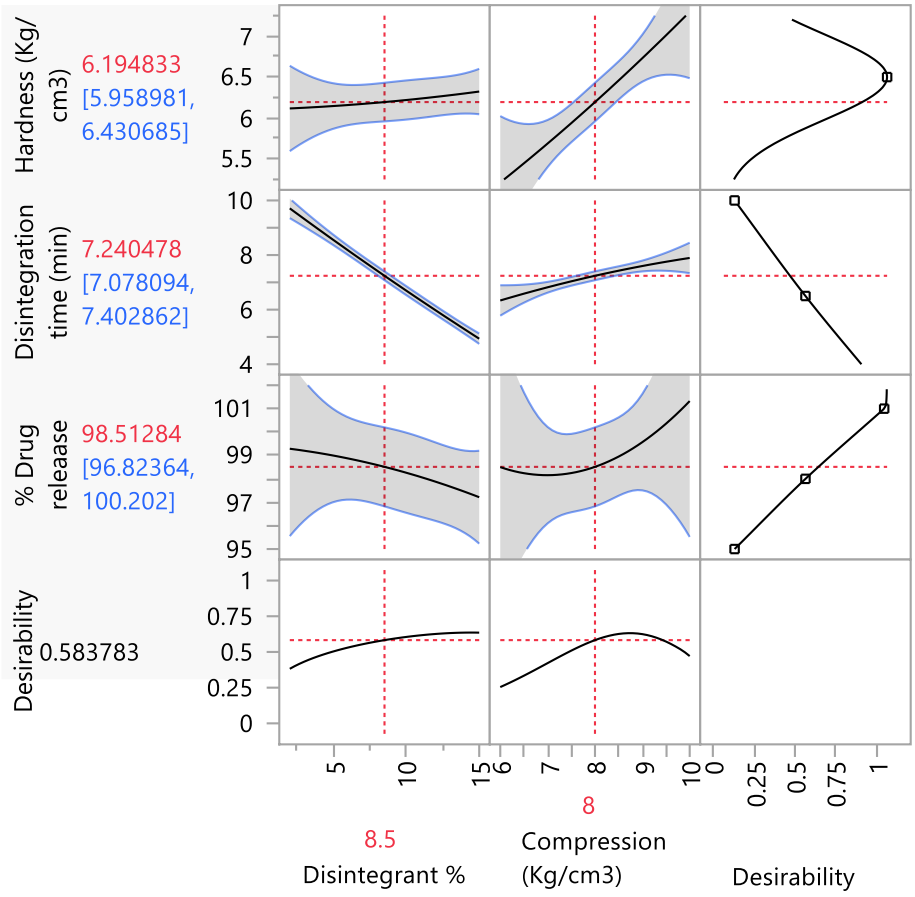


Figure 5.22. Prediction profile of atenolol IR core

Table 5.9 Optimal formula for atenolol IR core

Setting	Disintegrant %	Compression (Kg/cm3)	Hardness (Kg/cm3)	Hardness (Kg/cm3) Lower CI	Hardness (Kg/cm3) Upper CI	Disintegration time (min)	Disintegration time (min) Lower CI	Disintegration time (min) Upper CI	% Drug releaase	% Drug releaase Lower CI	% Drug releaase Upper CI	Desirability
Optimal	15	5	6.101199	5.8068	6.3956	4.7532	4.5505	4.9559	98.0921	95.9833	100.2010	0.6586

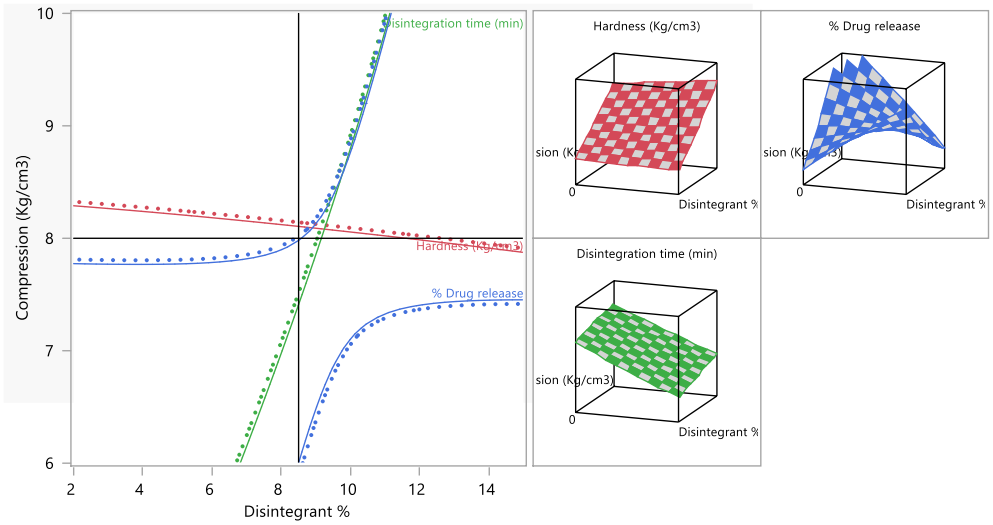


Figure 5.23. The plot of relationship between compression force and disintegrant with drug release % disintegration time and hardness

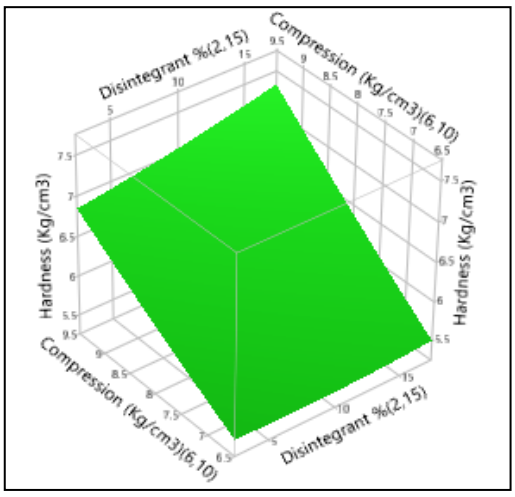


Figure 5.24.3D plot of hardness (Kg/cm3)

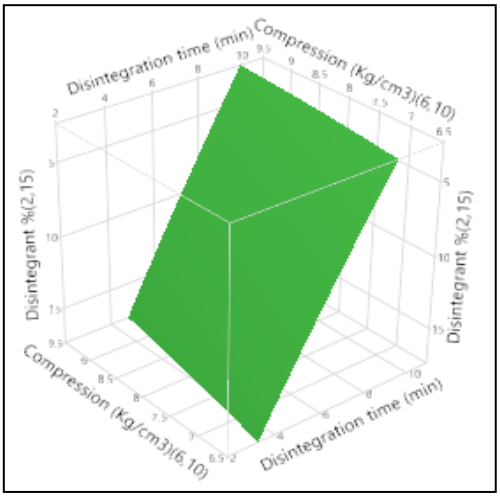


Figure 5.25.3D plot of drug release %

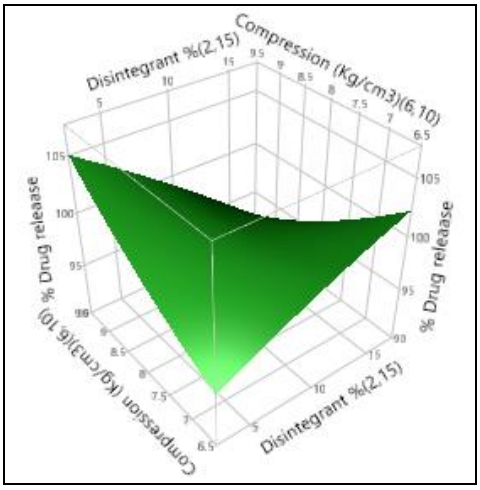


Figure 5.26. 3D plot of disintegration time

5.7.4.1.3 Least squares fit applied to atenolol SR plug

HPMC concentration is one of the major determinants in the drug release from SR formulation. HPMC concentration ranging from 5.857 to 34.1421was selected for the trials. The compression forces selected were 4.378, 5, 6.5, 8 and 8.621. 13 runs were performed to identify the optimized formula and to establish a relationship between the independent and dependent variables. The results indicated a linear relationship between the compression force and the hardness of the tablet. The HPMC concentration and drug release were inversely proportional. Maximum drug release with acceptable hardness was observed when the HPMC concentration was 5.857%, and the compression force was 6.5 kg/cm3. The prediction profiler recommended formula with 5 mg of HPMC and a compression force of 6.99 kg/cm3. (Table 5.10-5.15 and Figure-5.27-5.32).

Table 5.10 Optimization using least square method

Run	HPMC	Compression force (kg/cm3)	% drug release	Hardness kg/cm3
1	10	8	96.78	6.2
2	20	6.5	93.21	5.7
3	34.1421	6.5	80.28	5.8
4	20	6.5	84.12	5.7
5	30	8	83.31	6.2
6	20	8.62	88.89	6.36
7	20	4.37	92.19	4.6
8	30	5	84.69	4.9
9	10	5	98.73	4.8
10	20	6.5	91.59	5
11	20	6.5	94.01	5.9
12	5.85	6.5	100.58	5.8
13	20	6.5	91.72	5.9

Response drug release percentage

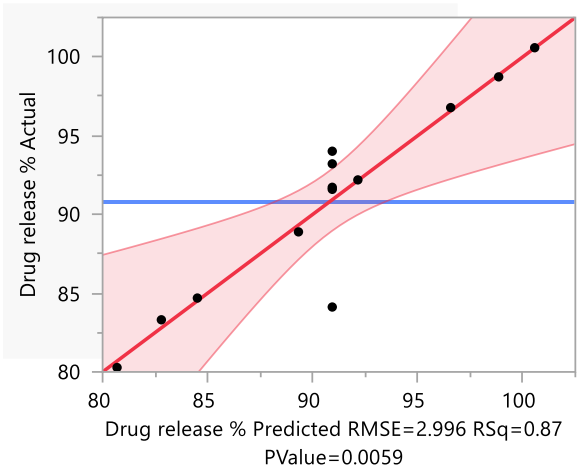


Figure 5.27. Actual by predicted plot of atenolol SR plug for drug release

Table 5.11 Lack of Fit: Drug release %

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack of Fit	3	0.03326429	0.011088	0.0803
Pure Error	4	0.55200000	0.138000	**Prob > F**
Total Error	7	0.58526429		0.9673
				Max RSq
				0.8643

Table 5.12 Parameter Estimates: Drug release %

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	90.93	1.339848	67.87	<.0001*
HPMC (5,35)	-10.54098	1.588865	-6.63	0.0003*
Compression (Kg/cm3)(4,9)	-1.666022	1.765404	-0.94	0.3767
HPMC *HPMC	-0.34173	2.555804	-0.13	0.8974
HPMC *Compression (Kg/cm3)	0.35625	3.744988	0.10	0.9269
Compression (Kg/cm3)*Compression (Kg/cm3)	-0.269092	3.155309	-0.09	0.9344

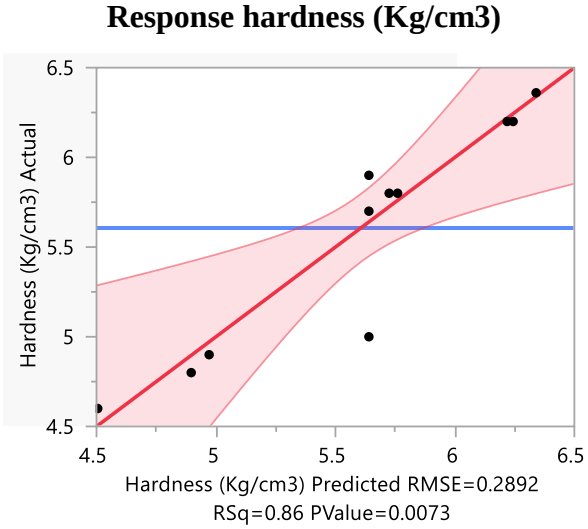


Figure 5.28 Actual by predicted plot of atenolol SR plug for hardness

Table 5.13 Lack of fit: Hardness

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack of Fit	3	0.03326429	0.011088	0.0803
Pure Error	4	0.55200000	0.138000	**Prob > F**
Total Error	7	0.58526429		0.9673
				Max RSq
				0.8643

Table 5.14 Parameter Estimate

Term
Intercept
HPMC(5,35)
Compression
HPMC *HPM
HPMC *Com
Compression (Kg/cm3)

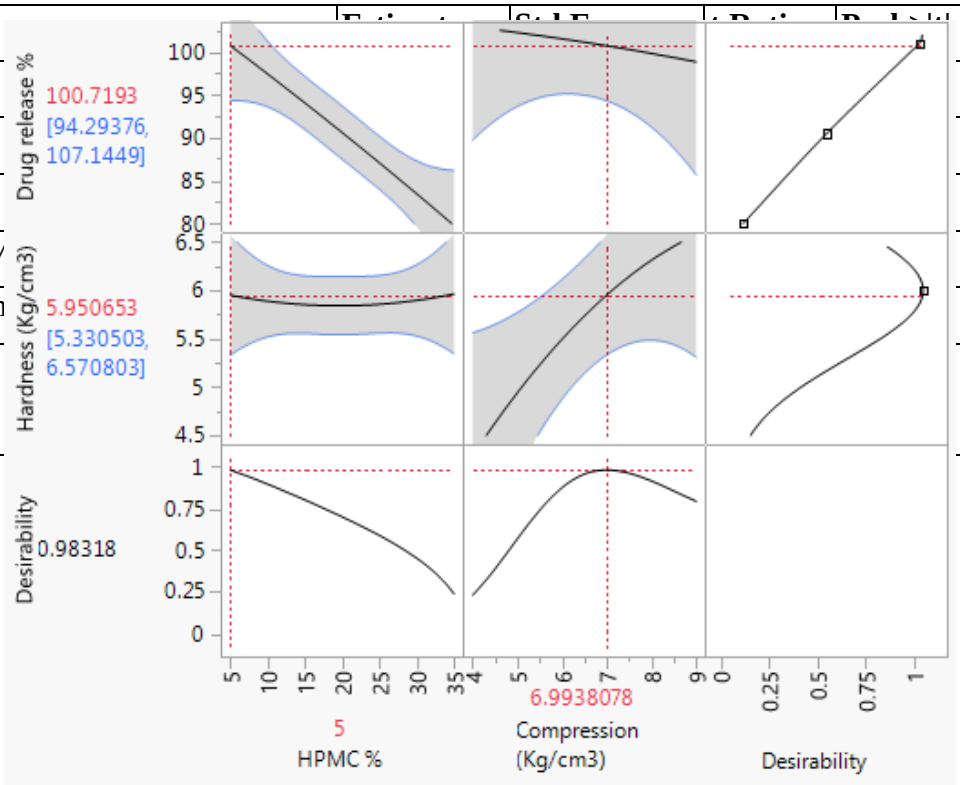


Figure 5.29 Prediction profile of atenolol SR core

Table 5.15 Optimal formula for atenolol SR core

Setting	HPMC	Compression (Kg/cm3)	Drug release %	Drug release % Lower CI	Drug release % Upper CI	Hardness (Kg/cm3)	Hardness (Kg/cm3) Lower CI	Hardness (Kg/cm3) Upper CI	Desirability
Optimal	5	6.634	100.202	94.207	100.198	6.001	5.799	6.204	0.994

Figure 5.30 Plot of relationship between compression force, HPMC and drug release % and hardness

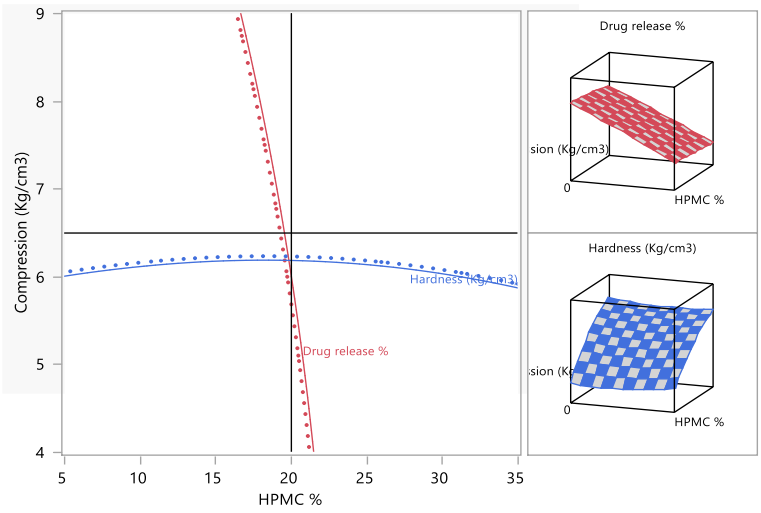
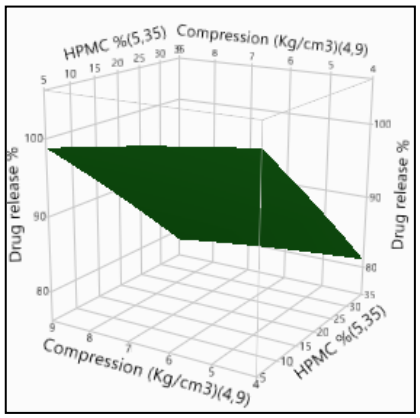
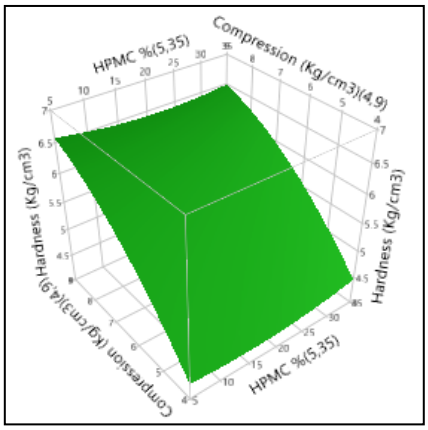


Figure 5.31 3D plot of drug release %

Figure 5.32 3D plot of hardness (kg/cm^3)





Optimized formula for core in cup tablet

The optimized formula for metoprolol core in cup tablet and the atenolol mucoadhesive core in cup tablet is as follows (Table 5.16-5.18).

Table 5.16 Composition of atenolol IR core

Composition	**Quantity (%)**
Drug	Cross carmellose sodium (CCS)
MCC	Sorbitol
Talc	Magnesium stearate
Total weight	Punch size
Compression force	13
15	10
25.1	0.325
0.325	63.75
6mm	5 kg/cm^3

Table 5.17 Composition of atenolol SR plug

Composition	**Quantity (%)**
Drug	12
HPMC	5
PVP in alcoholic solution	2.8
MCC	55
Lactose	32
Talc	0.5
Magnesium stearate	0.5
Total weight	107.8
Punch size	12 mm
Compression force	7 kg/cm^3

Table 5.18 Composition of atenolol Mucoadhesive cup layer

Formulation	Chitosan (mg)	Polycaprolctone (mg)
M1	1.1 (1%)	108.9
M2	2.2 (2%)	107.8
M3	3.3 (3%)	106.7

5.7.5 Study of flow property (angle of repose, bulk and tap densities, Carr's index, Hausner's ratio) of powder material

5.7.5.1 Angle of repose

The flow characteristics and compressibility of powder mixes are critical in defining the quality and reproducibility of direct compression tablets. According to the USP, a powder sample with an angle of repose of 25–30^0 has good flow properties. All the powder mixes had an angle of repose ranging from 28-30^0. The angle of repose of all the formulations is given in table 5.19.

5.7.5.2 Bulk density

The bulk density of the powder mix affects the flowability of the powder. Low bulk density associated with agglomerated particles negatively influences the flowability and of the powder. The observed bulk density of the formulations is given in table 5.19.

5.7.5.3 Tapped density

Tapped density is greater in the case of regularly shaped particles than irregularly shaped particles. Determined tapped density was mentioned in table 5.19. These values were used for the calculation of the Hausner's ratio and Carr's index.

5.7.5.4 Carr's index

Carr's compressibility index values have been used to define the flow properties of the powders. Carr's index for the studies was in the range of 10 - 14. These values indicate the excellent flowability of the powders (Table 5.19).

5.7.5.5 Hausner's ratio

Hausner's ratio is related to the interparticle friction between the particles. For a satisfactory flow property, Hausner's ratio should be less than 1.25. Table 5.19 lists the IR tablet's Hausner's ratio.

Table 5.19. Flow properties of immediate release formulations

Evaluation parameters	Atenolol	
	IR	SR
Angle of repose	28.25	29
Bulk density	0.3	0.21
Tapped density	0.34	0.29
Carr's index	12	10
Hausner's ratio	1.12	1

5.7.6 Evaluation of core in cup tablet

All the core in cup tablets passed the quality control tests performed. The thickness of the atenolol mucoadhesive core in cup tablet was 3.62±0.021mm, and the diameter was 6.04±0.01mm. The hardness was 4.99±0.01 kg/cm2, and the friability and average weight were <0.25% and 281±0.34 mg, respectively (Table 5.20).

Table 5.20 Quality control tests for Atenolol mucoadhesive core in cup tablet

Test	Result
Thickness	Diameter
Hardness	Friability
Average weight	3.62±0.021mm
6.04±0.01mm	4.99±0.01 kg/cm^3
<0.25%	281±0.34 mg

5.7.6.1 Formulation of core in cup tablet

The tablets were prepared as per the optimized formula. The images of the prepared tablets are provided below (Figure 5.33).

Figure 5.33. Prepared core in cup tablets

5.7.6.2 Disintegration time of the IR core tablet

The disintegration time for the immediate release core tablet was determined in water, 0.1 N HCl and pH 6.8 phosphate buffer. The disintegration of the core tablet was instantaneous in all three media. The disintegration time was less than 1 minute for atenolol immediate release core (figure 5.34).

Figure 5.34 Disintegration studies

5.7.7 In vitro drug release from IR core tablet and SR plug

5.7.7.1 In vitro drug release from IR core tablet

In vitro cumulative release of atenolol IR core tablet was studied in 900 mL 0.1N HCl at 37^0C and 50 rpm. To get an immediate release following the lag time, CCS was incorporated as a super disintegrant in the core tablet. Super disintegrants speed up the disintegration of direct compressible tablets and improve the solubility of medications that are difficult to dissolve. The drug release mechanism is dependent on the concentration of super disintegrants. The atenolol IR core tablet released 99.491% atenolol in 30 min (Table 5.21 and Figure 5.35 & 5.36).

Figure 5.35 Dissolution

Table 5.21 % Cumulative atenolol release from IR core tablet

Time (min)	Absorbance	Conc (mcg/ml)	Conc (mg/ml)	Concentration*dilution factor	Error	Bath Concentration	Drug release	% Cumulative drug release
0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.00
5	0.108	0.686	0.001	0.007	0.000	6.176	6.176	26.50
10	0.114	1.005	0.001	0.010	0.007	9.048	9.055	69.65
15	0.118	1.218	0.001	0.012	0.017	10.963	10.980	84.45
20	0.12	1.324	0.001	0.013	0.029	11.920	11.949	91.91
25	0.121	1.378	0.001	0.014	0.042	12.399	12.441	95.70
30	0.122	1.431	0.001	0.014	0.056	12.878	12.934	99.49

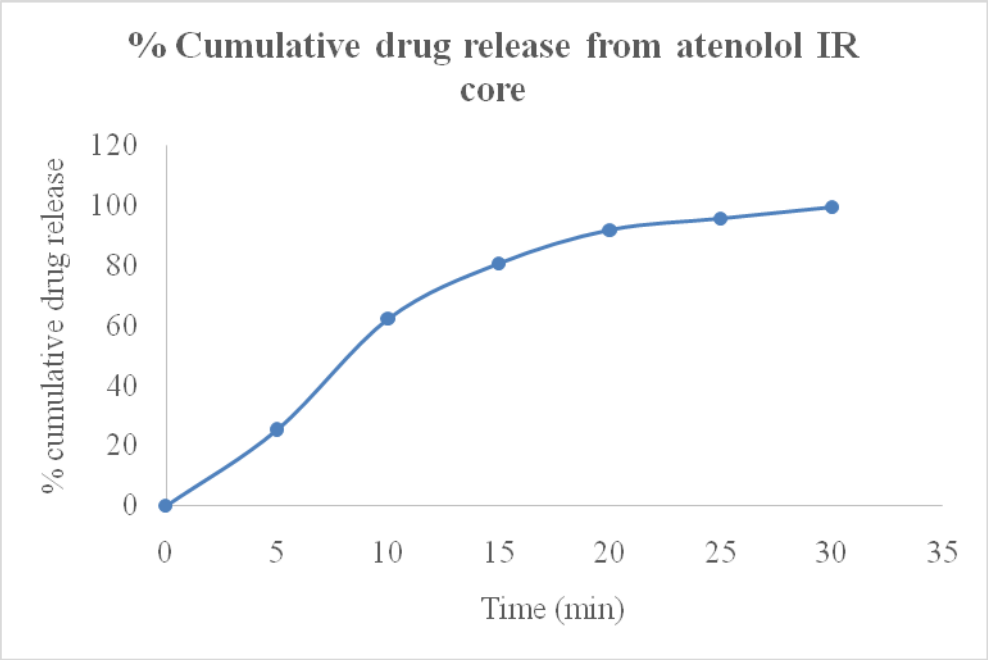


Figure.5.36 % of Cumulative atenolol release from IR core tablet

Drug release from the IR core of atenolol was compared with the IR formulations available on the market. Atenolol was compared with Devolol 50mg. The cumulative atenolol release from Devolol 50 mg was 99.450%. Drug release from atenolol core tablet was 99.44% in 30 min. The calculated f2 value for the formulation compared to their marketed formulation was more than 50. Hence the drug release was concluded to be similar (Table 5.22 and figure 5.37).

Table 5.22 % Cumulative drug release from atenolol marketed IR tablet (Devolol 50 mg)

Time (min)	Absorbance	Conc (mcg/ml)	Conc (mg/ml)	Concentration*dilution factor	Error	Bath Concentration	Drug release	% Cumulative drug release
0	0	0.000	0.00000	0.000	0.000	0.000	0.000	0.00
5	0.099	0.207	0.00021	0.002	0.000	1.867	1.867	14.36
10	0.114	1.005	0.00101	0.010	0.002	9.048	9.050	69.61
15	0.118	1.218	0.00122	0.012	0.012	10.963	10.975	84.42
20	0.119	1.271	0.00127	0.013	0.024	11.441	11.466	88.19
25	0.121	1.378	0.00138	0.014	0.037	12.399	12.436	95.66
30	0.122	1.431	0.00143	0.014	0.051	12.878	12.928	99.45

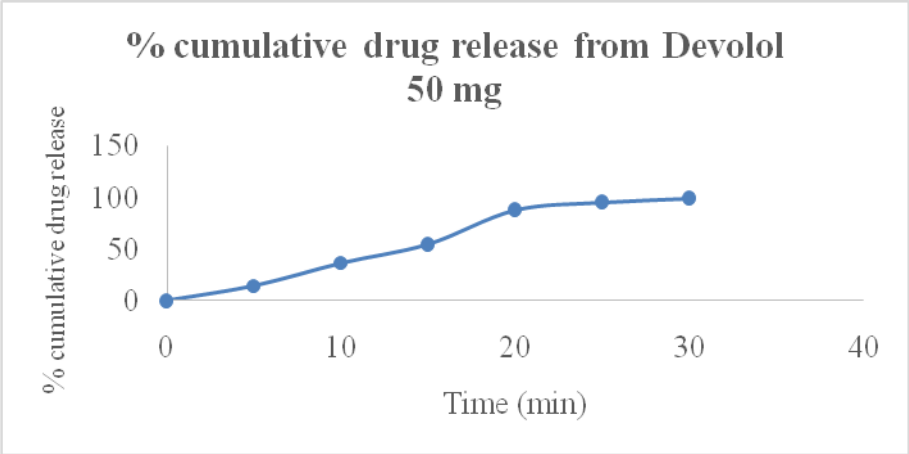


Figure 5.37 % Cumulative drug release from metoprolol marketed IR tablet (Devolol 50 mg)

5.7.7.2 In vitro drug release from SR plug

The sustained release polymeric plug contributes to the lag period prior to the onset of drug release from the IR core. The tablet plug was made with a SR polymer, HPMC, to maintain a low concentration of drug in the blood during the lag period and to reach the therapeutic concentration faster. When HPMC comes into contact with water, it produces a viscous gel layer that aids in the sustained release of the drug from the plug. The swelling expansion of the polymer prevents water from accessing the IR core, so HPMC produces a sustained drug release from the plug. As the swelling progresses, the plug's rigidity deteriorates, causing the plug layer to erode. Atenolol SR plugs were able to release the drugs for up to 360 minutes. Atenolol plug released 99.364% of the drug (Table 5.23 and Figure 5.38).

Table 5.23 % Cumulative atenolol release from SR core tablet

Time (min)	Absorbance	Conc (mcg/ml)	Conc (mg/ml)	Concentration*dilution factor	Error	Bath Concentration	Drug release	% Cumulative drug release
0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.00
60	0.098	0.154	0.000	0.002	0.000	1.388	1.388	10.67
120	0.107	0.633	0.001	0.006	0.002	5.697	5.698	43.83

180	0.112	0.899	0.001	0.009	0.008	8.090	8.098	62.29
240	0.115	1.059	0.001	0.011	0.017	9.527	9.543	73.41
300	0.118	1.218	0.001	0.012	0.027	10.963	10.990	84.54
360	0.122	1.431	0.001	0.014	0.040	12.878	12.917	99.36

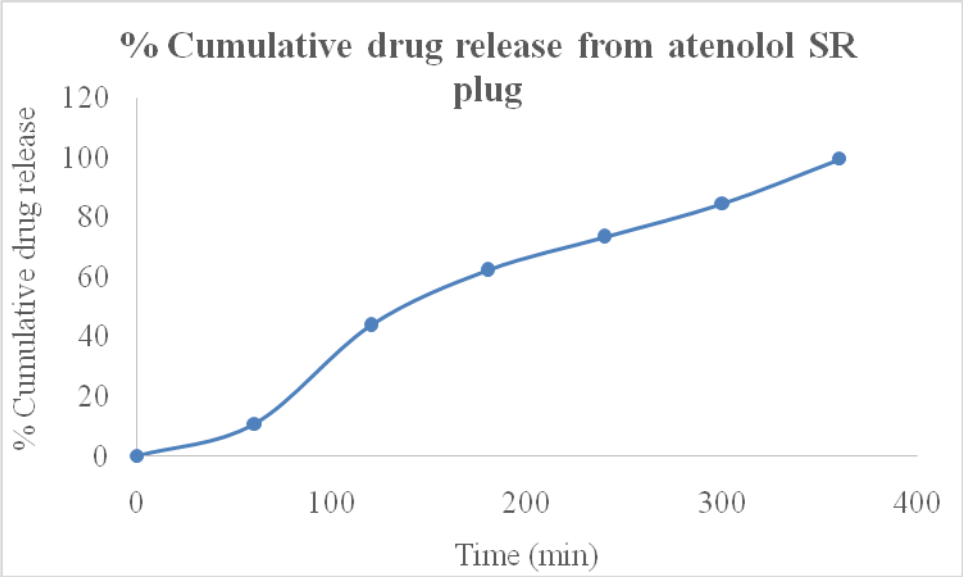


Figure 5.38 % Cumulative atenolol release from SR core tablet

5.7.8 Evaluation of cup layer

5.7.8.1 Effect of the cup in drug release

To identify the effect of the cup on drug release from the core in cup tablets of atenolol, drug release was studied with the 'core in cup' tablet without the plug layer (A1) and the IR core tablet (A2) alone. In both cases, the immediate release core was exposed to the dissolution media, which facilitates the leaching of water into the core and immediate disintegration of the IR tablets. Because the drug release profiles of A1 and A2 were similar, the influence of the cup layer (polycaprolactone + chitosan) on drug release was minimal (Figure 5.39).

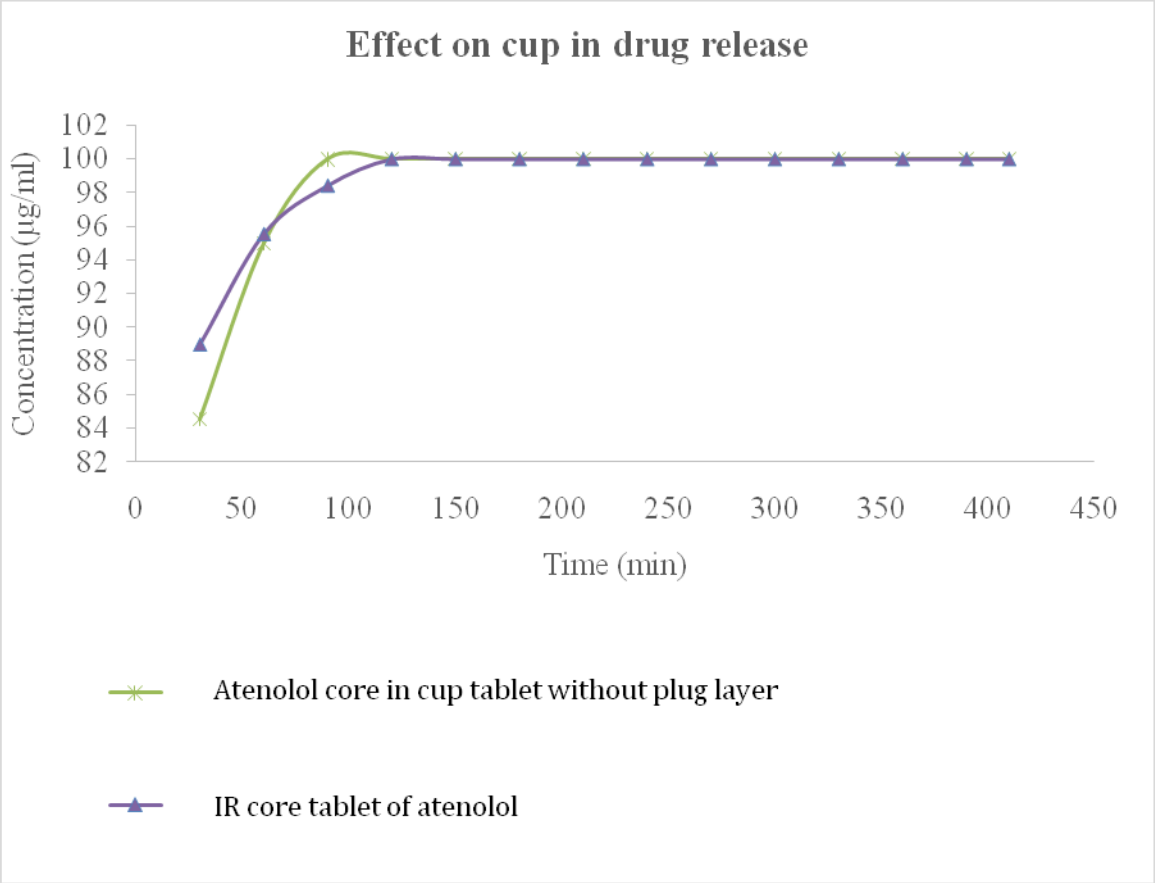


Figure 5.39 Effect of cup in drug release

5.7.8.2 Mucoadhesive strength of the atenolol cup

Figure 1 depicts the findings of the mucoadhesive strength study. It was established how much strength (g) was required to separate the tablet from the membrane. The mucoadhesive strength of the cup layer is determined by the chitosan content. The formulation with the lowest chitosan concentration (M1) detached faster than the formulation with the highest chitosan concentration (M2). Among the other formulations, M3 had the strongest mucoadhesive strength (figure 5.40).

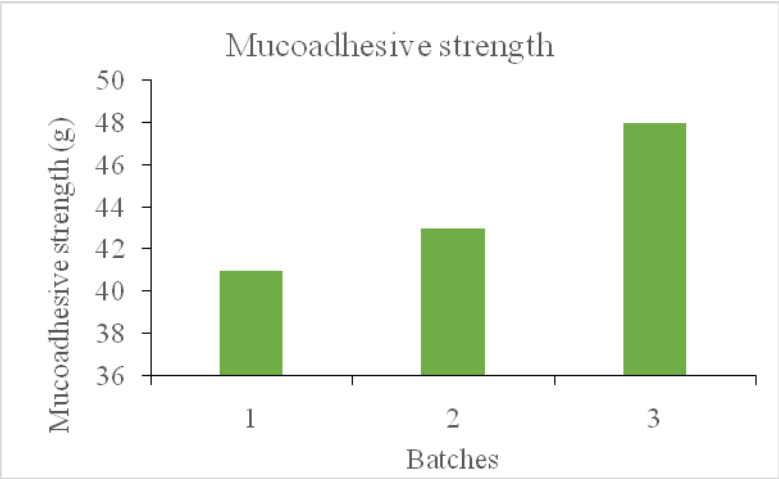


Figure 5.40 Mucoadhesive strength of cup layer

5.7.8.3 Mucoadhesion residence time for atenolol cup

All three formulations stayed adhered to the mucosal membrane at least for 10 h. The layer containing 3% of chitosan stayed unchanged in its adhesion properties till the end of the study compared to the formulations containing 1 and 2% chitosan.

5.7.8.4 Drug release from core in cup tablets of atenolol

A mucoadhesive core in cup tablet of atenolol was prepared using the M3 composition of the cup. The drug release was observed 29.698% of the drug was released from the 'core in cup' formulation till 270 min (Table and Figure). The formulation had no lag time till 270 mins. In 300 min, the drug release increased to 81.046% and reached maximum release (101.425 %) in 480 min (Table 5.24 and Figure 5.41). The drug release profile indicates that the created 'core in cup' tablet can be used as a pulsatile drug delivery system.

Table 5.24 % Cumulative drug release from atenolol core in cup mucoadhesive tablet

Time (min)	Absorbance	Conc (mcg/ml)	Conc (mg/ml)	Concentration*dilution factor	Error	Bath Concentration	Drug release	% Cumulative drug release
0	0	0.000	0.00000	0.000	0.000	0.000	0.000	0.00
30	0.094	0.059	0.00006	0.001	0.000	0.535	0.535	4.11
60	0.097	0.103	0.00010	0.001	0.001	0.924	0.925	7.11
90	0.098	0.157	0.00016	0.002	0.002	1.411	1.412	10.86
120	0.099	0.211	0.00021	0.002	0.003	1.897	1.900	14.6
150	0.1	0.265	0.00026	0.003	0.005	2.384	2.389	18.37
180	0.1	0.265	0.00026	0.003	0.008	2.384	2.392	18.39
210	0.101	0.319	0.00032	0.003	0.011	2.870	2.881	22.16
240	0.102	0.373	0.00037	0.004	0.014	3.357	3.371	25.92
270	0.103	0.427	0.00043	0.004	0.018	3.843	3.861	29.69
300	0.117	1.184	0.00118	0.012	0.022	10.654	10.676	82.12
330	0.118	1.238	0.00124	0.012	0.034	11.141	11.174	85.95
360	0.118	1.238	0.00124	0.012	0.046	11.141	11.187	86.05
390	0.119	1.292	0.00129	0.013	0.058	11.627	11.685	89.88
420	0.12	1.346	0.00135	0.013	0.071	12.114	12.185	93.72
450	0.121	1.400	0.00140	0.014	0.085	12.600	12.685	97.57
480	0.122	1.454	0.00145	0.015	0.099	13.086	13.185	101.42

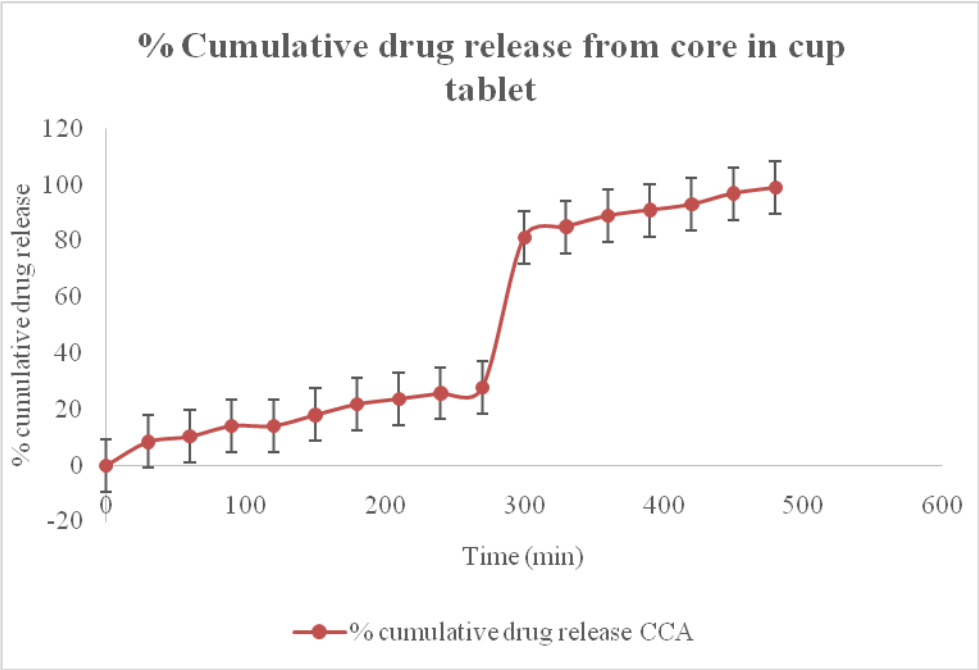


Figure 5.41 % Cumulative drug release from core in cup tablet

5.7.8.5 Modeling of drug release

The release of atenolol from the core in cup fits well into the Korsmeyer-Peppas model, as indicated by the value R2 value of 0.9937 for atenolol, and the profile is linear (Table 5.25). It can be concluded that the mechanism of drug release fromthe core in cup tablets follows diffusion and swelling. The exponential coefficient (n) value was found to be > 0.5, indicating a non-Fickian diffusion mechanism. In other models, linear profiles were not obtained (figure 5.42-5.46)

Table 5.25 The correlation coefficient values for dissolution kinetics data

Drug	**Zero order R^2**	**Higuchi R^2**	**Peppa's-Korsmeyer**		**First order R^2**	**Hixon Crowell model R^2**
			R^2	**N**		
Atenolol	0.7237	0.5606	0.9937	0.592	0.5778	0.6227

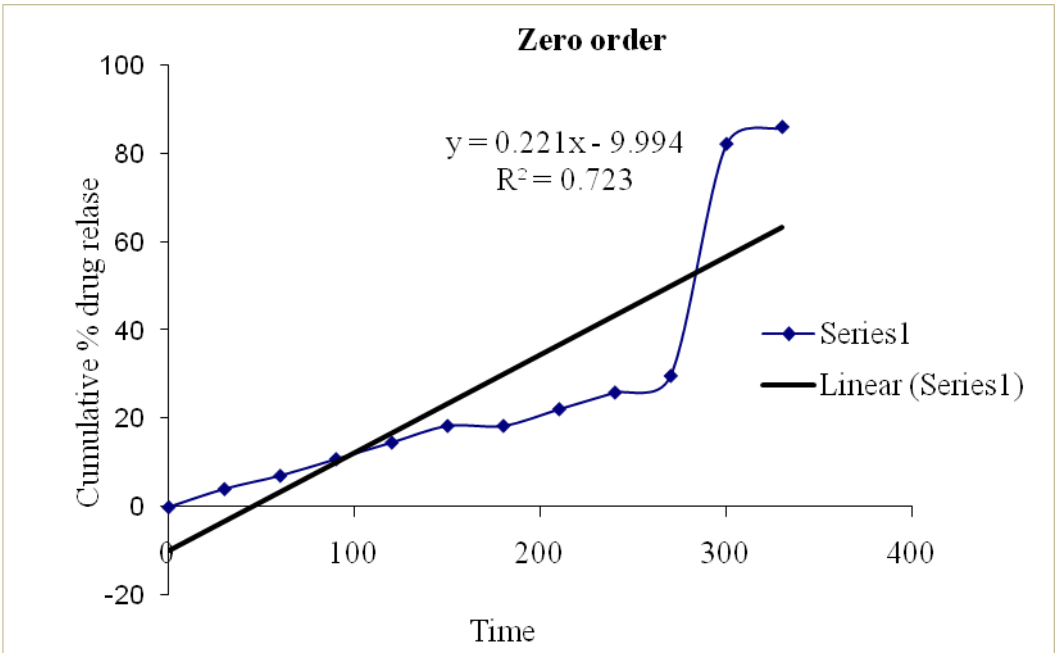


Figure 5.42 Zero order kinetic model for atenolol

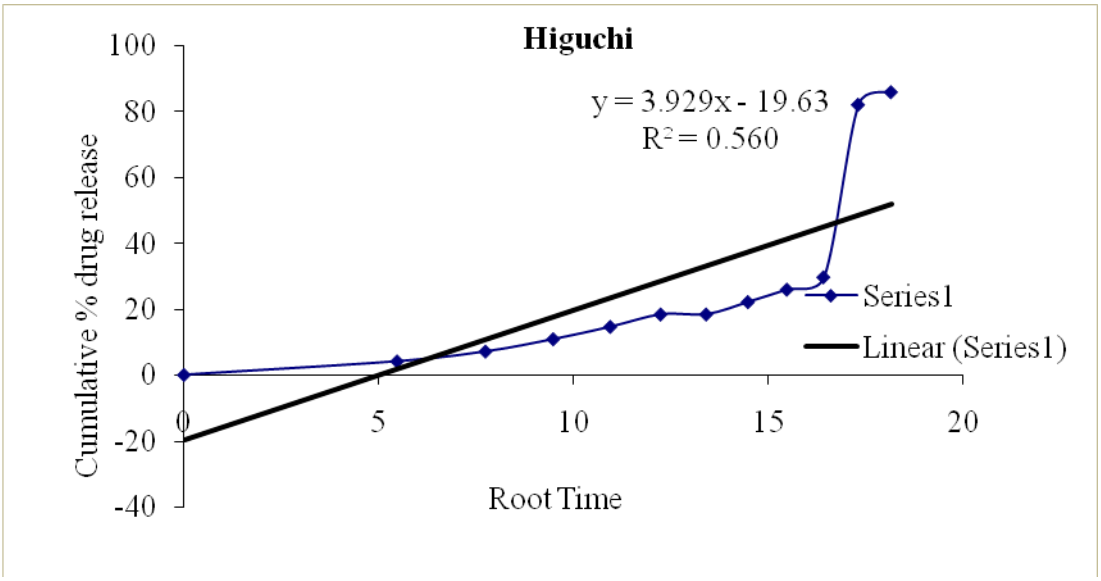


Figure 5.43Higuchi model drug release for atenolol

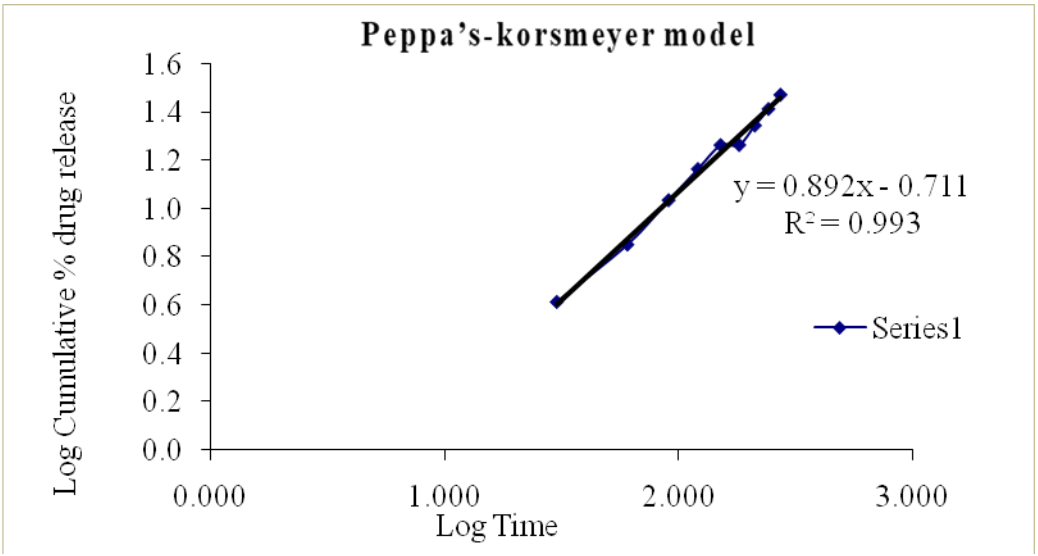


Figure 5.44. Peppa's-Korsmeyer model for metoprolol

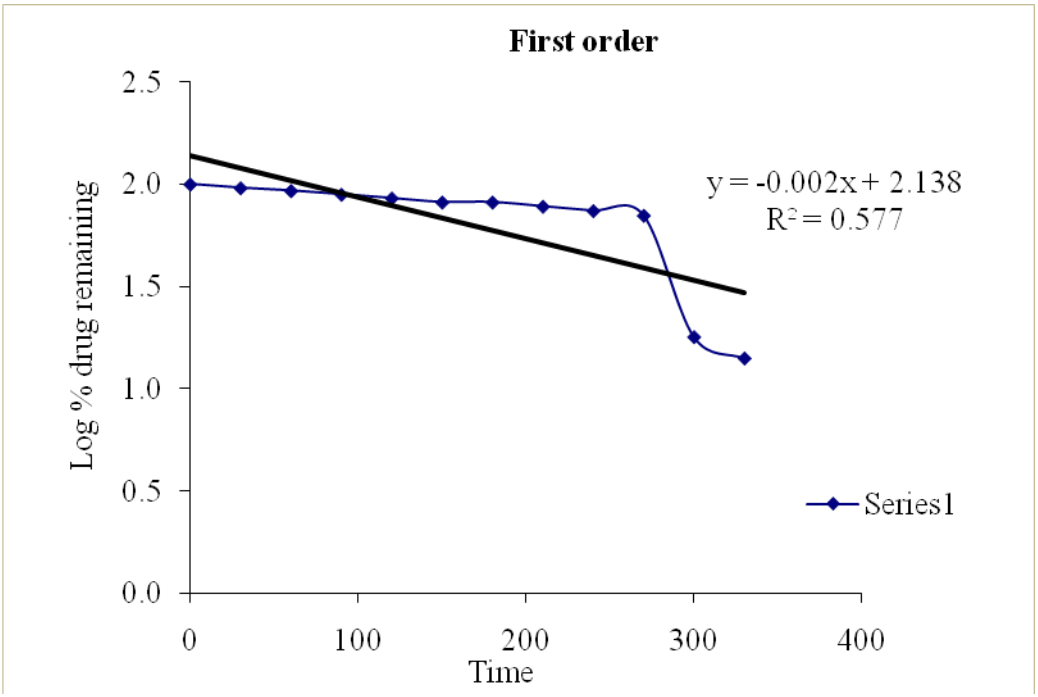


Figure 5.45. First order kinetic model for atenolol

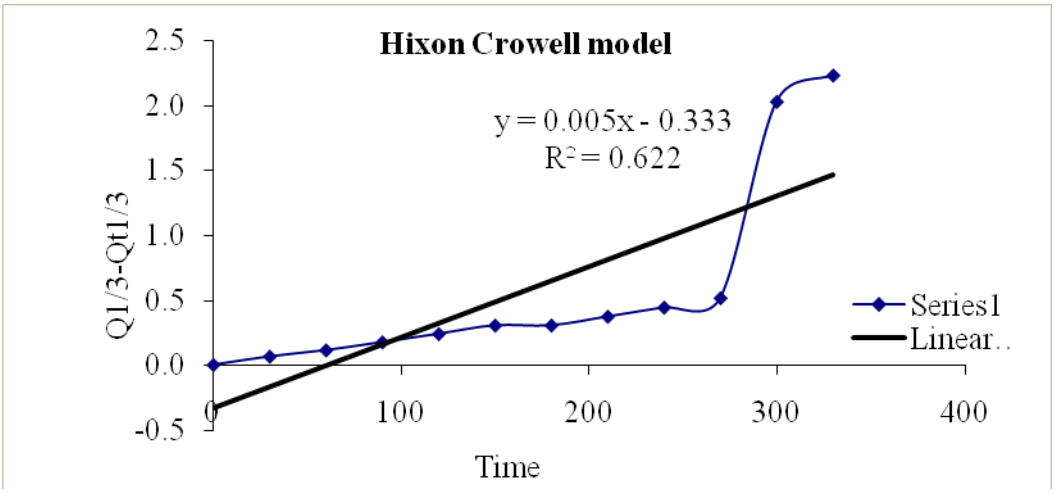


Figure 5.46. Hixon Crowell model for atenolol

5.7.8.6 Water uptake and drug erosion studies

The mucoadhesive atenolol core in cup formulation, the study was conducted in 0.1 N HCl since the tablets remain in the stomach during the drug release. Figure 5.74 depicts the relationship between polymer water uptake and polymer breakdown (Table 5.26 and Figure 5.47).

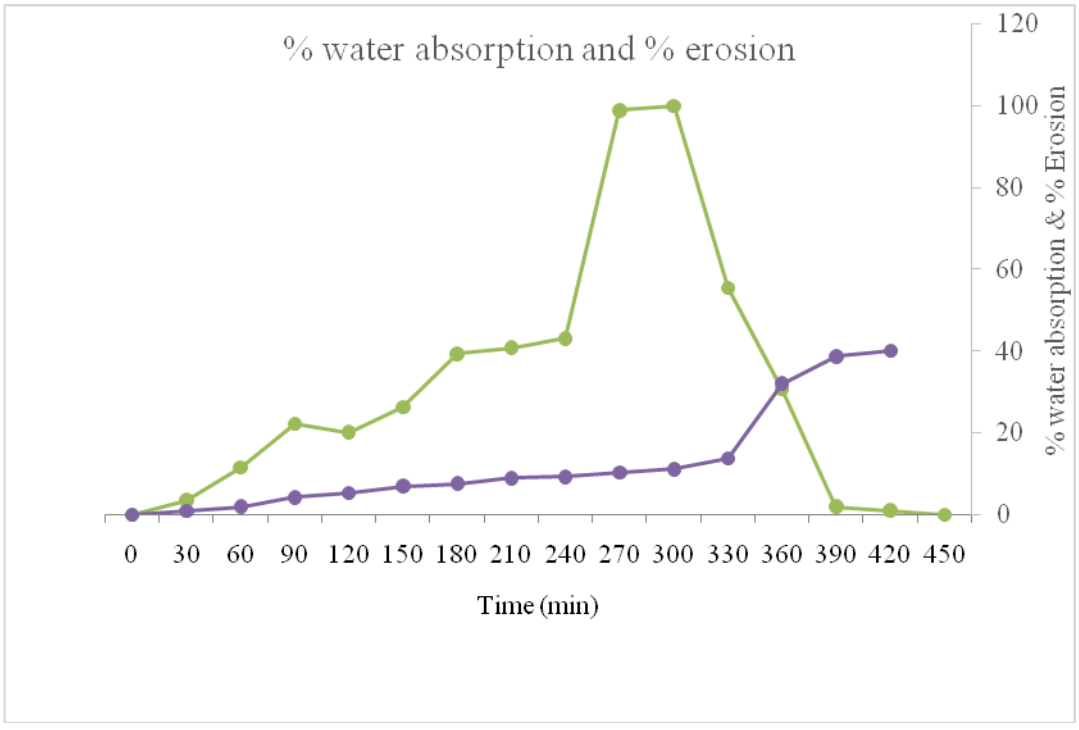


Figure 5.47. % of water absorption and % of erosion

Table 5.26 % of water absorption and % of erosion

Time (min)	% Water absorption (atenolol)	% Erosion (atenolol)
0	0	0
30	3.48	0.88
60	11.49	2.01
90	22.11	4.22
120	20.18	5.29
150	26.22	6.98
180	39.31	7.56
210	40.82	8.91
240	43.11	9.22
270	98.83	10.21
300	99.91	11.1
330	55.44	13.69
360	30.71	32
390	1.98	38.71
420	1.06	39.99
450	0	

5.7.9 Stability study

Formulation F3 was kept for three months in both standard long-term storage (20°C, 60% RH) and accelerated stability (40°C, 75% RH). After the trial period, the tablets were examined for drug release, friability, hardness, and product look. The findings of the tablets' early tests showed no significant differences.

5.7.10 In-vivo Studies

The kinetic parameters, including Cmax, Tmax and AUC were analyzed. [34, 35] The in-vivo concentration of atenolol with respect to time is shown in table 5.27, and their relative plasma drug concentration is illustrated in figure 5.48. The Tmax, Cmax, and AUC of atenolol were observed to be higher than those of metoprolol. This effect may

be due to the higher gastric retention time of the mucoadhesive system (Table5.27,5.28 and Figure 5.48).

Table 5.27*In-vivo* concentration of Atenolol

Time	Concentration (ng/ml)	Time	Concentration (ng/ml)
0	0	15	23.2
30	27.8	60	30.3
120	31.9	180	35.6
240	40.7	300	47.8
360	97.03	420	53.7
480	30.3	540	12.5

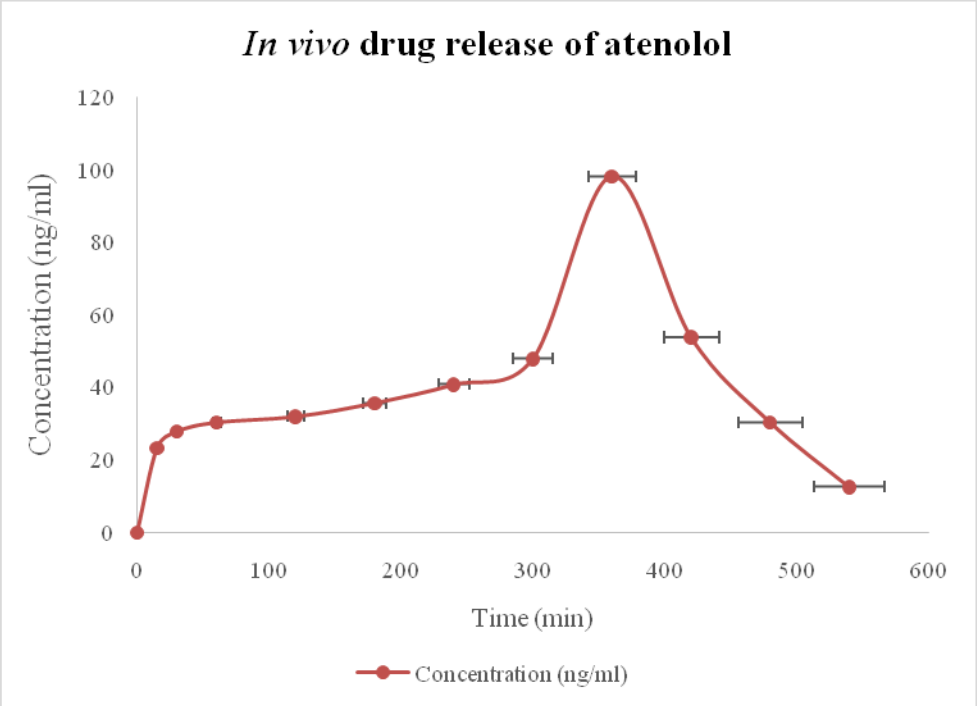


Figure 5.48. Plasma concentration profile of atenolol core in cup tablet

Table 5.28 Pharmacokinetic parameters

Parameters	**Atenolol**
Tmax	360 min
Cmax	97.3 ng/ml
AUC	22,759.8 min. ng/ml

CHAPTER-6

6 Summary and conclusion

Preformulation studies

Preformulation studies have been used for the characterization of excipients and the active pharmaceutical ingredients (API). The study identifies the influence of ingredients on the performance of a formulation. Calibration curves for atenolol and metoprolol were plotted by the UV spectrometric analysis of the samples. The calibration curves were linear and were used to determine the drug release from the designed dosage forms. Solubility analysis revealed that the drugs are sparingly soluble in water, 0.1N HCl, and pH 6.8 phosphate buffer. DSC and FTIR studies proved the compatibility of the excipients and the API.

Formulation of core in cup bilayer tablets

Metoprolol core in cup tablets and atenolol mucoadhesive core in cup tablets were designed to release the drug adequately when required, to avoid the presence of a high dose of metoprolol and atenolol throughout the day in blood circulation, and to reduce the adverse reactions. The core of the formulation was an immediate release system that delivers the drug immediately after a predetermined lag time. The lag time was contributed by the drug-loaded sustained-release plug, which releases the drug continuously during the lag period and helps to attain the peak concentration rapidly when the immediate core releases the drug. The formulation was designed as a time adjustable pulsatile release system containing atenolol/metoprolol as an active pharmaceutical agent for bedtime dosage administration and early morning release of the medicine to manage blood pressure. The core in cup tablets were prepared by direct compression method using different compression forces for each layer. The immediate-release layer was composed of atenolol/metoprolol, croscarmellose sodium, MCC, sorbitol, talc, and magnesium stearate. The SR plug was composed of HPMC, PVP, MCC, lactose, talc, and magnesium stearate. The formula was optimized using the least square method (JMP software).

Optimization of formulation

The core in cup tablet of metoprolol and the mucoadhesive core in cup tablet of atenolol were optimized using the least square method (JMP software). The independent variables for the immediate release core were compression force and the concentration of disintegrant used (croscarmellose sodium). The dependent variables were disintegration time, % drug release, and hardness. The least-square method generated the optimized concentration of the excipients and the required compression force to compose the IR core and SR plug to release the predetermined drug at the desired time. The compression force recommended for the IR core was 5 kg/cm^3 and the optimized disintegrant concentration was 15 mg. The HPMC concentration suggested was 5mg and the compression force proposed was 7 kg/cm3. The optimized metoprolol IR core tablet with 20 mg disintegrant concentration demonstrated a minimum hardness of 4.869 kg/cm^3, disintegration time of 3.475 min, and drug release percentage of 98.073. The desirability was 0.991. The disintegrant concentration and the compression force recommended for atenolol IR core tablets were 15 mg and 5 kg/cm^3, respectively. The predicted hardness was 6.10 kg/cm3, disintegration time was 4.75 min, and the drug release was 98.092 %. The recommended HPMC concentration for the SR plug of both the drugs were 5%, and the compression force was 7 kg/cm^3. The concentration of mucoadhesive polymer was selected by preparing a cup with three different concentrations of chitosan and evaluating the mucoadhesive strength and mucoadhesion time.

Preparation and characterization of metoprolol and atenolol core in cup tablet

Core in cup tablets of both atenolol and metoprolol were prepared as per the results of the least square method of optimization. The compressed core in cup tablets' hardness, thickness, diameter, friability, and average weight was evaluated. Further, the IR core, SR plug, and the influence of the cup on drug release were studied separately.

Metoprolol IR core released 98.379% drug in 30 mins, and atenolol released 99.491% of the drug in 30 min. To find any influence of the cup on the drug release from the core, studies were conducted with the 'core in cup' tablet without the plug layer (M1 and A1) and the IR core tablet (M2 and A2) alone. There was no significant difference in the drug release in the presence and absence of the cup layer. SR plug of both atenolol and metoprolol were able to release the drug for up to 360 minutes. Metoprolol SR plug released 98.341% of the drug at 360 min, and the atenolol plug released 99.364 % of the drug. The mucoadhesive strength of the atenolol mucoadhesive cup layer with 1%, 2%, and 3% chitosan was evaluated. The formula with 3% demonstrated the highest mucoadhesion strength. All three formulations stayed adhered to the mucosal membrane at least for 10 h. Hence the formula M3 with the highest concentration of chitosan was used to prepare atenolol mucoadhesive core in a cup tablet. The drug release from the metoprolol core in cup system was 22.89 % till 270 min when the plug was actively diffusing the drug. At 300 min, burst release of medication was observed, and there was a sudden drastic increase of drug release from 27.614-81.046%, and cumulative % release had reached 96.789 till 420 min, and drug release happened from the core formulation (IR) too. The cumulative drug release (%) data as obtained from the 'core in cup' tablet was compared to that of the marketed product Metatus 50 mg. The IR tablet showed a drug release of 98.396% metoprolol release in 30 min. A similar pattern of drug release was observed with atenolol mucoadhesive core in cup tablets. 29.698% of the drug has been released from the 'core in cup' formulation till 270 min. The formulation had no lag time till 270 mins. In 300 min, the drug release increased to 81.046% and reached maximum release (101.425 %) in 480 min. The drug release profile indicates that the created 'core in cup' tablet can be used as a pulsatile drug delivery device.

Drug release kinetics

The release kinetics of atenolol and propranolol from the core in cup fits well into the Korsmeyer-Peppas model as indicated by the value R2 value 0.962 for metoprolol and 0.9937 for atenolol, and the profile is linear. The

mechanism of drug release from the core in cup tablets follows diffusion and swelling. Water uptake and drug erosion studies demonstrated that the polymer swelling and erosion support the programmed drug delivery. The formulations proved their stability in accelerated stability studies.

In vivo drug release study in the rabbit model

In vivo studies were conducted in the rabbit model. The metoprolol core in cup system exhibits tmax at 300 min. While the tmax of the atenolol mucoadhesive system was 360 min, the Cmax of the metoprolol formulation was 95.76 ng/ml, and that of the atenolol delivery system was 97.3 ng/mL. The AUC of the atenolol mucoadhesive system was greater (22,759.8 min ng/ml) than that of metoprolol (19,628.6 min ng/ml). In the analysis, the atenolol mucoadhesive core in cup formulation exhibited a higher tmax, Cmax, and AUC compared to that of the metoprolol core in cup tablet.

The current study results suggested the suitability of the designed time adjustable core in cup formulation in the choropharmaceutical delivery of atenolol and metoprolol.

CHAPTER-7

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Research article

Preparation and Bio Pharmaceutical Evaluation of Chrono Pharmaceutical Drug Delivery System of Atenolol

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ABSTRACT

A time-adjustable pulsatile release system containing atenolol as an active pharmaceutical agent was developed for bedtime dosage administration and release of medicine in the early morning to manage elevated blood pressure. The system contained an immediate release (IR) core, a sustained-release (SR) plug, and a mucoadhesive cup layer and it was designed by the cup and core technique. The immediate-release layer was composed of atenolol, croscarmellose sodium, microcrystalline cellulose (MCC), sorbitol, talc, and magnesium stearate. The SR plug was composed of hydroxyl propyl methylcellulose (HPMC), polyvinyl pyrrolidine (PVP), MCC, Lactose, talc, and magnesium stearate. The mucoadhesive cup contains polycaprolactone and mucoadhesive polymer chitosan. Bilayer tablets were punched by applying different compressive forces. Based on the Release data of the drug from each layer F5 and S5 were selected for the preparation of the bilayer tablet. Cup composition containing 3% of chitosan provided the highest mucoadhesion time up to 10h. Pulsatile delivery of the drug from the formulation was observed at 300 min after the administration of the drug. The constructed pulsatile delivery systems were compared to that of commercially available atenolol IR tablet. The comparison demonstrated that the formulation is suitable for the intended chronopharmaceutical delivery of antihypertensive drugs.

Keywords: Chronopharmaceuticals, Pulsatile Drug Delivery, Hypertension, Mucoadhesive Polymer, Atenolol.

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INTRODUCTION

The goal of chronopharmaceutical medication delivery is to synchronize the illness's inherent chronological time and therapeutic administration [1]. When a disease exhibits chronological behavior, it is not required to maintain a constant drug concentration throughout the day, but it is necessary to deliver the optimum dose at a specific moment. Heart rate and blood pressure have a distinct diurnal pattern in both normotensive and hypertensive patients. The heart rate and blood pressure are higher in the early morning and lower at night while sleeping [2, 3, and 4]. According to previous studies, during the hours of 6:00 a.m. and 12:00 p.m., patients had a 29 %, 40 %, and 49 % greater risk of cardiac mortality, heart attack, and stroke respectively in the time span of 6.00 am and 12pm[5]. Conventional drug delivery systems release the medicine instantly and it has to be administered during the peak hours to maintain the drug concentration throughout the day. In such situations, chrono pharmaceutical drug delivery methods are user-oriented [6]. In the present study, a time adjustable pulsatile release system containing atenolol as an active pharmaceutical agent for bedtime dosage administration and early morning release of the medicine to manage blood pressure. Atenolol is a cardioselective beta-blocker used in normalizing the heart rate, reducing the strain on heart and blood pressure. Atenolol is a BCS class III drug with an elimination half-life of 6-7h, pKa Value of 9.6, and bioavailability of 45-50% [7]. This drug delivery system is aptly designed to release the drug adequately when required, to avoid the presence of a high dose of atenolol throughout the day in blood circulation, and to reduce the adverse reactions [8, 9]. The study involves designing of 'core in cup' drug delivery system in which the core tablet is an immediate-release tablet, the plug is a sustained atenolol release polymeric layer and the cup is a mucoadhesive polymeric layer. The mucoadhesive cup adheres to the mucosal membrane of the gastrointestinal tract lengthening the time of drug retention in the stomach. The direct compression method was used to make the core and the cup tablet. Objectives of the present study are preparation and evaluation of pulsatile drug delivery system of atenolol and comparison of the drug release profile with the marketed atenolol tablet.

MATERIALS AND METHODS

The drug atenolol was procured from Carbanio.com.

Microcrystalline cellulose [MCC), chitosan and sodium starch glycolate [SSG) were procured from Lobachemie. Talc, cross carmellose sodium [CCN) magnesium stearate, polycaprolactone, sorbitol, lactose and hydroxy propyl methyl cellulose (HPMC) were sourced from SD fine chemicals.

Pre-formulation studies

Pre-formulation is the primary step of product development in which the physicochemical properties of the active pharmaceutical ingredient and other excipients are studied before product development to check their physiochemical effects on the quality of product formulation and performance [10]. The pre-formulation studies conducted are as follows.

Analytical method development

Preparation of Calibration Curve

Spectrophotometer method is used to assay the drug. A standard calibration curve of pure drug atenolol was plotted with the concentration *Vs.* absorbance values measuring at λ_{max} of 224 nm. A series of diluted solutions were made with solvent methanol and diluent 0.2M phosphate buffer [11]. The linear regression curve is used to measure an unknown concentration of the drug.

Solubility studies

A saturation solubility study of atenolol was carried out according to BCS guidelines. In 500ml conical flasks, 25mg of atenolol was added to 250ml of each medium [0.1N HCl pH 1.2, distilled water pH 7.0, acetate buffer pH 4.5 and phosphate buffer pH 6.8). Flasks were shaken in an orbital shaker at 37°C for 48 hours. The samples were then filtered and diluted before being analysed with a UV spectrophotometer [12].

Melting point determination

The determination of the melting point aids in the identification of the material and its purity. The melting point device can be used to determine the melting point [13].

Drug – excipient compatibility studies using differential scanning calorimeter (DSC)

Using a differential scanning calorimeter, the thermograms of the drug and excipients were recorded. As a baseline, an empty pan was used. About 1 g of drug/excipients/drug and excipient mixture was weighed and sealed in a small aluminium pan and was heated to 20-400ºC at a rate of 10ºC/min with constant purging of dry nitrogen 50ml/min. A blank pan was sealed and used as a reference. DSC thermogram was obtained using automatic thermal analyzer equipment. DSC thermogram was analyzed to check the compatibility of the drug and the excipients [14].

Study of flow property Study of flow property (angle of repose, bulk and tap densities, Carr index, Hausner's ratio) of powder material

Angle of repose: The angle of repose was used to determine the frictional force in a loose powder, and the angle of repose was measured using the fixed funnel method. As a function of the powder flow property, the maximum angle (θ) permitted between the surface of the powder pile and the horizontal plane was determined [15] using the formula $\theta = \tan^{-1}(h/r)$, here, h is the height of the cone and r is the radius of the cone base.

Bulk density, ρ_b

It is the ratio of the mass of the powder (M) to the volume, V (including the inter-particle void volume) of an untapped powder sample, ($\rho_b = M/V_o$).

Tapped density (ρ_t) is higher than bulk density; it is obtained after mechanically tapping a cylinder containing the sample. It is calculated by the ratio of the mass of the powder (M) and tapped volume, V_t attained after tapping 100 times using mechanical tapped density tester ($\rho_t = M/V_t$).

Measure of powder compressibility

The Compressibility index (Carr's Index) is a measure of a powder's propensity to be compressed, based on bulk and tapped densities. In free-flowing powders, inter particle interactions are less, and bulk and tapped densities will be closer in value. Poor flowing materials are cohesive in nature, resulting in a wider gap between bulk and tapped densities [15]. The Compressibility Index, which is determined using the following formula,

$$Carr's\ index = \left[\frac{(\rho_t - \rho_b)}{\rho_t}\right] \times 100$$

Where, ρ_t is the tapped density ρ_b is the bulk density

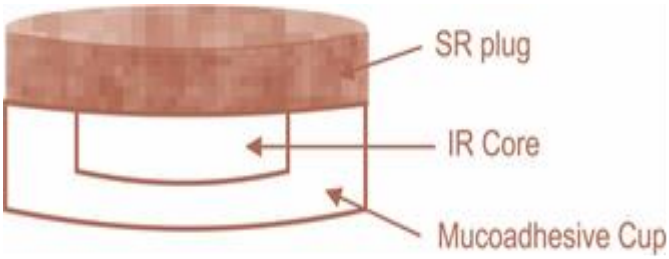
Hausner's Ratio: The flow ability of powders or granules is expressed by Hausner's ratio ($=V_o/V_t$), where V_o is the untapped apparent volume (cm^3) and V_t is the final tapped volume (cm^3) [15].

PREPARATION OF PULSATILE TABLETS

Formulation of core in cup tablet

A 'core in cup' tablet with a sustained-release (SR) plug layer, an immediate release (IR) core, and a mucoadhesive cup (figure 1) was prepared by direct compression method. The pulsatile core in the cup bilayer tablet was made with a 12 mm punch. With a low compression force (1 ton), the IR layer was compressed in a 6 mm punch. The cup material, a mixture of polycaprolactone and chitosan was filled into a 12 mm punch die and slightly pressed. The core tablet was placed in the slightly pressed bed and subsequently filled the gap between the die wall and tablet with polycaprolactone and chitosan mixture (110 mg). The top of the core tablet was then filled with the SR mixture and compressed to form a tablet (3 tone) [16, 17].

Figure: 1. Fabricated tablet for illustration



To achieve the desired drug release profile, a number of IR tablet trials with various concentrations of super disintegrants (cross carmellose sodium and sodium starch glycolate) were made (table 1). Various trials were also carried out to optimize the release profile of the SR layer and the mucoadhesive cup (Table 2 and 3).

Table 1: Different batches of immediate-release (IR) tablet

Ingredients	Composition of formulations (mg)					
	F1	F2	F3	F4	F5	F6
Atenolol	13	13	13	13	13	13
Cross carmellose sodium (CCS)	-	-	-	3	6	9
Sodium starch glycolate (SSG)	3	6	9	-	-	-
MCC	10	10	10	10	10	10
Sorbitol	37.1	34.1	31.1	37.1	34.1	31.1
Talc	0.325	0.325	0.325	0.325	0.325	0.325
Magnesium stearate	0.325	0.325	0.325	0.325	0.325	0.325
Total weight	63.75	63.75	63.75	63.75	63.75	63.75
Punch size	6mm					
Compression force	1 ton					

Table 2: Different batches of sustained release (SR) tablet

Ingredients	Composition of formulations (mg)					
	S1	S2	S3	S4	S5	S6
Atenolol	12	12	12	12	12	12
HPMC	10	15	20	10	15	20
PVP in alcoholic solution	2.8	2.8	2.8	-	-	-
MCC	60	55	50	62.8	57.8	52.8
Lactose	22	22	22	22	22	22
Talc	0.5	0.5	0.5	0.5	0.5	0.5
Magnesium stearate	0.5	0.5	0.5	0.5	0.5	0.5
Total weight	107.8	107.8	107.8	107.8	107.8	107.8
Punch size	12 mm					
Compression force	3 tons					

Table 3: Mucoadhesive cup layer

Formulation	Chitosan (mg)	Polycaprolctone (mg)
M1	1.1 (1%)	108.9
M2	2.2 (2%)	107.8
M3	3.3 (3%)	106.7

EVALUATION OF CORE IN CUP TABLET

Hardness test

The Monsanto hardness tester was used to determine the hardness of the tablets (force necessary to break the tablet). Hardness was measured by placing the tablet between the moving and fixed jaws of the apparatus and applying pressure. Six tablets were used in the experiment [18].

Friability test

Friability refers to a tablet's ability to endure mechanical stress during handling. It aids in determining the tablet's vulnerability to fragmentation, chipping, and other forms of damage. The tablets were placed in a friabilitor and spun at 25 rpm for four minutes.

Weight variation test

The average weight of 20 tablets was obtained after each tablet's weight was measured individually. The tablet passes the weight variation tests if not more than two tablets are beyond the prescribed limits, according to the USP specification [19].

$$Average\ weight = \frac{W_{total}}{n_{tab}}$$

Where W_{total} is the total weight of tablets and n_{tab} is the number of tablets

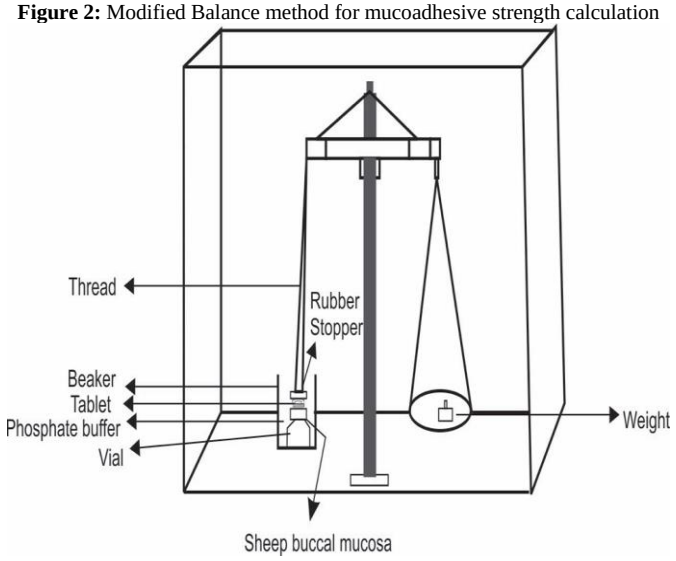
Disintegration test

The disintegration test apparatus was used to test the tablets' disintegration time. Water, 0.1 N HCl, were two distinct mediums used. The tablets were inserted in the apparatus's basket's six tubes. At a rate of 29 to 32 cycles per minute, the basket was pushed up and down in the media [20].

Mucoadhesive strength

The mucoadhesive strength of the 3 combinations of polycaprolactone and chitosan were measured. The force required to detach the drug from the attached membrane (porcine GI mucosa) was determined using the modified balance method. Porcine gastric mucosa with a thick layer of mucus was affixed to a rubber cork that had previously been attached to the vial in the bottom of a beaker with the relevant medium at a level slightly above the mucosa. The weight was attached to the tablet through a rubber beaker and brought into touch with the porcine mucosa and kept undisturbed for 5 minutes. The mucoadhesive strength was measured by adding weights to the right-side pan in incremental increments and recording the weight at which the tablet disengaged from the mucosa [21].

Figure 2: Modified Balance method for mucoadhesive strength calculation



Mucoadhesion residence time

The tablets must remain adherent to the gastro intestinal mucosa to retain the drug in GIT for certain period. Hence an *ex vivo* mucoadhesion test was used to determine the tablet's residence time in the gastro intestinal mucosa. Cyanoacrylate glue was used to adhere the sample of bovine GI mucosa on a stainless-steel plate measuring 8.5 cm x 5 cm. Further, each tablet was stuck to the mucosa with a specific pressure (500 g for 30 s). The prepared plate assembly was placed in a beaker containing 0.1N HCl at a 60° angle, then in a shaking water bath at 37°C and 15 rpm [22].

***In vitro* drug release studies**

Each drug layer (IR core, the SR plug, and the core in a cup tablet), was evaluated separately to study the drug release profile. The primary factor for selecting the optimal formulation among trials in each layer preparation was *in vitro* drug release. Each data is the average of three repetitions.

***In vitro* drug release from IR core tablet and SR plug**

In vitro drug release experiments for the atenolol core tablets and the SR plug layer were conducted individually using the USP dissolution apparatus II. The temperature was kept at 37ºC. 0.1 N HCl was chosen as the dissolution medium for IR tablets. The dissolution media was agitated at 50 rpm [23, 24]. At predetermined time intervals, a predetermined number of aliquots were removed for analysis and subsequently, replaced with an equal amount of fresh dissolution medium. A spectrophotometer set to 224nm wavelength was used to examine the aliquots.

***In vitro* drug release from bilayer tablet (core in cup tablet)**

In USP dissolution apparatus II, a dissolution test for the bilayer tablet was performed. The medium used for the first 300 min was 730 mL of 0.1M HCl solution. The tablet was then subjected to a 6.8 pH phosphate buffer solution by the addition of 0.2 M $Na_3PO_4.12H_2O$ into the existing media (25). A spectrophotometer set to 224nm wavelength was used to examine the withdrawn samples. Similarly, the drug release profile of the marketed atenolol IR tablet was investigated and compared with the core in cup formulation.

Modeling of drug release

To examine the drug release kinetics, the data were fitted into zero order, first order, Korsmeyer-Peppas, Higuchi, and Hixson-Crowell models (26).

Water uptake and erosion studies

Water absorption and erosion were studied using the USP dissolution apparatus type I. The weight of the empty basket and the tablets were recorded before starting the experiment. The tests were carried out in the same prescribed conditions as the dissolution test. At predetermined times, individual tablets were taken out of the medium. The basket and tablets were weighed following the completion of the test. The tablets were dried at 80ºC until they attain a constant weight. After they had cooled to room temperature, the tablets were weighed again. To compute the % erosion, the below formula was used (27,28).

$$\%\ erosion = \frac{W_i - W_f}{W_i} \times 100$$

Where W_i represents the initial weight of the tablet and W_f represents the final weight of the dried tablet.

The excess weight gain of the tablet at each time interval was used to calculate water uptake by the tablet.

$$Weight\ increased = \frac{W_{wet} - W_f}{W_f} \times 100$$

Where W_{wet} represents the weight of the wet tablet

Stability study

The stability tests on the tablets were carried out in compliance with ICH guidelines. The tablets were maintained in two different study conditions. One batch of tablets was kept in a desiccator for three months at 25°C and 60% RH in the presence of anhydrous calcium chloride in polypropylene containers. Another pair was kept in a stability chamber at 40°C and 75 RH for three months. Three months later, dissolution tests and the physical appearance of the tablets were evaluated [29].

In-vivo Studies: Preparation and biopharmaceutical evaluation of Chrono pharmaceutical drug delivery system of atenolol

Number of days each rabbit was housed for 3 weeks under standard conditions with a 12 h light/dark cycle with free access to water and a basal diet for 1 week. Animals weighing about 1 to 5 Kilograms was selected and divided into the above-mentioned groups. Further, they were subjected to fasting for 12 hours with access to drinking water. After 12 hours, the prepared formulations were administered (16mg/kg) through the oral route by keeping them in an upright position. Blood samples of 1 ml has been collected from the tail vein using a needle at 15, 30, 60, 120, 180, 240, 300, 360, 420, 480 and 540 minutes after administration. All blood samples were immediately put into heparin then it will be subjected to centrifugation at 4000 rpm for 10 min to separate the plasma. Then the plasma was frozen and stored at -20ºC until the analysis. The kinetic parameters including Cmax, Tmax, AUC was analysed. [35,36]

RESULTS AND DISCUSSION

Pre-formulation Studies

The graphical plot of absorbance (y) vs concentration (x) yielded the linear calibration curve equation (y = 0.0394x + 0.0204, R^2 =0.9971), which was used to calculate the amount of drug released in dissolution studies of different batches of products.

Solubility studies

Saturation solubility of atenolol was observed as 13.3±0.48, 12 ± 0.51, 10.12 ± 0.28 mg/mL at 25°C in double-distilled water (DDW), 0.1 N HCl, and pH 6.8 phosphate buffer. The drug was found to be sparingly soluble in water, practically insoluble in HCl and acetate buffer, and soluble in a pH 6.8 phosphate buffer.

Melting point determination

The melting point of atenolol was found to be 123-125ºC. It was comparable to the melting point reference provided by the USP.

Drug – excipient compatibility study using differential scanning calorimeter

DSC was used to determine the compatibility between drug and excipients used in the formulations. Individual components, such as atenolol, cross carmellose, sorbitol, MCC, HPMC, magnesium stearate, chitosan, and the physical mixture of drug and excipients,

were investigated using DSC. The thermograms of the physical mixture revealed no interactions, implying that there were no incompatibilities between drugs and excipients.

Study of flow property

(angle of repose, bulk and tap densities, Carr's index, Hausner's ratio) of powder material

Angle of repose

The flow characteristics and compressibility of powder mixes are critical in defining the quality and reproducibility of direct compression tablets. According to the USP, a powder sample with an angle of repose of 25-30º has a good flow property. All the powder mixes had angle of repose ranging from 28-30º. The angle of repose of all the formulations is given in table 4.

Bulk density

The bulk density of the powder mix affects the flowability of the powder. Low bulk density associated with agglomerated particles negatively influences the flowability and of the powder. The observed bulk density of the formulations is given in table 4.

Tapped density

Tapped density is more in the case of regularly shaped particles than irregularly shaped particles. Determined tapped density was mentioned in table 4. These values were used for the calculation of the Hausner's ratio and Carr's index.

Carr's index: Carr's compressibility index values have been used to define the flow properties of the powders. Carr's index for the studies was in the range of 10-14. These values indicate the excellent flowability of the powders.

Hausner's ratio

Hausner's ratio is related to the interparticle friction between the particles. For a satisfactory flow property, Hausner's ratio should be less than 1.25. Table 4 lists the IR tablet's Hausner's ratio [30].

Table 4: Flow properties of immediate release formulations

Evaluation parameters	Formulations					
	F1	F2	F3	F4	F5	F6
Angle of repose	29.36	28.01	28.69	29.05	28.25	29
Bulk density	0.25	0.27	0.28	0.25	0.30	0.21
Tapped density	0.29	0.28	0.29	0.31	0.34	0.29
Carr's index	13.8	10.69	12.88	13.31	12.89	10.70
Hausner's ratio	1.15	1.13	1.13	1.15	1.12	1.13

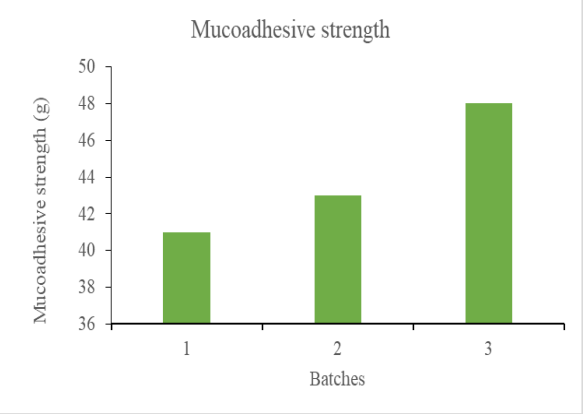
Evaluation of core in cup tablet

All of the quality control tests on the core tablet were successful. The tablet's thickness was 3.51±0.04mm, while its diameter was 6.08±0.01mm. The hardness (5.51±0.3 kg/cm^2) was within acceptable limits. The tested tablets had a friability of 0.25%. The average weight of 20 tablets was 284±0.9 mg. The core tablet disintegrated instantly in both alkaline and acidic environments. F5 disintegrated the fastest (1 minute) [31].

Mucoadhesive strength

Figure 1 depicts the findings of the mucoadhesive strength study. It was established how much strength (g) was required to separate the tablet from the membrane. The mucoadhesive strength of the cup layer is determined by the chitosan content. The formulation with the lowest chitosan concentration (M1) detached faster than the formulation with the highest chitosan concentration (M2). Among the other formulations, M3 had the strongest mucoadhesive strength.

Figure 3: Mucoadhesive strength of cup layer



Mucoadhesion residence time

All the three formulations stayed adhered to the mucosal membrane at least for 10 h. The layer containing 3% of chitosan stayed unchanged in its adhesion properties till the end of the study compared to the formulations containing 1 and 2% chitosan.

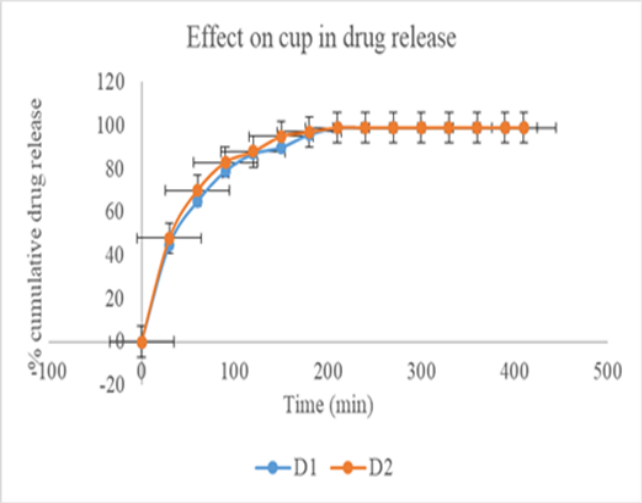
***In vitro* drug release studies**

The drug release from various formulations was measured using the USP dissolution apparatus II. The drug release from bilayer tablet components such as the IR core, the SR plug, and the influence of the cup on drug release were studied separately to identify the best formula. This study was successful in assessing the effects of polymers and identifying the best formulation for pulsatile drug release with desirable properties. The core tablet was initially studied to find the qualities of immediate drug release.

Effect of disintegrants on drug release from IR core

Figure 2 shows the *in vitro* cumulative release of atenolol from the IR core tablet in 1000 mL 0.1N Hcl at 37ºC and 50 rpm. To get an immediate release following the lag time, CCS/SSG was incorporated as a super disintegrant in the core tablet. Super disintegrants speed up the disintegration of direct compressible tablets and improve the solubility of medications that are difficult to dissolve. Formation of pore and swelling are two distinct mechanisms involved in the dissolution of CCS and SSG. The drug release mechanism is dependent on the concentration of super disintegrant. Disintegration will be minimal when swelling predominates (in case of 9 mg, Table 1). The formulation F5 with CCS (in case of 6 mg) delivered the medication in 30 min (94.62%). Whereas, formulation with SSG (F2) released 89.9% of atenolol after 30 min. The drug release from six trials of IR core tablets is depicted in the graph. From the release profiles, F5 was selected for further 'core in cup' tablet preparation [33].

Figure 4: Cumulative drug release (%) from IR core tablets

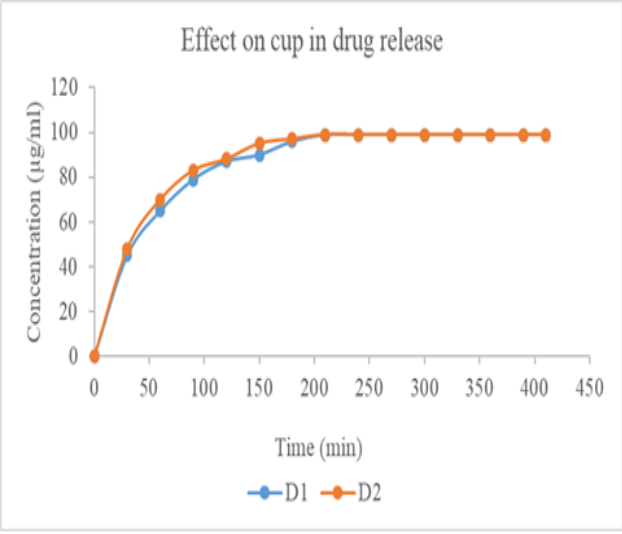


F1: 3mg SSG, F2:6% SSG, F3:9% SSG, F4: 3% CCS, F5: 6% CCS, F6: 9% CCS

Effect of the mucoadhesive cup in drug release

To identify the effect of the cup in drug release from the core in cup tablet drug release was studied with the 'core in cup' tablet without the plug layer (D1) and the IR core tablet (D2) alone. In both cases, the immediate release core was exposed to the dissolution media which facilitates the leaching of water into the core and immediate disintegration of the IR tablets. Because the drug release profiles of both D1 and D2 were similar, the influence of the polycaprolactone and chitosan layer on drug release was minimal, and the effect of the plug layer had to be investigated (Figure 5).

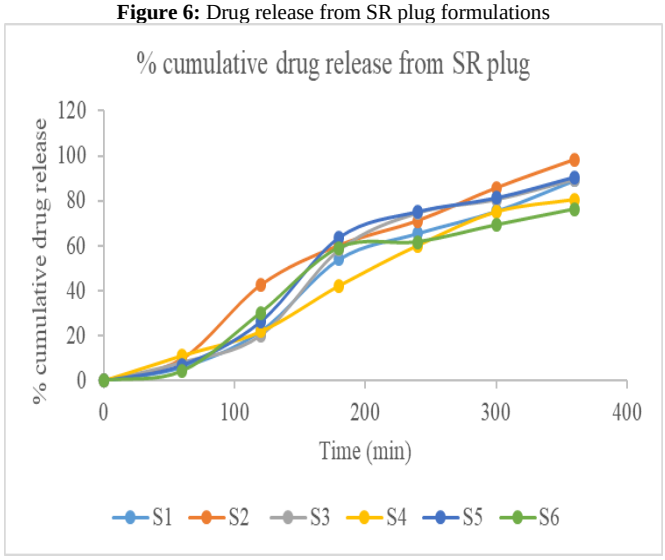
Figure 5: Effect of cup in drug release profile



Study of drug release from SR plug

The sustained release polymeric plug contributes to the lag period prior to the onset of drug release from the IR core. To maintain a low concentration drug in the blood during the lag period and to reach the therapeutic concentration faster, the tablet plug was made with a sustained-release polymer HPMC and atenolol. With different concentrations of HPMC and MCC, six distinct formulations were prepared. When HPMC comes into contact with water, it produces a viscous gel layer that aids in the sustained release of atenolol from the plug. The swelling expansion of the polymer prevents water from accessing the IR core, so HPMC produces a sustained drug release from the plug. As the swelling progresses, the plug's rigidity deteriorates, causing the plug layer to erode. All six plug formulations were able to release the drug for up to 360 minutes. Figure 4 depicts the influence of HPMC concentration on medication release. Formulation S2 released 98% of the drug at 360 min. Hence S2 was selected for the plug layer preparation.

Figure 6: Drug release from SR plug formulations



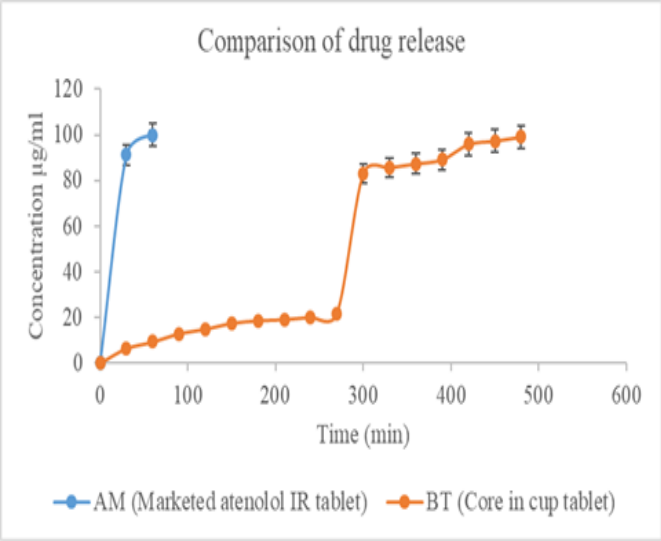
S1 (10mg HPMC and 2.8mg PVP), S2 (15 mg HPMC and 2.8mg PVP), S3 (20 mg HPMC and 2.8mg PVP), S4 (10% HPMC), S5 (15% HPMC), S6 (20% HPMC)

Drug release from core in cup tablet and comparison with marketed atenolol IR tablet

To make the final 'core in cup' tablet, compositions of F5 core tablet, S2 plug, and polycaprolactone and chitosan (M3) cup were used. Accordingly, a 'core in cup' tablet (BT) was prepared. It was observed that 22.89 % of the drug was released from the 'core in cup' tablets till 270 min when the plug was actively diffusing the drug. At 300 min, burst release of medication was observed, and there was a sudden drastic increase of drug release from 22.89-85.68%, and, cumulative % release had reached 99.1% till 420 min, as drug release happened from the core formulation (IR) too. The cumulative drug release (%) data as obtained from the 'core in cup' tablet (BT) was compared to that of marketed product (Devolol 50mg) atenolol IR (AM) tablets. The IR tablet AM showed drug release of 90.19 % of atenolol in 30 min in medium 0.1N HCl, whereas, 22.89% of the drug was released from the 'core in cup' formulation till 270 min in 0.1N HCl. The AM had no lag time, whereas the BT had a lag period of up to 270 min **(Figure 7)**. The calculated f2 value for AM and BT was 7. Since the f2 value was lesser than 50 the drug release profiles are confirmed different for

both the formulations. The drug release profile indicates that the created 'core in cup' tablet can be used as a pulsatile drug delivery device.

Figure 7: Drug release from IR tablet and 'core in cup' tablet

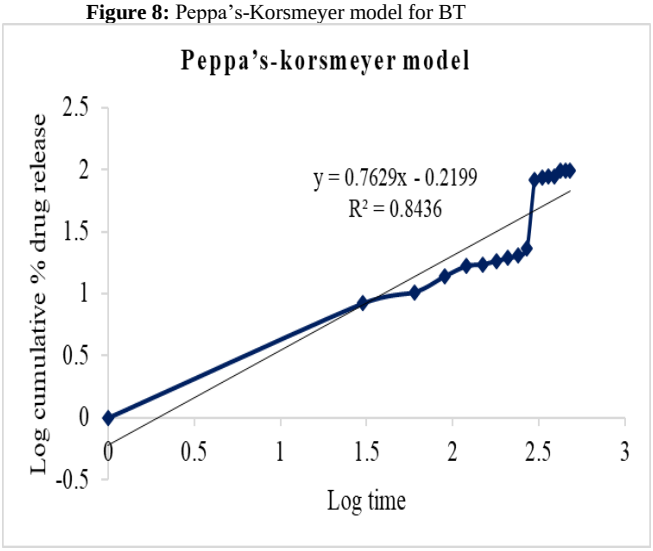


Drug release kinetics

Table 5: The correlation coefficient values for dissolution kinetics data (formulation BT)

Zero order R^2	Higuchi R^2	Peppa's-Korsmeyer		First order R^2	Hixon Crowell model R^2
		R^2	N		
0.6319	0.4867	0.9895	0.592	0.5248	0.5563

Figure 8: Peppa's-Korsmeyer model for BT



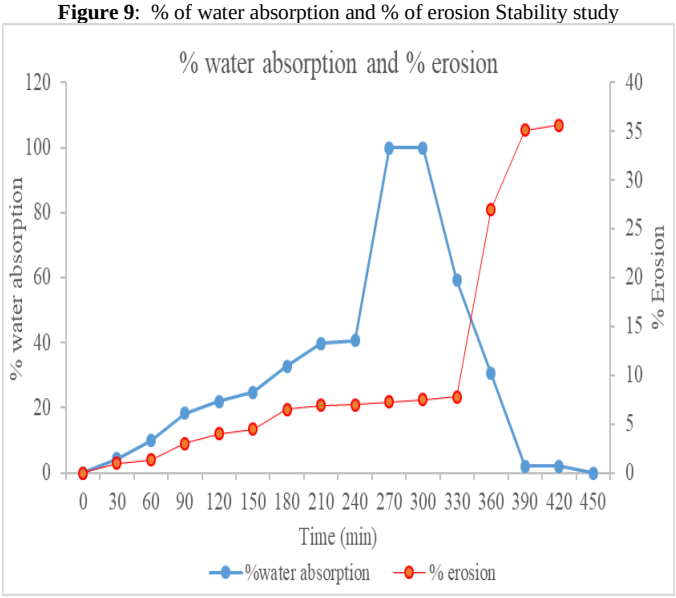
The release of atenolol from BT fits well into Korsmeyer-Peppas model as indicated by the value R^2 value (0.9895) and the profile is linear (Table 5 and Figure 8). It can be concluded that the mechanism of drug release from BT follow diffusion and swelling mechanism. The exponential coefficient (n) value was found to be in >0.5 indicating that non Fickian diffusion mechanism.

The release profiles exhibit biphasic stages as it involves release of drug from SR system initially at 0.1M HCl medium up to 270 min which is followed by burst release of drug and finally drug was released steadily in the last stage. In other models, linear profiles were not obtained.

Water uptake and drug erosion studies

The atenolol cup and core tablet were tested for water uptake and erosion in both phosphate buffer pH 6.8 and 0.1 N Hcl. When the tablet was placed in 0.1 N Hcl, HPMC plug layer absorbed the most water. The rate of water absorption increased gradually, reaching a maximum at time 300 min. The amount of water absorbed remained constant until the pH was raised in the phosphate buffer solution. There was a rapid drop in water absorption after 300 min, followed by the layer's quick disintegration. Figure 9 depicts the relationship between polymer water uptake and polymer breakdown in various pH solutions [34].

Figure 9: % of water absorption and % of erosion Stability study



Formulation F3 was kept for three months in both standard long-term storage (20°C, 60% RH) and accelerated stability (40°C, 75% RH). After the trial period, the tablets were examined for drug release, friability, hardness, and product look. The findings of the tablets' early tests showed no significant differences.

Table: 6 *In-vivo* concentration of Atenolol

Time	Concentration (ng/ml)
0	0
15	23.2
30	27.8
60	30.3
120	31.9
180	35.6
240	40.7
300	47.8
360	97.03
420	53.7
480	30.3
540	12.5

In-vivo Studies

Preparation and biopharmaceutical evaluation of Chrono pharmaceutical drug delivery system of atenolol

The kinetic parameters including Cmax, Tmax, AUC was analysed. [35,36] *In-vivo* concentration of atenolol with respective to the time was shown in the table 6 and their relative plasma drug concentration was illustrated in figure 10.

Figure 10: Plasma Drug Profile of Chrono pharmaceutical drug delivery system of atenolol

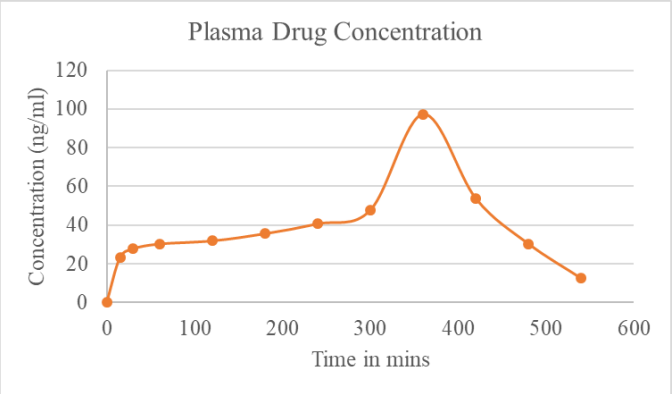


Table 7: Pharmacokinetic parameters

Tmax	360 mins
Cmax	97.3 ng/ml
AUC	22,759.8 mins. ng/ml

CONCLUSION:

A promising pulsatile core in cup drug delivery system was successfully produced with the desired lag time. The mucoadhesive chitosan polymeric cup proved effective in extending the drug's GI retention period up to 10 h. Throughout the lag period, the formulation was continually releasing atenolol at a low concentration (22.89%) from the sustained release plug (270 min). At 300 min, the drug was released quickly and instantly from the IR core, with the release of atenolol reaching 85.64 % in 30 min. Core in cup drug release profile was compared with the commercial atenolol immediate release tablet. The marketed atenolol tablet released 90.19 % of atenolol in 30 min without any lag time. The drug release profile from the core in cup tablet demonstrated that it is suitable for the chronological delivery of atenolol.

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Contemporary Technologies in Chronopharmaceutic Drug Delivery System

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Traditional drug delivery methods aimed for a consistent or sustained medication output to maximize treatment efficacy while minimizing side effects. These dosage forms release medications in a controlled or varied manner. Illnesses are treated by administering drugs to patients in a variety of traditional dose patterns. All these dosage patterns should always be administered monotonously for retaining the drug concentration in a therapeutically effective spectrum. Chronotherapeutics, a type of drug delivery system, has become increasingly important in the treatment of chronic diseases in recent years. Today's environment necessitates chronopharmaceutical formulations that increase patient compliance, optimize medicine distribution at the target site, and minimize side effects to reduce mortality rates. A mechanism in which a medicament has been distributed rapidly after a specified lag interval or time gap in compliance with the circadian rhythm of sickness conditions is known as pulsative drug release. Pulsatile medication delivery is becoming more prevalent these days. The main benefit of this method of the medication delivery system is that the substance is only aired when it is required. Because of this, the risk of developing drug resistance, which is common in both preparations for both conventional and sustained release, is minimized. In addition, certain anticancer medications are quite hazardous. In both traditional and sustained release therapy, these medicines cause serious

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complications. There are now a plethora of FDA-approved chronotherapeutic medications on the market. This treatment is most useful when a long-term effect is just not necessary and medications are harmful. The most important aspect of this formulation's development is determining the circadian rhythm or an appropriate criterion that would set off the drug's release.

Keywords: Chronotherapeutics; hypertension; circadian rhythm; pulsatile devices; OROS delivery; formulations.

1. INTRODUCTION

Oral administration of drugs to accomplish systemic impacts of disease such as hypertension is the most efficient remedy of administration. Systems for administering drugs orally account for around half of all drug delivery systems now on the market, and these approaches have a distinct value in terms of patient compliance and convenience of delivery. Traditional drug delivery methods aimed for a consistent or sustained medication output to maximize treatment efficacy while minimizing side effects. These dosage forms release medications in a controlled or varied manner. Illnesses are treated by administering drugs to patients in a variety of traditional dose patterns [1]. All these dosage patterns should always be administered monotonously for retaining the drug concentration in a therapeutically effective spectrum. Controlled distribution medication keeps the drug at a steady level rather than delivering it as and when it's needed. Conventional dosage formulations are inadequate to meet the needs of conditions if disease symptoms arise in a specific duration of day or night. To minimize the frequency of dosing and enhance patient adherence, modified-release formulations are required. Resistance, tolerance to drugs, and activation of the physiological systems because of the prolonged consistent drug concentration in the body are some of the issues associated with modified dosage form formulations.

In both health and disease, all functions in human are highly ordered in time as biological rhythms of various periods. Chronobiology deals with the internal biological clocks and the time. Chronopharmacology study the effect of biological timing on drug. Advances in chronobiology and chronopharmacology recommended drug delivery design based on the circadian rhythm. The chronopharmaceutical drug delivery systems deliver the drug over a set period of time based on the underlying activities of a disease. These drug delivery systems are designed to deliver the drug at a predetermined point of time. Pulsative dose formulations can help in the time programmed delivery of a drug. When steady plasma drug levels are not sought, nor is an ideal therapy regimen impact is obtained from a regularly varying drug concentration, pulsatile dose forms are beneficial [2]. Arthritis, asthma, cardiovascular diseases, peptic ulcer, hypercholesterolemia, diabetes mellitus, attention deficit syndrome are some of the diseases necessitate chronotherapeutic approaches in management [3]. Here we discuss about the diseases and the effect of circadian rhythm and the chronopharmaceutical drug delivery systems.

2. NEED OF THE CHRONOKINETIC DRUGS

The circadian time structure has received the greatest attention and is crucial in medical practice and patient therapy. Circadian rhythms play a role in the incidence of significant medical events such as myocardial infarction and stroke, as well as the expression and severity of chronic illness symptoms such as allergic rhinitis, asthma, and arthritis. Both physiological and biochemical rhythms have an impact on how patients respond to drugs, diagnostics, and therapy. The synchronization of disease pathophysiology and drug delivery is the basis of chronotherapy. Time specified delivery of drugs synchronized with biological rhythm significantly improves the therapy outcome. Control of adverse effects of the drug is one of the advantages of chronokinetic drugs, especially narrow therapeutic window drugs [4]. They reduce the frequency of drug administration and minimize the risk of toxicity. Patient compliance and a better therapeutic outcome id the benefits of chronokinetic drugs [5].

3. CHRONOPHARMACEUTICS DRUG DELIVERY SYSTEM INTENDED FOR ANTIHYPERTENSIVE AGENTS

A rise in blood pressure is thought to be responsible for 7.5 million deaths worldwide, accounting for approximately 12.8 percent of overall deaths. This translates into 57 million

DALYs (disability-adjusted life years), or about 3.7 percent of all DALYs. High blood pressure is, therefore, a significant potential component for coronary heart diseases, such as ischemic and hemorrhagic stroke. Blood pressure values have been correlated to a greater incidence of stroke and coronary heart disease consistently and beneficially. The probability of cardiovascular disease multiplies with each 20/10 mmHg elevation in blood pressure in specific age groups, starting at 115/75 mmHg. In conjunction to coronary heart disease and stroke, the consequences of high blood pressure include heart disease, peripheral arterial disease, renal disease, ocular hemorrhage, and vision deterioration. The lowering of cardiovascular problems is linked to managing diastolic and systolic blood pressure till it is less than 140/90 mmHg [6].

According to the World Health Organization, Hypertension affects 1.13 billion people globally, about two-thirds of them residing in low- and middle-income countries. Hypertension was diagnosed in one out of every four men and one out of every five women in 2015. Only around one out of every five hypertensive persons has their health in check. Worldwide, high blood pressure is the major driver of death. One of the worldwide non communicable disease targets is to reduce the incidence of hypertension by 25% by 2025.

Today's environment necessitates Chrono pharmaceutical formulations that increase patient compliance, optimize medicine distribution at the target site, and minimize side effects in order to reduce mortality rates. A mechanism in which a medicament has been distributed rapidly after a specified lag interval or time gap in compliance with the circadian rhythm of sickness conditions is known as pulsatile drug release [7]. Long-term treatment can lead to drug resistance, which can have harmful repercussions. The possibilities are limited since the proper medication concentration for particular times is available [2,3]. This technique is favorable as a result of the drug's significant metabolic first-pass and is directed to a particular location in the gastrointestinal track to accomplish the therapy objective.

4. PULSATILE DRUG DELIVERY SYSTEM (PDDS)

Pulsatile devices are getting prominence as they administer the medication to the optimal targeted site at the appropriate period and in the appropriate dose, allowing for spatial and temporal administration while also boosting patient compliance. In order to meet the requirement of quick medication release after a lag time, a pulsatile delivery system was designed. Within establishment of pulsatile medication release, the primary factor to consider is the circadian rhythm. When consistent drug release, zero order release of drugs, for example, also isn't preferred, the pulsatile delivery method can be highly useful. Because it is unaffected by pH, enzymes, or gastrointestinal motility, the pulsatile delivery system has been identified as a time-controlled release mechanism [8].

PDDS can be used to treat diseases that have a circadian rhythm underlying pathogenesis. It has been discovered that diseases have characteristic cyclic rhythms, and that pacing treatment regimens correctly can improve therapeutic outcomes in certain chronic disorders. Chronotherapeutic is a therapy strategy in which drug availability in vivo is scheduled in accordance with cyclic rhythms of drug-related biological processes to maximize benefit while limiting risk.

Time-controlled pulsatile release (regime with a solitary or several components), intrinsic stimulation driven release, as well as pulsatile release systems driven by external stimuli are the three primary classes of PDDS based on the pulse regulation of drug release. PDDS can also be divided into three categories based on the dosage form: tablets, capsules, and pellets. The cup and the core pills are suitable for the chronotherapeutics system.

Vascular reactivity and capillary resistance are significantly greater in the early hours and reduces later during the day in cardiovascular disease; platelet agreeability is enhanced, resulting in blood hypercoagulability; as a result of this reaction, the probability of myocardial infarction and unexpected cardiac death is higher early in the morning. Heart attacks are five to six fold higher to strike between the hours of 1 to 5 in the early morning and studies indicate that early heart attacks are more serious than those that strike later in the day. High blood pressure is usually the first symptom of cardiovascular disease.

Pulsatile delivery methods are divided into two categories: time-controlled and site-specific. The first group's release is mostly influenced by the

system, whereas the second group's release is primarily influenced by the physiological conditions in the gastro intestinal tract, such as pH or enzymes [9]. The word "Chrono pharmaceutics," which is a mix of chronobiology and pharmaceutics, is related to pulsatile medication delivery. Biological rhythms as well as its processes have been discussed in chronobiology. There are three sorts of mechanical rhythms in our bodies. The term "circadian" comes from the Latin words "circa" which means "around" and "dies" which means "day." [10,11]. Circadian rhythms in digestive, liver, kidney, and other body systems and their functions are crucial for treatments, such as deciding when to deliver medication based on pharmacokinetics, effect duration, efficacy, side effects, and favorable results [12].

5. DISEASES WITH EXISTING CIRCADIAN RHYTHMS

The disorders that have subsequently been targeted for pulsatile medication delivery have enough empirical support to justify the use of a timed pharmaceutical drug delivery system over traditional drug administration [13]. These illnesses include, among others, nighttime asthma, arthritic, duodenal ulcer, cancer, cardiovascular disease, diabetic, hyperlipidemia, and neurological issues.

6. BRONCHIAL ASTHMA

It's distinguished by airway inflammation, which causes the lower respiratory tract to be hyper responsive to numerous environmental stimuli [14].In asthmatic individuals, airway resistance rises gradually during night. This type of asthma, also known as nighttime asthma, is marked by an increase in symptoms, airway reactivity, and/or lung function [15]. During the day, antigen activates mast and eosinophil cells to release pro-inflammatory mediators, which leads to exacerbation of edema, smooth muscle bronchospasm, contraction, and over activation of mucus glands, resulting in mucus hypersecretion of the lung's restricted airways. Because bronchoconstriction and clinical signs intensification follow a circadian pattern, it is a great candidate for chronotherapy [16].

Bronchial asthma has a diurnal cycle, with symptoms peaking at night and early in the morning. Anti-asthmatic medicine dosing at night has been shown to be beneficial in bronchial asthma investigations. A solitary dosage of the majority of the pharmaceuticals now used for asthma chronotherapy at night helps patients adhere to their medications and manage their asthma better. Chronotherapy is necessary and helpful for Nocturnal Bronchial Asthma because of the day-night cycle and circadian rhythm interdependence [16,17]. Chronotherapy is gaining popularity because it allows doctors to provide drugs at certain times based on the disease's pathophysiology, which improves patient treatment efficacy and compliance. The purpose of bronchial asthma chronotherapy is intended to produce the optimal prospective outcomes: less asthma indications, the peak flow levels at their finest, the least number of medicine adverse effects, and the retention of regular or slightly elevated pulmonary function, lifestyle, exercise, and sleep in asthmatic patients [18-20]. Anti-inflammatory medication to manage airway inflammation, bronchodilator 2 agonist to ease bronchospasm and bronchoconstriction, and corticosteroid medication for particularly extreme forms of BA are some of the medications typically used for chemotherapy for asthma [21-23]. Synthetic glucocorticoids have been used as a short-term "burst" to achieve swift control of an asthmatic exacerbation, as well as protracted avoidance of symptoms in serious persistent asthma; inhibition, regulation, and inversion of inflammation [24]. Chronotherapy with inhaled corticosteroids (ICS) is a highly efficient anti-inflammatory treatment for the protracted management of chronic asthma. Inhaled corticosteroids can lessen or abolish the requirement for oral steroids [25].

The most widely administered corticosteroids for adult bronchial asthma are first-generation aerosol corticosteroids, such as beclomethasone, budesonide, flunisolide, fluticasone, and triamcinolone [26]. These medications minimize hyper responsiveness of the airways to antigenic as well as other asthma stimuli by inhibiting the production of cytokines that cause airway inflammation [27,28]. Tulobuterol, a long-acting beta2-adrenergic receptor agonist transdermal patch (Hokunalin Tape, patent: WO2005046600A2) for chronotherapy, was designed and developed by Abbot Japan Co., Ltd. This transdermal patch provides delayed release of the medicine for nocturnal asthma chronotherapy when placed at night. This 2 agonist's transdermal preparation was created to avert a quick elevated serum

tulobuterol levels, as well as adverse symptoms such as palpitation and tremor that can occur when taken orally [29,30]. Uniphyl® extended-release tablets manufactured by Purdue Pharmaceutical Products L.P. (patent; US 8431,553 B2) containing theophylline is used as an oral bronchodilator in asthma patients.

7. ALLERGIC RHINITIS

Sneezing, sinus rhinorrhea, itchy red eyes, nasal itching, and difficulty breathing are all allergic rhinitis indicators [31]. Each symptom was shown to happen more consistently preceding breakfast as well as in the morning, and less frequently in the afternoon. Allergic rhinitis can exhibit itself in two stages: early (In a few minutes) & late (within hours) (sustaining beyond 12-16 hours).The production of Prostaglandins, histamine, cytokines, TNF-A, chemotaxis factors and other substances causes sneezing, nasal irritation, and rhinorrhea in the early stages. Late phase is characterized by circulating leukocytes, T cells, and eosinophils proliferation, adherence, and incursion resulting in nasal obstruction and blockage as a result of the worsening of nasal, sinus inflammatory conditions as well as upper respiratory tissues [32].

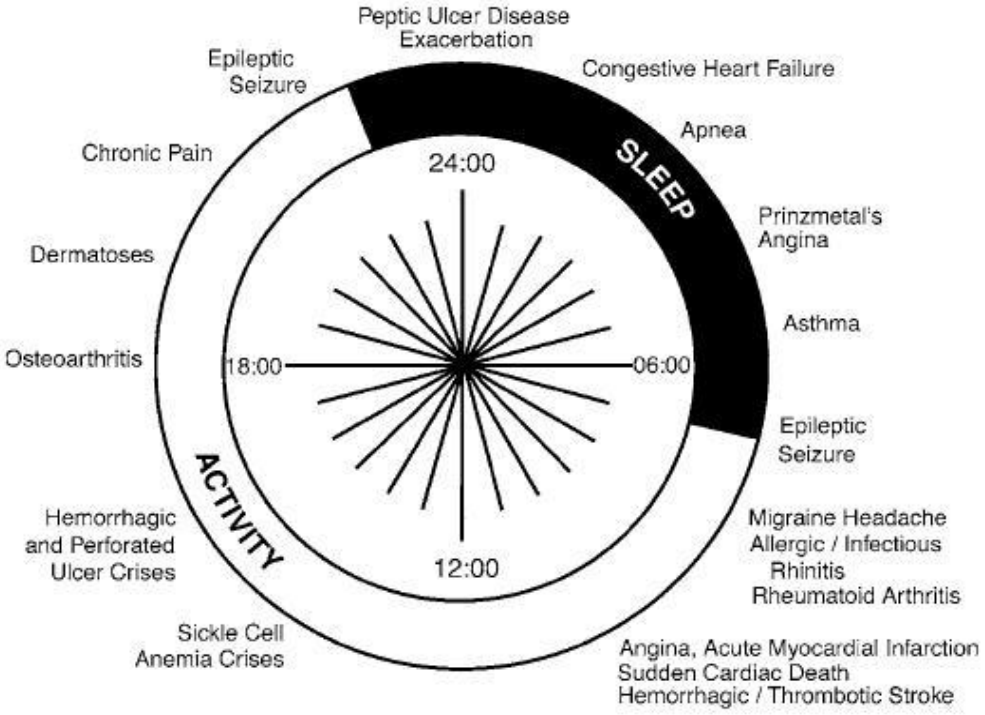


Fig. 1. Diseases that necessitate PDDS are depicted in a circadian rhythm diagram [4]

Table 1. The effect of circadian rhythm on physiological activity

S. No	Physiological activity	Influences in activity
1	Thermoregulation	Sleep deprivation, elevated cognition
2	Respiration	Sleep deprivation, elevated cognition
3	Blood pressure	Sleep deprivation, elevated cognition
4	Growth hormone	11 p.m. rise in secretion
5	Adrenaline	11 p.m. rise in secretion
6	Heart rate	Sleep deprivation, elevated cognition
7	Catecholamines	Significant raise in the morning
8	Agreeability of plasma	Significant raise in the morning
9	Activation of fibrinolytic enzymes	Reduced in the morning
10	Secretion of gastric acid	Significant raise in the evening
11	Gastric emptying	Significant raise in the morning

8. PAIN

One amongst the most significant treatment goals is pain management [33]. The greatest threshold was recorded at the completion in terms of resting time, whereas the lowest had been recorded well at conclusion of a interaction phase. The plasma concentrations of individuals having rheumatoid arthritis had higher levels of interleukin-6 and C- reactive protein follow a diurnal cycle [34]. In addition, nociceptors are activated by opioid peptides such as 5-hydroxytryptamine, bradykinin, glutamate, NO, substance P, cytokines, and prostanoids [33]. In a rat model, the proportion of substance P in the brain is greatest at night as contrasted to daytime. Endogenous opioid peptide levels are greater in the morning and significantly decrease in the late afternoon in those of neonates and human adult participants, according to research. Osteoarthritis patients experience reduced discomfort in the daytime and more anguish at bedtime. Patients suffering from rheumatoid arthritis experience discomfort which rises during early hours and gradually diminishes in the later period of the day. Finger swelling and joint soreness are two of the symptoms [12]. Patients with gastroesophageal reflux disease experience agony at night [33]. Renal colic, on the other hand, has a morning peak regardless of gender or the existence or lack of noticeable kidney stones. The type of analgesics used and how they are administered are determined by the nature and length of the pain. Anti-epileptic, local anaesthetics, and tricyclic antidepressants is employed to treat nociceptive pain, whereas anticonvulsants, paracetamol, NSAIDs, and morphinomimetics are used to treat neurogenic pain.

9. DUODENAL ULCER

In most duodenal ulcer patients, secretion of stomach acid is greatest in the late afternoon while decreases mostly in early hours [12, 34, 35]. The occurrence of perforation of an ulcer was evaluated (circadian), weeklong (circaseptan), and annual (circannual) period impact by one group of investigators [36]. Overall, a consistent and rather constant circadian rhythm has been discovered throughout seasons, decades, and days of the week. Perforations in the intestines had a primary rise about midday, as well as a subsequent rise towards fortnight, while duodenal perforations had the largest percentage in the afternoon. In most subgroups, the circannual pattern for duodenal ulcer perforation was defined by the 6-month cycle, with considerably greater prevalence during May to July as well as November and December. Although no circaseptan rhythm was discovered, Thursday – Friday had a much greater occurrence than Sunday – Monday. Gaster® tablet, a Famotidine delivery system manufactured by LTL Pharma Co., Ltd. support the chronotherapy in ulcer patients [37].

10. CANCER

There are several clock genetic traits associated in the stimulation of transcription and post transcription processes, as well as the inhabitation of regulatory loops in mammalian cells that create circadian oscillation [38]. CLOCK: BMAL1 or NPAS2:BMAL1 protein dimers, in particular, are important for the transcription of the clock genes Per and Cry. In a few mouse models, although, the clock genes Per1, Per2, Bmal1, and Rev-erb have been reported to be expressed. The desynchronization of specific tumor cells that form a solid tumor may cause clock gene-related rhythm changes at the tissue level.

Such a situation is caused by a variation of minutes or hours in each cell's intrinsic rhythm from its neighbours. Reduced expression of Per1, Per2, or Per3 genes at a single time point in contrast to reference tissue further supports a change in the molecular clock of human malignancies. Furthermore, cancer patients have a larger blood flow to the diseased location than the rest of the body [13]. Animal studies have also revealed that the rate of survival fluctuates depending on the anticancer medications' circadian dosage schedule [38]. When a combination of 6-mercaptopurine and methotrexate was given in the evening instead of the morning, the percentage of patients who survived was nearly quadrupled [12]. Another group of researchers investigated the effect of continuous 5-flourouracil (5-FU) infusion with circadian regimens of 5-FU administration, which exhibit peaks at 4 a.m., 10 a.m., 4 p.m., or 10 p.m. [9,39]. According to the findings, the cytotoxic effect of 5-FU on circadian delivery is minimal. Blood flow fluctuation at the rat's subcutaneous tumour location was also studied on a daily basis [40].

The results showed that tumour arterial circulation had been considerably maximum at night when compared during the day. While there

was no significant variations between the day and night groups of rats in average arterial pressure, cancer growth, or body mass. Normal tissue blood flows such as the subcutis, liver, kidney, cortex, bone marrow, and tumour tissues (SLC) had been examined during the day and at night [41]. Rats were employed as the test animals. There were no substantial differences in average blood flow between two distinct time zones in all normal tissues. Blood flow in tumour tissue, on the other hand, was much greater during the late night when compared to during the dawn. These data indicate that blood flow at the tumour site has a circadian pattern.

11. CARDIOVASCULAR DISEASES

Vascular reactivity and capillary resistance were more prevalent in the dawn and decline eventually during the day in cardiovascular disease. In the morning, platelet aggregability increases and fibrinolytic activity decreases, resulting in hypercoagulability of the blood. As a result, the risk of myocardial infarction and sudden cardiac death is higher between the hours of 10 a.m. and 12 p.m. [41]. The described blood pressure in ambulatory blood pressure measurement exhibits a considerable diurnal fluctuation. Peripheral variables like race, sexuality, tone of the autonomic nervous system, vasoactive substances, hematological, and nephro variables all influence this variation. Increased heart rate, blood pressure, autonomic tone imbalances, and circulating levels of catecholamines that control cardiac arrhythmias all display significant diurnal variation and contribute to the emergence of the circadian pattern of cardiac arrhythmias [42]. Atrial arrhythmias appear to have a diurnal pattern, with higher frequency during the day and lower frequency at night, and the aberrant foci appear to be subjected to the same long-term autonomic regulation as normal pacemaker tissue. According to the research, ventricular tachyarrhythmias have a late morning peak in individuals who have had a myocardial infarction in the distant past and an afternoon peak in those who have had a recent myocardial infarction. Myocardial ischemia, angina pectoris, acute myocardial infarction, and sudden cardiac death are likewise unevenly distributed throughout the course of a 24-hour period, with higher-than-expected incidents occurring in the late afternoon or early evening [43]. The circadian time of administration has been demonstrated to affect the pharmacokinetics and pharmacodynamics of several oral nitrates, calcium channel blockers, and -adrenoceptor antagonist medicines. Some of the available chronotherapneutic delivery systems available in the market for the therapy of hypertension are specified here. Verelan® PM (verapamil) extended-release capsules manufactured by RECRO Gainesville (US6500459B1) are prescribed for hypertension. It reduces the elevated blood pressure during the morning period [44]. Cardizem® LA-extended-release tablets contain Diltiazem HCL and Verapamil HCL, is used in the chronotherapeutic treatment of hypertension. InnoPran® XL-extended-release capsules of Propranolol HCL Verapamil HCL is a circadian aligned formulation for night time administration and to provide maximum effect in the morning [45].

12. DIABETES

Circadian cycles of insulin requirement and its activities are commonly asked questions in the instance of type I diabetes from a physiological and clinical standpoint [46, 47]. Insulin is usually delivered in a pulsatile pattern, however it can also be irregular. Insulin can have a cyclic rhythmicity of 8-30 minutes, indicating that it is performing at its best. Insulin release in the basal mode has both stimulatory and inhibitory effects on B cells. Stress hormones, cortisol, epinephrine, and growth hormone may impair target cell sensitivity to insulin action and hyperglycemia, while intrinsic rhythmicity, dehydration, and sustained insulin cessation may stimulate a secondary feedback signal on insulin release, that can aim to boost blood sugar levels. The modulators of insulin release and action are segregated in a circadian pattern, and the mode of insulin release is influenced secondarily. As a result, any change in plasma insulin concentration between a daily maximum and minimum, aside from its short-term rhythmicity, must be considered a complex secondary circadian rhythm. It's because of the variable secondary insulin resistance that occurs early in the morning and late in the afternoon [48].

13. HYPERCHOLESTEROLEMIA

During cholesterol production, a circadian rhythm occurs. Cholesterol synthesis is often higher at night than during the day. It differs from person to person at times. The maximum production occurs in the early morning, 12 hours after the last meal. Evening dose of 3-hydroxy-3methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors was found to be more

effective than morning dosing in studies. The rate-limiting enzyme HMG-CoA has a greater activity at night [49]. The diurnal changes, on the other hand, are caused by the regulating enzyme's periodicity or deterioration. Zocor® tablets, a controlled release simvastatin is prescribed for patients with hypercholesterolemia helps in the chronologically aligned drug delivery. Lipovas® tablet is another formulation of simvastatin (Cipla Ltd) used in hypercholesterolemia chronotherapy [50].

14. SLEEP DISORDER

Many biological signalling systems, such as sleep disorders in the central and autonomous nervous systems, have a complicated time structure with rhythm and pulsatile changes in multiple frequencies.

Each person's sleep requirements are usually consistent. Despite the fact that there is a lot of variance between people [51]. A rhythmic (circadian) mix of changes in physiological biochemical and physiological processes characterises sleep. A range of illnesses can occur when a circadian rhythm is disrupted or when particular processes during sleep are aberrant. Delayed sleep phase syndrome, for example, is characterised by severe sleep-onset insomnia [11]. Sleep is normally not feasible until 3 a.m. or later, or until waking up at the customary time is extremely difficult. The ability to adjust to the circadian rhythm varies among individuals as well. In managing with various sleep disorders, identifying individual variation is half the battle [52].

15. EPILEPSY

Some types of epileptic seizures may also be influenced by the circadian rhythm [53]. In several experimental animal models, the impact of the circadian rhythm on epilepsy of some partial seizures has been discovered. The approach for measuring the human circadian rhythm is also looked into. Behavioral chronobiology allows for the detection of possible new regulatory processes relating to epilepsy's core mechanisms [54]. Because of this, the circadian psychophysiological pattern of epilepsy reveals dynamic biological systems that suggest certain endogenous processes of intermodulation between observation and seizure susceptibility. Furthermore, the application of chronobiologic principles to epileptic behaviour suggests the emergence of new heuristic elements in comparative Psychophysiology [55].

16. ALZHEIMER'S DISEASE

Patients with alzheimer's disease experience changes in their circadian rhythm [56]. Individuals with Alzheimer's disease had lower diurnal motor behavior, a greater proportion of nighttime activity, worse inter-day motor activity stability, and a later activity acrophase (peak time) than healthy people. Alzheimer's disease causes neurodegeneration in the suprachiacsatic nucleus, which disturbs the brain's circadian cycles. Patients with this condition have a greater core body temperature. In this condition, the circadian irregularities are evident alongside cognitive and functional decline. There have been no other modifications assessed [57].

17. PARKINSON'S DISEASE

Parkinson's disease causes autonomic dysfunction, which causes many changes in the circadian rhythm of blood pressure, as well as increased diurnal blood pressure fluctuation and postprandial hypotension [58]. However, the presence of a circadian rhythm in this condition has yet to be determined. Clinical data demonstrate daily oscillations in motor activity patterns, but it's impossible to predict the impact on the course of the disease and the following functions of medications [59].

18. COAGULATION DISORDER AND THROMBOSIS

Life depends on the fluidity and preservation of blood inside the circulatory system [60]. The hemostatic system is formed by the actions and interactions of multiple variables that combine to generate these dual roles. Many components of the circulatory and haemostatic systems, such as muscle cells, the aorta, peripheral vascular muscle, and endothelium, have been revealed to have a circadian rhythm [61].

Circadian rhythm changes in time structure can cause hypercoagulability and thrombosis, or hypocoagulability and bleeding. Peripheral resistance, blood flow, blood viscosity, blood pressure, and heart rate are all factors that affect haemostasis. During the afternoon, peripheral vascular resistance reduced, resulting in an increase in blood flow in diurnally active personnel. In the morning, the vasomotor tone of coronary and peripheral arteries, as well as the vasoconstrictor response to adrenaline, are higher than in the afternoon. - thromboglobulin has a peak concentration at 6 a.m. and a low

concentration between noon and midnight. Factor VII has a strong diurnal fluctuation, peaking between 8 a.m. and noon, while its antigen level does not [62].

Factor IX is likewise said to be at its height around 9 a.m. Natural coagulation inhibitors such as protein C, protein S, and antithrombin have their highest concentrations at 6 a.m. and their lowest concentrations between noon and midnight. Although fibrinolytic systems show rhythmic changes, these may differ at the local tissue level [63].

19. INFECTIOUS DISEASE

Changes in the occurrence of infectious diseases over time are well-known [64]. Fever related to bacterial illnesses is more likely in the evening, whereas fever owing to viral infections is much more likely in the daytime, and influenza is epidemic throughout the winter season. In both the northern and southern hemispheres, it was found that morbidity and mortality were highest in the winter and lowest in the summer. The weight of nasal discharges is peak in the morning in cold patients, then decreases during the day until increasing slightly in the late evening. Though the cause of individual infectious disease seasonal patterns is complicated because several factors are implicated, seasonal cycles in infectious diseases are commonly credited to seasonal variation in weather/atmospheric circumstances, pathogenic or incidence of casual pathogens, and/or variations in host behavior [65].

Furthermore, the immune system, as well as the central nervous system, autonomous nervous system, endocrine glands, peripheral endocrine tissues, such as the intestinal tract and adipose tissue, and the immune system, have a variable time configuration with rhythms and pulsatile variants in multiple frequencies [66]. Biological indices such as cortisol, catecholamines, and melatonin have all changed. In diurnally active people, intraocular pressure, which would be a diagnostic hallmark of glaucoma, is high around 2 and 4 a.m. and least in the late afternoon [11].

Many endocrine factors require rhythmicity to have an effect. The rhythmic variability of a hormone or associated messenger determines its impacts and potency on a target tissue at multiple places [67]. Melatonin promotes the nighttime drop in body temperature that may be caused by vascular melatonin receptor stimulation, which causes peripheral vasodilation. Because of the nighttime reduction in body temperature, sleep is easy to come by. Plasma concentrations of prolactin show pulsatile episodic hormone secretion characteristics that are dominant after ultradian rhythms and circadian oscillation. During rapid eye movement sleep, the majority of the hormone is separated.

Regardless of the time of day, sleep beginning is accompanied with an upsurge in prolactin production during daytime sleep, but the intensity of the prolactin increase during daytime sleep is usually lower than during nocturnal sleep. Thyroid stimulating hormone is released in separate pulses as well. The incidence and extent of diseases have a circadian rhythm as well. At night, gout, gallbladder, and peptic ulcer attacks are more common [68].

In the morning, depression is more intense. Migraine headaches are usually brought on by rapid eye movement episodes that occur during sleep or early in the morning. The following markers of various diseases are useful in the design of a pulsatile drug delivery system because they will trigger the release of the medicine after a predetermined time interval. It can be used for single dose as well as multiple dosing. The importance of rhythms, particularly circadian rhythms, in physiology, pharmacology, molecular biology, and health sciences has grown dramatically in recent years [69]. When drugs are swallowed, injected, infused, or administered in any other way, the circadian time when they are taken, injected, infused, or applied in any other way seems to be a very significant indicator of its efficacy and safety. The circadian timing of drugs may even play a role in patient survival in life-threatening situations.

20. FORMULATIVE CHRONOPHARMACOLOGY REVIEW

20.1 Osmosis-Based System

The capsule is covered with a selectively permeable membrane in these systems. The capsule contains an insoluble plug, an osmotically active agent, and a medication formulation. When the partially permeable of the capsule shell comes into touch with GI fluid, gastric fluid can pass through. As a result, the plug swells, causing osmotic pressure.

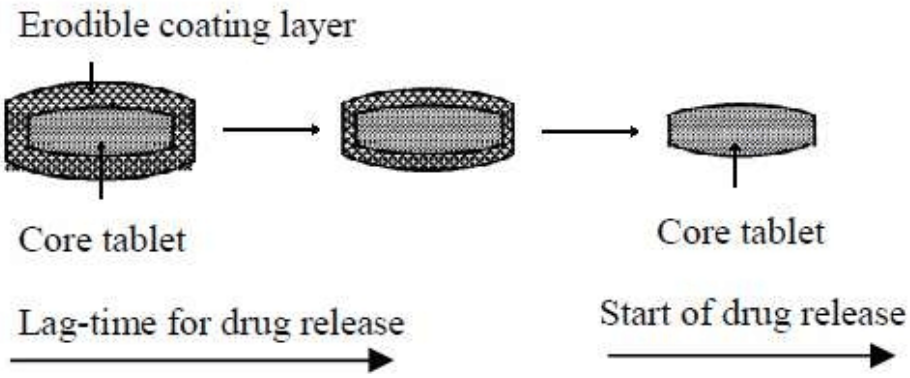


Fig. 2. Drug delivery with an erodible coating layer in a pulsatile drug delivery system is depicted schematically [70]

When such pressure surpasses the membrane's tensile properties, it bursts, and the time it takes for the membrane to rupture is characterized as lag period. After a certain amount of time has passed, the plug is ejected, allowing the medicine to be released. Barzegar-Jalali and colleagues Hard gelatin capsules loaded containing acetaminophen, sorbitol as just an osmotic agent, and sodium dodecyl sulphate as a release promoter were developed as an osmotic capsule. The capsule shell was sealed with white bees wax and covered with partially permeable cellulose acetate comprising lipophilic plasticizer (castor oil) [70] when a semipermeable membrane comes into touch with water, it allows water to pass through and increases the osmotic pressure within the shell. It raises hydrostatic pressure, causing the plug to fall outside the shell and the medicine to be released.

Linkwitz et al. used expandable orifice technology in their research [71] the medicine is given through the capsule's wall via this device that is in the type of a capsule. It is so tiny that the drug flow through the aperture is almost zero in a relaxed state. The flexible barrier is expanded when pressure is created within the shell. As a result, the orifice widens significantly from time to time allowing the medicine to be released in a pulsatile way. Up a different osmotic delivery capsule demonstrated pulsatile active medication release [72].

Rather than chemical compositions, the structure of pulsatile medication administration can be constructed. Within the capsule, a moveable barrier has been placed to separate the medicine from the osmotically active substances. Inside the capsule, impediments are put at regular intervals. Until enough pressure develops within the chamber to counteract the resistance, every barrier keeps the partition immobile. The partition then proceeds to the next point, where it is immobilised once more. During the advancing movement of the partition, the medication is released. Niwa et al. employed ethyl cellulose capsules for osmotic-based time-specific medication release with in colon [73, 74]. The impact of internal diameter on drug release was investigated by varying the thickness of the ethyl cellulose capsule body. Towards the base of the capsule structure, there were countless tiny holes. The hydroxyl propyl cellulose with low substituents was retained at the bottom of the body. A mixture of medication, bulking agent, and fluorescein was put above the hydroxyl propyl cellulose. After that, a powerful ethyl cellulose solution was used to cap and seal the capsule. Water permeates the capsule through micro pores when it comes into touch with G.I. fluid, causing the hydroxyl propyl cellulose to swell. As a result, internal osmotic pressure rises, producing capsule shell rupture.

The development of a once-daily controlled-onset extended-release (COER-24) dosage of verapamil hydrochloride depends on osmotic pumping [109]. COER-24 was a bipartite core tablet with an expandable polymeric compartment and a medication compartment, designed using OROS® Push-pullTM technology. A semi-permeable layer was applied to the core, with laser-drilled orifices linking the medication tablet to the outer medium. A hydrophilic layer is placed across the center as well as the outside membrane to extend the time between release and the commencement of the

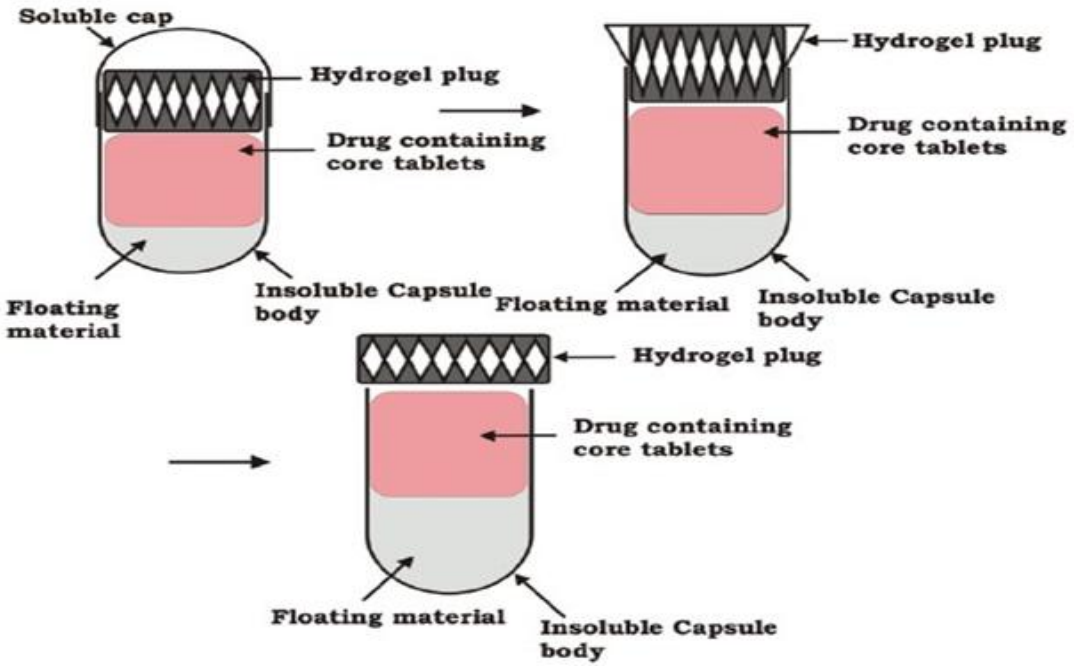
delay (Fig. 2). The active substance dissolved when exposed to water, and the push chamber began to enlarge. As a result, the medication solution was continuously pumped out via the semipermeable film's orifices. Verapamil was found to be released in a sustained manner over a period of 4-6 hours, with an excellent in vitro-in vivo connection [120]. Clinical investigations verified COER-24's potential for satisfying the well-established chronotherapeutic criteria of cardiovascular disease [121]. This technology is used in the chronopharmaceutical product Covera-HS (Pfizer, Patent: US6500459B1), which is currently on the market.

20.2 System Based on Capsule

The Pulsincap system is a capsule-based pulsatile system that is widely utilised. R.P. Scherer International Corporation, based in Michigan, developed it [74]. It comprises of a drug reservoir enclosed in an insoluble capsule. The drug substance is sealed inside the capsule body with swellable hydrogel plugs. Hydrogels of various viscosity grades, such as hydroxypropyl methyl cellulose, poly methyl methylacrylates, poly vinyl acetate, and poly ethylene oxide, are utilised to create such plugs. Lag time is determined by the length of the plug. The capsule shell's soluble cap, which is dissolved in the presence of dissolving or gastric juice, locks the entire system together. After ingesting gastric media, the plug swells as well as exerts itself beyond the capsule, releasing the medication.

Bussemer et al. created and tested a pulsatile drug delivery device based on a drug-filled hard gelatin capsule with a swelling layer and an exterior water-insoluble but porous coating [75]. The lag time increases as the diameter of the external coating film increases. With the inclusion of a hydrophilic pore forming and an increase in the diameter of the bulging layer, it can be reduced to a minimum.

Krogel and Bodmeier tested a pulsatile delivery system relying on an impervious capsule shell with an erodible plug [76]. The medication and excipients were first poured into the insoluble capsule body. Simultaneous interpretation or coagulating a meltable plug inside the capsule aperture were used to make the Plug. The lag time is determined by the plug's disintegration or erosion time. They changed the plug's composition later on [77]. Pectin and pectinase enzyme are used to make this plug. Because the enzyme is present in the plug, it degrades it. Super disintegrants have been employed in the formulation of capsule-based systems that include a medication, a swelling agent, and a rupturable polymer matrix [78]. The swelling compound is swelled to the point where the polymeric film is totally ruptured. All solid and liquid medication formulations can be delivered using this technique [79, 80].



Fig;3 System based on capsule

20.3 System with Eroidable, Soluble or Rupturable Membrane

A different notion has been used in this case. A dissolving or prone to erosion barrier is applied to a drug reservoir. The medicine is discharged from the reservoir when the barrier dissolves or erodes. The time clock ® system is made up of a solid dosage form covered with lipid barriers made up of carnuba wax and bees' wax, as well as surfactants like polyoxyethylene sorbitan monolate [81, 82]. At the aqueous environment, the layer erodes or emulsifies in a timeframe proportionate to the diameter of the coating, exposing the core for dispersion. In existence of intestinal enzymes, mechanical action of the stomach, or gastro-intestinal pH did not appear to alter the lipophilic film redispersion in a research with human volunteers, and the lag time seemed irrespective of gastric residence duration [73]. Because the lag time rises as the layer thickness increases, such approaches are ideal for water soluble drugs.

The main benefit of this system is that it is simple to manufacture and does not require any special equipment; yet, lipid-based systems can have a lot of in-vivo variation (e.g. food effects). Since water permeates and breaks the medicine in erosion-controlled systems, the drug may be released prematurely. The solubilized medication disperses out via the barrier layers as well as releases over time without eroding or dissolving the barrier layer completely. As a result, the medication release is slowed in a pulsatile manner.

A drug-containing core is covered with lipophobic swellable hydroxypropylmethyl cellulose (HPMC) in the chronotropic system [83]. After a predetermined amount of time, the barrier coating dissipates or diminishes. The thickness of the surface coating as well as the viscosity grade of HPMC can actually affect the lag time before release of drug out from reservoir-type [84]. Furthermore, using an exterior gastric resistance enteric membrane frequently can help to address the variation in gastric emptying and generate colon-specific release [85]. The drug was released following a lag time through the in vitro release profiles of enteric coated anti-prime tablet, and the pharmacokinetic results corroborated a lag period earlier to the availability of significant levels of drug in saliva. The lag times and the amount of hydrophilic retarding polymer applied had a good correlation. Both pills and capsules can be used with this technique [86, 87, 88].

Two drug-containing layers were separated by a drug-free gellable polymeric matrix in a three-layered tablet [89, 90]. Mostly in existence of dissolution media, the initial dose out from uncoated layer is released swiftly. The dosage is wrapped on three sites with impervious ethyl cellulose as well as the top section was left uncoated. The gellable barrier layer then makes contact with the dissolving media. Because of the contact with water, this barrier transforms into a viscous gel, decreasing its resistance qualities. The length of time it takes for water to travel through the barrier layer is determined not only by its composition, but also by its thickness [91].

Due to consistent gastric emptying, reduced chance of dose dumping, adjustable release patterns, and enhanced bioavailability with reduced inter and intra structural variability, multiparticulate systems are attracting a lot of interest in pulsatile drug administration [93]. The core containing the medicine is coated with a polymeric coating that erodes over period as a lag time [92]. This approach has been used to coat the core of pellets containing pharmaceuticals with hydroxypropyl methyl cellulose (HPMC). Ethyl cellulose (EC), hydroxuypropyl methyl cellulose phthalate (HPMCP), and a plasticizer diethylphthalate made up the outermost erodible layer. Because the EC to HPMCP ratio was 1:1, holes formed as a result of HPMCP flushing inside the intestinal region. To obtain a lag period of 3.5 to 5 hours and, most likely, to prevent early drug release, a significant covering level (weight gain of about 40%) is required. Patients improved significantly in a clinical trial using capsules containing pellets and the medication nizatidine. Mini tablets were made in a different way by pressing elevated viscosity HPMC and microcrystalline cellulose in varying ratios to achieve different lag times [94, 95].

To achieve the appropriate lag durations, eroding systems typically require thick eroding layers, particularly when such pellets are small. The issue is that water-soluble drugs can sometimes leak through the inflated barrier. On either hand, due to persisting barrier components, medication release after a lag period is sustained. In contrast to a erodible or swellable coating system, a rupturable membrane system relies on the coating disintegration to release the medication. Effervescent excipients, swelling agents, or osmotic pressure can all be used to provide the pressure required for the coating to rupture. This system is protected by a membrane that is water insoluble but permeable.

Many gadgets have been coated with an interior swellable layer and an outside rupturable layer in recent years. The thickness of the swellable layer controls drug release, however the coating layer's elasticity as well as tensile strength may also play a role. In a tablet core coated with ethyl cellulose, Krogel et al. utilised an effervescent combination of citric acid with sodium bicarbonate [96]. When carbon dioxide reacts with water, it causes the outer layer to break, allowing the medication to escape. A drifting pulsatile system is another name for this system. Tartaric acid can be used in place of citric acid. The lag time is determined by the firmness of its core tablet, not the coating thickness. This method can be applied to systems with several units. The swellable layer is applied to non-pareil sugar seeds in multiparticulate systems, accompanied by a water-resistant bur porous layer [97]. Based on the overall weight of the core, 5 to 60% of the medication is contained in the core. To quickly break the outer membrane, super disintegrants such as sodiumcarboxy methyl cellulose, sodium starch glycolate, L-hydroxypropyl cellulose, and polymers such as poly vinyl acetate, poly acrylic acid, and polyethylene glycol are commonly utilised. Varying the coating thickness and adding high volumes of lipid soluble plasticizer with in the outer layer might modify the lag time. A considerable portion of osmotic agent is used to achieve rapid medication release.

20.4 System with Change in Membrane Permeability

Pellet cores covered with amino-methylacrylate copolymer and containing medication and succinic acid yielded a sigmoid kind of releasing pattern [98, 99]. The succinic acid is dissolved by the water in the medium. The polymer film's permeability is increased by the medication and the acid environment inside. Actually, the presence of various counter-ions in the medium can alter the porosity as well as water uptake by acrylic polymers carrying quaternary ammonium groups [100]. The polymer side chain of Eudragit RS 30D comprises a positively polarised quaternary ammonium group, which is invariably accompanied by negative HCl counterions. The hydrophilic character of the ammonium group improves the contact of the polymer with water, resulting in a change in permeability and regulated water penetration of the active core. The drug permeability of the Eudragit film is also influenced by a little volume of sodium acetate in the pellet core. This causes the complete dose to be released in a matter of minutes. This system is utilised to create a core that contains acid.

21. DIFFERENT CHRONOPHARMACEUTICAL TECHNOLOGIES THAT HAVE RECENTLY BEEN MADE AVAILABLE

21.1 OROS Technology

Chronset™ is now a patented OROS delivery device that delivers a bolus medicine dose to the gastrointestinal system in a time- or site-specific way. It's purely an osmosis-based mechanism. The active medication is stored in a reservoir that is enclosed by a selectively permeable membrane that has been laser perforated with a delivery aperture and then formed into a tablet. This tablet is made up of two layers: one medication surface as well as another osmotically active ingredient. The osmotic agent transforms between non-dispensable onto dispensable viscosity when it comes into contact with GI fluid. As a result of the osmotic agent's pump effect, active medication is pushed out of the channel. It is commonly employed in the development of extended-release tablets [121]. Procardia XL®, Ditropan XL® and Concerta® are some of the examples of OROS systems.

22 OROS ORAL DRUG DELIVERY TECHNOLOGY

22.1 Ceform Technology

It creates pharmaceutical substance microspheres that are equally sized and formed. This method is based on "melt-spinning," which involves processing solid substrate (i.e. biodegradable polymer/bioactive agent combination) using a mixture of temperature, heat fluxes, mechanical stimuli, flow, and flow rates. The microspheres are virtually completely spherical, with a diameter of roughly 150-180mm, and may hold a large amount of medication. Pills, capsules, suspensions, effervescent tablets, and sachets are just a few of the dosage forms that the microspheres can be employed in. The microspheres can be coated including an enteric coating for controlled release or mixed as a fast/slow release mixture.

22.2 Contin Technology

A molecular coordination complex is formed between cellulose polymer and then a nonpolar solid aliphatic alcohol. A polar solvent is used to

dissolve the polymer at first. An aliphatic group may be substituted with alcohol if desired. This alcohol is preferably applied as a melt to the solvated polymer. It then forms a coordination complex that can be used as a scaffold in controlled release formulations because of its consistent porosity that can be modified. It can also be used to create controlled-release tablets. This method has a high level of control over drug delivery into the bloodstream, lowering the risk of undesired side effects.

22.3 Diffucaps Technology

This technique consists of a capsule-based system including one or even many drug-containing components (e.g. beads, pellets, granules etc.). Each bead has a pre-programmed quick or sustained release profile, as well as a lag time. It has already been addressed in a system having a membrane part that is erodible, soluble, or rupturable. AMRIX (Cyclobenzaprine) is a delayed release capsule developed based on Diffucaps technology which is used in muscle spasms.

22.4 Chronotopic Technology

This is also considered in the perspective of a membrane system that's erodible, soluble, or rupturable. It's basically a drug-filled core with a release-controlling coating on top. The internal drug formulation has included solitary as well as various dosage types including tablets as well as capsules, minitablets and pellets.

22.5 Egalet Technology

It has a delayed release since it is made up of an impenetrable layer containing two lag plugs enclosing an active drug plug in the centre. The medication is released after the inner plugs have eroded. The lag time is determined by the time it takes to dissolve the inner plugs. The shells are made of a mixture of pharmaceutical excipients, including polymers like poly-ethylene oxide, and plasticizers (such as cetostearyl alcohol). The matrix of the plugs is made of a mixture of gradually compostable polymer (e.g. ethylcellulose) as well as plasticizers (such as cetostearyl alcohol) (PEO).

22.6 Codas Technology

The CODAS (Chronotherapeutic Oral Drug Absorption Device) is a miltiparticular approach for bedtime dosage. A non-enteric coating is put to drug-loaded beads in this case to postpone the release of the medicine for up to 5 hours. A blend of water-soluble as well as water-insoluble polymers is used in this release control. When the water-soluble polymer in this dosage pattern comes into contact with fluid, it progressively dissolves and pores form on the coated layer. The medicament diffuses thru the holes that arise. The controlled release method of verapamil is maintained by a water-insoluble polymer serving as just a barrier. pH, posture, and diet have no effect on the rate of release. Verelan® PM release verapamil after 4-5 h of administration. The dosage form was designed based on the Codas technology.

22.7 Geoclock Technology

Geomatrix technology is used to develop the concept. For continual medication release in this technique, a multilayered technology was initially advocated. One or both bases are partly covered with an active core or hydrophilic matrix. The core hydration mechanism is adjusted, and the outer layer susceptible for drug release is reduced. Its barrier layer swells to become a gel in the presence of dissolution media. Its gelling layer is not degraded; instead, it functions as a regulating membrane that regulates the release process. Instead, the erodible surface is gradually removed from the dissolving solvent. As the active core erodes, more planar surface of the active core is exposed to the outside environment for longer periods of time, facilitating drug release. LodotraTM developed based on Geoclock technology is Nitec's single-pulse delayed-release tablet available.

22.8 Port Technology

The PORT (Programmable Oral Release Technology) system is a specially coated, encapsulated technology that allows for numerous medication releases. It has a polymeric core that is coated with a rate-controlling, semipermeable polymer. To provide consistent controlled release from the dosage form, poorly soluble medicines can indeed be coated with solubilizing agents. The gelatin capsule is treated with a semipermeable, rate-controlling polymer in capsule form. Inside the capsule shell, the active medication is combined with an osmotic agent. The capsule shell is sealed by a water-insoluble stopper. Depending on the situation, an immediate release chamber can be incorporated [101, 102, 103,104].

22.9 Three Dimensional Printing (3dp) Technology

It's a complicated oral dose administration technique that's new. It's made using a solid free-form construction technique. Internal geometries with complex internal geometries, changing densities, diffusivities, and chemical properties are beneficial in the design of such a device. Three-dimensional printing technology has been utilised to create complex dosage forms such as immediate-extended-release tablets, pulse release, breakaway tablets, and twin pulsatory tablets. Diclofenac sodium was printed in two separate regions on the enteric dual pulsatile tablets, which were made up of one uninterrupted enteric excipient phase. In vitro, these samples revealed two surges of releases with a 4 hour lag time between them. TheriForms' technology is based on this technique. It also is a microfabrication technique that functions similarly to a "inkjet" printer. It's a computer-aided design and production process that's totally integrated. Before the actual application of the preparation process, products can be designed as three-dimensional models on a computer screen.

22.10 Timerx Technology

It's a controlled release device made of hydrogel. With zero order to chronotherapeutic release, this technology can help. By changing molecular interactions, it can provide varied release kinetics. The "molecular engine," according to the author, eliminates the need for sophisticated processing or unique excipients, allowing desired drug release patterns to be "factory configured" after a straightforward formulation creation process. Basically, xanthan and locust bean gums are combined with dextrose in this technology. In the presence of water, the physical interaction between these components creates a strong, binding gel. Its degree of water penetration from the gastrointestinal tract into the TIMERx gum matrix, which swells to create a gel and then releases the active therapeutic component, controls drug release.

22.11 Physico-Chemical Modification of the API

To achieve a chronopharmaceutical effect, physico-chemical properties of the API (active pharmaceutical ingredient) such as solubility, drug lipophilicity, partition co-efficient, crystalline form, membrane permeability, melting point, and so on can be modified by introducing new substitutions to the original structure. The maximum plasma concentration of a medication (Tmax) changes depending on the parent compound's physiochemical modification. The Tmax of lovastatin is 2 hours, but the Tmax of simvastatin is 4 hours. Pulsatile medication delivery can also be achieved using the pro drug method. Lactone prodrugs like lovastatin & simvastatin are converted to potent hydroxyl acid molecules in the liver. Lactones are indeed water soluble than various statins since they are lactones. Pepcid® tablets, Zocor® tablets, and Gaster® tablets are some of the chronotherapeutic systems work based on Physico-chemical modification of the API [122].

22.12 Chronomodulated Infusion Pump

These devices are light in weight and have high accuracy drug delivery values. An insulin-containing implantable infusion pump is surgically implanted inside the left top or bottom quadrant of the abdomen's subcutaneous tissue (above or below the belt). The insulin is delivered intraperitoneal via a catheter that runs from the pump thru the muscle layer and into the peritoneal cavity, where it floats freely. It is replenished once monthly or every three months by putting a needle into the pump via the skin under the supervision of a physician. Melodie™, Rhythmic™, Panomat™ V5 and Synchromed™ are some of the examples.

22.13 Microchip with Controlled Release

The solid-state silicon microchip is a microfabrication process that uses micrometre scale pumps, valves, and flow channels to distribute active medication in a pulsatile way. It can release single or several chemical substances in a controlled manner depending on the situation. The electro-chemical dissolution of anode membranes covering a micro reservoir packed with chemicals in solid, liquid, or gel form provides the basis for the release mechanism. Proof-of-principle release investigations with a prototype microchip employing gold as the electrode material and saline as the release medium indicated regulated, pulsatile release of chemical compounds with this device.

22.14 Chronopharmacokinetics: The Drugs Circadian Rhythm

Chronopharmacokinetics is the analysis of observed changes in pharmacokinetics and

hence considers the impact of administration time on these several processes. Sequential differences in absorption of drugs as from gastro-intestinal tract (because of changes in gastric acid output and pH, mobility, gastric emptying time, and digestive blood circulation), plasma protein binding and drug distribution, drug metabolism (temporal variation in enzyme activity, hepatic blood flow), and renal drug excretion can all be affected by temporal variations (due to variation in glomerular filtration, renal blood flow, urinary pH tubular resorption.) As a result, the timing of drug administration is a major source of variance that must be included in kinetic investigations, necessitating the use of specific chronokinetics methodology [105, 106, 107].

23. WHEN DO WE NEED CHRONOKINETIC STUDIES?

There are some instants in which chronokinetic study is needed:

- Whenever necessary, each day pharmacokinetic fluctuations may be to blame for time-dependent alterations within medication effects (For example, some antimicrobials are much more efficient at certain times of the day),
- Whenever the drug's therapeutic range is limited, or when disease symptoms are clearly related to the 24-hour clock. (Examples include nocturnal asthma, angina pectoris, myocardial infractions, and ulcer disease)
- When it comes to therapeutic action of a medicine is well connected with its plasma concentrations, even though the latter is circadian phase dependent.
- Whenever a medicine has a major detrimental effect which can be prevented or diminished by changing the time of delivery (e.g. aminoglycosides, nephrotoxicity).

23.1 Chronokinetic Drugs

23.1.1 Antihypertensive drugs

Within 24 hours of the day, nearly all physiological functions and pathological events, including the cardiovascular system, show repeatable rhythmic alterations. Antihypertensive drug effects on blood pressure and heart rate rhythms in addition varies with the duration of day, according to clinical chronopharmacological investigations. Daily variations in the kinetics of propranolol, oxprenolol, nifedipine, verapamil, and other drugs were also identified in chronopharmacokinetic studies. Cmax was greater and/or Tmax was narrower following dawn administration of these lipophilic medicines than nighttime dosing. Regardless than whether or not daily fluctuations in the kinetics were discovered, the dose-response association has mostly been time-dependent [108, 109].

23.1.2 Antibiotics

The kinetics of antimicrobial medicines have been found to vary over time in many investigations. The period that perhaps the levels of antibiotics remain higher than the MIC (T>MIC) is the greatest critical element to ascertain the invivo effectiveness of antibiotics such as beta-lactams which have lethal effects independent of concentration in vitro, according to experimental animal models. As a result, regular changes in pharmacokinetics may be to blame for chemotherapeutic effect impairment. When low-susceptibility microorganisms are engaged in the infectious process, this is critical [110]. Another crucial component of antibiotic chronokinetics is that, as we saw with aminoglycosides, not just efficacy of the medicine but even its toxicity can change influenced by the time during the day.The following are the most notable findings from antibiotic chronokinetics studies:

23.1.3 Aminoglycosides

While aminoglycosides were administered in the midst of the experimental animals' rest time, the toxicity was at its peak, whereas as they were medicated in the course of the active process, the toxicity was at its lowest. The renal function toxic effects of aminoglycosides can be decreased by delivering the medicine with a single shot per day while Patients are engaged, according to numerous studies and evidence in the current literature (at day time). The working mechanics underlying the temporal variance in aminoglycoside kidney toxicity remain unknown [111].

23.1.4 Gentamicin

The efficacy and toxicology of gentamicin differed over course of a 24-hour period, with the efficacy being highest when the drug's toxicity was lowest. Thus, giving gentamicin early or late

in the day in humans may lower kidney toxicity and boost the effectiveness of such antibiotics [111].

23.1.5 Tobramycin

As tobramycin is given at 0200 h (night period), the CLT and AUC were much greater than that as tobramycin being administered at 1400 h (day period) [110].

23.1.6 Amikacin

In humans, amikacin revealed greater kel values in the day than that of the night.

23.1.7 Ceftriaxone

In rats, total clearance of ceftriaxone fluctuates throughout the day, peaking during dark (active) period and declining during the light (relaxation) period [110].

23.1.8 Ciprofloxacin

The proportion of ciprofloxacin removed in urine in people was higher when the antibiotic was administered at 1000 hours as while it was delivered at 2200 hours.

23.1.9 Valporic acid

At the absorption phase after oral treatment, mean absolute VPA levels in plasma were considerably greater in the daytime than the night. In the morning, Cmax was greater, tmax was shorter, and the absorption rate constant (ka) was larger than in the evening, despite no differences in other pharmacokinetic variables between the morning and evening trials.

23.1.10 Sumatriptan

Sumatriptan is the medicine of choice for migraine treatment, because migraine is an illness with symptoms that occur at regular intervals, chronotherapy may be helpful in addressing the problem. Following the 0700h injection, the mean peak serum concentration Following the administration at 7:00 a.m. and 01:00 pm, the mean area under serum concentration time curve from zero to the last time point (AUC0-t), the area under the serum concentration time curve from zero to infinity (AUC0-infinity), and the area under the first movement curve (AUMC) were considerably larger after the 1900 h treatment. The mean oral clearance and apparent volume of distribution were considerably lower after the 0700 h administration than after the 1900 h administration. The differences could be attributable to changes in the degree of absorption over time and/or changes in hepatic flow on a daily basis [112].

23.1.11 Cyclosporine

One study looked at the pharmacokinetics of cyclosporine in five pancreatic transplant patients. The reduced apparent clearance between the night time over the day time results in a somewhat enlarged area under the concentration-time curve in these patients. After the dusk treatment, there was a considerable delay in mean residence time, as well as the night time area under the moment curve was greater than the dawn value. We suggest three chrono pharmacokinetics dosing approaches that change the dusk dosage delivery schedule or transfer the regular amount to achieve equal cyclosporine exposure during activity and resting times. These trends and discrepancies point to the need for a more advanced time-dependent cyclosporine dosage strategy to balance dawn and dusk medication exposure and optimize immune suppression [113].

23.1.12 Methotrexate

Six children with leukemia were given a methotrexate dose at 10 in the morning and 9 in the night in a study. As a result, plasma clearance dropped significantly at night. The fundamental reason of these changes (among others) would be differences in passive tubular reabsorption caused by the urine pH rhythm. A strong diurnal regularity of methotrexate serum levels was reported in two pigs at 01:00 in the afternoon in another animal (4 Pigs) investigation [114].

23.1.13 Nsaid

Ketoprofen: When ketoprofen was given in the morning, the rate of absorption was likewise observed to be increased [115].

Indomethacin: When the medicine was administered at 07:00 or 11:00 h instead of 15:00, 19:00, or 23:00 h, the peak concentration was significantly greater and earlier [116].

24. CURRENT SITUATION AND FUTURE SCOPE

Pulsatile medication delivery is becoming more prevalent these days. The main benefit of this method of the medication delivery system is that the substance is only aired when it is required. Because of this, the risk of developing drug resistance, which is common in both preparations for both conventional and sustained release, is minimized. In addition, certain anticancer medications are quite hazardous. In both traditional and sustained release therapy, these medicines cause serious complications [117, 118, 119]. There are now a plethora of FDA-approved Chrono therapeutic medications on the market. This treatment is most useful when long-term effect is just not necessary and medications are harmful. The most important aspect of this formulation's development is determining the circadian rhythm, or a appropriate criterion that would set off the drug's release. Another issue is the lack of adequate rhythmic biomaterial that must be degradable, compatible, and rhythmically sensitive to certain biomarkers. Another significant issue is regulatory. It is challenging to demonstrate Chrono therapeutic benefits in clinical settings during the preapproval phase. The FDA now largely depends upon the establishment as well as execution of risk mitigation systems as a strategy for allowing pulsatile medication administration to uncover circadian rhythm with an appropriate system anywhere throughout the universe to be approved. Due to some unique characteristics such as low dumping of doses, medication adherence, as well as the preceding criteria, such administration becomes a pioneer technique to provide restorative drugs in the future.

25. CONCLUSION

Oral pulsatile delivery has gotten a lot of attention in the last decade, notably due to its propensity applicability for satisfying chronopharmaceutical needs connected to prevalent infection with varied circadian symptoms. The literature's description of a wide range of oral pulsatile delivery systems demonstrates the presences of a keen curiosity about the branch of pharmaceutics. True, the study of periodic rhythms in a growing variety of diseases, the convergence of Chrono therapeutics techniques, and a rising recognition of the importance of medication adherence are all probably to bolster endeavors in research in the organization, execution as well as assessment of these systems. However, for the proposed delivery systems to succeed, flexibility, novelty, a complete absence of stringent regulatory requirements, and the existence of human proof-of-concept the outcomes also seem to be believed to be critical. To ascertain the drug-delivery strategy, dosage, and delivery time, optimize favored and/or minimize negative impact, knowledge of I circadian period configuration as well as dials which govern this, (ii) cycles in disease mechanisms or there are 24 hour rhythms in disorder frequency of underlying health circumstances, and (iii) Chrono pharmacology (chronokinetics and chromodynamics) of treatments is typically required for disease chronotherapy.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Preparation And Bio Pharmaceutical Evaluation Of Chronopharmaceutical Drug Delivery System Of Metoprolol

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ABSTRACT

The basic purpose of constructing drug delivery systems is to design when and where the drug will be released. The episode of many biological events is really important for such knowledge. Metoprolol pulsatile drug delivery system was developed for this purpose, which can release the drug when blood pressure needs to be modulated in the early morning. The Cup and core techniques were used to build this system, which included immediate release (IR), sustained-release (SR), and a polycaprolactone plug layer. The formulation of the ingredients was facilitated by various preformulation studies. The IR and SR tablets were bilayered, with polycaprolactone entirely coating the IR layer. The IR and SR tablet release profiles were optimised for the F5 batch, which was then used to construct a pulsatile drug delivery system. Clinical trials were conducted with the prepared tablet, which included the use of BaSO4 tagged tablets for X-ray examinations. All of the findings indicated the optimal drug release of metoprolol, which can be used for individuals who are more prone to blood pressure abnormalities in the morning.

Keywords: Chronopharmaceutial, Metoprolol, BaSO4 labelled, Pulsatile Drug Delivery

1. INTRODUCTION

Time is an important variable in drug development and delivery. Biological processes have been shown to have cycles of various scales, ranging from very brief (ultradian) rhythms to rhythms with a period of about one day (circadian) to rhythms with longer cycles, such as a week, a month, or even longer. Endogenous biological clocks, or time keeping systems, generate these rhythms rather than being a passive response to environmental changes. As a result, there is an abundance of data of chronopharmacotherapeutics, ranging from basic chronobiology to clinical applications (chronotherapy). (1).

The chronotherapeutical is a unique medication delivery system that synchronises drug availability with disease-related rhythms. If disease symptoms appear at a given time of day or night, regular dosage forms are inadequate to meet the needs of the situation. The substance in a controlled release medication is released at a steady rate rather than as and when needed. In recent years, the chronopharmaceutical drug delivery system has become increasingly important in the treatment of chronic diseases such as hypertension, allergic rhinitis, rheumatoid arthritis, nocturnal asthma, osteoarthritis, peptic ulcer, and cancer. (2).

Pulsatile Drug Delivery System (PDDS) can be defined as the drug gets released after a particular lag time in relation to the circadian rhythm of the disease. Time-controlled pulsatile release (single or multiple unit system), internal stimuli induced release, and external stimuli induced pulsatile release systems are the three primary classes of PDDS based on the pulsed regulation of drug release. PDDS can also be divided into three categories based on the dosage form: capsules, pellets, and tablets. The ‘core and cup' tablet is suitable for the chronotherapeutics system in tablet dosage form. A appropriate polymer/polymer coating controls the release of medication in flux at a specific time after a lag. (3).

Capillary resistance and vascular reactivity are higher in the morning and decrease later in the day in cardiovascular diseases; platelet aggregation is increased, resulting in blood hypercoagulability; as a result of this action, the risk of myocardial infarction and sudden cardiac death is higher in the early morning. Heart attacks are five to six times more likely to strike between the hours of 1 and 5 a.m., and studies show that morning heart attacks are more severe than those that occur later in the day. Typically, cardiovascular disorders begin with high blood pressure, which is a key risk factor for coronary heart disease and ischemic and hemorrhagic stroke, as well as peripheral vascular disease, renal impairment, retinal haemorrhage, and vision impairment. Raised blood pressure is probably responsible for 7.5 million fatalities worldwide, or 12.8 percent of all deaths (4).

Many hypertension patients, especially those at high risk for cardiovascular disease, are prescribed beta-blockers as first-line therapy. They're also used to treat various heart problems like congestive heart failure and post-myocardial infarction. β -blockers have a greater impact on diurnal blood pressure reduction, which matches well with the circadian pattern in sympathetic tone. Rather than giving patients the maximum tolerable dose of -blocker on a continuous basis, it may be preferable to timing the β-blockade to coincide with the highest levels of sympatho-excitation. By permitting periods of reduced β-blockade during lower-risk periods, this may lead to the increased exercise tolerance in persons with chronotropic incompetence (inadequate heart rate response) (5). Metoprolol is an antihypertensive cardioselective competitive beta-1 adrenergic receptor antagonist with no intrinsic sympathomimetic action. Metoprolol works by blocking beta 1-adrenergic receptors in the myocardium, lowering the frequency and force of myocardial contraction and lowering cardiac output. This drug may also inhibit renin secretion, resulting in lower levels of angiotensin II, reducing vasoconstriction and aldosterone secretion (6). Metoprolol was classified as a ‘Class I’ drug in the Biopharmaceutics Classification System with high solubility and permeability.

The aim of the proposed work is to develop suitable ‘Pulsatile dosage form’ which falls under the category of Chronopharmaceutical drug delivery system’. Model drug chosen was Metoprolol with an objective of delivering drug during specific time of day or night and reducing the dosing frequency, minimizing adverse drug reaction and to improve patient compliance by enhancing the therapeutic efficacy of the drug.

2. MATERIALS AND METHODS

2.1. MATERIALS

The Pure Drug Metoprolol was purchased from Carbanio.com. Polymers and excipients such as Polycaprolactone, HPMC, Lactose, Sorbitol, Magnesium stearate and Talc was obtained from SD fine chemicals. Micro Crystalline Cellulose and Sodium Starch Glycolate were received from Lobachemie.

2.2. METHODS

2.2.1. Preformulation Studies

Preformulation is the primary level of product development where the physiochemical properties of the active pharmaceutical ingredient and excipients which has the effect on the product formulation and performance can be studied (7). The preformulation studies are follows

2.2.1.1. Analytical Method Development

Preparation of Calibration Curve

50mg of Metoprolol was dissolved in 50ml of methanol [stock-I (1000µg/ml)]. 1ml was taken from the above solution and made up with 10 ml of methanol [stock-II (100µg/ml)]. Using 0.2M Phosphate buffer (pH 6.8), the above stock-II solution was diluted, to obtain series of dilutions containing 2, 4, 6,8,10 µg/ml of solution. The absorbance of the above dilutions was measured at 220nm using UVSpectrophotometer taking 0.2M phosphate buffer (pH 6.8) as blank. Then a graph was plotted by taking Concentration on X-Axis and Absorbance on Y-Axis which gives a straight line Linearity of standard curve, which was assessed from the square of correlation coefficient (R^2).

2.2.1.2. Flowability Studies

Angle of repose

It is defined as, the maximum angle possible between the surface of the pile of the powder and the horizontal plane. Angle of repose measures the frictional force in a loose powder, in which fixed funnel method was used to measure the angle of repose.A funnel was fixed at a given height, above the graph sheet, which was placed on a flat horizontal surface. The blend was carefully pored through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius (r) of the base of the conical pile was measured (8). The angle of repose was calculated using the following formula:

$\theta = Tan^{-1}(h / r)$

Where,

h = Height of the cone

r = Radius of the cone base

Bulk density

Bulk density, is defined as the mass of the powder divided by the bulk volume and is expressed as gm/cm3. Particle size distribution, Particle shape and adherence of particles determine bulk density of powders. 10 gm powder blend was sieved and introduced into measuring cylinder, without compacting (9). Bulk density was calculated using,

Bulk Density = M / V_o

Where,

M = weight of sample

V_o = apparent volume of powder

Tapped density

Tapped density is defined as the mass of the powder divided by the bulk volume after the mechanically tapping. 10g of powder blend was placed in measuring cylinder and tapped for 100 times using mechanical tapped density tester (10). Tapped density was calculated using,

Tapped density = M / V

Where,

M = Weight of sample

V= Tapped volume of powder

Measures of powder compressibility

The Compressibility Index (Carr's Index) is a measure of the propensity of a powder to be compressed, which is determined using bulk and tapped densities. Inter particulate interactions are generally less significant in free flowing powders and bulk and tapped densities will be closer in value.

For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed (11). These differences are reflected in the Compressibility Index which is calculated using the following formulas:

Carr's Index = [(tap - b) / tap] × 100

Where,

b = Bulk Density

Tap = Tapped Density

Hausner's Ratio

Hausner's ratio expresses the flowability of the powders or granules (12). Hausner ratio was calculated by using the following equation:

Hausner Ratio = V_0/V_f

where, V_o = unsettled apparent volume (cm^3),

V_f = final tapped volume (cm^3)

2.2.1.3. Drug – excipient Compatibility Studies

Differential scanning Calorimetry

The thermograms of drug and excipients were recorded on model differential scanning calorimeter. An empty pan was used as a standard. About 1 gram of sample including pure drug (metoprolol), physical mixture (drug, excipients) were separately weighed and sealed in a small aluminium pan and

it was heated up to 20-400^{o}C at the heating rate of 10^{o}C/min with constant purging of dry nitrogen 50ml/min.For the reference, a blank pan was sealed and used. Using automatic thermal analyzer system, DSC thermogram was obtained. The DSC thermo gram obtained from this study was used for determining the compatibility between the drug and the excipients.

2.2.1.4. Solubility Studies

As per BCS guidelines, saturation solubility studies of MetaprololSuccinate was conducted. 25mg of Metoprolol Succinate was added to 250ml of each medium (0.1N HCl pH 1.2, Acetate Buffer pH 4.5, Distilled water pH 7.0 and Phosphate buffer pH 6.8) placed in 500ml Conical flask.

2.2.1.5. Melting Point Determination

Melting point determination helps in specifying the solid crystalline materials by thermal analysis. The melting point can be determined by using the melting point apparatus.

2.2.2. PREPARATION OF PULSATILE METOPROLOL TABLETS

2.2.2.1. Preparation of metoprolol core tablet

Table 2 shows the ingredients of a metoprolol tablet. Separately, all of the ingredients were sieved with no. 45. The geometric addition approach was used to combine metoprolol succinate, microcrystalline cellulose, sorbitol, and talc. The Core in Cup tablet was made as a bilayered tablet, with a prolonged release layer as the first layer and an immediate release layer with direct compression as the second layer. It was designed with the required amount of ingredients for the immediate and sustained release layers. An 8mm punch was used to punch the bilayered tablet.

To make a bilayered tablet, the immediate release layer was punched first with a low compression force, and then the sustained release layer was inserted and punched above it. Various batches of immediate and sustained release tablets have been developed in order to obtain an optimal drug release profile.

Table 1: Composition of Metoprolol Core Tablet

Ingredients	F1	F2	F3	F4	F5	F6
Immediate Release Tablet						
Metoprolol	14	14	14	14	14	14
Cross carmellose sodium	-	-	-	3	6	9
Sodium Starch Glycolate	3	6	9	-	-	-
MCC	10	10	10	10	10	10
Sorbitol	37.1	33.85	30.6	37.1	33.85	30.6

Talc	0.325	0.325	0.325	0.325	0.325	0.325
Magnesium Stearate	0.325	0.325	0.325	0.325	0.325	0.325
Sustain Release Polymeric Layer						
Metoprolol	12	12	12	12	12	12
HPMC	10	15	20	10	15	20
PVP in alcoholic solution	2.8%	2.8%	2.8%	-	-	-
MCC	60	55		60	57	50
Lactose	22	22	22	22	22	22
Talc	0.5	0.5	0.5	0.5	0.5	0.5
Magnesium Stearate	0.5	0.5	0.5	0.5	0.5	0.5
Coating						
Polycaprolatone	QS	QS	QS	QS	QS	QS

2.2.2.2. Coating of core in cup tablet

The drug was coated on a bilayer tablet to achieve pulsatile release. Polycaprolactone (hydrophobic polymer) was dissolved in chloroform to generate a viscous solution for the coating. This solution was used to spray coat the immediate release layer of a bilayered tablet on all sides, leaving the sustained release layer untreated.

2.2.3. EVALUATION OF PREPARED CORE TABLETS

2.2.3.1. Disintegration test

For tablets, the first important step towards drug dissolution is breakdown of the tablets into granules or primary powder particles, a process known as disintegration. All USP tablets must pass a test for disintegration, which is conducted *in vitro* using a disintegration test apparatus. The apparatus consists of a basket-rack assembly containing six open-ended transparent tubes of USP-specified dimensions, held vertically upon a 10-mesh stainless steel wire screen. During testing, a tablet is placed in each of the six tubes of the basket, and through the use of a mechanical device, the basket is raised and lowered in a bath of fluid (e.g. water, or as prescribed in the individual drug monograph) at 29 to 32 cycles per minute, the wire screen always below the level of the fluid.

2.2.3.2. Hardness test:-

Monsanto hardness tester was employed for this test. Force required to break the tablet is determined in this test. The tablet was placed between two pieces. The strength necessary to break the tablet was considered. The crushing strength test was executed on 8 tablets from the formulation.

2.2.3.3. Weight Variation Test

20 tablets were weighed individually and average weight was calculated. The tablet pass the U.S.P. test if no more that 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit.The weight variation was calculated and results were reported.

Average weight (mg) = Total Weight /20

2.2.3.4. Friability test

It is an important parameter to know whether the drug undergoes any capping, lamination etc during shifting of the products. Friability test helps in identifying the robustness of the tablets. The friability can be determined by

$$\text{Friability (\%)} = \frac{Initial\ weight - final\ weight}{Initial\ weight} \times 100$$

2.2.3.5. In vitro drug release studies

The core tablets were tested in vitro using a USP dissolution apparatus II at 37 ° C with 900 ml of phosphate buffer (pH 6.8) and a stirring rate of 50 revolutions per minute. The USP Dissolution device II was used to test in vitro drug release for immediate release, sustained release, and bilayered tablets (Paddle). The experiment was carried out with 900 ml of PBS 6.8 and a 37°C temperature. The dissolution medium and the paddle were set up with the formulation. The shaft was fixed and rotated at 50 revolutions per minute. At various time intervals, 1ml of sample was extracted from the basket. Following the removal of the sample, the same amount of PBS was replaced with new PBS. The filtered sample was measured with UV absorbance at 220nm. The following calculation was used to compute the cumulative percentage drug release.

Concentration (µg/ml) = Absorbance ± intercept × slope
Amount release (mg) = Concentration × Bath Volume × Dilution factor / 1000
Percentage drug release = Amount released × label claim
Cumulative release (%) = volume withdraw × PDR (t-1) + PDR Bath volume.
Whereas,
PDR = percentage drug release at time t
PDR (T-1) = previous percentage drug release at time t

2.2.4. Modeling of release profiles

The release data was treated according to Higuchi, korsmeyer-peppas, Hixson-crowell, and weibll models, as well as zero and first order patterns using add in DD solver software to establish Metoprolol release kinetics for the specified formulas.

2.2.5. Water uptake and erosion studies

Type 1 dissolution apparatus was used for the water uptake and erosion studies. Prior to the experiment, tablets and empty baskets were weighed. Individual tablets were withdrawn at regular

time intervals. To remove the excess liquid, basket and tablet were blotted and then reweighed. At 80°C, wetted tablets were dried until a constant weight is reached. Once after drying, tablets were allowed to cool in a desiccator and then reweighed. The experiment was done in duplicate. The extent of erosion, E (%), was determined as follows:

$E\% = 100 \times (W_i - W_f) / W_i$,

Where,

W_i - initial starting dry weight

W_f - final dry weight of the same dried and partially eroded tablet

The increase in weight (uptake) due to absorbed water A (%), was calculated at each time point using:

$A\% = 100 \times (W_w - W_f) / W_f$,

Where,

W_w - Weight of the wet tablet before drying.

W_f - Final dry weight of the same dried and partially eroded tablet.

2.2.6. Stability studies

Under both standard long-term and accelerated storage conditions, the selected formulations were assessed for stability according to the International Council of Harmonization (ICH) standards. Petri dishes made of polypropylene were used to hold the tablets. Half of the tablets were placed in a desiccator with anhydrous calcium chloride and stored at 25°C and 60% relative humidity for three months. For three months, the other half was stored in a stability cabinet (Climacell-MMM, Germany) set at 40°C and 75 % relative humidity. The tablets were tested for physical appearance and in vitro drug release at predetermined intervals.

2.2.7. Floating tests

An in-vitro experiment was carried out to estimate the floating time. The buoyancy of tablet formulations was examined in a 100ml beaker containing 80ml 0.1M Hydrochloric acid. The floating time, or the point at which the tablet of each formula begins to sink, was recorded. Each experiment was repeated three times. The image of the tablets was captured using a digital camera in each case.

2.2.8. Verification of tablets buoyancy in healthy volunteers:

After obtaining informed written consent, two healthy male volunteers took part in the study. The participants were 30 and 34 years old, with heights of 157 and 164 cm and weights of 57 and 63 kg respectively. The Nandha Medical College and Hospital Protection of Human Subjects Committee approved the study, and the methodology follows the hospital's ethical committee's requirements.

A full medical history, physical examination, and haematological and biochemical laboratory investigations were used to establish the health state of the volunteers. The participants were told not to take any medications for a week before and throughout the trial.

All other ingredients were preserved in the same quantities and one of the selected formulations was made X-ray opaque by replacing 30 mg of Sorbitol with barium sulphate (barium sulphate labelled tablets). This amount was discovered through experimentation to allow X-ray opacity without compromising tablet buoyancy. The alternate version, which has 40 mg barium sulphate in the cup, was x-ray opaque by default.

The volunteers were offered a low-calorie meal after fasting for the night. One F Tablet was given to one individual and one barium sulphate labelled F tablet was given to the second subject with 200 ml of water half an hour later.
Before and after drug delivery, the volunteers were subjected to abdomen X-ray imaging in a standing position. For all photos, the distance between the X-ray source and the subject was kept constant.

2.2.9. Statistical analysis

The data is presented as a mean standard deviation. The student's t-test was used for all statistical comparisons, and a p-value of 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1. Flowability studies

It is necessary to have good flow and compacting qualities for direct compression of materials. Angle of repose of 31-35 °, according to USP, indicates good flow quality. Fair flow is indicated by a Hausner ratio of less than 1.25 and a vehicles index of 16-20.

Metoprolol express the angle of repose of 20.22±0.24 – 27.12±0.22, showing excellent flow properties. Table 3 depicts the pre-compression parameters for Immediate Release Layer.

Table 2: Pre-compression Parameters for Immediate Release Layer

Batch	Angle of Repose (°)	Bulk density (g/cc)	Tapped density (g/cc)	Carr's Index (%)	Hausner's Ratio
F1	26.8	0.25	0.29	13.79	1.16
F2	25.6	0.25	0.28	10.71	1.12
F3	29.8	0.27	0.31	12.90	1.14
F4	25.1	0.26	0.30	13.33	1.15
F5	27.8	0.31	0.35	12.90	1.12
F6	26.2	0.25	0.28	10.71	1.12
F7	29.1	0.30	0.34	11.76	1.13
F8	28.5	0.26	0.29	10.34	1.11

Carr's index and Hausner ratio of metoprolol formulations were 10.34 to 13.79 and 1.11 to 1.16 respectively. This showed good flow properties of the formulation blend.

3.2. DSC Studies

The DSC investigations were carried out to see if metoprolol and excipients were compatible. The resulting thermogram clearly demonstrated that metoprolol and the excipients don't have any physical contact.

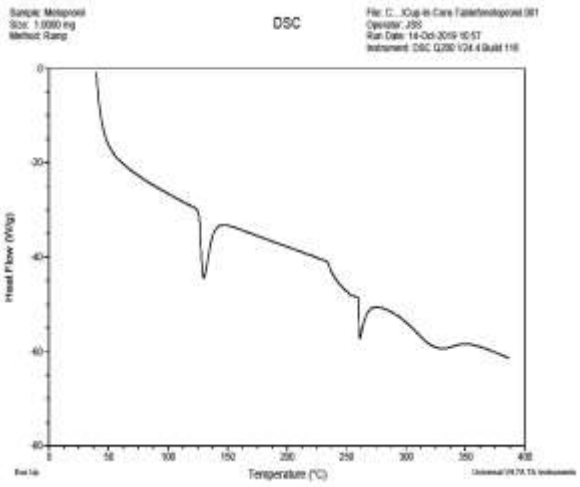


Fig.no.3.2.1: Metoprolol

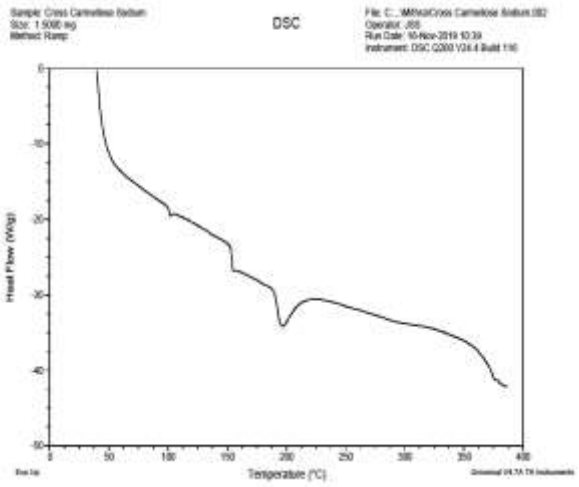


Fig.no.3.2.2: Cross carmellose

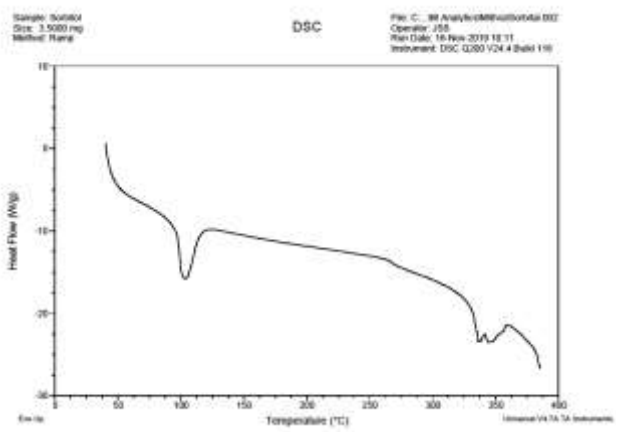


Fig.no.3.2.3: Sorbitol

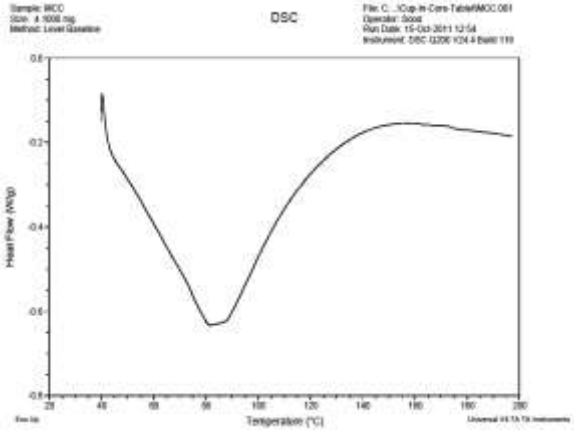
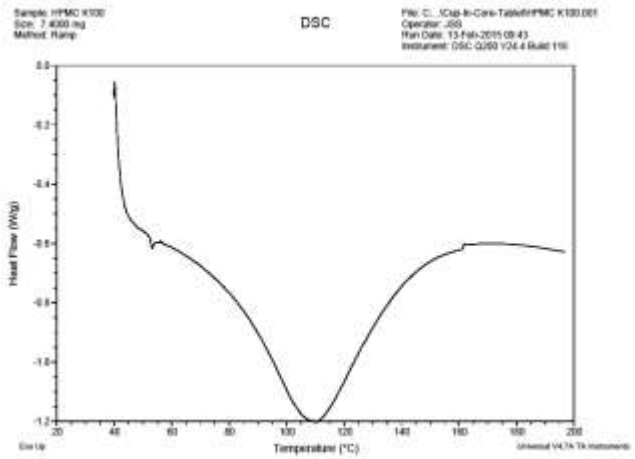


Fig.no3.2.4 :MCC



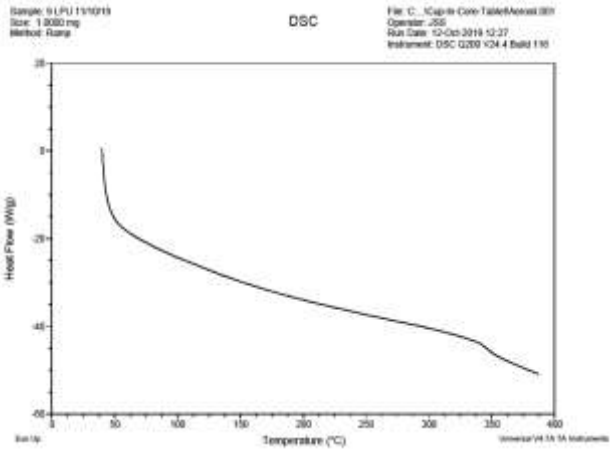
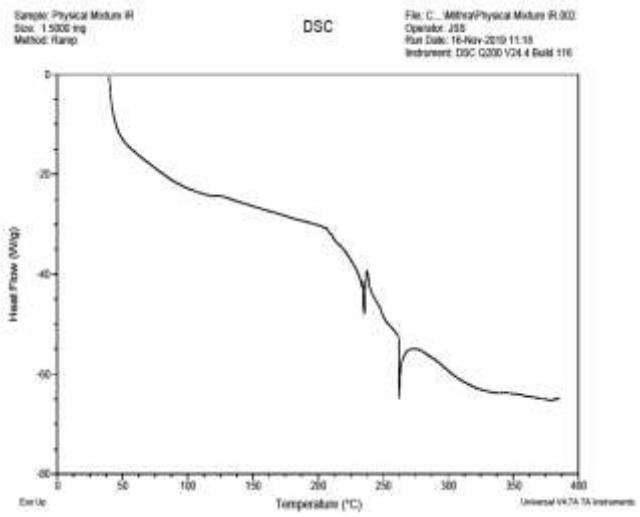


Fig. no.3.2.5: HPMC

Fig. no.3.2.6: Magnesium Stearate



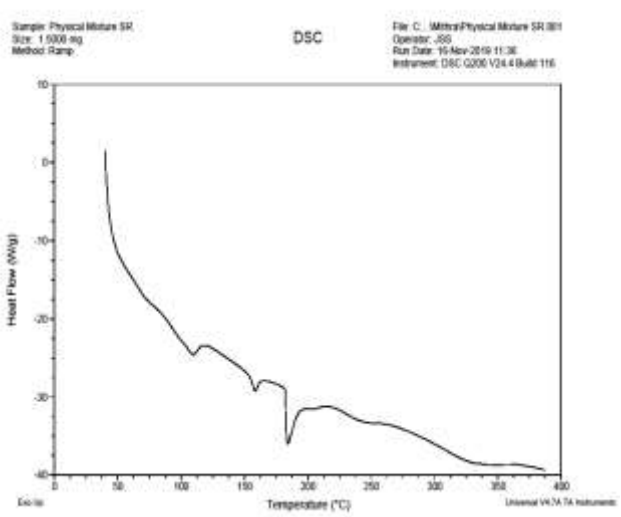


Fig.no.3.2.7: PM IR

Fig. no.3.2.8: PM SR

3.3. Solubility study

In 0.1 N HCl, phosphate buffer (pH 6.8), and distilled water, metoprolol solubility was 0.067, 42.7, and 0.18 g/L, respectively. These solubility values are associated with metoprolol's mildly acidic nature. In acidic media, it has low solubility, but in alkaline media, it has high solubility.

3.4. Evaluation of the prepared core tablet

All of the tests for core tablets that were conducted were successful. The thickness (3.48 ± 0.04mm) and diameter (6.07 ± 0.01mm) of the tablets were also consistent. The hardness of the material was determined to be 5.0 ± 0.3 kg/cm2. The percentage weight loss was 0.19 percent in terms of friability. The weight of 20 tablets was 164 ± 0.9mg on average. In all medium, the disintegration time of the core tablet formulation was instantaneous, but the disintegration period of the F5 formulation was less than 1 minute. As a result, it was determined that cup compression has no effect on the disintegration time of the core tablet.

3.5. Assay method validation

The linearity, inter-day precision, and intra-day precision data were used to validate the spectrophotometric assay's parameters.

Linearity:

Metoprolol showed a linear relationship in 0.1 N HCl & pH 6.8 over a concentration range of (1-3 mg%) in both media with a value of coefficient of determination (R^2) 0.998 & 0.9995 respectively.

PRECISION:

i) Inter-day Precision:

In 0.1 M HCl, % CV is not more than 5.13%,

In pH 6.8, % CV is not more than 6.44%.

ii) Intra-Day Precision:

In 0.1 M HCl, % is not more than 2.49%,

In pH 6.8, CV is not more than 1.81%.

No interference was reported from the other components of the tablets under the selected π_{max} in both media.

3.6. *In-vitro* drug release studies

In vitro release profile of metoprolol from the core tablet in 100mL.buffer with pH 6.8 for 3 hours at 37 ± 0.5® and 50rpm was conducted to investigate the release profile of metoprolol from the core tablet in a medium.

The release of metoprolol from the core tablet was compared from a core-in-cup formulation without the plug layer to see how the cup affected drug release. The difference in metoprolol release between the C and bilayer tablets was not statistically significant (P> 0.05). As a result, the EC cup has no effect on metoprolol release.

The plug layer, which is made up of polycaprolactone, absorbs water when the core-in-cup tablet comes into contact with the medium which leads to the polymer stretches and swells. With time, the plug swells and expands, providing a barrier that prevents liquids from coming into contact with the core tablet's surface. The type of polymer employed can have an influence on this technique. It's also possible that the plug layer's enlargement will destabilize the plug, causing it to erode or be removed over time. As a result, the rate at which the plug layer polymer erodes is controlled by the plug polymer's swelling and instability. Finally, the plug is totally eroded and water entry into the core increases dramatically, resulting in rapid medication release, depending on the properties of each polymer. Moreover, in the same formulation strategies, to provide a quick release following the lag period, CCNa was added as a superdisintegrant to the core formulation. CCNa reduces the time to disintegrate and improves the solubility of poorly soluble medicines. Priority and severe swelling are the two disintegration mechanisms linked with CCNa, with the latter being the most critical. When the percentage of CCNa does not reach the point where disintegration time is the least (7.6%), the swelling process takes over.

As will be demonstrated, the analysis of water uptake-erosion in the Polycaprolactone plug layer can fairly explain the behavior of the top plug layer. With the passage of time, the Polycaprolactone layer swelled and expanded rapidly, reaching its maximum water uptake (121.7%) after 2 hours. When the medium pH was changed from 1.2 to 6.8, there was a significant drop in water intake, as well as significant erosion. Polycaprolactone erosion accelerated from 10.4 ± 2.2 percent after 2 hours to 27.2 ± 1.9 percent after 2.5 hours. As a result, Polycaprolactone had a faster release after the lag time. It is clear that our findings are consistent with those of Efentakis et al.

The utilized HPMC grade, S Methocel®K4M, was distinguished by its viscosity, which was 4000cp. The gel layer was sufficiently resistant to widespread erosion even after hydration, which was followed by an increase in route length. As a result, drug release from the core-in-cup tablet technology does not match the pulsatile release rate profile.

After adjusting the pH from 1.2 to 6.8, the in vitro release rate of metoprolol was found to be the most convenient among previously examined tablet formulations, with an optimal lag period followed by rapid pulsatile drug release.

The marketed tablet had no lag time and 25 percent drug release after 2 hours followed by entire drug release after 3.5 hours. As a result, the core-in-cup system's delayed impact is clarified.

All of the generated core-in-cup tablets were floating due to the low density of the EC utilized (0.4 g/cm3). The suppression of floating of the F5 tablet formulation was necessary because these tablets had a long floating time (3 hours & 40 minutes, as evidenced by the results of the in vitro floating time measurement), which resulted in an increase in gastric retention and a delay in the arrival of the tablets to the absorption window. % metoprolol release from F5 in 0.1MHCl was about 17 percent during this floating duration (the drug release exceeded 10 percent & the lag time has ended before leaving the stomach). As a result, F5 tablets should be allowed to sink for 2 hours (lag time) before leaving the stomach.

Preliminary testing was conducted to determine the best high density material to be integrated into the EC cup to prevent pill drifting. ZnO and BASo4, which have densities greater than that of stomach contents (1.004 g/cm3), were used in 1:2, 1:2.75, 1:3, and 1:4 ratios to EC powder in the studies. The lowest possible ratio of 1:2.75 was chosen and integrated with the EC cup of f2 to generate an in-core-cup single pulse tablet formulation (f5), whose composition was displayed, thus limiting stomach retention of the tablets and hence accelerating the pulsatile release of metoprolo at 6.8 pH. After a 2-hour lag period in 0.1M HCl, F5 tablets released metoprolol quickly.

When a tablet is swallowed, it instantly sinks into the stomach's pylorus until gastric emptying (after 2-3 hours), as evidenced by X-ray radiography. No drug is released as long as the tablet is inside the stomach. Polycaprolactone rapidly erodes and metoprolol is released once the tablet moves from the stomach acidity to the higher pH of the duodenum (6.8), the metoprolol window of absorption.

To achieve good patient compliance and less frequent dosage ingestion, several multi-layered tablet designs are used to obtain two or more drug release pulses.

Metoprolol is released in two pulses by F5 tablet. The tablet floats in 0.1M HCl, and the first pulse is released immediately. The tablet is swept into the pH 6.8 (corresponding to the metoprolol absorption window) as soon as it descends, allowing the second pulse to be released.

To allow for an appropriate lag time between the two consecutive pulses, F5 must be floated. It's also crucial to avoid as early release of the second pulse, which could circumvent the duodenum's absorption window, lowering metoprolol bioavailability.

Floating lag time and duration of floating of F5 tablets were 76 ± 11 s and 162 ± 17 min respectively.

Finally, F5 is the met's chosen single pulse core-in-cup tablet information. After a lag time equal to the time it takes for the stomach to empty, the drug's pulse is released (2 hours). Meanwhile, metoprolol is released by F5 in two consecutive pulses with a fair lag time in between because of good floating property.

3.7. Modeling of release profiles

The value of R2-0.8503 indicated that metoprolol was released into the Korsmeyer-Peppas model. The “n” exponent value was employed by Peppas in 1985 to characterise the various release processes. The associated release mechanism is quasi-Fickain diffusion with partly chain relaxation regulated process, since the n value for F5 formulation is 0.0887.

Metoprolol's release fits into the Weibull model. The shape of the curve becomes sigmoidal with a turning point as b, the shape parameter, is >1 ;(3.679). Complex release is indicated by the sigmoidal curve form. A mechanism, since the rate of release does not alter in a predictable manner. In fact, the release rate rises nonlinearly until it reaches the inflection point, then falls asymptotically.

Table 3: *In-vitro* Drug Release of Immediate Drug Release

Time (hrs)	F1	F2	F3	F4	F5	F6
	% Cumulative Drug Release					
1	22.35	25.36	19.5	0.23	0.82	0.37
2	38.12	45.25	35.69	6.98	4.95	10.26
3	49.5	56.27	48.67	35.64	42.15	43.98
4	67.58	69.15	71.25	45.32	54.59	53.27
5	89.92	95.64	85.63	76.25	81.51	79.61
6	95.25	97.46	94.35	89.32	98.13	95.62

Table 4: *In-vitro* Drug Release of Sustain Drug Release

Time (hrs)	F1	F2	F3	F4	F5	F6
	% Cumulative Drug Release					
1	2.63	1.35	1.23	0.82	0.99	1.23
2	10.12	6.54	9.64	4.95	5.32	3.21
3	60.14	66.21	61.36	65.15	63.68	68.67
4	65.62	71.14	70.19	75.59	75.12	71.32
5	75.51	79.68	75.12	81.51	86.21	89.21
6	89.23	91.26	86.61	98.13	96.51	95.32

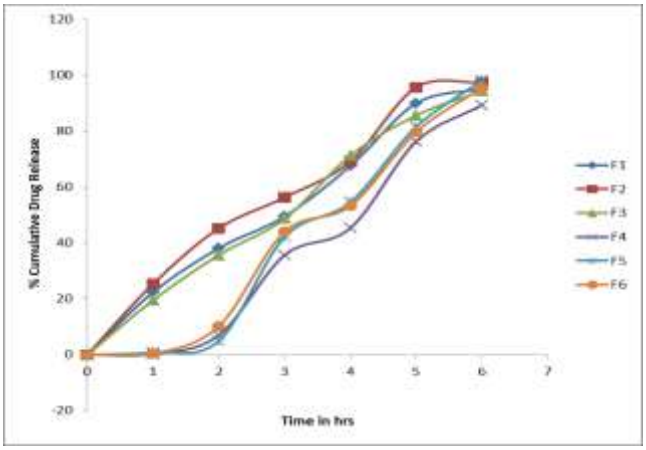


Figure. Release profile of Immediate Drug Release

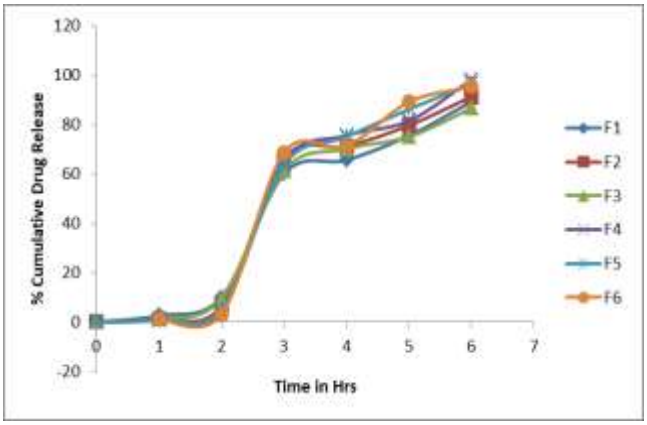


Figure. *In-vitro* Drug Release of Sustain Drug Release

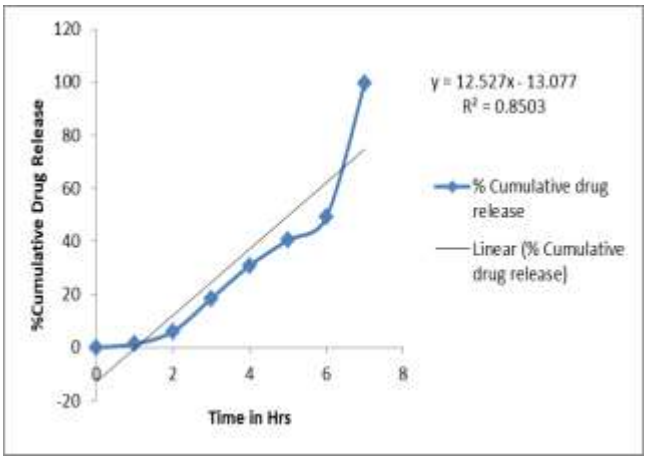


Figure 3: Invitro Drug Release of Bilayered Tablet

3.8. Water uptake and erosion studies

Since the Polycaprolactone plugged core-in-cup tablets of metoprolol showed the fastest pulsatile drug release after a lag time, a study of water uptake and Polycaprolactone was conducted. The amount of water absorbed and the amount of erosion of the Polycaprolactone layer are reflected in lag time value. When Polycaprolactone polymer is exposed to water, it swells and forms a viscous gel at the top layer surface, according to thorough observation. The maximum swelling was obtained during the trials, followed by disintegration of the polymeric layer.

When the pH of the medium was changed from 1.2 to 6.8, the Polycaprolactone layer showed a rapid liquid uptake with a maximum between 1.5 and 2 hours, followed by a rapid decline owing to erosion. After 3.5 hours, the erosion was complete (31 percent erosion, calculated as a percentage of the initial core-in-cup tablet weight). The inflated layer disintegrated quickly after 2 hours due to the low viscosity of Polycaprolactone.

3.9. Stability studies

F5 and F6 tablets were stored at 25 degrees Celsius and 60% relative humidity for 3 months (normal long-term storage settings) and 40 degrees Celsius and 75% relative humidity for 3 months (accelerated storage condition). After storage under both regular and accelerated settings, the rate of valsartan release from F5 did not alter significantly ($P > 0.05$).

Furthermore, during the study period, F5 and F6 did not demonstrate any significant changes in appearance. Under accelerated storage circumstances, there was a small plug layer swelling in both formulations at the conclusion of the study period, but it had no effect on the drug release profile ($P > 0.05$).

3.10. Verification of tablets buoyancy in healthy volunteers

Figure 8 shows radiographic images taken at various times after oral delivery of one tablet of each of the F5 and barium sulphate labelled B6 tablets. The high density $BaSO_4$ in the B6 tablet causes it to sink in the pylorus, as seen on the radiographs. The tablet went somewhat downwards after 2 hours, but it remained sinking in the pylorus until stomach emptying was complete. For the first 3 hours, the barium sulfate-labeled B6 tablet appears to be floating in the same position in the stomach. This could be due to the EC content's low density.

4. Conclusion

Promising pulsatile single pulse and floating double pulse core-in-cup tablets of metoprolol were successfully formulated. They provided a desirable lag time followed by rapid and complete drug release. This pulsatile drug delivery mechanism is clearly designed to release drugs in a chronological manner. For example, the sympathetic nervous system, heart rate variability, and the rapid rise in blood pressure have all been linked to the early morning hours. In this instance, pulsatile drug delivery techniques may be preferable. As a result, the medicine will only release when it is needed.

REFERENCE

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